2019

NORM
NORM-VET

Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

ISSN: 1502-2307 (print) / 1890-9965 (electronic)

Suggested citation: ***NORM/NORM-VET 2019. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2020. ISSN:1502-2307 (print) / 1890-9965 (electronic).***

This report is available at www.vetinst.no and www.antibiotikaresistens.no

CONTRIBUTORS AND PARTICIPANTS

Editors:

Gunnar Skov Simonsen	NORM, Univ. Hosp. North Norway	gunnar.skov.simonsen@unn.no	NORM, Univ. Hosp. North Norw.
Hege Salvesen Blix	Norw. Inst. of Pub. Health	hege.salvesen.blix@fhi.no	Norw. Inst. of Pub. Health
Kari Grave	Norwegian Veterinary Institute	kari.grave@vetinst.no	Norw. Vet. Inst.
Anne Margrete Urdahl	NORM-VET, Norwegian Veterinary Institute	anne-margrete.urdahl@vetinst.no	NORM-VET, Norw. Vet. Inst.

Authors:

Per Espen Akselsen	Antibiotic usage in humans	per.akselsen@helse-bergen.no	KAS, Haukeland Univ. Hosp.
Cecilie Torp Andersen	*Candida* spp.	ceanders@ous-hf.no	Oslo Univ. Hosp.
Hege Salvesen Blix	Antibiotic usage in humans	hege.salvesen.blix@fhi.no	Norw. Inst. of Pub. Health
Dominique Caugant	Gonococci and meningococci	dominique.caugant@fhi.no	Norw. Inst. of Pub. Health
Petter Elstrøm	MRSA in humans	petter.elstrom@fhi.no	Norw. Inst. of Pub. Health
Hege Enger	MRSA in humans	hege.enger@stolav.no	St. Olav Univ. Hosp.
Frode Width Gran	MRSA in humans	frode.gran@stolav.no	St. Olav Univ. Hosp.
Kari Grave	Antibiotic usage in animals	kari.grave@vetinst.no	Norw. Vet. Inst.
Einar Heldal	Tuberculosis	einar.heldal@fhi.no	Norw. Inst. of Pub. Health
Kari Olli Helgesen	Antibiotic usage in animals	kari.helgesen@vetinst.no	Norw. Vet. Inst
Petter Hopp	Antibiotic usage in animals	petter.hopp@vetinst.no	Norw. Vet. Inst.
Sigurd Høye	Antibiotic usage in humans	sigurd.hoye@medisin.uio.no	ASP, Univ. of Oslo
Gro Johannessen	Bacteria from food and feed	gro.johannessen@vetinst.no	Norw. Vet. Inst.
Aleksandra Jakovljev	Group B streptococci	aleksandra.jakovljev@stolav.no	St. Olav Univ. Hosp.
Umaer Naseer	Enteropathogenic bacteria in humans	mohammed.umaer.naseer@fhi.no	Norw. Inst. of Pub. Health
Marion Neteland	Antibiotic usage in humans	marion.iren.neteland@sav.no	KAS, Haukeland Univ. Hosp.
Madelaine Norström	Bacteria from animals, food and feed	madelaine.norstrom@vetinst.no	Norw. Vet. Inst.
Gunnar Skov Simonsen	Bacteria from humans	gunnar.skov.simonsen@unn.no	NORM, Univ. Hosp. North Norw.
Jannice Schau Slettemeås	Bacteria from animals, food and feed	jannice.schau-slettemeas@vetinst.no	Norw. Vet. Inst.
Marianne Sunde	Animal clinical isolates	marianne.sunde@vetinst.no	Norw. Vet. Inst.
Anne Margrete Urdahl	Bacteria from animals, food and feed	anne-margrete.urdahl@vetinst.no	NORM-VET, Norw. Vet. Inst.

Institutions participating in NORM-VET:

Norwegian Food Safety Authority	Kjell Hauge / Gerda Ingrid Heglebäck / Solfrid Åmdal
Norwegian Veterinary Institute	Agathe Vikre Danielsen / Gro Johannessen / Madelaine Norström/ Jannice Schau Slettemeås / Marianne Sunde / Anne Margrete Urdahl

Institutions participating in NORM:

Akershus University Hospital, Lørenskog, Department of Microbiology	Nora Nyquist / Marit Vattøy
Fürst Medisinsk Laboratorium, Oslo	Trond Egil Ranheim / Nina Beate Johansen
Førde Hospital, Department of Microbiology	Reidar Hjetland / Astrid Vedde
Haugesund Hospital, Department of Microbiology	Liv Jorunn Hafne / Pirrko-Liisa Kellokumpu
Haukeland Univ. Hospital, Bergen, Dep. of Microbiology	Paul Christoffer Lindemann / Helge Kolstad
Innlandet Hospital, Lillehammer, Department of Microbiology	Tine Nilsen Dons / Kari Ødegaard
Levanger Hospital, Department of Microbiology	Angela Kümmel / Berit Harbak
Molde Hospital, Department of Microbiology	Einar Nilsen / Haakon Espelund
Norwegian Institute of Public Health, Ref. Lab. for Enteropathogenic Bacteria	Mohammed Umaer Naseer / Ina Haagensen
Norwegian Institute of Public Health, Ref. Lab. for *M. tuberculosis*	Anne Torunn Mengshoel / Annika Reichman
Norwegian Institute of Public Health, Ref. Lab. for *N. gonorrhoeae*	Dominique Caugant / Lene Kolstad
Norwegian Institute of Public Health, Ref. Lab. for *N. meningitidis*	Dominique Caugant / Lene Kolstad
Nordland Hospital, Bodø, Department of Microbiology	Sandra Åsheim / Hege Elisabeth Larsen
Oslo University Hospital, Radiumhospitalet, Laboratory of Microbiology	Gorm Hansen / Gøril Aaslund
Oslo University Hospital, Rikshospitalet, Institute of Medical Microbiology	Jørgen Vilderhøj Bjørnholt / Marcela Zamudio
Oslo University Hospital, Rikshospitalet, Ref. Lab. for Mycology	Cecilie Torp Andersen / Aina Myhre
Oslo University Hospital, Ullevål, Department of Microbiology	Gaute Syversen / Thea Bergheim
Stavanger University Hospital, Department of Microbiology	Iren Løhr / Anita Løvås Brekken
St. Olav University Hospital, Trondheim, Department of Microbiology	Aleksandra Jakovljev / Alexander Husby Albertsen
St. Olav University Hospital, Trondheim, Ref. Lab. for MRSA	Hege Enger / Anette Skjærvik
St. Olav University Hospital, Trondheim, Ref. Lab. for *S. agalactiae*	Aleksandra Jakovljev / Randi Valsø Lyng
Sørlandet Hospital, Kristiansand, Department of Microbiology	Ståle Tofteland / Lise Hulløen-Orø
Unilabs Telelab A/S, Skien	Krisztina Papp / Anne Ragnhild Oseid
University Hospital of North Norway, Tromsø, Department of Microbiology	Karina Olsen / Elin Rydningen Elstad
University Hospital of North Norway, Nat. Adv. Unit on Detection of AMR	Ørjan Samuelsen / Bjørg C. Haldorsen
Vestfold Hospital, Tønsberg, Department of Microbiology	Åshild Marvik / Ann Kristin Berg
Vestre Viken - Bærum Hospital, Department of Medical Microbiology	Annette Onken / Harald Landa
Vestre Viken - Drammen Hospital, Department of Medical Microbiology	Einar Tollaksen Weme / Hanne Fanuelsen
Østfold Hospital, Kalnes, Department of Microbiology	Martin Steinbakk / Anne Cathrine Hollekim
Ålesund Hospital, Department of Microbiology	Einar Nilsen / Elisabeth V. Hjelle

NORM reference group in 2019:

Didrik Frimann Vestrheim	Norw. Inst. Pub. Health	Kjersti Wik Larssen	St. Olav Univ. Hosp.
Heidi Cecilie Villmones	Vestfold Hosp. Trust	Aasmund Fostervold	Norw. Soc. Med. Microbiol.
Brian Guennigsman	Norw. Soc. Engineers and Technologists	Jon Birger Haug	Norw. Soc. Inf. Dis.
Linda Rui	Norw. Coll. Gen. Pract.		

CONTENTS

INTRODUCTION

Antimicrobial resistance is an emerging problem worldwide. It reduces the effectiveness of antimicrobial treatment of infectious diseases in humans and animals thereby leading to increased morbidity and mortality, as well as higher costs. It is well established that there is a strong association between the usage of antimicrobial agents and the occurrence of resistance. The selective pressure exerted following use of antimicrobial agents is a key issue in the epidemiology of resistance. Moreover, resistance can be disseminated through the spread of resistant pathogenic bacteria themselves or by horizontal transfer of resistance genes from one type of bacteria to another. Such transfer is not limited to closely related bacteria; it can also take place between bacteria of different evolutionary origins and/or ecological niches. Thus, antimicrobial drug usage and resistance in one ecological compartment can have consequences for the occurrence of resistance in another compartment. When addressing antimicrobial resistance – the occurrences, causes, consequences and preventive measures – a holistic approach is needed, encompassing both data on usage and resistance in human and veterinary medicine, as well as microbes in the food production chain.

In response to the growing concern about antimicrobial resistance, the Norwegian Ministry of Health and Social Affairs issued a national action plan against antimicrobial resistance in March 2000. The importance of monitoring the human and animal health sectors as well as food production, was emphasised. The action plan recognised the need for ongoing surveillance as a fundamental component of the strategy. The NORM and NORM-VET programmes were consequently established in order to provide and present data on the occurrence and distribution of antimicrobial resistance over time. The national action plan formally expired by the end of 2004. However, the need for continued surveillance of both resistance and antimicrobial usage was emphasised at subsequent consultations and an integrated national strategy for prevention of infections in the health service and antibiotic resistance (2008-2012) was issued in the summer of 2008. Following the renewed effort of the WHO in recent years, the Norwegian government launched a new national strategy (2015-2020) in June 2015 including an explicit target of 30% reduction in antibiotic consumption in human medicine by 2020 compared to 2012. For food-producing terrestrial animals and companion animals the target is 10% and 30% reduction in the usage, respectively, by 2020, with 2013 as reference year. Additional specific targets in the food production chain are that livestock associated MRSA will not be established in the Norwegian pig population, and that ESBL in the poultry production will be reduced to a minimum. Mapping of reservoirs of antimicrobial resistant bacteria will also be carried out in the most relevant animal populations and plants important to food safety. As the present strategy will expire this year, the government has initiated the process to develop a new framework for the coming years.

The NORM surveillance programme for antimicrobial resistance in human pathogens was established in 1999 and is coordinated by the Department of Microbiology and Infection Control at the University Hospital of North Norway in Tromsø. The NORM-VET monitoring programme for antimicrobial resistance in animals, food and feed was established in 2000 and is coordinated by the Norwegian Veterinary Institute commissioned by the Norwegian Food Safety Authority. The NORM/NORM-VET reports also present data on the usage of antimicrobial agents in humans and animals in Norway. The NORM and NORM-VET programmes are valuable tools for setting policies, assessing risks and evaluating interventions.

This report, which is the twentieth annual joint report from NORM and NORM-VET, presents data on resistance and usage for 2019. The editors would like to thank all those who contributed to data collection and the writing of this report, for excellent work.

Tromsø / Oslo, September 2020

SAMMENDRAG

Dette er den tyvende felles rapporten fra Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM) og Norsk overvåkingsprogram for antibiotikaresistens i mikrober fra fôr, dyr og næringsmidler (NORM-VET). Rapporten presenterer data om forekomst av antibiotikaresistens og forbruk av antibiotika til mennesker og dyr i 2019. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkingsprogrammene, presenteres også.

NORM og NORM-VET ble etablert som deler av Regjeringens tiltaksplan mot antibiotikaresistens som ble offentliggjort i 2000. NORM koordineres av Avdeling for mikrobiologi og smittevern, Universitetssykehuset Nord-Norge i Tromsø. NORM-VET koordineres av Veterinærinstituttet. Programmene utgir en felles årsrapport.

Forbruk av antibiotika til dyr

I 2019 utgjorde salget av antibakterielle veterinærpreparater til landdyr totalt 5008 kg, som er en nedgang på 3 % sammenlignet med 2018.

Salget av antibakterielle veterinærpreparater til matproduserende landdyr, inkludert hest, var på 4673 kg. Data rapportert til Veterinært legemiddelregister (VetReg) viser at til gris, storfe, sau, geit og fjørfe ble det i all hovedsak brukt penicilliner og av disse var det nesten utelukkende beta-laktamasefølsomme penicilliner (benzylpenicillin-prokain) som ble benyttet. Fra 2013 til 2019 var det en nedgang i salget av antibakterielle veterinærpreparater som i hovedsak benyttes til de viktigste matproduserende artene (storfe, gris, sau, geit og fjørfe), på 21 % målt i kg aktivt stoff. Når salget relateres til dyrepopulasjonen, var nedgangen i forbruket 18 %.

Salget av antibakterielle veterinærpreparater som kan benyttes til flokkbehandling, er fortsatt lavt; i 2019 representerte salg av slike preparater 4 % av totalsalget. Til hest ble det i hovedsak brukt trimetoprim-sulfa (oralpasta). Forbruket av veterinære antibakterielle midler til oppdrettsfisk (forbruk til rensefisk inkludert) var fortsatt svært lavt i 2019 og utgjorde 222 kg. Dette representerer en nedgang på over 99 % sammenlignet med 1987 da forbruket var på sitt høyeste. I 2019 ble det foretatt behandling med antibiotika av laks og regnbueørret i 1,3 % av sjølokalitetene.

Til kjæledyr (hund og katt) ble det i 2019 solgt 335 kg veterinære antibakterielle midler. Dette er en nedgang på 37 % sammenlignet med 2013. Data rapportert til VetReg for perioden 2015-2019 viser en gradvis reduksjon av forskrivningen av antibakterielle humanpreparater til hund og katt, noe som indikerer at redusert salg av veterinære antibakterielle midler ikke har blitt erstattet med forskrivning av antibakterielle humanpreparater.

Det Europeiske legemiddelbyrået (EMA) har anbefalt å begrense bruken av enkelte antibakterielle midler til dyr på grunn av den potensielle risikoen for folkehelsa, som 3.-4. generasjon cefalosporiner, kinoloner (fluorokinoloner og andre kinoloner) og polymyksiner. I Norge er salget av slike antibakterielle midler til dyr svært lavt og omfatter bare kinoloner.

Narasin ble faset ut som fôrtilsetningsmiddel til slaktekylling sommeren 2016. Bruken av antibiotika til behandling av slaktekylling er fortsatt svært lavt; i 2019 ble det foretatt behandling i < 0,1 % av slaktekylling-flokkene, og det ble kun brukt beta-laktamasefølsomme penicilliner.

Forbruk av antibiotika hos mennesker

Siden 2012 har det vært nedgang i den totale antibiotikabruken, men i 2019 ble det observert en liten økning. Bruken er gått ned med 22 % siden 2012. Med total antibiotikabruk mener vi her alt salg i Norge av antibakterielle midler til systemisk bruk hos mennesker (J01 ekskl. metenamin) dvs. i primærhelsetjenesten og til institusjoner. Det totale salget var 13,2 definerte døgndoser (DDD)/1000 innbygger/døgn i 2019. Dette er på samme nivå som i 2017. Andelen smalspektrede penicilliner (J01CE) var stabil og utgjør 27 % av totalt salg (J01, ekskl. metenamin). Salg av metenamin var kraftig redusert i 2019 på grunn av mangelsituasjon i vårsemesteret.

Rundt 84 % av totalt antall DDD av antibakterielle midler brukes i primærhelsetjenesten, dvs. utenfor helseinstitusjoner. I 2019 var penicilliner (J01C) mest brukt i primærhelsetjenesten; 54 % av alle DDD for antibakterielle midler til systemisk bruk (J01, ekskl. metenamin), etterfulgt av tetracykliner, J01A (26 %). De tre hyppigst brukte antibiotika i 2019 var fenoksymetylpenicillin, doksycyklin og pivmecillinam. Disse tre representerte 50 % av alle forskrevne resepter og 54 % av alle solgte DDD. Tannleger forskriver rundt 5% av alle DDD i primærhelsetjenesten.

Antibiotikasalg (i DDD) til sykehus utgjorde 8 % av totalt salg av antibakterielle midler til mennesker i 2019. I norske sykehus ble det gjennomsnittlig brukt 75 DDD/100 liggedøgn i 2019. Dette er en økning på 12 % siden 2012. Forbruket målt som DDD/sykehusinnleggelse (i 2019; 3,1 DDD/innleggelse) økte med 3 % i samme periode. Terapimønster av antibakterielle midler i sykehus endres ikke mye fra et år til et annet, men det er en tydelig trend til mer bruk av antibiotika anbefalt i retningslinjene. Bruken av bredspektrede antibiotika er redusert med 16 % fra 2012 (målt i DDD/100 liggedøgn). I sykehus ble penicilliner (J01C) mest brukt (ca halvparten av bruken målt i DDD), mens cefalosporiner er den nest største antibiotikagruppen med 17 % av alle DDD. Det er store variasjoner mellom sykehus, både målt i volum (DDD/100 liggedøgn) av antibiotika og i terapiprofil. Variasjonene kan ikke forklares med forskjeller i aktivitet eller pasientsammensetning alene.

Resistens hos kliniske isolater fra dyr

I 2019 ble det undersøkt kliniske isolater fra infeksjoner med *Escherichia coli* (både urinveisinfeksjon og andre infeksjoner), *Staphylococcus pseudintermedius* og *Streptococcus canis* hos hund. Dette var første gang *S. canis* ble undersøkt, og vurdering av resultatene må gjøres med forsiktighet. Nedsatt følsomhet for erytromycin, klindamycin og azithromycin var mest vanlig. Hos *E. coli* fra urinveisinfeksjoner var 70,8 % fullt følsomme, mens bare 46,5 % av isolatene fra andre typer *E. coli* infeksjoner var fullt følsomme for de antibiotika det ble testet for. Resistens mot ampicillin, kinoloner og tetracyklin var det

vanligste. Blant de undersøkte *S. pseudintermedius*, var kun 17,2 % fullt følsomme. Resistens mot benzylpenicillin, sulfamethoxazol, tetracyklin og fusidinsyre var det vanligste funnet. Det var en høyere andel av resistens hos kliniske isolater av *E. coli* og *S. pseudintermedius* enn i tilsvarende isolater fra friske bærere (se under).

Resistens hos indikatorbakterier fra dyr, fôr og mat

Resultatene fra 2019 bekrefter at situasjonen i Norge er god med tanke på antibiotikaresistens hos bakterier fra dyr, fôr og mat. Forekomsten av multiresistens (resistens mot ≥ 3 antibakterielle klasser) og spesielle resistente bakterier/ resistensmekanismer av særlig interesse er fremdeles lav.

NORM-VET følger de krav til overvåking av antibiotikaresistens som er satt i EU-regelverket (2013/652/EU). *E. coli* og *Enterococcus* spp. benyttes som indikatorbakterier, dvs. sensitivitetstesting av *E. coli og Enterococcus* spp. benyttes som indikator for forekomst av antibiotikaresistens i bakteriepopulasjonen. I tillegg overvåkes/ kartlegges bakterier og resistensformer ut i fra nasjonale hensyn. Selektive metoder benyttes til overvåking av *E. coli* som er resistente mot ekstendert-spektrum cefalosporiner (ESC), kinolonresistente *E. coli* (QREC), karbapenemaseproduserende *Enterobacteriaceae* (CPE), kolistinresistente (COL-R) *E. coli*, vankomycinresistente *Enterococcus* spp. (VRE), meticillinresistente *Staphylococcus aureus* (MRSA), og *S. pseudintermedius* (MRSP).

I 2019 ble det undersøkt blindtarmsprøver fra storfe under ett år og slaktegris, samt avføringsprøver fra geit og hund for isolering og sensitivitetsundersøkelse av *E. coli* og *Enterococcus* spp., samt isolering av ESC resistente *E. coli*, CPE og COL-R *E. coli.* Fra besetninger med geit ble nesesvabre og miljøprøver undersøkt for MRSA. Hos hund ble svabre fra munn-/neseslimhinne/perineum benyttet for isolering av både MRSA, *S. pseudintermedius* og MRSP. Av prøver fra mat ble det undersøkt storfe- og svinekjøtt, samt bladsalat og krydderurter. Rått hundefôr ble også undersøkt.

Svært få *Enterococcus* spp. ble isolert fra storfe, og kun tetracyklinresistens ble påvist fra disse. Tetracyklinresistens var også det eneste som ble funnet i *E. faecalis* fra gris (29 av 46 isolater), og var mest vanlig forekommende hos de resistente *E. faecium*. Majoriteten (> 90 %) av *E. coli* fra storfe og gris, samt alle de 62 isolatene fra geit, var fullt følsomme. Resistens mot sulfamethoxazol, trimetoprim (gris), tetracyklin og ampicillin var mest vanlig. Kun 0,3 % av isolatene fra storfe og 2,9 % fra gris var multiresistente. Resultatene for storfe er i samsvar med resultatene fra tidligere år, mens andel av *E. coli* isolater som er fullt følsomme har økt siden 2015 (fra 78,9 % i 2015 og 83,6 % i 2017, til 90,9 % i 2019).

Forekomsten av QREC hos geit var 1,7 %, noe som indikerer en noe lavere forekomst hos geit enn hos de andre drøvtyggerne hvor forekomsten er ~7 %.

ESC resistente *E. coli* ble påvist fra 4,4 % av prøvene fra storfe, ikke fra noen av geiteprøvene, og fra 18,9 % av prøvene fra gris. Kun hos fire av de totalt 14 storfeisolatene var resistensen mot ESC forårsaket av plasmidbårne gener (ESBL fenotype, genotype $bla_{\text{CTX-M-55}}$). Blant de ESC resistente *E. coli* isolatene fra gris, hadde fem ESBL fenotype med genotype $bla_{\text{CTX-M-15}}$, to av disse hadde også $bla_{\text{TEM-1b}}$. Det har vært en økning av ESC resistente *E. coli* hos storfe siden 2015. En økning kan også sees hos gris disse årene, men den er ikke statistisk signifikant. Denne økningen hos storfe og gris er hovedsakelig på grunn av isolater med kromosomale mutasjoner. Det er fortsatt lav forekomst av ESC resistente *E. coli* med ESBL fenotype, men det observeres en økning i forskjellige varianter av plasmidbårne gener, noe som kan indikere at disse spres i storfe- og grisepopulasjonene.

Funn av MRSA i den norske dyrepopulasjonen er sjelden. Ingen av de 94 undersøkte geitebesetningene var positive for MRSA. Kun én besetning ble funnet positiv for MRSA CC398 i det årlige MRSA overvåkingsprogrammet av MRSA hos gris. Ytterligere syv besetninger ble funnet positive ved kontaktsporing eller undersøkelser av andre grunner.

Fra avføringsprøver fra hund, var 59,7 % av de isolerte *E. faecalis* og 85,3 % av *E. faecium* fullt følsomme. Det var imidlertid kun 34 *E. faecium* isolater. Resistensen hos *E. faecalis* var hovedsakelig forårsaket av resistens mot tetracyklin. Blant *E. coli*, var 86,8 % fullt følsomme. Resistens mot ampicillin var vanligst, fulgt av resistens mot sulfamethoxazol og tetracyklin. Kun 3,7 % av isolatene var multiresistente. ESC resistente *E. coli* ble kun påvist hos 1,3 % av hundene. Hos to av disse tre isolatene var resistensen mot ESC forårsaket av plasmider (ESBL fenotype, hhv. genotypene $bla_{\text{CTX-M-1}}$ / $bla_{\text{OXA-1}}$, og $bla_{\text{CTX-M-55}}$ / $bla_{\text{TEM-1B}}$). QREC ble påvist hos 8,2 % av hundene. Noen av disse QREC-isolatene var multiresistente, og resistens opp mot hele syv antimikrobielle klasser ble påvist.

S. pseudintermedius isolert fra de samme hundene ble også sensitivitetstestet. Av disse var 21,1 % av isolatene fullt følsomme. Resistens mot sulfamethoxazol var det vanligste, fulgt av resistens mot benzylpenicillin, fusidinsyre og tetracyklin. Det ble ikke påvist MRSA og MRSP med selektive metoder fra hundene. Det er en lavere andel av resistens hos både *E. coli* og *S. pseudintermedius* isolater fra friske bærere enn i tilsvarende isolater fra syke hunder (se over).

Fra rått hundefôr, var 69,6 % av *E. faecalis* og 83,9 % av *E. faecium* fullt følsomme. Resistens mot tetracyklin var mest vanlig forekommende hos *E. faecalis*, kun 1,8 % var multiresistente. VRE ble ikke påvist. Over 80 % av de 65 undersøkte *E. coli* var fullt følsomme, men fire av de resistente *E. coli* isolatene ble kategorisert som multiresistente. ESC resistente *E. coli* ble påvist fra 4,0 % av prøvene med selektiv metode, og kun en av de tre isolatene hadde en ESBL fenotype forårsaket av plasmidbårne gener ($bla_{\text{CTX-M-15}}$). QREC ble isolert fra 17 av 65 prøver, og blant disse var seks isolater multiresistente med resistens mot inntil syv antibakterielle klasser. Ingen COL-R *E. coli* eller CPE ble påvist.

I prøver fra mat ble det ikke påvist ESC resistente *E. coli* fra svinekjøtt, og kun fra 0,9 % av prøvene av storfekjøtt. De tre isolatene hadde en ESBL fenotype, henholdsvis genotypene $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-1}}$/$bla_{\text{CTX-M-36}}$/$bla_{\text{TEM-1b}}$ og $bla_{\text{CTX-M-1}}$/$bla_{\text{TEM-1b}}$. Ingen kjøttprøver var positive for CPE. Undersøkelsene av bladsalat og krydderurter påviste hverken ESC resistente *E. coli*, CPE eller COL-R *E. coli* i 2019. Antall prøver per kategori (importert / norskprodusert bladsalat, og importerte krydderurter) er imidlertid lav, og resultatene fra 2017, 2018 og 2019 bør derfor sees under ett. Majoriteten av indikator *E. coli* isolatene var fullt

følsomme, og kun noen få isolater var multiresistente. Totalt fem ESC resistente *E. coli* isolater ble påvist disse årene, alle var fra importerte produkter, og alle hadde ESBL fenotype med genotypene *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{CTX-M-15}}$ og *bla*$_{\text{SHV-12}}$. Noen isolater hadde også plasmidbåren kinolonresistens, samt plasmidbåren kolistin-resistens. Sammenlikning mellom de forskjellige kategorier bør gjøres med forsiktighet. Imidlertid viser resultatene at importerte bladgrønnsaker og urter kan være kontaminert med spesielle resistensmekanismer som ikke er vanlig å påvise fra produksjonsdyr i Norge eller norskprodusert mat.

CPE har ikke blitt påvist fra dyr eller mat i Norge. Dette gjelder også for 2019.

Resistens hos zoonotiske bakterier og andre enteropatogene bakterier

Zoonosebakterier isolert fra dyr, fôr og mat

Den norske husdyrpopulasjonen er regnet som fri for *Salmonella*. I 2019 ble det sensitivitetstestet åtte *Salmonella* isolater fra henholdsvis kalkun, kylling, hund, svin, tre katter, og rått hundefôr. Alle isolatene ble vurdert som fullt følsomme.

Blant *Campylobacter coli* fra gris, var 53,4 % fullt følsomme. Resistens mot streptomycin var mest vanlig (41 %), fulgt av resistens mot kinoloner (15,7 %). Redusert følsomhet for erytromycin og gentamicin ble ikke påvist. Ni patogene *Yersinia enterocolitica* isolater fra kjøttdeig av svin ble sensitivitetstestet. Alle isolatene var fullt følsomme.

Kliniske isolater av tarmpatogene bakterier fra mennesker

Etter omorganiseringen av Referanselaboratorium for Enteropatogene Bakterier (NRL) ved Folkehelseinstituttet (FHI), og det midlertidige opphøret i følsomhetstesting for antimikrobiell resistens i 2018, har NRL gjenopptatt anti-mikrobiell følsomhetstesting for de enteropatogene bakterier for 2019.

Hos *Salmonella* Typhimurium og den monofasiske varianten av *S.* Typhimurium var det totale resistensnivået høyere for reiseassosierte stammer sammenlignet med innenlands ervervede stammer. Antimikrobiell resistens var høyest blant *Salmonella* Typhi, med en økende trend av resistens mot ciprofloxacin, tetracyklin, kloramfenikol og utvidet spektrum cefalosporiner. Multiresistens (MDR) var også en karakteristisk egenskap hos et betydelig antall av *S.* Typhi stammer (54%). Seks *Salmonella* isolater ble karakterisert som ESBL-produserende.

Hos *Campylobacter jejuni* var det totale resistensnivået mot ciprofloxacin og tetracyklin høyere for reiseassosierte stammer sammenlignet med innenlands ervervede stammer. En fortsatt oppadgående trend i resistens mot ciprofloxacin og tetracyklin for innenlands ervervede stammer ble observert.

For *Shigella sonnei* ble det observert en økende trend av resistens mot cefalosporiner med utvidet spektrum og ciprofloxacin. Seksten *Shigella* spp. ble bekreftet som ESBL produserende. Antimikrobiell resistens i *Yersinia enterocolitica* er fortsatt lav.

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterie-isolater fra mennesker var fortsatt lav i 2019. Det ble påvist 12 tilfeller av methicillinresistente *Staphylococcus aureus* (MRSA) blant 1492 blodkulturisolater (0,8 %) som ble inkludert i NORM-protokollen. Dette samsvarer godt med tall fra laboratorienes datasystemer som rapporterte 19 MRSA-isolater blant 2158 *S. aureus* (0,9 %) fra blodkultur og spinalvæske i 2019. Andelen er på samme nivå som i 2017 og 2018 (0,8 % begge år). Meldesystemet for infeksjonssykdommer (MSIS) registrerte 945 tilfeller av MRSA-infeksjon i 2019 mot 763 i 2017 og 905 i 2018. De fleste tilfellene var fra pasienter med overfladiske sårinfeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus* isolater fra sårprøver (13 av 1014; 1,3 %) slik de har gjort i tidligere år (1,2 % i 2017 og 1,7 % i 2018). MSIS registrerte videre 1499 tilfeller av MRSA-kolonisering i 2019 mot 1529 i 2017 og 1631 i 2018. I alt ble det meldt funn av MRSA hos 2444 personer i 2019. Dette utgjør en insidensrate på 46/100 000 personår mot 48/100 000 i 2018. Overvåkingen viser at det totale antallet MRSA-registreringer er stabilt. Det påvises fortsatt svært få alvorlige MRSA-infeksjoner. En høy andel er smittet i utlandet, og det påvises svært få tilfeller av landbruks-assosiert MRSA.

Blodkulturisolater av *E. coli* viste stort sett uendret forekomst av resistens mot bredspektrede antibiotika i 2019. Forekomsten av gentamicinresistens var 5,9 % i 2019 sammenliknet med 7,0% i 2017 og 5,4 % i 2018, mens forekomsten av resistens mot ciprofloxacin ble redusert fra 11,7 % i 2018 til 11,3 % i 2019. *Klebsiella* spp. har fortsatt lavere forekomst av resistens mot gentamicin (4,4 %) og ciprofloxacin (6,1 %) enn *E. coli*, men forskjellen er mindre enn tidligere.

Produksjon av bredspektrede beta-laktamaser (ESBL) er blitt et utbredt problem i mange land, og forekomsten har også vært økende i Norge. Til sammen 167/2350 *E. coli* (7,1 %) og 58/1017 *Klebsiella* spp. (5,7 %) fra blodkultur ble rapportert som ESBL-positive i 2019. Forekomsten er svakt økende for *E. coli* (6,6 % i 2017 og 6,5 % i 2018) men stabil for *Klebsiella* spp. (5,3 % i 2017 og 6,6 % i 2018). Andelen av ESBL-positive isolater var fortsatt høyere blant *E. coli* fra blodkulturer (7,1 %) enn fra urinprøver (3,0 %). Det ble funnet lav forekomst av resistens mot beta-laktam antibiotika (3-5 %) og aminoglykosider (1-2 %) blant *Pseudomonas aeruginosa* isolater fra blod og urin.

Karbapenemaseproduserende *Enterobacterales* (CPE), *Pseudomonas aeruginosa* og *Acinetobacter* spp. har vært meldepliktige til MSIS siden juli 2012. Antallet pasienter meldt med CPE økte fra 54 i 2018 til 75 i 2019, og antallet pasienter med karbapenemaseproduserende *P. aeruginosa* (n=5) og *Acinetobacter* spp. (n=23) var også svakt økende.

Det ble ikke gjennomført overvåking av resistens hos systemiske isolater av *Haemophilus influenzae* i 2019 på grunn av begrenset kapasitet på referanselaboratoriet ved Nasjonalt folkehelseinstitutt (FHI). *Neisseria meningitidis* fra systemiske infeksjoner (n=16) var stort sett følsomme for alle aktuelle antibiotimikrobielle midler. *Neisseria gonorrhoeae* (n=623) viste utbredt resistens mot penicillin G (18,6 %), og bare 1,3 % var følsomme for standard dosering svarende til villtype-populasjonen. Hele 59,1 % var resistente mot ciprofloxacin. To isolater (0,3 %) var

resistente mot ceftriaxon mens i alt syv isolater (1,1 %) var resistente mot det perorale cefalosporinet cefixim.

Det ble påvist to enterokokkisolater fra blodkultur med klinisk signifikant vankomycinresistens (VRE) i 2019 (begge VanB *E. faecium*). Forekomsten av resistens mot ampicillin i *E. faecium* ligger stabilt rundt 70-80 %. Høygradig gentamicinresistens ble påvist i 13,6 % av *E. faecalis* og 32,4 % av *E. faecium*. Dette er på samme nivå som henholdsvis 14,1 % og 32,0 % i 2018, og det sees dermed en avflating av den fallende tendensen for aminoglykosidresistens hos enterokokker. Nesten alle *E. faecium* med høygradig gentamicinresistens var også resistente mot ampicillin. Det ble ikke funnet linezolidresistente enterokokker (LRE) i NORM-overvåkingen i 2019. Både VRE og LRE er meldepliktige til MSIS, og det ble bekreftet funn av 204 VRE og 16 LRE på referanselaboratoriet ved Nasjonal kompetansetjeneste for påvisning av antibiotrikaresistens (K-res) ved UNN. Forekomsten av VRE varierer med utbrudd fra år til år, mens antallet LRE er gradvis økende. To isolater var kombinert VRE og LRE.

Resistensforholdene i systemiske isolater av *Streptococcus pneumoniae* og *Streptococcus pyogenes* (beta-hemolytiske gruppe A streptokokker) ble ikke overvåket i 2019 på grunn av begrenset kapasitet på referanselaboratoriet ved FHI. *S. pyogenes* fra sår og luftveisprøver viste stabil forekomst av resistens mot erytromycin og tetracyklin sammenliknet med 2013. Det ble ikke påvist nedsatt følsomhet for penicillin G. Systemiske isolater av *Streptococcus agalactiae* (beta-hemolytiske gruppe B streptokokker) hadde høy forekomst av resistens mot erytromycin (22,8 % i 2018 og 25,5% i 2019) og tetracyklin (75,4 % i 2018 og 77,7% i 2019).

I alt 165 tilfeller av tuberkulose ble meldt til MSIS i 2019. Det ble utført resistensbestemmelse av 126 *Mycobacterium tuberculosis* isolater. To isolater (1,6 %) fra pasienter smittet i Asia ble klassifisert som multiresistente.

Det ble utført resistensbestemmelse av 199 *Candida* blodkulturisolater av ni ulike species fra 185 ulike pasienter. De vanligste artene var *C. albicans* (n=116), *C. glabrata* (n=29), *C. parapsilosis* (n=18), *C. tropicalis* (n=17) og *C. dubliniensis* (n=11). Alle *C. albicans* var følsomme for de undersøkte midlene med unntak av ett enkelt micafunginresistent isolat. Det ble kun påvist enkelte non-albicans isolater med ervervet resistens mot anytimykotika, men som forventet var det høy forekomst av resistens mot azoler hos *C. glabrata*. Nøyaktig species-bestemmelse er avgjørende for å forutsi iboende resistens og velge effektiv behandling. Resultatene er i samsvar med tidligere studier fra Norge.

Konklusjon

I Norge er forekomsten av antibiotikaresistens fortsatt lav i bakterier fra mennesker og dyr. Dette skyldes lavt forbruk av antibiotika, et fordelaktig forbruksmønster og effektive tiltak mot spredning av resistente bakterier. Resultatene som presenteres i rapporten, viser at strategiene mot antibiotikaresistens har vært vellykkede både i husdyr-holdet og i helsevesenet. Det er imidlertid nødvendig med kontinuerlig innsats for å bevare den gunstige situasjonen slik at antibiotika også i fremtiden vil være effektive for dem som trenger det. NORM/NORM-VET-rapporten er viktig for å dokumentere utviklingen av antibiotikaforbruk og resistens hos mennsker og dyr, og for å evaluere effekten av tiltak.

SUMMARY

This is the twentieth joint report from the NORM surveillance programme for antimicrobial resistance in human pathogens and the NORM-VET monitoring programme for antimicrobial resistance in bacteria from feed, food and animals. The report presents data on the occurrence of antimicrobial resistance and the usage of antimicrobial agents in humans and animals for the year 2019. The NORM and NORM-VET programmes were established as part of the Norwegian Government's Action Plan against Antimicrobial Resistance issued in 2000. NORM is coordinated by the Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø. NORM-VET is coordinated by the Norwegian Veterinary Institute. A joint NORM/ NORM-VET report is issued annually.

Usage of antimicrobial agents in animals

The total sales of antibacterial veterinary medicinal products (VMPs) for terrestrial animals in Norway were 5,008 kg antibacterial ingredients in 2019.

Sales of antibacterial VMPs for use in terrestrial food producing animals, including horses, were 4,673 kg in 2019. Penicillins continued to be the most-selling antibacterial class for the major species – i.e. cattle, pigs, goat, sheep and poultry - and were almost exclusively accounted for by beta-lactamase sensitive penicillins. From 2013 – 2019, the estimated sales of antibacterial VMPs for cattle, pigs, poultry, sheep and goat declined by 21% when measured in kg and 18% when measured in mg/PCU (population correction unit). For horses, the usage was mainly accounted for by trimethoprim-sulfa (oral paste).

The sales (kg) of antibacterial VMPs for group treatment of terrestrial food producing animals in Norway continued to be very low; in 2019 such products accounted for only 4% of the total sales.

In 2019, the sales (kg) of antibacterial VMPs for farmed fish (cleaner fish included) were 222 kg. This is a reduction of more than 99% compared to 1987, when the sales were at its highest. For Atlatic salmon and rainbow trout, fish in only 1.3% of the on-grower locations were subjected to antibacterial treatment in 2019.

The sales (kg) of antibacterial VMPs marketed for companion animals were 335 kg in 2019. From 2013 to 2019 the sales of such VMPs for use in companion animals have been reduced by 37%. The prescriptions of human antibacterial medicinal products reported to the Veterinary Prescription Register declined gradually from 2015 to 2019. This indicates that the decline in the sales of antibacterial VMPs for companion animals has not been substitutet by prescribing of human products.

The European Medicines Agency (EMA) has suggested to restrict the use of some antbacterial classes in animals due to the potential risk to public health including 3rd and 4th generation cephalosporins, quinolones (fluoroquinolones and other quinolones) and polymyxins. In Norway, very low quantities of these antibacterial VMPs are sold and only includes quinolones.

In February 2015, the Norwegian poultry industry launched a project aiming at phasing out use of narasin as coccidiostat feed additive in broilers, a goal that was reached in June 2016. The usage of therapeutic antibiotics for broilers continues to be very low; in 2019, < 0.1% of the broiler flocks were subjected to such treatment and only beta-lactamase sensitive penicillins were used.

Usage of antimicrobial agents in humans

Since 2012 there has been a decline in total antibiotic use, but in 2019 a slight increase was observed. The use is reduced by 22% since 2012. By total antibiotic use we mean all sales of antibacterial agents for systemic use in humans (J01 excl. methenamine) i.e. in primary care and to institutions. The total sales were 13.2 Defined Daily Doses (DDD)/1,000 inhabitants/day in 2019. This is at the same level as it was in 2017. The proportion of narrow-spectrum penicillins (J01CE) was stable and accounted for 27% of total sales (J01, excl. methenamine). Sales of methenamine were reduced in 2019 due to a shortage situation in spring 2019.

Around 84% of the total human sales of antibacterials are used in primary care, i.e. outside health institutions. For ambulatory care, the most important antibiotic group in 2019 was penicillins, J01C; 54% of all DDDs for systemic antibacterials (J01, excl. methenamine), followed by tetracyclines, J01A (26%). The three most prescribed antibiotics for outpatients in 2019 were phenoxymethyl-penicillin, doxycycline and pivmecillinam. These three substances represented 50% of all prescriptions and 54% of all DDDs sold. Dentists prescribe around 5% of all DDDs in primary care.

In 2019, the antibacterial sales (in DDDs) to hospitals represented 8% of total sales of antibacterials for human use in the country. In 2019, a mean use of 75 DDD/100 bed days was observed, an increase by 12% since 2012. The amount by DDD/admission (2019; 3.1 DDD/admission) increased by 3% in the same period. Therapy pattern of antibacterials in hospitals does not change much from one year to another but there is a clear trend towards more use of antibiotics recommended in the guidelines. The use of broad-spectrum antibiotics was reduced by 16% compared to 2012 (measured in DDD/100 bed days). In hospitals, around half of the use, measured in DDDs, is penicillins (J01C). The second largest group is the cephalosporins; 17% of all DDDs. There are large variations between the hospitals in volume of antibiotics used, measured in DDD/100 bed days, and in therapy profile. The variations cannot be accounted for by differences in activity or patient composition alone.

Resistance in animal clinical isolates

The clinical isolates included in NORM-VET 2019 were from infections in dogs; *Escherichia coli* from both urinary tract infections (UTI) and other infections, *Staphylococcus pseudintermedius* and *Streptococcus canis*. This was the first time *S. canis* was included, and the results obtained need to be considered with care. Decreased susceptibility to erythromycin, clindamycin and azithromycin were, however, most common. Among *E. coli*, 70.8% and 46.5% of the isolates originating from UTI and other infections,

respectively, were susceptible to all antimicrobial classes included in the susceptibility testing. Resistance towards ampicillin, quinolones and tetracycline were most common. In total, 17.2% of *S. pseudintermedius* isolates were susceptible to all antimicrobial agents included in the susceptibility testing. Resistance towards benzylpenicillin, sulfamethoxazole, tetracycline and fusidic acid were most common. There was a higher proportion of resistance in clinical *E. coli* and *S. pseudintermedius* than in corresponding isolates from healthy dog carriers (see below).

Resistance in indicator bacteria from animals, food and feed

The 2019 data confirm that the situation regarding antimicrobial resistance in bacteria from animals, food and feed in Norway is good. The occurrence of multi-drug resistance (MDR), i.e. resistance to three or more antimicrobial classes, and specific emerging resistant bacteria/mechanisms are still low.

NORM-VET is following the requirements set in Commission implementing decision of 12. Nov 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU). *E. coli* and *Enterococcus* spp. are used as indicator bacteria, i.e. susceptibility testing of *E. coli* and *Enterococcus* spp. is used as an indicator for occurrence of antimicrobial resistance in the bacterial population. In addition, antimicrobial testing of bacteria from other sources than those included in this decision, or investigation of presence of specific antimicrobial resistant bacteria/resistance mechanisms by selective methods, are included. Selective methods are used for detection of *E. coli* resistant to extended-spectrum cephalosporins (ESC), quinolone resistant *E. coli* (QREC), carbapenemase-producing *Enterobacteriaceae* (CPE), colistin resistant (COL-R) *E. coli*, vancomycin resistant *Enterococcus* spp. (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), and *S. pseudintermedius* (MRSP). The use of selective methods is especially relevant for low prevalent sources, as it enables early detection of specific emerging resistant bacteria or mechanisms, such as for instance ESC resistant *E. coli* and CPE; thereby enabling these to be monitored. Some of the antimicrobials are defined by the WHO as critically important for treatment of human infections. Significant reservoirs of such resistant bacteria in animals and the food production chain are of concern as they may interact with the human bacterial populations and thus have an impact on resistance development in these.

In 2019, animal samples included caecal samples from cattle less than one year of age and fattening pigs, faecal samples from goats, and faecal swabs from dogs for susceptibility testing of *E. coli* and *Enterococcus* spp., and detection of emerging resistant bacteria/resistance mechanisms such as ESC resistant *E. coli*, CPE and COL-R *E. coli*. In addition, nasal swabs and environmental cloths from goat herds were included for detection of MRSA, and swabs from oral/nasal mucosa and perineum of dogs for both MRSA, *S. pseudintermedius* and MRSP. Food samples included beef and pork, as well as leafy greens and leafy herbs, while feed samples included raw dog feed.

Very few *Enterococcus* spp. were isolated from cattle, and only tetracycline resistance was detected from these. Tetracycline resistance was the only resistance determinant detected from pig isolates of *E. faecalis* as well (29 of 46 isolates), and the most commonly detected among the resistant *E. faecium*. The overall majority (> 90%) of *E. coli* isolates from cattle and pigs were fully susceptible to all antimicrobial agents in the test panel, while all the 62 goat *E. coli* isolates were fully susceptible. Among the isolates showing decreased susceptibility, resistance to sulfamethoxazole, trimethoprim (pig isolates), tetracycline and ampicillin were most frequently identified. Only 0.3% of the cattle isolates and 2.9% of the pig isolates were MDR. The susceptibility results from cattle are in concordance with previous years, while the proportion of pig isolates fully susceptible has increased since 2015 (from 78.9% in 2015 and 83.6% in 2017, to 90.9% in 2019).

QREC was detected from only one goat (1.7%) sample by selective methods, indicating an even lower occurrence in goats than in previous surveys of cattle and sheep (~7%).

ESC resistant *E. coli* were detected from 4.4% of the cattle, none of the goats and 18.9% of the pig samples. Only four of the 14 ESC resistant *E. coli* from cattle were resistant due to plasmid encoded resistance genes (ESBL phenotype, genotype $bla_{\text{CTX-M-55}}$). Among the pig isolates, five displayed an ESBL phenotype and were genotyped as $bla_{\text{CTX-M-15}}$, two of these also harboured $bla_{\text{TEM-1b}}$. Since 2015, there has been an increase in overall occurrence of ESC resistant *E. coli* in cattle (i.e. both those displaying a AmpC beta-lactamase phenotype and an ESBL phenotype). A slightly increasing trend, though not statistically significant, was noted in pigs the same years. This overall occurrence of *E. coli* resistant to ESC in cattle and pigs is mainly due to isolates with chromosomal mutations. However, there has been a change regarding *E. coli* displaying an ESBL phenotype due to plasmid encoding genes. Though the prevalence is low, the variation in genes detected from both animals (and meat) is increasing, indicating a possible dissmination of these genes within the cattle and pig populations.

Findings of MRSA in the Norwegian animal population are rare. None of the 94 investigated goat herds were positive for MRSA. The yearly MRSA surveillance programme in pigs detected one herd with MRSA CC398 in 2019. Seven additional herds were found positive through contact tracing or investigations due to other reasons.

From the dog faecal samples, 59.7% of the isolated *E. faecalis* and 85.3% of the *E. faecium* isolates were fully susceptible. However, there was only 34 *E. faecium* isolates in total. Resistance to tetracycline accounted for most of the identified resistance in *E. faecalis*. Among the *E. coli* isolates, 86.8% were fully susceptible. Resistance to ampicillin was the most frequently identified resistance determinant, followed by resistance to sulfamethoxazole and tetracycline. Only 3.7% of *E. coli* isolates were MDR. ESC resistant *E. coli* were detected by selective methods from only three (1.3%) of the dogs. One isolate displayed an AmpC beta-lactamase phenotype due to chromosomal mutations. The last two displayed an ESBL phenotype, genotypes $bla_{\text{CTX-M-1}}$/$bla_{\text{OXA-1}}$, and $bla_{\text{CTX-M-55}}$/$bla_{\text{TEM-1B}}$, respectively. QREC was detected by selective methods from 8.2% of the dogs. Among these QREC, some isolates were MDR and resistance to up to seven antimicrobial classes were detected.

S. pseudintermedius isolated from the same dogs were also susceptibility tested. Among these, 21.1% of the isolates were fully susceptible. Resistance to sulfamethoxazole was

most frequently identified, followed by resistance to benzylpenicillin, fusidic acid and tetracycline. MDR was detected in 26.7% of the *S. pseudintermedius* isolates. Neither MRSA nor MRSP was detected from any of the samples using selective methods.

Results from analyses of raw dog feed showed that 69.6% of the *E. faecalis* and 83.9% of the *E. faecium* isolated were fully susceptible. Resistance to tetracycline accounted for most of the detected resistance in *E. faecalis*, and 1.8% of all isolates were MDR. VRE was not detected. Among the 65 *E. coli* > 80% were fully susceptible, and four isolates among the resistant isolates were MDR. ESC resistant *E. coli* was detected from three (4.0%) of the samples by selective methods, of which one isolate displayed an ESBL phenotype, genotype *bla*$_{\text{CTX-M-15}}$. Selective methods for isolation of QREC identified QREC in 17 of the 65 samples, of which six isolates were MDR to up to seven antimicrobial classes. Neither COL-R *E. coli* nor any CPE were detected from the samples.

ESC resistant *E. coli* was not detected in any of the pork samples in 2019, and in only three (0.9%) of the beef samples. These three displayed an ESBL phenotype, genotypes *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{CTX-M-36}}$/*bla*$_{\text{TEM-1b}}$ and *bla*$_{\text{CTX-M-1}}$/*bla*$_{\text{TEM-1b}}$, respectively. Investigations of leafy greens and leafy herbs in 2019 did not detect any ESC resistant *E. coli*, CPE or COL-R *E. coli*. However, the number of samples per category, i.e. domestic leafy greens, imported leafy greens and imported leafy herbs, is small and the results from 2017, 2018 and 2019 should therefore be considered together. The majority of indicator *E. coli* isolates were fully susceptible to all the antimicrobial agents tested for, and only a few were MDR. Altogether, only five ESC resistant *E. coli* isolates were obtained these years, all from imported products, and all displaying an ESBL phenotype with genotypes *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{CTX-M-65}}$, *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{SHV-12}}$, respectively. A few isolates also harboured plasmid-encoded quinolone resistance and plasmid-encoded colistin resistance. Comparison of results between the different sample categories should be done with caution. However, the results do show that imported leafy greens and leafy herbs can be contaminated with some emerging resistant bacteria carrying genes that are not commonly identified among production animals in Norway, nor from domestically produced food.

CPE has never been isolated in samples from animals or food in Norway. This still applies for the 2019 results.

Resistance in zoonotic bacteria and non-zoonotic enteropathogenic bacteria

Animal, feed and food isolates

The Norwegian animal production population is considered virtually free from *Salmonella* spp. In 2019, seven *Salmonella* spp. isolates from animals and one from raw dog feed were susceptibility tested. The animal isolates included one each from turkey, poultry, dog, and pig, and three cats, respectively. Due to differences in natural susceptibility to colistin among serovars, there is no general *Salmonella* ECOFF available for colistin, and all the isolates were regarded fully susceptible.

Among *Campylobacter coli* from pigs, 53.4% of the isolates were fully susceptible. Resistance to streptomycin was most frequently identified (41.0%), followed by resistance to ciprofloxacin and nalidixic acid (15.7%). Reduced susceptibility to erythromycin and gentamicin was not detected. None of the isolates were MDR.

Nine pathogenic *Yersinia enterocolitica* isolates were obtained from a survey of minced pork meat investigating a total of 152 samples. All nine isolates were fully susceptible to all antimicrobial agents included in the test panel.

Human clinical enteropathogenic isolates

Following the reorganisation of the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) and the paused antimicrobial susceptibility testing in 2018, the NRL resumed antimicrobial susceptibility testing for enteropathogenic bacteria in 2019.

For *Salmonella* Typhimurium and its monophasic variant, overall resistance levels were higher for travel-associated strains compared to domestically acquired strains. Antibiotic resistance was highest among *Salmonella* Typhi, with an observed increasing trend for resistance against ciprofloxacin, tetracycline, chloramphenicol, and extended-spectrum cephalosporins. Multi-drug resistance (MDR) was also a characteristic trait for a considerable proportion of the *S.* Typhi isolates (54%). Six *Salmonella* isolates were characterised as ESBL producers.

Also for *Campylobacter jejuni*, overall resistance levels for ciprofloxacin and tetracycline were higher for travel-associated strains compared to domestically acquired strains. A continued rising trend in resistance to ciprofloxacin, and tetracycline for domestically acquired strains was observed.

An increasing trend of resistance towards extended-spectrum cephalosporins and ciprofloxacin was observed for *Shigella sonnei*. Sixteen *Shigella* spp. were confirmed as ESBL producers. Antimicrobial resistance in *Yersinia enterocolitica* remains low.

Resistance in human clinical isolates

The prevalence of antimicrobial resistance in human clinical isolates was still low in Norway in 2019. Only twelve methicillin resistant *Staphylococcus aureus* (MRSA) blood culture isolates were detected among 1,492 strains included in the NORM protocol (0.8%). During 2019, the total number of systemic *S. aureus* isolates from blood cultures and cerebrospinal fluids was 2,158 including 19 MRSA strains (0.9%). This is at the same level as in 2017 and 2018 (0.8% both years). The Norwegian Surveillance System for Communicable Diseases (MSIS) registered 945 cases of MRSA infections in 2019 compared to 763 in 2017 and 905 in 2018. The majority of MRSA cases were reported as superficial wound infections and/or abscesses. The proportion of MRSA among non-invasive *S. aureus* isolates is still very low at 1.3% (13/1,014), as it was in 2017 (1.2%) and 2018 (1.7%). Furthermore, MSIS registered 1,499 MRSA colonisations compared to 1,529 in 2017 and 1,631 in 2018. A total of 2,444 persons were reported with MRSA in 2019, corresponding to an incidence rate of 46/100,000 person years (48/100,000 in 2018). The results indicate a relatively stable rate of MRSA notifications. The incidence of invasive disease has remained stable at a low level. A large proportion of cases

are infected abroad, and very few cases of livestock-associated MRSA are detected.

The rate of resistance to broad-spectrum antimicrobials in *E. coli* blood culture isolates remained essentially unchanged in 2019. The prevalence of gentamicin resistance was 5.9% in 2019 compared to 7.0% in 2017 and 5.4% in 2018, while the prevalence of ciprofloxacin resistance decreased from 11.7% in 2018 to 11.3% in 2019. *Klebsiella* spp. still demonstrates lower rates of resistance to gentamicin (4.4%) and ciprofloxacin (6.1%) than *E. coli*, but the difference is reduced compared to previous years.

Extended-spectrum beta-lactamases (ESBL) have emerged as a significant clinical problem in many countries, including Norway. A total of 167/2,350 (7.1%) *E. coli* and 58/1,017 (5.7%) *Klebsiella* spp. blood culture isolates were reported with this phenotype in 2019. The prevalence was slightly increasing for *E. coli* (6.6% in 2017 and 6.5% in 2018) but remained stable for *Klebsiella* spp. (5.3% in 2017 and 6.6% in 2018). The proportion of ESBL positive isolates is still higher among *E. coli* from blood cultures (7.1%) than in urinary tract isolates (3.0%). The prevalence of resistance to beta-lactam antibiotics (3-5%) and aminoglycosides (1-2%) was low in *Pseudomonas aeruginosa.*

Carbapenemase-producing *Enterobacterales* (CPE), *P. aeruginosa* and *Acinetobacter* spp. have been notifiable to MSIS since 2012. The number of reported patients increased from 54 in 2018 to 75 in 2019, and the number of patients with carbapenemase-producing *P. aeruginosa* (n=5) and *Acinetobacter* spp. (n=23) was also slowly increasing.

There was no surveillance of resistance in systemic isolates of *Haemophilus influenzae* in 2019 due to limited capacity at the reference laboratory at the Norwegian Institute of Public Health (NIPH). *Neisseria meningitidis* from systemic infections (n=16) was generally susceptible to all relevant antibiotics. *Neisseria gonorrhoeae* isolates (n=623) displayed resistance to penicillin G (18.7%), and only 1.3% were susceptible to standard dosage corresponding to the wild-type population. Ciprofloxacin resistance was detected in 59.1% of isolates. Two isolates (0.3%) were resistant to ceftriaxone and seven (1.2%) were cefixime resistant. All isolates remained susceptible to spectinomycin.

Two enterococcal blood culture isolates (0.3%) with clinically significant vancomycin resistance (VRE) were detected in 2019 (both VanB *E. faecium*). The prevalence of ampicillin resistance in *E. faecium* has stabilised around 70-80%. High-level gentamicin resistance (HLGR) was detected in 13.6% of *E. faecalis* and 32.4% of *E. faecium* isolates. This is at the same level as 14.1% and 32.0% in 2018, respectively, thus the downward trend for aminoglycoside resistance in enterococci was not continued. Almost all HLGR *E. faecium* isolates were also resistant to ampicillin. There were no linezolid resistant isolates (LRE) in the NORM surveillance programme in 2019. Both VRE and LRE should be reported to the national notification system (MSIS), and 204 VRE and 16 LRE were confirmed at the reference laboratory at K-res/UNN in 2019. The prevalence of VRE varies over time due to outbreaks, whereas there is a gradually increasing number of LRE from one year to another. Two isolates were combined VRE and LRE.

Resistance in systemic isolates of *Streptococcus pneumoniae* and *Streptococcus pyogenes* (beta-haemolytic group A streptococci) was not monitored in 2019 due to limited capacity at the reference laboratory at NIPH. *S. pyogenes* throat and wound isolates displayed stable prevalences of resistance to relevant antibiotics compared to 2013. No isolates with reduced susceptibility to penicillin G were detected. Systemic *Streptococcus agalactiae* isolates (beta-haemolytic group B streptococci) were commonly resistant to erythromycin (22.8% in 2018 and 25.5% in 2019) and tetracycline (75.4% in 2018 and 77.7% in 2019).

A total of 165 cases of tuberculosis were reported to MSIS in 2019. Susceptibility testing was performed on 126 *Mycobacterium tuberculosis* isolates. Two isolates (1.6%) originating from Asia were classified as multi-drug resistant (MDR).

Susceptibility testing was performed on 199 *Candida* spp. blood culture isolates of ten different species from 185 unique patients. The most common species were *C. albicans* (n=116), *C. glabrata* (n=29), *C. parapsilosis* (n=18), *C. tropicals* (n=17) and *C. dubliniensis* (n=11). All *C. albicans* were susceptible to the substances examined with the exception of a single micafungin resistant isolate. Only single non-albicans isolates with acquired fluconazole resistance were detected, but as expected there was a high prevalence of resistance to azoles among *C. glabrata*. Precise species identification is essential to predict inherent resistance and select appropriate antifungal therapy. The results are in accordance with previous studies from Norway.

Conclusion

Antimicrobial resistance is still a limited problem among humans and food-producing animals in Norway. This reflects the low usage of antibacterial agents in human and veterinary medicine, a favourable usage pattern, as well as effective infection control measures. The data presented in the report show that strategies for containment of antimicrobial resistance have been successful both in the food-producing animal sector and in the healthcare sector. Continuous efforts and awareness rising are needed to preserve the favourable situation and ensure the effectiveness of antibacterials when needed. The NORM/ NORM-VET report is vital in order to document the trends in antibiotic usage and occurrence of resistance in humans and animals, and to evaluate interventions.

POPULATION STATISTICS

Population statistics for human and animal populations are presented in order to facilitate comparison of Norwegian data with corresponding figures from other countries. The data are collected by Norwegian authorities as shown in the various tables below.

TABLE 1. Human population in Norway as of 01.01.2020. Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 4 years	290 063	149 413	140 650
5 to 14 years	640 059	328 165	311 894
15 to 24 years	659 329	340 136	319 193
25 to 44 years	1 447 780	742 037	705 743
45 to 64 years	1 386 922	707 689	679 233
65 years and older	941 780	438 113	503 667
All age groups	5 365 933	2 705 553	2 660 380

TABLE 2. Livestock population in Norway in 2019. Data provided by the Register of Production Subsidies as of 01.03.2019.

	Number* of	
Animal category	Herds	Animals
Cattle	13,500	862,000
Dairy cows only**	7,900	215,000
Suckling cow only**	5,900	92,700
Combined production (cow)**	900	42,000
Goat	1,300	71,000
Dairy goat**	350	36,000
Sheep	14,000	93,700
Breeding sheep > 1 year**	14,000	93,700
Swine	2,000	761,000
Breeding animal > 6 months**	1,100	45,200
Fattening pigs for slaughter**	1800	420,000
Laying hen flocks > 250 birds	580	4,252,000
Broilers	600[1]	65,517,000[2]
Turkey, ducks, geese for slaughter (flock > 250 birds)	42	440,000

* Numbers > 100 rounded to the nearest ten, numbers > 1000 rounded to the nearest hundred. ** Included in above total. [1] Included in the official surveillance programme of *Salmonella*, [2]Figures from the Norwegian Agriculture Agency (based on delivery for slaughter).

TABLE 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2019. Data provided by the Norwegian Directorate of Fisheries updated by 09.06.2020.

Year	Atlantic salmon (tonnes)	Rainbow trout (tonnes)	Cod (tonnes)	Arctic char (tonnes[2])	Halibut (tonnes[2])	Blue mussels (tonnes)	Scallops[1] (tonnes)	Oysters (tonnes)
1992	141,000	-	-	-	-	-	-	-
1993	170,000	-	-	-	-	-	-	-
1994	204,686	14,571	569	262	63	542	-	-
1995	261,522	14,704	284	273	134	388	-	-
1996	297,557	22,966	191	221	138	184	-	-
1997	332,581	33,295	304	350	113	502	-	-
1998	361,879	48,431	203	200	291	309	-	-
1999	425,154	48,692	147	498	451	662	67	41
2000	440,061	48,778	169	129	548	851	38	8
2001	435,119	71,764	864	318	377	920	22	3
2002	462,495	83,560	1,258	319	424	2,557	5	2
2003	509,544	68,931	2,185	272	426	1,829	1	2
2004	563,915	63,401	3,165	365	648	3,747	46	3
2005	586,512	58,875	7,409	352	1,197	4,885	3	2
2006	629,888	62,702	11,087	897	1,185	3,714	4	1
2007	744,222	77,381	11,104	394	2,308	3,165	6	4
2008	737,694	85,176	18,052	468	1,587	2,035	4	3
2009	862,908	73,990	20,924	421	1,568	1,649	7.7	3.8
2010	939,575	54,451	21,240	492	1,610	1,930	10.3	2.1
2011	1,064,868	58,472	15,273	276	2,767	1,743	13	2
2012	1,241,482	70,364	10,033	309	1,741	1,967	21	2
2013	1,168,324	71,449	3,770	281	1,385	2,328	23	5
2014	1,258,356	68,910	1,213	285	1,257	1,983	13	4
2015	1,303,346	72,921	5	257	1,243	2,731	21	10
2016	1,233,619	87,446	0	330	1,461	2,231	12	11
2017	1,236,353	66,902	117	339	1,623	2,383	29	17
2018	1,282,003	68,216	495	285	1,843	1,649	28	18
2019[3]	1,357,307	82,855	0	365	1,524	2,149	0	7

[1]From the wild population. [2]After 2001 in numbers of 1,000 individuals. [3] Preliminary numbers.

Import of live animals

There was no import of live animals (excluding fish and companion animals) to Norway in 2019 except for 18,279 day old chicks of hen, broiler, turkey and duck according to the yearly report from KOORIMP and KIF; https://www.animalia.no/no/Dyr/koorimp---import/arsmeldinger-koorimp-og-kif/.

USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave, Kari Olli Helgesen and Petter Hopp

Sales data for 1993-2019 for antibacterial veterinary medicinal products (VMP) for terrestrial animal species, obtained at wholesaler's level, have been stratified into sales of antibacterial VMPs approved for terrestrial food producing animals including horses and approved solely for companion animals, respectively (see Appendix 1). The data are based on sales to Norwegian pharmacies from medicine wholesalers of VMPs. This includes all pharmaceutical formulations approved for food producing terrestrial animals, including horses, and for companion animals as well as VMPs used on special permit (products approved in another EEA country). In addition, data obtained from the Veterinary Prescription Register (VetReg) have been used for some data analysis, including for supplementary information (see Appendix 1).

Usage of veterinary antibacterial agents

Overall, the sales in Norway of antibacterial veterinary medicinal products (VMPs) for therapeutic use in food producing terrestrial animals, including horses, and companion animals in 2019 were 5,008 kg. A decline of the annual sales of such VMPs of 46% in the period 1993-2019 is observed (Figure 1-3).

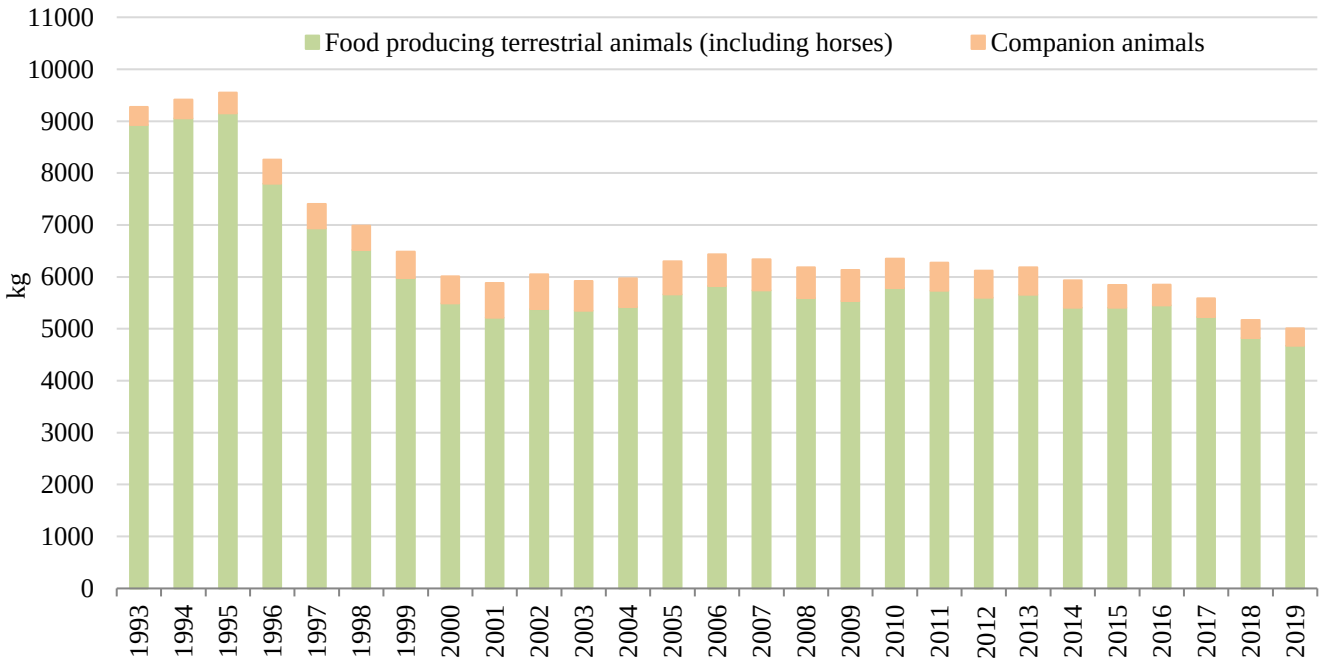


FIGURE 1. Total sales, in kg active substance, for food producing terrestrial animals (including horses) and companion animals, of antibacterial veterinary medicinal products for therapeutic use in Norway in 1993-2019.

Food-producing terrestrial animals, including horses

In 2019 the sales, in kg active substance, of antibacterial VMPs for use in terrestrial food producing animals, including horses, were 4,673 kg, and compared to 1993 a decrease in the sales of such VMPs of 48% is observed (Figure 2). In total, 61% of the sales (kg) of antibacterial VMPs for this animal category contained penicillins only, of which 93% was accounted for by beta-lactamase sensitive penicillins; of the total sales, 28% was accounted for by combination VMPs with trimethoprim-sulfa; of this combination, 89% was sold as orale paste for horses.

The proportion of sales of VMPs containing only penicillins for this animal category increased from 19% to 61% during the period 1993-2019. This is mainly due to reduced sales of injectable and intramammary combination VMPs of penicillins and aminoglycosides (dihydro-streptomycin) that has gradually been replaced by products containing antibacterials belonging to penicillins as the sole antibacterial agents.

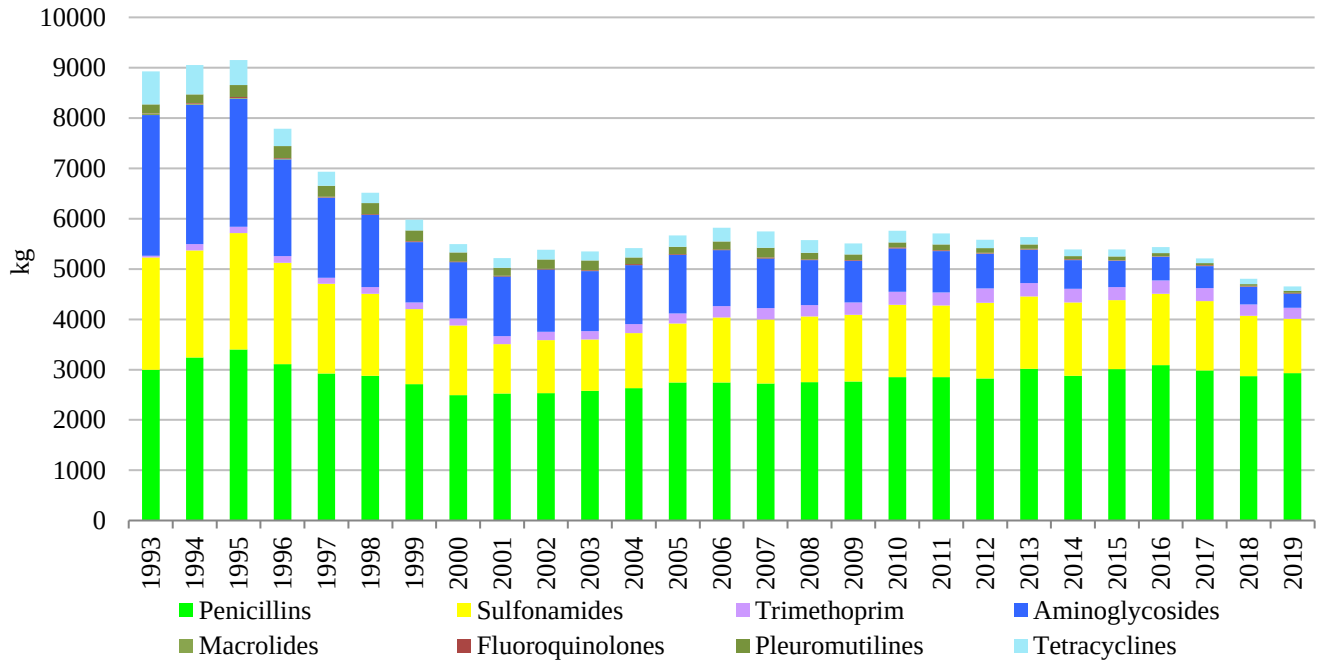


FIGURE 2. Sales, in kg active substance, of antibacterial veterinary medicinal products (VMPs) for therapeutic use in food producing terrestrial animals (including horses) in Norway in 1993-2019. In addition, minor amounts of amphenicols VMPs were sold in 2008-2019 (range 16-27 kg). Minor amounts of baquiloprim were sold annually in 1994-2000.

Of the antibacterials for which restriction of use in animals is recommend at EU/EEA level due to potential public health risks – i.e. 3rd and 4th generation cephalo-sporins, polymyxins and quinolones (fluroquinolones and other quinolones) (see text box) – only fluoroquinolones are marketed in Norway for food producing terrestrial animals. From 1993 to 2019, the proportion of sales of fluoro-quinolones for food producing terrestrial animals has been very low and stable varying between 0.1% to 0.3% of total sales (see also Figures 4-6). During 1993-2019 no VMPs containing 3rd and higher generations of cephalosporins has been approved for food producing animals in Norway via national procedures. Two 3rd generation products have been approved via community procedures, but these are not marketed in Norway. Applications for special permits to use such VMPs marketed in other EEA countries for food producing animals are normally not approved, an approval would only be given for specific animals if sensitivity testing precludes all other options. This is the case also for polymyxins (Tonje Høy, Norwegian Medicines Authority, personal communication). Glycopeptides are not allowed for food producing animals in EU/EEA countries; this is the case also for carbapenems.

In Norway, sales of antibacterial VMPs for treatment of food producing terrestrial animals are dominated by pharmaceutical forms for treatment of individual animals (Figure 3), primarily injectables. This reflects that livestock is characterised by small herds, but it can also partly be explained by therapeutic traditions. In 2019, only 4% of sales of antibiotic VMPs for food producing terrestrial animals was for VMPs for group treatment (oral treatment).

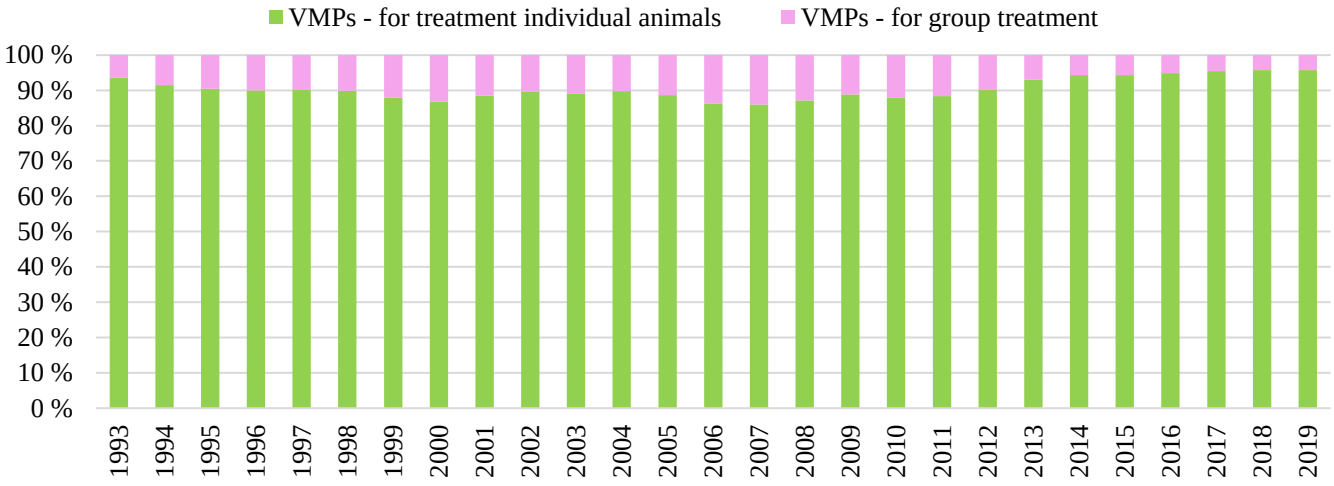


FIGURE 3. Sales in Norway, in kg active substance, of antibacterial veterinary medicinal products (VMPs) marketed for treatment of individual food producing terrestrial animals (bolus, injectables, intramammary preparations, intrauterine preparations, oral paste and some tablet VMP presentations – see Appendix 1) and for group treatment through feed or drinking water (oral solution and oral powder; no premixes are marketed for terrestrial food producing animals).

Usage patterns - major terrestrial food producing animals (VetReg data)

The usage patterns presented represent the data reported to VetReg (see Appendix 1) for 2019. Of the reported amounts (kg) of antibacterial VMPs for cattle, pigs, sheep and goat, only 0.3% was for goat and therefore data for this species are not presented. Of the amounts antibacterial VMPs and human medicinal products reported to VetReg for which EMA advice restriction of use due to potential public health risks, the proportion accounted for by for cattle, pigs and sheep was 0.1%, 0.04% and 0.01%, respectively, and of these only fluoroquinolones were used (Figures 4-6).

Cattle

Of the prescriptions (VetReg data) of antibacterial veterinary and human medicinal products for cattle in 2019, 91.4 % was for penicillins (kg active substance); 90.1% were for beta-lactamse sensitive penicillins (intra-mammaries not included) (Figure 4). These proportions were increasing slightly from 2015 to 2019. Of the prescriptions of intramammaries reported to VetReg, 99% (kg) was for cattle. For intramammaries the sales data are used to document the prescribing patterns (see explanation Appendix 1); the sales of intramammaries containing penicillins only were 42% in 2019, and for combinations of penicillins and aminoglycosides this figure was 58%.

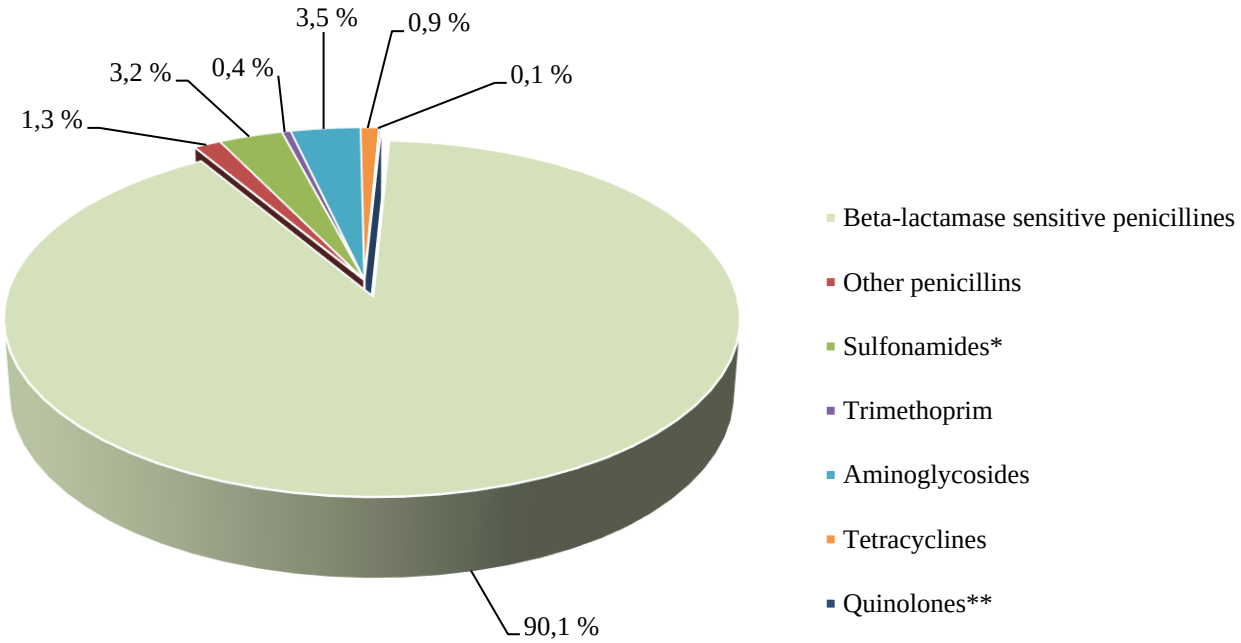


FIGURE 4. Prescribing patterns, in kg active substance, of antibacterial veterinary and human medicinal products for cattle in Norway in 2019. Data were obtained from the Veterinary Prescription Register (intramammaries not included in data in the figure). * In combination with trimethoprim only. ** Fluoroquinolones only. In addition, 0.06% of the prescribed amounts was for macrolides and 0.4% for amphenicols, lincosamides and pleuromutilins.

Pigs

Of the antibacterial veterinary and human medicinal products reported to VetReg as prescribed for treatment of pigs (Figure 5), 90.8% of the toal amount reported to VetReg was accounted for by penicillins, 85.7% was for beta-lactamse sensitive penicillins only (Figure 5). These proportions were increasing slightly from 2015 to 2019.

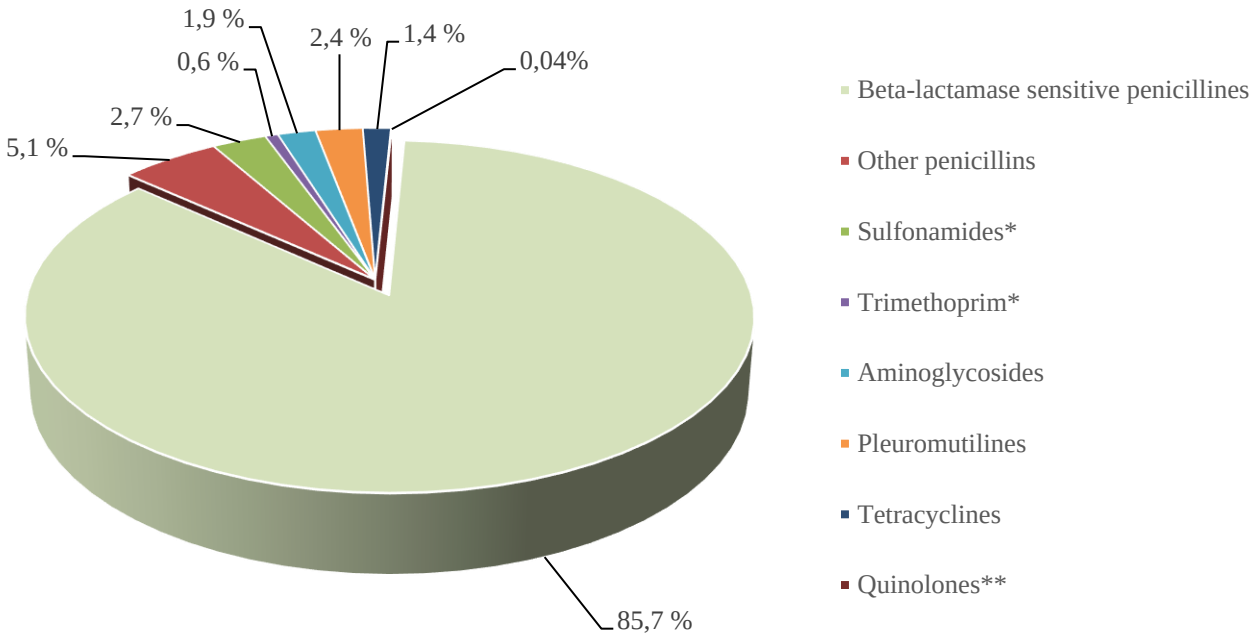


FIGURE 5. Prescribing patterns, in kg active substance, of antibacterial veterinary and human medicinal products for pigs in Norway in 2019. Data are obtained from the Veterinary Prescription Register. *In combination with trimethoprim only. **Fluoroquinolones only. In addition, 0.1 % of the prescribed amounts was for macrolides.

Sheep

Of the antibacterial veterinary and human medicinal products reported to VetReg as prescribed for treatment of sheep (Figure 6), 83.4% of the toal amount reported to VetReg was accounted for by penicillins, 82.8% were for beta-lactamse sensitive penicillins only (Figure 6). These proportions were increasing slightly from 2015 to 2019.

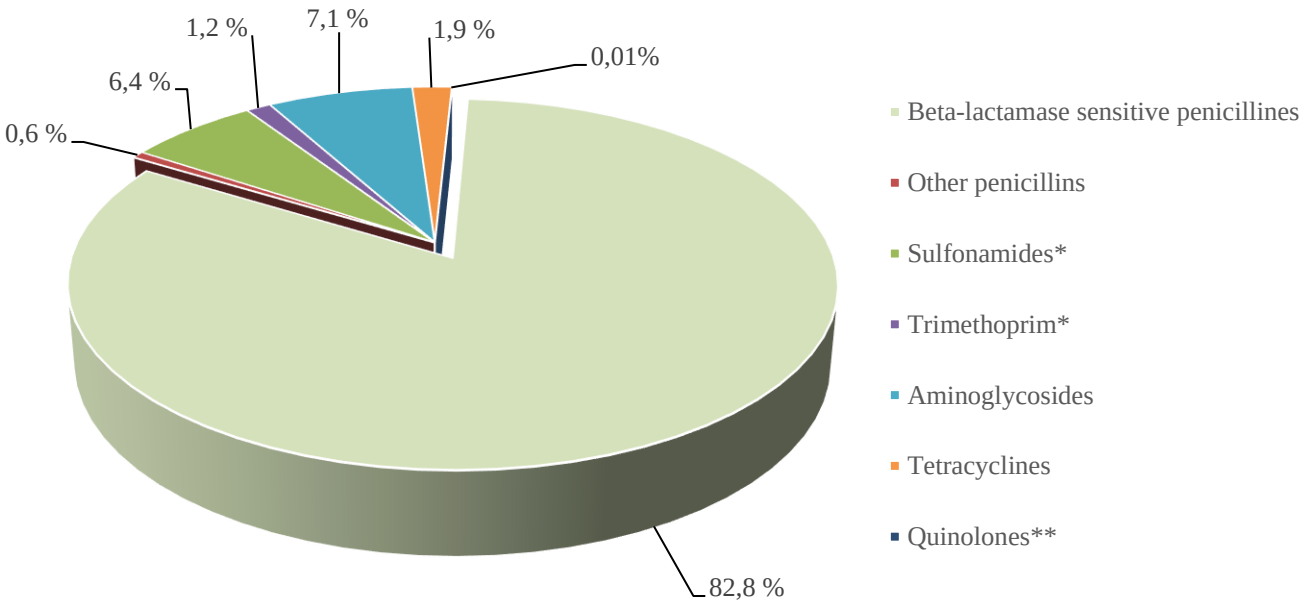


FIGURE 6. Prescribing patterns, in kg active substance, of antibacterial veterinary and human medicinal products for sheep in Norway in 2019. Data are obtained from the Veterinary Prescription Register. *In combination with trimethoprim only. **Fluoroquinolones only. In addition, 0.06% was for amphenicols and pleuromutilines.

Farmed fish

In 2019, the total amounts of antibacterials prescribed for use in aquaculture in Norway was 222 kg (Table 4); of this 175 kg were prescribed for farmed fish intended for human consumption (cleaner fish excluded). This level of usage was approximately at the same level as in 2015 and 2016. Compared to 2015 and 2016, there was an increase in the amounts (kg) of antibacterials prescribed for farmed fish in 2017 and 2018. This was not due to an increase in the number of treatments of farmed fish with antibacterials for these years as the number of prescriptions for 2015-2019 was 61, 63, 63, 43 and 45, respectively (Figure 7). The reason for the observed increase in prescriptions measured in kg active substance is that both in 2017 and 2018 a few sea-site locations with Atlantic salmon with high weight were subjected to treatment with antibiotics while in 2015, 2016 and 2019 such cases were not reported.

Of the antibacterials for which restriction of use in animals is recommend at EU/EEA level due to potential public health risk (see text box), only "other quinolones" are used for farmed fish. From 2010 to 2019, the proportion of sales of "other quinolones" has fluctuated. In 2018 and 2019, this proportion was 6% and 30%, respectively.

TABLE 4. Usage, in kg of active substance, of antibacterial veterinary medicinal products for farmed fish in Norway in 2010-2019. For 2010-2012 the data represent sales data from feed mills and wholesalers collected by the Norwegian Institute of Public Health; for 2013-2019 data represent prescription data obtained from the Veterinary Prescription Register (See Appendix 1). Note that data include antibacterials for use in cleaner fish.

Active substance	2010	2011	2012	2013	2014	2015[1]	2016[1]	2017	2018[1]	2019
Tetracyclines										
Oxytetracycline	10	1	1	0	0	0	0	0	20	0
Amphenicols										
Florfenicol	275	336	191	236	399	188	136	269	858	156
Quinolones										
Flumequine	0	0	0	25	25	< 0.05	< 0.05	< 0.05	0	0
Oxolinic acid	308	212	1,399	599	99	84	66	343	54	66
Combinations										
Spectinomycin + lincomycin (2+1)	57	0	0	0	0	0	0	0	0	0
Total	**649**	**549**	**1,591**	**860**	**523**	**273**	**201**	**612**	**931**	**222**

[1] The total amount (kg) given is deviating due to rounding of the individual values

For the years 2013 to 2019, the major proportion of prescriptions was for farmed fish in the pre-ongrower phase (Figure 7). The number of prescriptions of antibacterial VMPs for Atlantic salmon ongrowers was negligible during the period 2013-2019, despite that Atlantic salmon represents more than 95% of the biomass farmed fish produced in Norway. This is a strong indication that the vaccines used are efficient and that the coverage of vaccination of fingerlings is very high.

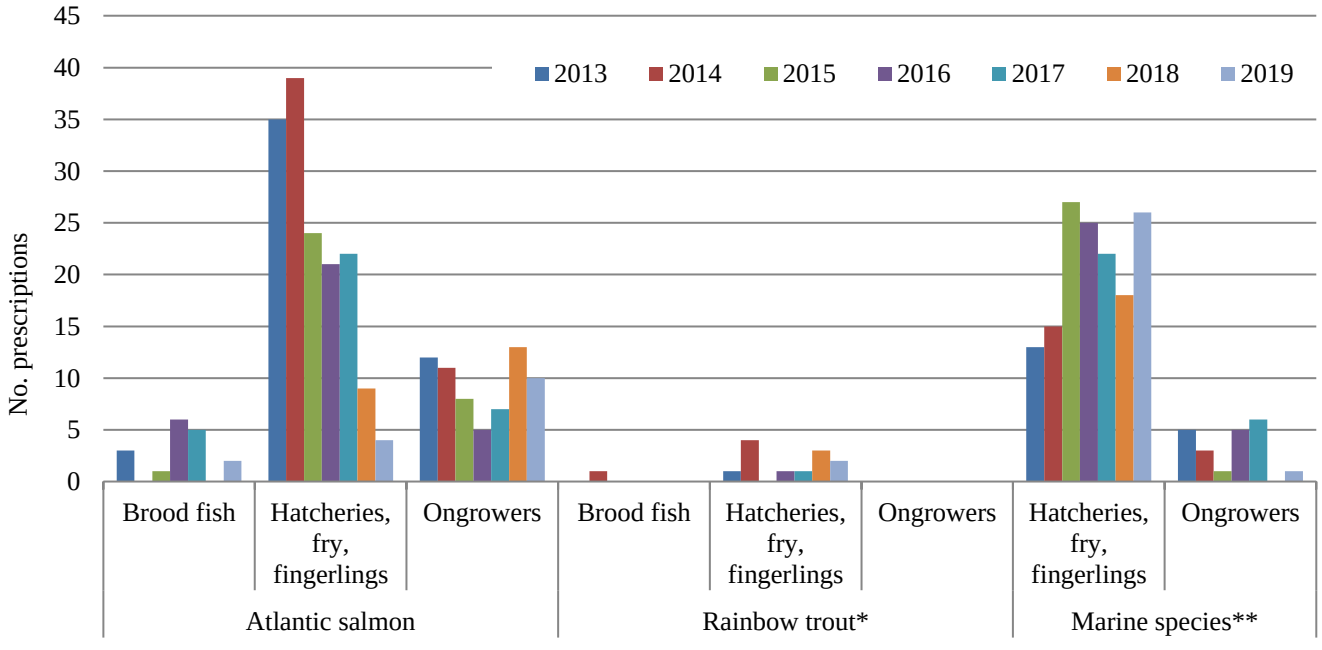


FIGURE 7. Number of prescriptions of antibiotics by fish species, split into production stages/types, in Norway in 2013-2019. Data were obtained from the Veterinary Prescription Register. *Includes two prescriptions for trout (*Salmo tutta*) fingerlings. **Cod, halibut, pollack, turbot and/or wolfish. Note that cleaner fish is not included.

The annual sales of antibacterial VMPs for use in aquaculture peaked in 1987 when it amounted to 48 tonnes (Figure 8) – i.e. 876 mg/PCU; the corresponding figure in 2019 was 0.15 mg/PCU. Thus, the sales in mg/PCU have declined by 99.9% (Table 4). The significant decrease in the usage of antibacterial agents in Norwegian aquaculture from 1987 is mainly attributed to the introduction of effective vaccines against bacterial diseases in Atlantic salmon and rainbow trout but also prevention of bacterial diseases and their spread.

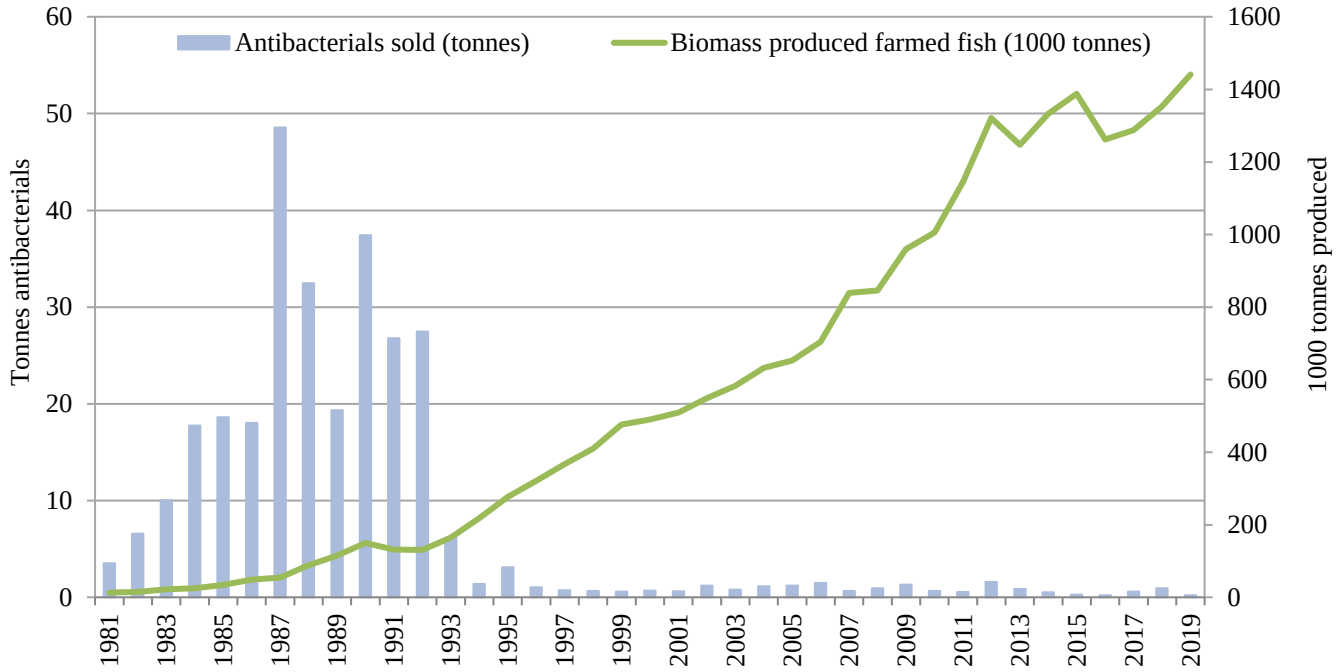


FIGURE 8. Sales, in tonnes of active substance, of antibacterial veterinary medicinal products for therapeutic use in farmed fish (including cleaner fish) in Norway in 1981-2019 versus produced biomass (slaughtered) farmed fish. For 1981-2014 the data represent sales data provided by the Norwegian Institute of Public Health; for 2013-2019 data represent prescription data obtained form the Veterinary Prescription Register. Data on slaughtered biomass farmed fish were obtained from Statistics Norway.

In a report from 2019 (1) it was shown that for Atlantic salmon and rainbow trout, only 1.5%, 1.4%, 1.0%, 0.6% and 0.8% of the ongrowers locations were subjected to treatment in the years 2013-2017, respectively. For 2018 and 2019 these figures were 1.6% and 1.3%, respectively.

Companion animals (dogs and cats)

The sales in 2019 of antibacterial VMPs approved solely for companion animals (including VMPs formulated as tablets, oral solution, injectable and oral paste) was 335 kg; in 2018 this figure was 347 kg. As shown in Figure 9, a steady increase in the sales from 1993 to 2001 was observed. This can in part be explained by changes in the number of antibacterial VMPs marketed for dogs and cats during that period. When the availability of VMPs for dogs and cats was lower, antibacterial human medicinal products (HMPs) were likely prescribed for dogs and cats. In 1993, only eight antibacterial VMP presentations (name, pharmaceutical form, strength and pack size) were authorised in Norway for dogs and cats, while in 2001 the corresponding number was 36. The number of VMP presentations for dogs and cats amounted to 49 in 2015; in 2019 this figure had decreased to 36.

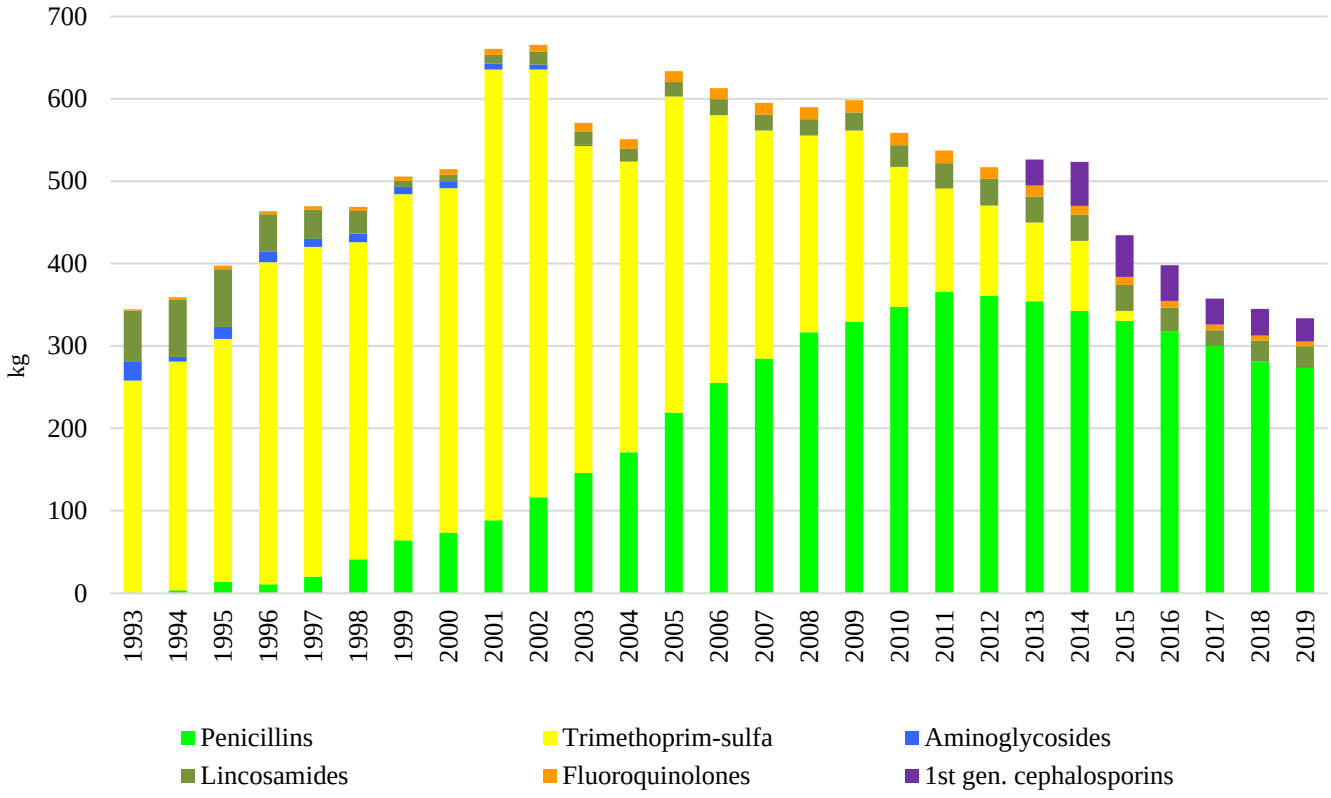


FIGURE 9. Sales, in kg active substance, of antibacterial veterinary medicinal products marketed solely for use in companion animals (injectables, oral paste, oral solution and tablets; note the exceptions for tablets: see Appendix 1) in Norway for the period 1993-2019. Minor sales of a 3rd generation cephalosporin injectable VMP (range 0.4-1.1. kg) in 2008-2019 and of macrolide VMPs (0.4-5 kg) in 1996-2003 were observed.

The sales patterns of antibacterial VMPs marketed solely for companion animals (dogs and cats) have changed significantly during the period 1993-2019 (Figure 9). The first penicillin VMP as tablets were marketed for companion animals in 1994; since then the proportion belonging to the penicillins sold of total sales of antibacterial VMPs approved for companion animals has increased from 1% to 82% (Figure 9).

Of the sales of antibacterials belonging to the penicillins VMPs approved for dogs and cats, the proportion of the combination amoxicillin and clavulanic acid increased steadily from its introduction in 1997 (Figure 10). The proportion of this combination peaked in 2017 accounting for 84% of the sales of these combination VMPs while in 2019 this figure had declined to 79 % (Figure 9).

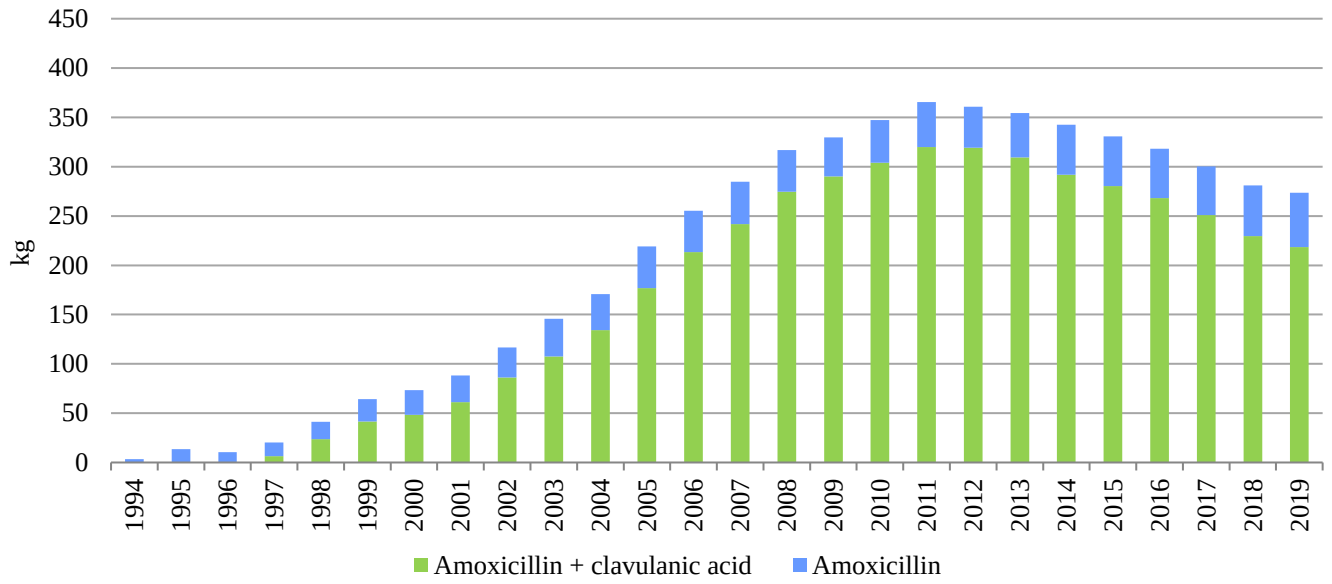


FIGURE 10. Sales, in kg active substance, of antibacterials belonging to penicillin veterinary medicinal products for companion animals (dogs and cats), in Norway in 1994-2019.

Of the antibacterials for which restriction of use in animals is recommend at EU/EEA level due to public health concerns – i.e. 3rd and 4th generation cephalosporins, polymyxins and quinolones (see text box), only 3rd and 4th generation cephalosporins and quinolones (fluoroquinolones) are marketed in Norway for dogs and cats. From 1993 to 2019, the proportion of sales of fluoroquinolones for this animal category has been very low, accounting for 0.5% in 1993 increasing to 2.8% in 2011. Since then, this proportion has gradually decreased to 1.7% in 2019 (Figures 9 and 11).

Antibacterials for which use in animals is adviced to be restricted

The text box on page 25 summarises categorisation of antibiotics for use in animals for prudent and responsible use at EU/EEA level, recently published by the Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the European Medicines Agency (EMA). For certain classes – i.e. quinolones (fluoroquinolones and other quinolones), 3rd and 4th generation cephalosporins and polymyxins it is advised that the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions. Figure 11 shows the amounts sold, in kg of the antibacterials, belonging to the categories that AMEG advices to restrict the use of, compared to the total sales of antibacterial VMPs, stratified by animal categories. In total, 1.5% of the sales of antibacterial VMPs was accounted for by the AMEG category adviced to restrict use and was primarily accounted for by use in farmed fish. Of note is that apart from one VMP for local ear treatment, other pharmaceutical forms of VMPs containing polymyxins are not marketed in Norway.

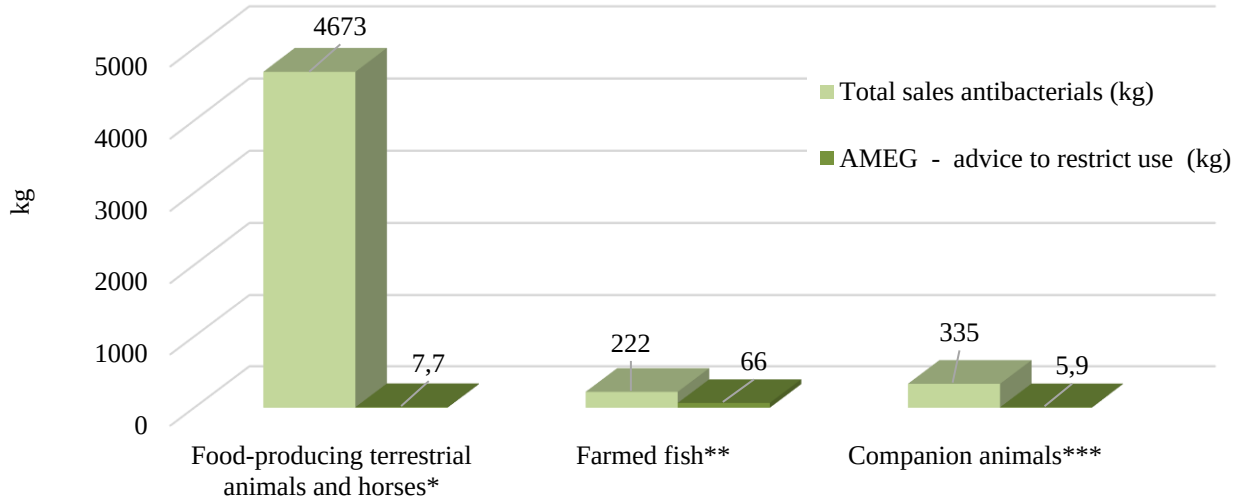


FIGURE 11. Total sales and sales of antibacterial veterinary medicinal products (VMPs) in 2019, for which the Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the European Medicines Agency advises that the use needs to be restricted, stratified by animal category. Of note, VMPs for topical treatment are not included. *Fluoroquinolones. ** Other quinolones. *** 3rd generation cephalosporins and fluoroquinolones.

Categorisation of antibiotics for use in animals for prudent and responsible use at EU/EEA level

The Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the European Medicines Agency has recently updated the categorisation of antibiotics based on the potential consequences for public health of increased antimicrobial resistance, when used in animals and the need for their use in veterinary medicine (1). This categorisation does not directly translate into a treatment guideline for use of antibiotics in veterinary medicine but can be used as a tool by those preparing national guidelines.

Veterinarians are encouraged to check the AMEG categorisation before prescribing any antibiotic for animals in their care. The AMEG categorisation does not replace national treatment guidelines, which also need to take account of other factors such as supporting information in the Summary of Product Characteristics for available medicines, constraints around use in food producing animal species, regional variations in the occurrence of diseases and antibiotic resistance, and national prescribing policies. The brief categorisation (2) as well as the antibiotic classes and substances included in Category A – Avoid and Category B – Restrict (2) are shown below.

TABLE 5. Categorisation according to the Antimicrobial Advice Ad Hoc Expert Group (AMEG) of the European Medicines Agency (AMEG) (2).

Category A **Avoid**	Category B **Restrict**
• antibiotics in this category are not authorised as veterinary medicines in the EU • should not be used in food-producing animals • may be given to companion animals under exceptional circumstances	• antibiotics in this category are critically important in human medicine and use in animals should be restricted to mitigate the risk to public health • should be considered only when there are no antibiotics in Categories C or D that could be clinically effective • use should be based on antimicrobial susceptibility testing, wherever possible
Category C Caution	**Category D Prudence**
• for antibiotics in this category there are alternatives in human medicine • for some veterinary indications, there are no alternatives belonging to Category D • should be considered only when there are no antibiotics in Category D that could be clinically effective	• should be used as first line treatments, whenever possible • as always, should be used prudently, only when medically needed

TABLE 6. Antibiotic classes and substances included in Category A – Avoid and Category B – Restrict (2).

A	**Amdinopenicillins** mecillinam pivmecillinam	**Carbapenems** meropenem doripenem	**Drugs used solely to treat tuberculosis or other mycobacterial diseases** isoniazid ethambutol pyrazinamide ethionamide	**Glycopeptides** vancomycin	AVOID
	Ketolides telithromycin	**Lipopeptides** daptomycin		**Glycylcyclines** tigecycline	
	Monobactams aztreonam	**Oxazolidinones** linezolid		**Phosphonic acid derivates** fosfomycin	
	Rifamycins (except rifaximin) rifampicin	**Riminofenazines** clofazimine	**Other cephalosporins and penems (ATC code J01DI), including combinations of 3rd-generation cephalosporins with beta lactamase inhibitors** ceftobiprole ceftaroline ceftolozane-tazobactam faropenem	**Pseudomonic acids** mupirocin	
	Carboxypenicillin and ureidopenicillin, including combinations with beta lactamase inhibitors piperacillin-tazobactam	**Sulfones** dapsone		**Substances newly authorised in human medicine following publication of the AMEG categorisation** to be determined	
		Streptogramins pristinamycin virginiamycin			
B	**Cephalosporins, 3rd- and 4th-generation, with the exception of combinations with β-lactamase inhibitors** cefoperazone cefovecin cefquinome ceftiofur	**Polymyxins** colistin polymyxin B	**Quinolones: fluoroquinolones and other quinolones** cinoxacin danofloxacin difloxacin enrofloxacin flumequine ibafloxacin marbofloxacin norfloxacin orbifloxacin oxolinic acid pradofloxacin		RESTRICT

Category B includes the quinolones, 3rd and 4th generation cephalosporins and polymyxins. Of note is that these are the same antibiotics as those listed by WHO (3) as the highest priority critical important antimicrobials (HP CIAs) for human medicine. WHO also list macrolides in this category. For the AMEG Category B antibiotics, it is advised that the risk to public health resulting from veterinary use needs to be mitigated by specific restrictions (1).

TABLE 7. Antibiotic classes and substances in Category C – Caution and Category D – Prudence (2).

C – CAUTION

Aminoglycosides (except spectinomycin)
amikacin
apramycin
dihydrostreptomycin
framycetin
gentamicin
kanamycin
neomycin
paromomycin
streptomycin
tobramycin

Aminopenicillins, in combination with beta lactamase inhibitors
amoxicillin + clavulanic acid
ampicillin + sulbactam

Cephalosporins, 1st- and 2nd-generation, and cephamycins
cefacetrile
cefadroxil
cefalexin
cefalonium
cefalotin
cefapirin
cefazolin

Amphenicols
chloramphenicol
florfenicol
thiamphenicol

Lincosamides
clindamycin
lincomycin
pirlimycin

Pleuromutilins
tiamulin
valnemulin

Macrolides
erythromycin
gamithromycin
oleandomycin
spiramycin
tildipirosin
tilmicosin
tulathromycin
tylosin
tylvalosin

Rifamycins: rifaximin only
rifaximin

D – PRUDENCE

Aminopenicillins, without beta-lactamase inhibitors
amoxicillin
ampicillin
metampicillin

Tetracyclines
chlortetracycline
doxycycline
oxytetracycline
tetracycline

Aminoglycosides: spectinomycin only
spectinomycin

Anti-staphylococcal penicillins (beta-lactamase-resistant penicillins)
cloxacillin
dicloxacillin
nafcillin
oxacillin

Sulfonamides, dihydrofolate reductase inhibitors and combinations
formosulfathiazole
phthalylsulfathiazole
sulfacetamide
sulfachlorpyridazine
sulfaclozine
sulfadiazine
sulfadimethoxine
sulfadimidine
sulfadoxine
sulfafurazole
sulfaguanidine
sulfalene
sulfamerazine
sulfamethizole
sulfamethoxazole
sulfamethoxypyridazine
sulfamonomethoxine
sulfanilamide
sulfapyridine
sulfaquinoxaline
sulfathiazole
trimethoprim

Natural, narrow-spectrum penicillins (beta lactamase-sensitive penicillins)
benzathine benzylpenicillin
benzathine phenoxymethylpenicillin
benzylpenicillin
penethamate hydriodide
pheneticillin
phenoxymethylpenicillin
procaine benzylpenicillin

Cyclic polypeptides
bacitracin

Steroid antibacterials
fusidic acid

Nitroimidazoles
metronidazole

Nitrofuran derivatives
furaltadone
furazolidone

Administration routes

In order to limit exposure of the microbiome, AMEG advices, among others, that the antimicrobial selection pressure should be as local as possible. A suggested listing of routes of administration and formulations, ranked in order from those with in general lower effect on the selection of AMR to those that would be expected to have higher impact on resistance, is shown below (2):

Other factors to consider

The **route of administration** should be taken into account alongside the categorisation when prescribing antibiotics. The list below suggests routes of administration and types of formulation ranked from the lowest to the highest estimated impact on antibiotic resistance.

Local individual treatment (e.g. udder injector, eye or ear drops)
Parenteral individual treatment (intravenously, intramuscularly, subcutaneously)
Oral individual treatment (i.e. tablets, oral bolus)
Injectable group medication (metaphylaxis), only if appropriately justified
Oral group medication via drinking water/milk replacer (metaphylaxis), only if appropriately justified
Oral group medication via feed or premixes (metaphylaxis), only if appropriately justified

This subsection is based on a simple review of literature (1).

References:

1. EMA/CVMP/CHMP/682198/2017, 2019. Categorisation of antibiotics in the European Union https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-european-union-answer-request-european-commission-updating-scientific_en.pdf
2. EMA, 2020. Categorisation of antibiotics for use in animals for prudent and responsible use https://www.ema.europa.eu/en/documents/report/categorisation-antibiotics-use-animals-prudent-responsible-use_en.pdf
3. WHO, 2019. 'Critically Important Antimicrobials for Human Medicine (6th revision) 2018 - Ranking of medically important antimicrobials for risk management of antimicrobial resistance due to non-human use', (https://www.who.int/foodsafety/publications/antimicrobials-sixth/en/).

Kari Grave, Marianne Sunde and Kari Olli Helgesen, Norwegian Veterinary Institute, Oslo, Norway, and Hans Kristian Østensen, Norwegian Medicines Agency, Oslo, Norway.

Sales of antimicrobial and coccidiostat feed additives

Due to reported association between use of avoparcin as antimicrobial growth promoter and the occurrence of vancomycin resistant enterococci in 1995, the Norwegian livestock industry immediately decided phasing out all use of antimicrobial growth promoters (AGPs) with instant stop of using of avoparcin May 1995 (Table 8). In 1996 and 1997, the sales of zinc bacitracin were only 64 kg and 27 kg, respectively, and since 1997 no AGPs have been used for animals in Norway. Data in Table 8 on sales of AGPs in 1995 are given as historical reference.

TABLE 8. Sales, in kg of active substance, of ionophore coccidiostat feed additives in Norway in 2010-2019. Data for 1995 include antimicrobial growth promoters and are given for historical reference. Data were obtained from the Norwegian Food Safety Authority.

Active substance	1995	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019
Avoparcin	419*	0	0	0	0	0	0	0	0	0	0
Zincbacitracin	129	0	0	0	0	0	0	0	0	0	0
Total antimicrobial growth promoters	**548**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
Lasalocid	996	0	0	0	0	0	164	0	0	0	0
Monensin	3,422	805	1,060	1,080	1,174	1,313	1,081	874	875	820	504
Salinomycin	214	0	0	0	0	0	0	0	0	0	0
Narasin	24	9,080	9,394	10,378	12,345	12,409	9,126	562	92**	52**	92**
Total ionophore coccidiostats	**4,656**	**9,885**	**10,454**	**11,458**	**13,519**	**13,722**	**10,371**	**1,436**	**967**	**872**	**596**

* Sold only part of the year; ** Used for control of necrotic enteritis (*Clostridium perfringens*).

References:

1. Kari Grave and Kari Olli Helgesen. Antibacterials for farmed fish – prescribing, usage and diagnoses 2013 - 2017 (In Norwegian: Antibakterielle midler til oppdrettsfisk – rekvirering, forbruk og diagnose 2013 - 2017). Rapport 5: Veterinærinstituttet, 2019.

National Strategy against Antibiotic Resistance (2015-2020)
Targets for reduction of antibiotic usage in animals and farmed fish – Changes according to targets

Previous targets for food-producing terrestrial animals

In 1996, the Norwegian livestock industry set a target for reduction of the usage of antibacterial VMPs, in weight of active substance, by 25% within five years with 1995 as the reference year. This target was reached already after two-three years (Figure 12). After five years the observed reduction was 40% and since then the usage for this animal category has been on approximately the same level – i.e. on average the sales for the period 1999 to 2012 were 39% lower than in 1995 (Figure 2, Figure 12).

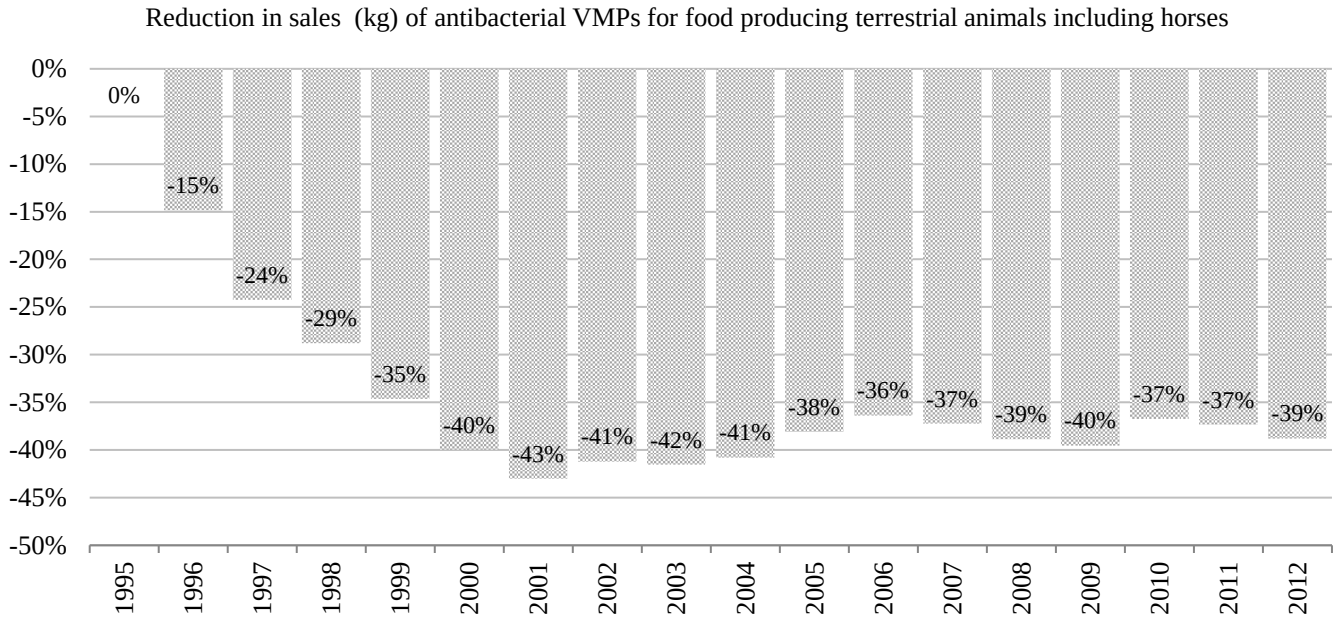


FIGURE 12. Changes in sales (kg active substance) in Norway of antibacterial veterinary medicinal products (VMPs) approved for use in food producing terrestrial animals, including horses, 1995 being the reference year.

Targets 2015 – 2020

In 2015, a National Strategy against Antibiotic Resistance (2015-2020) was agreed upon. Among others, this strategy has set four targets for reduction of usage of antibacterials in terrestrial animals and farmed fish:

1. To reduce the usage of antibacterials in food producing terrestrial animals by 10% by 2020, with 2013 as reference year.
2. In 2020, usage of antibacterials in farmed fish should be at the same level or lower than the average for the period 2004-2014.
3. To reduce the usage of antibacterials in companion animals by 30% by 2020, with 2013 as reference year.
4. Phasing out use of narasin and other coccidiostat feed additives with antibacterial properties in the broiler production without
    a. compromising animal health or animal welfare
    b. increasing the therapeutic use of antibacterials

Approach – assessment of changes

To evaluate progress in terms of reaching the goals set down in the national strategy, sales data for 2013-2019 have been further refined in order to obtain estimates on the usage that are more accurate in terms of identifying changes across time by sector. Data on prescribing per animal species obtained from the Veterinary Prescription Register (VetReg) have been used as supportive information for this refinement (see Appendix 1).

Food-producing terrestrial animals

In order to achieve Target 1 of the national strategy, Animalia, whose role is to provide Norwegian farmers with knowledge and expertise (https://www.animalia.no/en/animalia-in-a-brief/about-animalia/), initiated and coordinated the development and implementation of a joint action plan against antibiotic resistance (1). The suggested key measures to reduce the usage of antibacterials in the livestock industry are prevention of diseases and biosecurity as well as optimising the use of antibiotics. This action plan covers cattle, pigs, sheep, goat and poultry. The indicators used to express the usage are: kg (active substance) and mg (active substance)/PCU (population correction unit) (see Appendix 1).

The result of this analysis shows that the reduction in the usage of antibacterial VMPs for cattle, pigs, sheep, goat and poultry from 2013 to 2019 was 21% and 18% when measured in kg and in mg/PCU, respectively (Figure 13). The sales patterns (data from wholesalers) have been stable across the period 2013 to 2019, both in terms of proportion by antibacterial substances and by pharmaceutical forms. The figures are therefore assumed not to be biased by changes towards products/antibacterial classes with higher or lower dosing per treatment. The sales of injectable antibacterial VMP are included in sales for food producing terrestrial animals (horses excluded) but as the proportion of prescribing of such products for horses and companion animals (VetReg data) was relatively stable (and very low) across 2015-2019, the impact on the trends is thought to be minor. Antibacterial human medicinal products (HMPs) are allowed to be used for animals according to the so-called cascade (Directive 2001/82/EC, Article 10) – i.e. if there is no VMP authorised for the condition a HMP is allowed to be used. For food producing species it requires that a maximum residue level (MRL) has been established for the antibacterial substance in question or that it is shown that MRL is not nessecary. Estimates based on VetReg data show that for cattle, pig, sheep and goat (see Appendix 1 for estimation methodology; Table 9 on treatment of broilers) the usage of HMPs was very low for the years 2015-2019 (68 kg, 38 kg, 32 kg, 40 kg and 50 kg, respectively) and was mostly accounted for by benzylpenicillin for injection and primarily used in sheep.

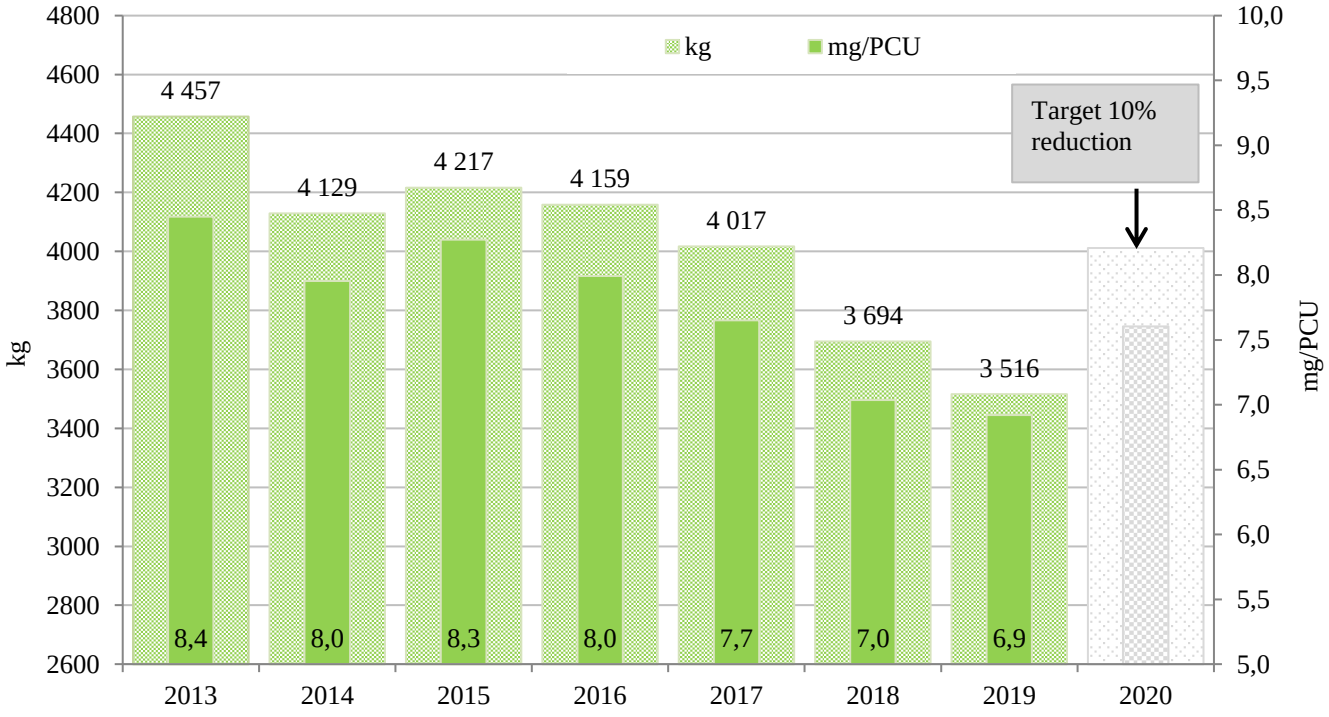


FIGURE 13. Estimated sales, in kg active substance and in mg/PCU, of antibacterial veterinary medicinal products for cattle, pigs, sheep, goat and poultry in Norway from 2013 to 2019 and the target according to the National Strategy. Sales data were obtained from the Norwegian Institute of Public Health. Note that antibacterial human medicinal products are not included. Note the starting points and the differences in the scales of the Y-axes.

Farmed fish

For farmed fish the goal is that the usage of antibacterials should be at the same level or lower in 2020 than the average for the period 2004 to 2014 – i.e. the usage should not be above 1,003 kg or 1.14 mg/PCU (maximum levels).

Figure 14 shows that sales of antibacterial VMPs for farmed fish have been below the maxium level set for the years 2015-2019.

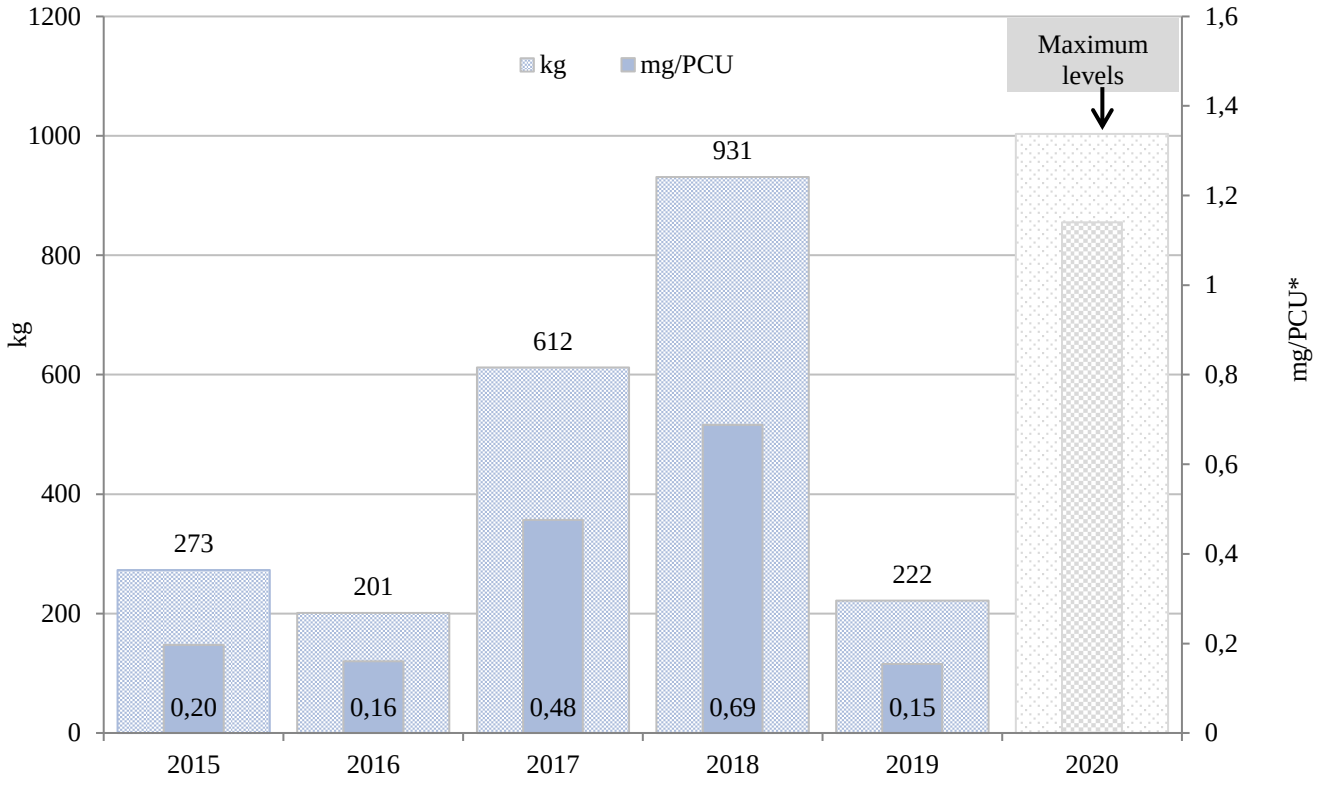


FIGURE 14. Prescription, in kg active substance and in mg/PCU, of antibacterial VMPs for farmed fish, in Norway in the period 2015 to 2019 and the target according to the National Strategy. Maximum levels are based on average for the period 2004-2014. Prescription data were obtained from the Veterinary Prescription Register and include prescription for cleaner fish. Note the differences in the scales of the Y-axes.

Companion animals (dogs and cats)

Sales of antibacterial VMPs for companion animals include tablets, oral solution, injectable and oral paste approved for dogs and cats only (see Appendix 1 for exception for tablets). From 2013 to 2019 a reduction in the sales of such antibacterial VMPs for companion animals of 37% is observed (Figure 15). The usage of antibacterial HMPs for dogs and cats for 2015 to 2019, estimated by use of VetReg data, declined gradually from 269 kg to 220 kg (see Appendix 1 for estimation methodology). This indicates that prescribing of antibacterial HMPs has not substituted antibacterial VMPs for companion animals.

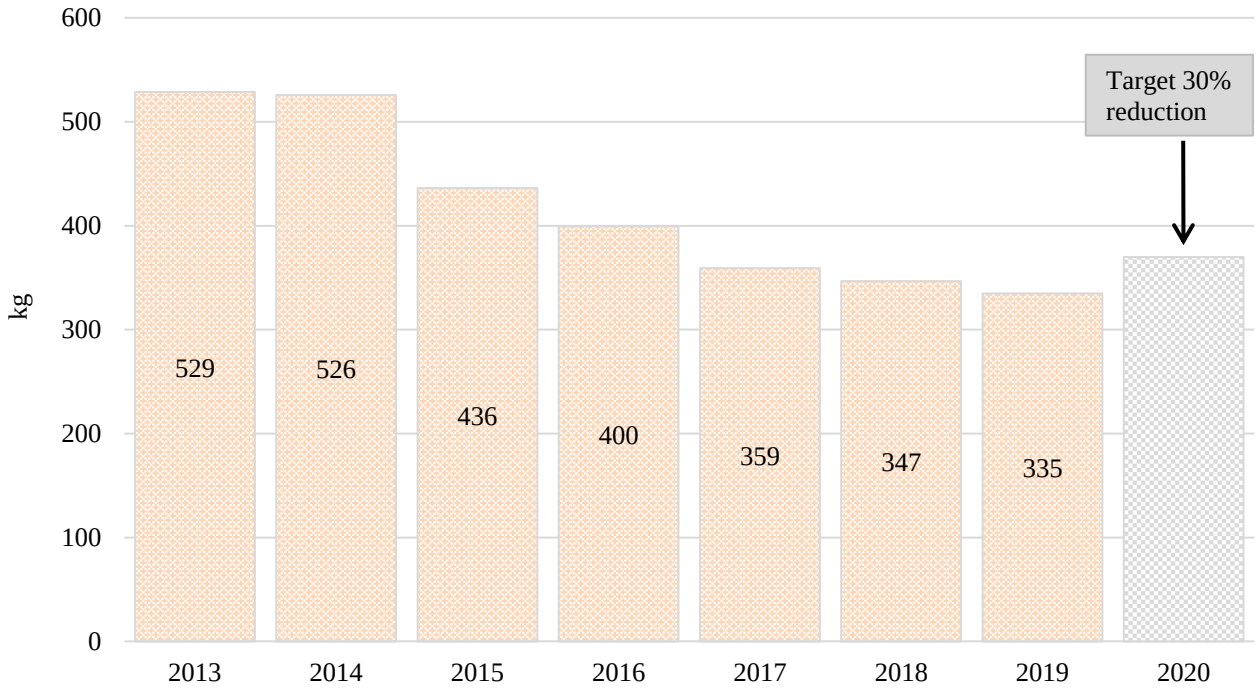


FIGURE 15. Sales in Norway, in kg active substance, of antibacterial veterinary medicinal products (VMPs) marketed for therapeutic use in companion animals only (oral paste, oral solution and tablets; exceptions for tablets - see Appendix 1) in the period 2013-2019 and the target according to the National Strategy.

Phasing out narasin in the broiler production

Narasin was gradually phased out as coccidiostat feed additive by the Norwegian broiler industry during the period from February 2015 to June 2016 (Table 8). One of the targets stated in the National Strategy against Antibiotic Resistance is phasing out use of narasin as coccidiostat feed additive in the Norwegian broiler industry, without increasing the usage of antibacterials for therapeutic use.

Due to the quality of the VetReg data for poultry in general – i.e. it was not possible to report to VetReg the VMP typically used for broilers; data on number of treatments with antibiotics were obtained from Animalia (Thorbjørn Refsnes, personal communication). Table 9 shows that the annual percentage of broiler flocks treated with antibiotics has been very low during the years 2013 to 2019.

TABLE 9. Number and percentages (in brackets) of broiler flocks, by production stage, treated with antibacterial veterinary medicinal products (VMPs)[1] in Norway in the period 2013-2019. Data were obtained from HelseFjørfe, Animalia.

	2013	2014	2015[3]	2016[4]	2017	2018	2019
Broiler production	No of flocks treated (%)	No of flocks treated (%)	No of flocks treated (%)	No of flocks treated (%)	No of flocks treated (%)	No of flocks treated (%)	No of flocks treated (%)
Breeders P[5] (Rearing)	1 (1.1)	2 (2.2)	1 (1)	0 (-)	0 (-)	0 (-)	0 (-)
Breeders P[5] (Layers)	1 (1.1)	0 (-)	1 (1)	2 (2.1)	0 (-)	1 (1.4)	1[2] (1.3)
Broiler	8 (0.16)	2 (0.04)	1 (0.02)	3 (0.07)	7 (0.18)	4 (0.10)	2 (0.05)
No. flocks treated	10	4	3	5	7	5	3

[1] Mostly phenoxymethylpenicillin VMPs; minor use of amoxicillin VMPs up to 2017. [2]One flock treated with oxytetracycline. [3]Phasing out narasin as coccidiostat feed additive started February 2015. [4]Out-phasing of narasin finished June 2016. [5]Parents.

Narasin has been used in some cases of necrotic enteritis (*Clostridium perfringens*). In 2017, 2018 and 2019, a few of the broiler flocks were given narasin in 5-7 days, with the same daily dose as when used as coccidiostat feed additive and a withdrawal period of two days was applied (Bruce David, Nortura, personal communication).

References

1. Animalia, 2017. The Norwegian livestock industry's joint action plan on antimicrobial resistance. (https://www.animalia.no/contentassets/05c57591f69d4e1da9bb5c44668bd0c1/eng_husdyrnaringas-hplan-amr-endelig-enkeltsider_220617.pdf).

USAGE IN HUMANS

Hege Salvesen Blix, Marion Neteland, Per Espen Akselsen and Sigurd Høye

Overall antibiotic sales

In 2019, the total sales of antibacterials for systemic use in humans (J01, excluding methenamine) increased by 2% compared to 2018 from 12.9 to 13.2 Defined Daily Doses (DDD)/1,000 inhabitants/day (Table 10). The use has decreased every year since 2012 and the small increase from 2018 to 2019 – back to approximately the level of 2017 – may indicate that the use has stabilised at a new and more appropriate level for the three last years. Antibiotics are prescription-only drugs in Norway and probably there are still areas of improvement, e.g. in choice of antibiotics or duration of course length, so one should expect that it is possible to achieve an even better narrow-spectrum profile and a lower consumption rate.

Overall antibiotic consumption includes all sales of antibiotics to humans in Norway i.e. in primary care, in hospitals and in long-term care institutions. Around 84% of the human use of antibacterials is used by patients outside health institutions. Hospitals cover 8% of total DDDs of antibiotics and long-term care institutions probably around 6-7%.

The overall consumption (J01, excl. methenamine) has decreased by 22% since 2012, when a *Mycoplasma pneumoniae* epidemic caused a high prescription rate of macrolides and tetracyclines. In the latest years, decreased sales are observed for all main antibiotic subgroups, with a small increase of penicillins and tetracyclines in 2019 (Figure 16). The proportion of narrow-spectrum penicillins (J01CE) of the total sales (J01, excl. methenamine) has been quite stable around 27%, but it was higher 20 years ago; in 1997, the proportion was 35%. The small increase in total use of antibiotics in 2019 was mainly due to increased use of antibiotics for respiratory tract infections observed for all age groups. Antibiotics for urinary tract infections also increased, but to a lesser extent. The latter may be due to the shortage of methenamine in spring 2019, leading to a large decrease in DDDs for methenamine (Table 10, Figure 16).

TABLE 10. Human usage of antibacterial agents in Norway 2012-2019 by ATC groups. The usage is presented as DDD (Defined Daily Doses)/1,000 inhabitants/day and in % change 2018-2019. Methodology for collection of data on human usage of antimicrobial agents is presented in Appendix 2.

ATC	Groups of substances	2012	2013	2014	2015	2016	2017	2018	2019	Change (%) 2018-2019
J01A	Tetracyclines	3.87	3.54	3.46	3.39	3.16	3.01	2.86	2.96	+ 3
J01B	Amphenicols	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-
J01CA	Penicillins with extended spectrum	2.79	2.82	2.90	2.73	2.62	2.47	2.46	2.53	+ 3
J01CE	Beta-lactamase sensitive penicillins	4.31	4.09	3.88	3.88	3.73	3.61	3.43	3.56	+ 4
J01CF	Beta-lactamase resistant penicillins	0.90	0.79	0.91	0.89	0.90	0.84	0.90	0.93	+ 3
J01CR	Combination of penicillins	0.04	0.05	0.07	0.08	0.10	0.07	0.08	0.10	+ 25
J01D	Cephalosporins, monobactams, carbapenems	0.53	0.50	0.46	0.43	0.42	0.38	0.39	0.37	- 5
J01E	Sulfonamides and trimethoprim	0.87	0.86	0.88	0.88	0.85	0.84	0.88	0.93	+ 6
J01F	Macrolides, lincosamides and streptogramins	2.26	1.94	1.68	1.51	1.33	1.18	1.05	1.04	- 1
J01G	Aminoglycosides	0.08	0.07	0.08	0.08	0.08	0.09	0.09	0.10	+ 11
J01M	Quinolones	0.74	0.71	0.67	0.60	0.53	0.45	0.42	0.36	- 14
J01X*	Other antibacterials	0.47	0.45	0.43	0.41	0.38	0.36	0.32	0.32	-
J01	**Total excluding methenamine**	**16.9**	**15.8**	**15.4**	**14.9**	**14.1**	**13.3**	**12.9**	**13.2**	**+ 2**
J01XX05	Methenamine	3.57	3.70	3.86	3.99	4.09	4.11	4.08	3.39	- 17
J01	**Total all antimicrobial agents**	**20.4**	**19.5**	**19.3**	**18.9**	**18.2**	**17.4**	**16.9**	**16.6**	**- 2**

*J01X includes glycopeptides, colistin, fusidic acid, metronidazol (i.v.), nitrofurantoin, fosfomycin, linezolid, daptomycin and tedizolid. Methenamine is excluded.

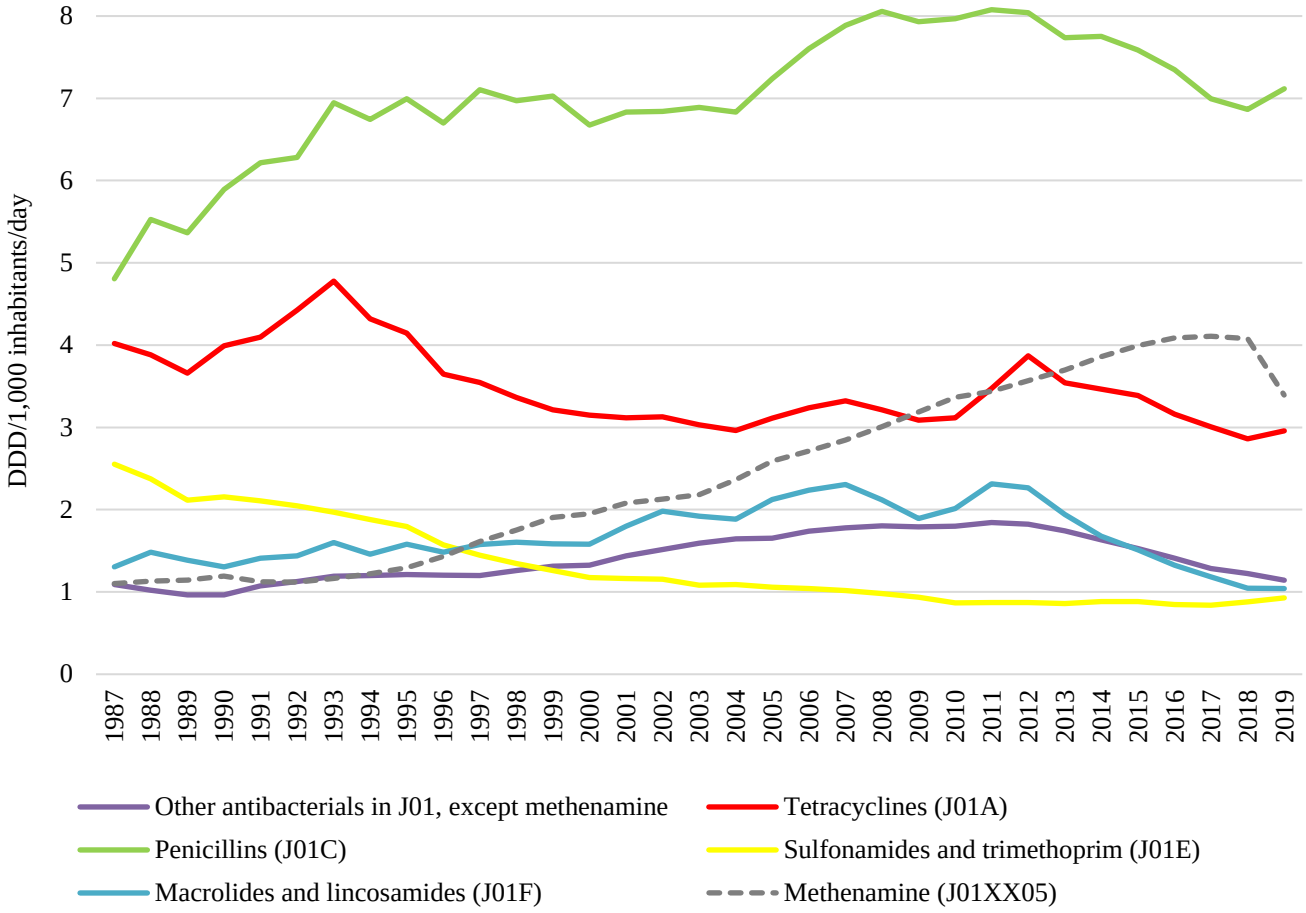


FIGURE 16. Sales of penicillins (J01C), tetracyclines (J01A), macrolides, lincosamides and streptogramines (J01F), sulfonamides and trimethoprim (J01E), methenamine and other antibacterials in Norway 1987-2019. Other types of antibacterials include all other antibacterials in ATC group J01, except methenamine (J01XX05).

The beta-lactamase sensitive penicillin-group (J01CE), the tetracyclines (J01A) and penicillins with extended spectrum (J01CA) were the three most used antibacterial groups in Norway in 2019. The use of the urinary prophylactic agent methenamine seemed to have reached a stable level in 2016, but due to the shortage in spring 2019 it is difficult to evaluate the current trend (Figure 17, Table 11). Methenamine has the largest amount of DDDs of all antibiotics and accounted for 20% of total antibacterial use in 2019. Of the tetracyclines (J01A), doxycycline is most frequently used, followed by lymecycline, a drug mainly indicated for acne (Table 11).

In 2019, the penicillins (ATC group J01C) accounted for 43% of the total antibacterial use in Norway (Figure 17). Over the years there has been a shift towards use of more broad-spectrum penicillins. In 2019, beta-lactamase sensitive penicillins accounted for half of the penicillin group (50% share) measured in DDDs, and this picture has been stable since 2012. Penicillins with extended spectrum (J01CA) represent 35% of the J01C group compared to 23% in 1999 (Figure 17). This is mainly due to increasing use of amoxicillin and pivmecillinam. An increased use of penicillins with beta-lactamase inhibitors has been observed in the latest years (Table 11). In May 2017, oral co-amoxiclav was approved in Norway, since then a significant increase is observed. Pivmecillinam is the main antibiotic used for urinary tract infections, at the expense of trimethoprim and possibly due to increasing resistance in *E. coli*. The subgroup of sulfonamides and trimethoprim as a whole has decreased over the years, but the combination - co-trimoxazole - is increasing; since 2012 by 58% (Figures 16-17, Table 11).

Since 2012 the use of macrolides has dropped markedly (Tables 10-11 and Figures 16-17). The use of of the group J01F macrolides, lincosamides and streptogramins has followed a wavy pattern over the years. The shifts in use could be explained to some degree by the recurrent epidemics of *M. pneumoniae* in Norway, occurring with four- to six-year intervals. Furthermore, the decreased use since 2012 can partly be explained by a change in treatment guidelines for sexually transmitted diseases as azithromycin is no longer first line treatment.

In the latest years, sales of ATC group J01D (cephalosporins, monobactams and carbapenems) have decreased, mainly due to decreased use of 1st and 2nd generation cephalosporins (Tables 10-11 and Figure 17).
The quinolones represent only a small fraction (2%) of total antibacterial sales (Tables 10-11 and Figure 17), and the use has steadily decreased since 2012. Focus has been on the resistance driving effect of the quinolones, and in combination with “dear doctor” letters on severe adverse effects of fluoroquinolones, this has probably caused the decrease. Ciprofloxacin is the main substance accounting for 92% of the quinolone group in 2019.

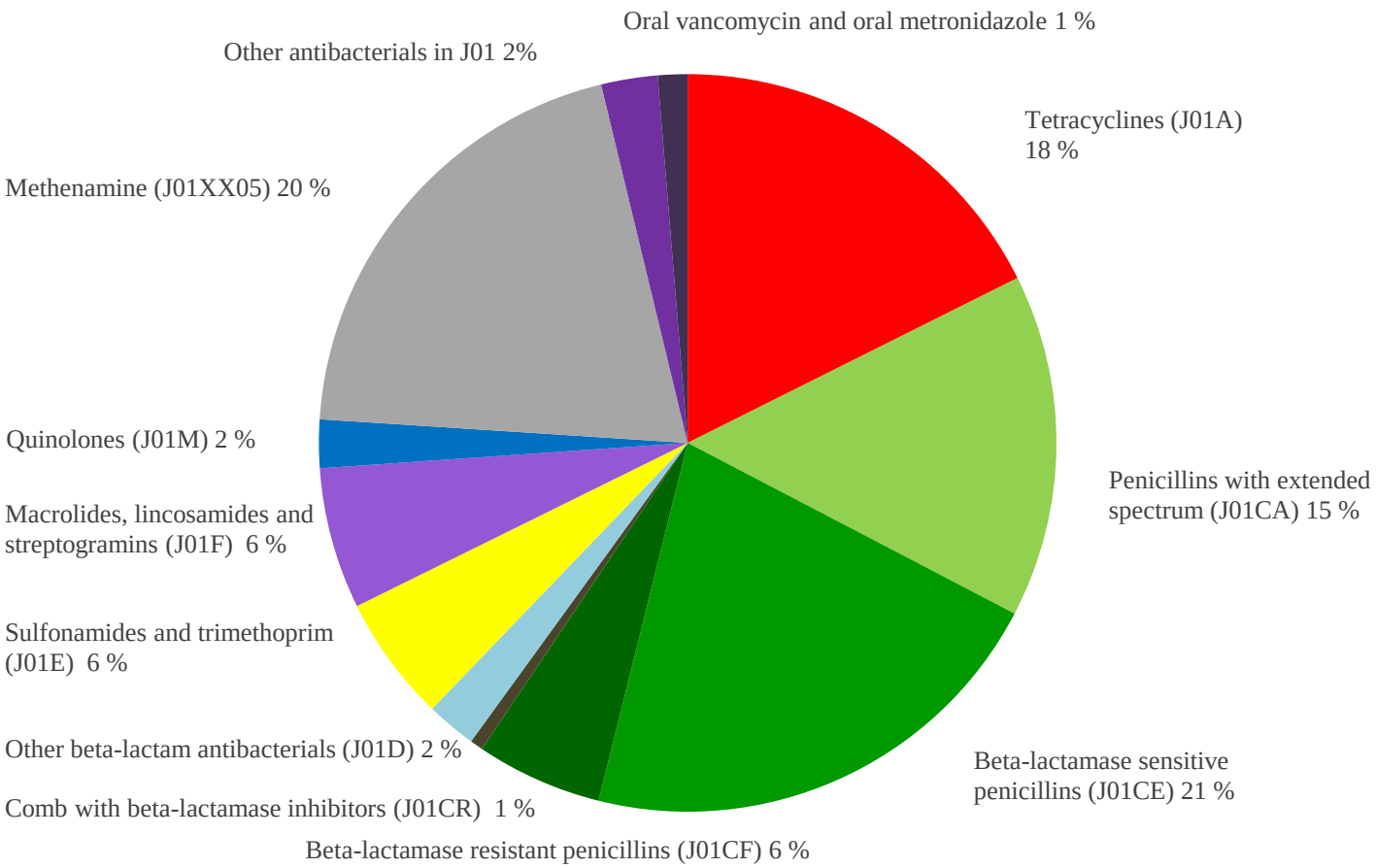


FIGURE 17. Relative amount of antibacterial agents for systemic use in 2019 in Defined Daily Doses (DDD) (total sales in the country).

TABLE 11. Total human usage of single antibacterial agents for systemic use in Norway. Sales for overall use are given in DDD/1,000 inhabitants/day. The methodology for collection of data on human usage of antibacterial agents is presented in Appendix 2.

ATC group	ATC code	Substance	2012	2014	2016	2018	2019
J01A - Tetracyclines	J01A A02	Doxycycline	2.36	1.99	1.82	1.60	1.67
	J01A A04	Lymecycline	0.90	0.96	0.94	0.93	0.98
	J01A A06*	Oxytetracycline	-	<0.001	<0.001	<0.001	<0.001
	J01A A07	Tetracycline	0.62	0.50	0.40	0.32	0.31
	J01A A08*	Minocycline	0.006	0.003	0.002	0.001	0.001
	J01A A12	Tigecycline	<0.001	<0.001	<0.001	<0.001	<0.001
J01B - Amphenicols	J01B A01	Chloramphenicol	<0.001	<0.001	<0.001	<0.001	<0.001
J01CA - Penicillins with extended spectrum	J01C A01	Ampicillin	0.03	0.04	0.04	0.05	0.05
	J01C A04	Amoxicillin	0.97	0.97	0.88	0.84	0.89
	J01C A08	Pivmecillinam	1.78	1.87	1.69	1.57	1.58
	J01C A11	Mecillinam	0.008	0.008	0.005	0.002	0.003
J01CE - Beta-lactamase sensitive penicillins	J01C E01	Benzylpenicillin	0.24	0.24	0.23	0.24	0.23
	J01C E02	Phenoxymethyl-penicillin	4.07	3.64	3.50	3.18	3.33
	J01C E08*	Benzathine benzylpenicillin	<0.001	<0.001	<0.001	<0.001	<0.001
J01CF - Beta-lactamase resistant penicillins	J01C F01	Dicloxacillin	0.76	0.72	0.74	0.74	0.76
	J01C F02	Cloxacillin	0.14	0.19	0.17	0.16	0.17
	J01C F05*	Flucloxacillin	<0.001	<0.001	<0.001	<0.001	<0.001
J01CR - Combination of penicillins, incl. beta-lactamase inhibitors	J01C R02	Amoxicillin and enzyme inhibitor	0.002	0.008	0.011	0.028	0.042
	J01C R05	Piperacillin and enzyme inhibitor	0.03	0.07	0.09	0.05	0.05
J01DB – first gen. cephalosporins	J01D B01	Cefalexin	0.18	0.14	0.10	0.09	0.07
	J01D B03	Cefalotin	0.08	0.09	0.09	0.07	0.02
	J01D B04	Cefazolin				0.03	0.09
J01DC – second gen. cephalosporins	J01D C02	Cefuroxime	0.08	0.06	0.04	0.03	0.03
J01DD – third gen. cephalosporins	J01D D01	Cefotaxime	0.12	0.12	0.12	0.12	0.12
	J01D D02	Ceftazidime	0.01	0.01	0.01	0.01	0.01
	J01D D04	Ceftriaxone	0.03	0.02	0.02	0.02	0.02
	J01D D08*	Cefixime			<0.001	<0.001	<0.001
	J01D D52	Ceftazidime and avibactam				<0.001	<0.001
J01DF - Monobactams	J01D F01	Aztreonam	<0.001	0.001	0.001	<0.001	<0.001
J01DH - Carbapenems	J01D H02	Meropenem	0.03	0.03	0.03	0.02	0.02
	J01D H03	Ertapenem	0.002	0.002	0.002	0.002	0.002
	J01D H51	Imipenem and enzyme inhibitor	0.002	0.002	0.002	0.002	0.002
J01DI – Other cephalo-sporins and penems	J01D I02	Ceftaroline fosamil		<0.001	<0.001	<0.001	<0.001
	J01DI54	Ceftolozane and enzyme inhibitor			<0.001	<0.001	<0.001
J01E - Sulfonamides and trimethoprim	J01E A01	Trimethoprim	0.51	0.46	0.38	0.34	0.36
	J01E C02*	Sulfadiazine			0.001	<0.001	
	J01E E01	Sulfamethoxazole and trimethoprim	0.36	0.40	0.44	0.53	0.57

ATC group	ATC code	Substance	2012	2014	2016	2018	2019
J01F - Macrolides, lincosamides and streptogramins	J01F A01	Erythromycin	1.06	0.75	0.60	0.44	0.44
	J01F A02	Spiramycin	0.01	0.005	0.003	0.002	0.002
	J01F A06*	Roxithromycin		<0.001	<0.001	<0.001	<0.001
	J01F A09	Clarithromycin	0.39	0.23	0.14	0.11	0.11
	J01F A10	Azithromycin	0.48	0.35	0.30	0.24	0.24
	J01FS15	Telithromycin	<0.001	<0.001	<0.001		
	J01F F01	Clindamycin	0.33	0.34	0.28	0.25	0.25
J01G - Aminoglycosides	J01GA01*	Streptomycin	<0.001	<0.001	<0.001	<0.001	<0.001
	J01G B01	Tobramycin	0.03	0.02	0.02	0.01	0.01
	J01G B03	Gentamicin	0.05	0.05	0.06	0.08	0.09
	J01G B06*	Amikacin	0.001	0.001	0.001	0.001	<0.001
J01M - Quinolones	J01M A01	Ofloxacin	0.02	0.01	0.01	0.01	0.01
	J01M A02	Ciprofloxacin	0.71	0.64	0.51	0.39	0.33
	J01MA12	Levofloxacin	0.002	0.002	0.003	0.004	0.004
	J01MA14*	Moxifloxacin	0.004	0.007	0.009	0.011	0.011
J01X - Other antibacterials	J01X A01	Vancomycin	0.01	0.02	0.02	0.02	0.02
	J01X A02	Teicoplanin	0.001	<0.001	<0.001	<0.001	
	J01X B01	Colistin	0.004	0.005	0.006	0.006	0.007
	J01X C01	Fusidic acid	0.005	0.004	0.003	0.003	0.002
	J01X D01	Metronidazole	0.07	0.05	0.03	0.04	0.04
	J01X E01	Nitrofurantoin	0.37	0.35	0.31	0.25	0.24
	J01XX01	Fosfomycin	<0.001	<0.001	<0.001	<0.001	<0.001
	J01X X05	Methenamine	3.57	3.86	4.09	4.08	3.39
	J01XX08	Linezolid	0.01	0.007	0.010	0.009	0.010
	J01XX09	Daptomycin	0.001	<0.001	0.001	0.001	0.001
	J01X X11	Tedizolid			<0.001	<0.001	<0.001
Antibiotics in other ATC groups	A07A A09	Vancomycin	0.002	0.002	0.002	0.002	0.003
	A07A A11	Rifaximin	0.004	0.012	0.043	0.076	0.090
	A07A A12	Fidaxomicin	<0.001	<0.001	<0.001	<0.001	<0.001
	P01A B01	Metronidazole	0.23	0.24	0.23	0.21	0.22
	D06A X09/ R01A X06*	Mupirocin (grams)[1]	145	174	186	247	315

*Drugs not licensed at the Norwegian marked in 2019. [1]Given as the total amount grams (g) mupirocin per year.

Antibiotic usage in primary care

Around 84% of the total human sales of antibacterials are sold as prescriptions from pharmacies - that is prescribed to persons in primary care, mainly those living at home. The data are captured from the Norwegian prescription Database (NorPD) that includes all prescriptions of antibacterials dispensed to persons living in Norway (including those antibiotics prescribed from hospitals to discharged patients and out-patients), see Appendix 2.
The increase in total use of antibacterials was mainly due to increased use in primary care. An increase of 3% was seen from 2018 to 2019 as measured in DDD/1,000 inhabitants. For primary care, the most important antibiotic group in 2019 was the penicillins, J01C (54% of DDDs and 59% of prescriptions in ATC group J01, excl. methenamine). Tetracyclines, J01A was the second most used group (26% of DDDs and 12% of prescriptions) followed by macrolides and lincosamides, J01F (8% of DDDs and 11% of prescriptions). The three antibiotic substances most often prescribed for outpatients in 2019 were phenoxymethyl-penicillin, doxycycline and pivmecillinam. These three antibiotics represented 50% of all prescriptions and 54% of all DDDs of the antibacterial group J01, excluding methenamine. Of the whole ATC group J01 antibacterials for systemic use in primary care, the urinary antiseptic methenamine represented 22% of the DDDs and 7% of the J01 prescriptions – a decrease since 2018.

Geographical variation
The usage of antibacterials varies among the Norwegian counties. The county using the least is using around 73% in DDDs and 75% in prescriptions of the county using the most (Figures 18-20). Over the years, and measured in DDDs, the same counties seem to be high-use counties and low-use counties, respectively. Antibiotic use has decreased in all counties the latest years, but with certain differences between the counties. Oslo is the county with the largest decrease in use of antibiotics (J01) - 26% reduction since 2012 (orange dots in Figure 20).
Females use more antibiotics than males; 23% of the females purchased at least one antibiotic prescription (methenamine excluded) in 2019 compared to 16% of the males. The prevalence of antibiotic use has decreased over the years, more so in the young children than in the elderly. The gender pattern is similar in all regions in the country. Young children, young women and the elderly are high users of antibiotics (Figure 22). Among those who use antibacterials, the elderly population use more; for those above 75 years; 2.1 prescriptions/male user and 2.2 prescriptions/female user are dispensed every year compared to around 1.5 prescriptions/user for younger persons (men and women together) (Figure 23). The number of DDDs/user has increased in 2019; by 1-2% compared to 2018, while the number of prescriptions per user is approximately the same as in 2018 in all age groups except for the youngest and the oldest, where an increase was observed. Mean number of DDDs/prescription is 11.5 DDDs, wich indicates a mean treatment length of 11-12 days.

Antibiotics prescribed by dentists
Physicians are the main prescribers to humans, but dentists prescribe around 5% (measured in DDDs) of antibiotics (J01) to humans in ambulatory care. Moreover, they prescribe 18% of all DDDs of metronidazole oral forms. In 2019, dentists most often prescribed phenoxymethyl-penicillin (76% of all antibiotic DDDs prescribed by dentists) followed by amoxicillin (9%), clindamycin (5%) and oral metronidazole (4%) (Figure 26).

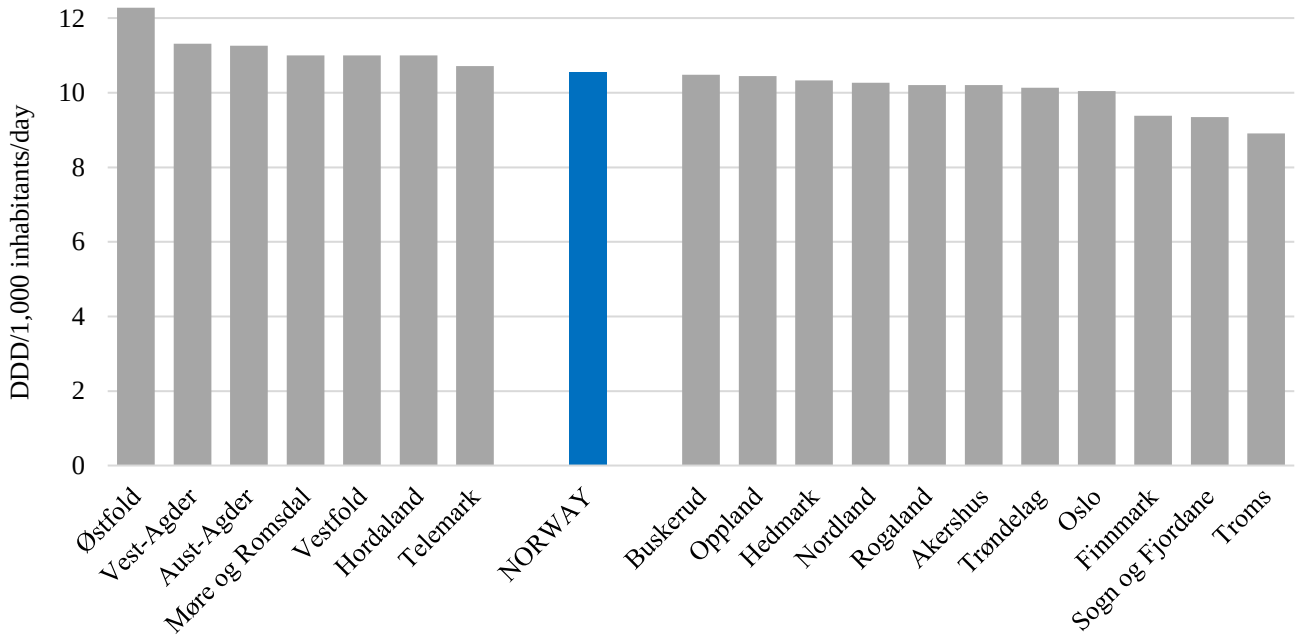


FIGURE 18. Consumption of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2019. Measured as number of DDD/1,000 inhabitants/day. Data from NorPD (i.e. health institutions and sales to prescribers´ own practice not included).

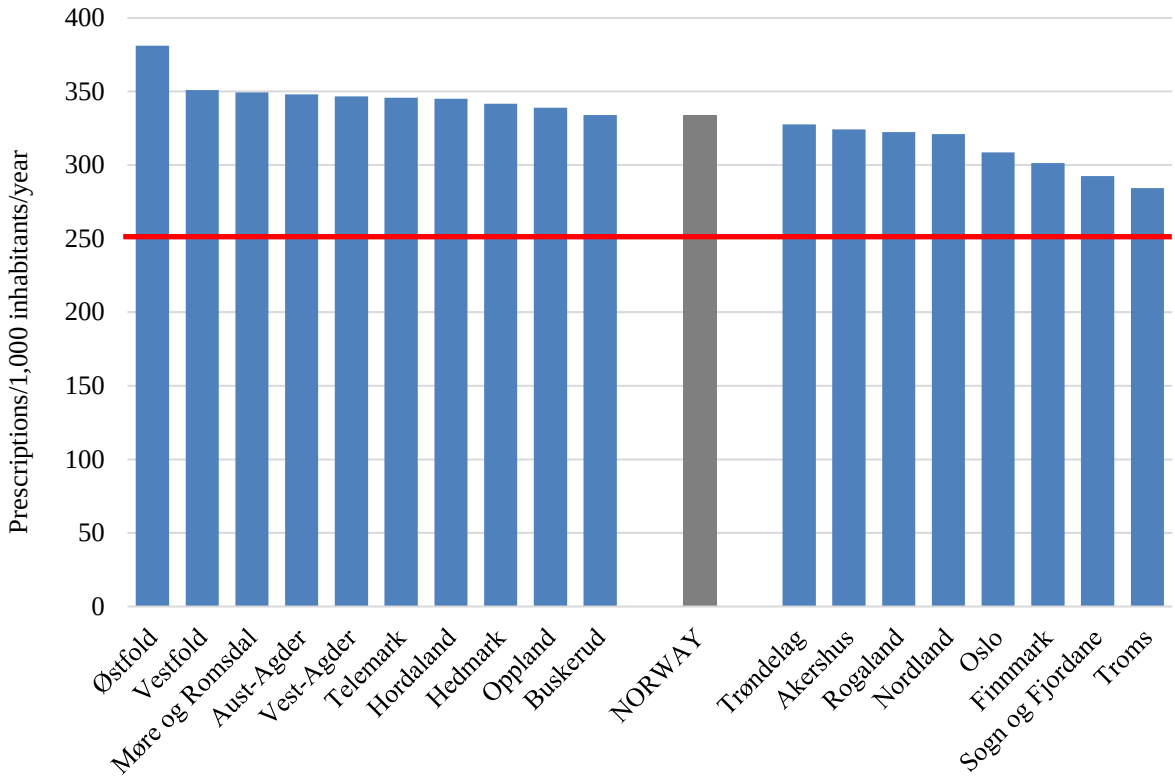


FIGURE 19. Consumption of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2019. Measured as number of prescriptions/1,000 inhabitants. Data from NorPD (excl. health institutions). Red line; goal set by the National Strategy against Antibiotic Resistance 2015-2020.

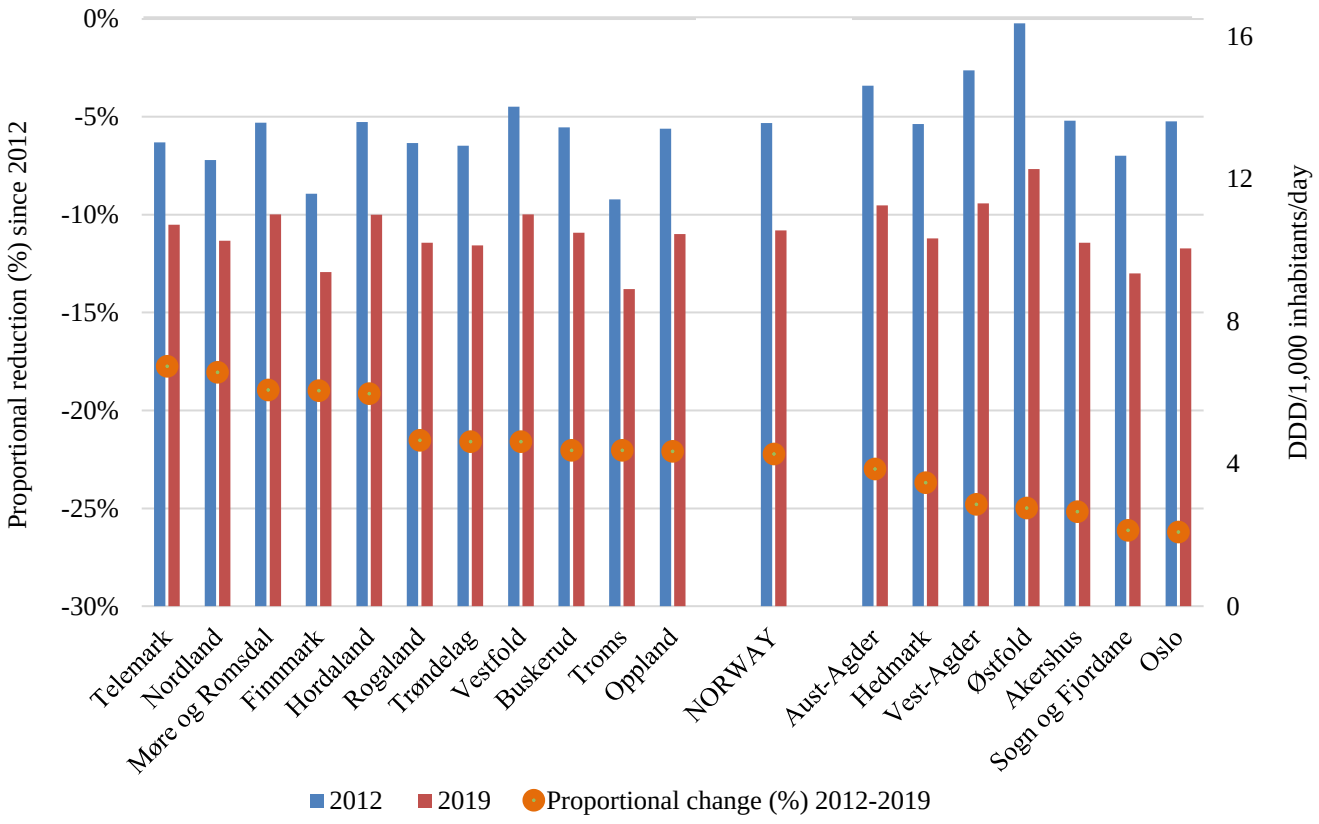


FIGURE 20. Consumption of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2012 and 2019 and proportional change (reduction in %). Measured as number of DDD/1,000 inhabitants/day. Data from NorPD (i.e. health institutions and sales to prescribers´ own practice not included).

RAK – Promoting appropriate use of antibiotics among general practitioners

The Antibiotic Center for Primary Care (ASP) was in 2016, through the Government's Action Plan against Antibiotic Resistance in the Health Services, assigned responsibility for Measure 4.2 *Review of antibiotic prescription at group level* (aimed at general practitioners (GPs)). This has resulted in the quality improvement course *More appropriate use of antibiotics in the municipalities* (RAK – Riktigere Antibiotikabruk i Kommunene).

The course is based on a quality improvement model where participants set goals for change, gain access to measurements of their own quality, and select measures to achieve desired goals. RAK is designed as a 15-hour clinical course, and consists of three e-learning modules and three group meetings that are conducted in the GPs' continuing education groups. Participating GPs receive two individual prescription reports based on data from the the Norwegian Prescription Database, one at the start of the course period and a new one after six months, and they are to be presented and discussed at the group meetings. The reports show individual figures, as well as figures for the entire continuing education group, the municipality, the county and the country.

The e-learning modules provide an introduction to possible measures that GPs may use to reach their goals, such as delayed prescriptions, enhanced communication skills and patient leaflets. It also contains up-to-date knowledge on the most common infections in general practice, with special emphasis on respiratory tract infections.

The course is introduced county by county. The continuing education group leaders in each county are invited to a start-up meeting, after which they guide their group through the course. The course is free of charge, and the participants are rewarded with course points needed to obtain or maintain specialty in general practice/family medicine. Project partners are the Center for Quality in GPs' Offices (SKIL – Senter for kvalitet i legekontor), the Norwegian Institute of Public Health, and the municipal chief medical officers.

As of June 2020, RAK is introduced in 18 out of 19 counties (old county division), and 2,217 GPs participate or have participated, i.e. around 48% of the GPs in these counties. Figure 21 displays the number of prescriptions pr 1,000 listed patients for participating GPs in the counties that have finished the project. Overall, the participants reduced their number of prescriptions with 9.8% during the intervention year, compared to a 4.1% reduction in the country in the same period. Participants also increased the relative use of narrow-spectrum penicillin for respiratory tract infections with 10.3%, compared to a 2.3% increase in the country.

Given the popularity and the impact of the project, ASP is now preparing to make RAK a permanent quality improvement system in Norwegian general practice.

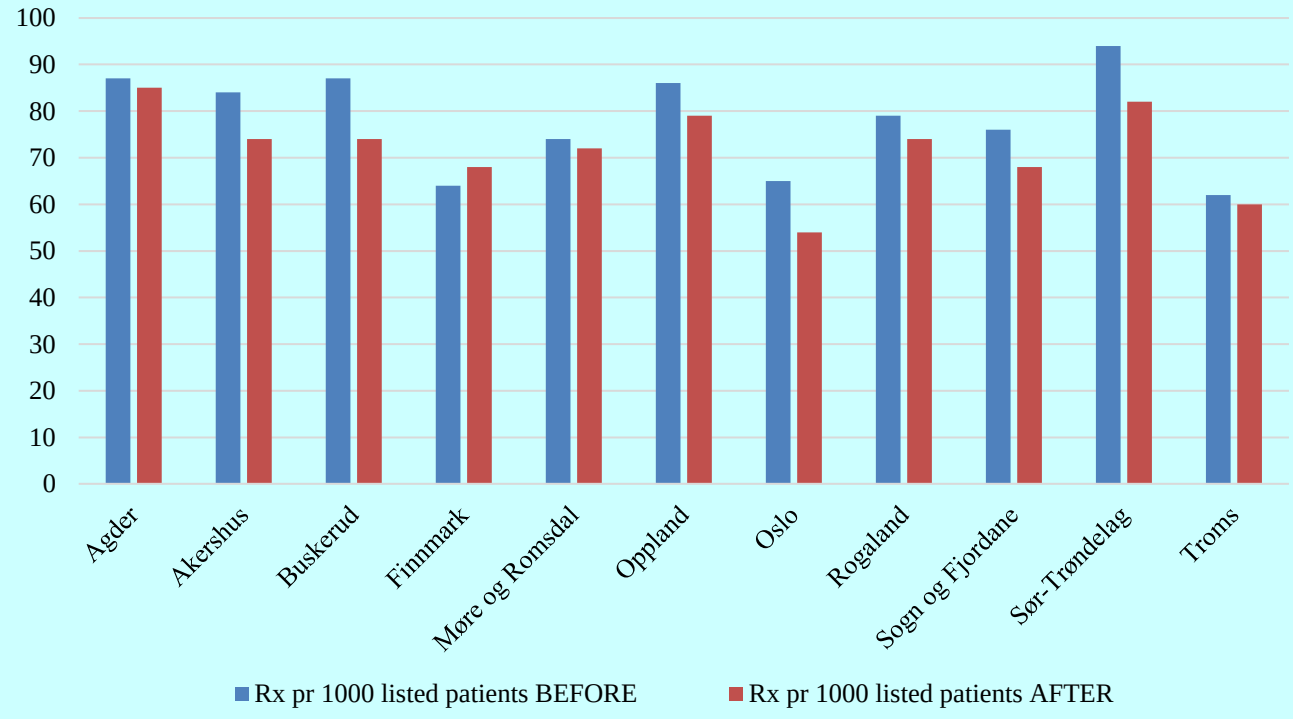


FIGURE 21. RAK participating GPs´ average number of prescriptions (Rx) per 1,000 listed patients (per year).

Sigurd Høye and Morten Lindbæk, The Antibiotic Centre for Primary Care (ASP), General Practice Medicine Unit, University of Oslo, and Hege Salvesen Blix, Department of Drug Statistics, Norwegian Institute of Public Health.

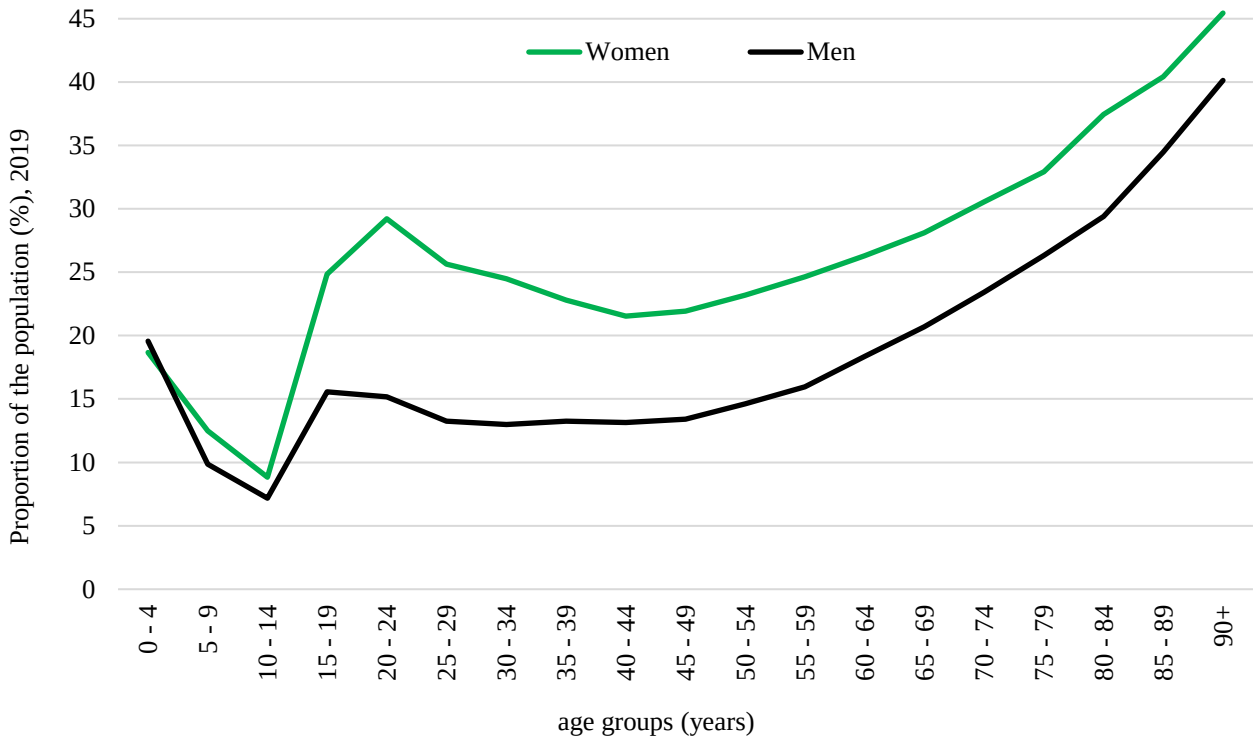


FIGURE 22. Proportion (%) of the population having dispensed at least one prescription of antibacterials (one year prevalence) in primary care by gender and age in Norway, 2019. Antibacterials included are antibacterials for systemic use (ATC group J01), vancomycin (A07AA09), fidaxomicin (A07AA12) and metronidazole (P01AB01). Prevalence in age groups above 65+ is adjusted according to persons from these age groups living outside institutions.

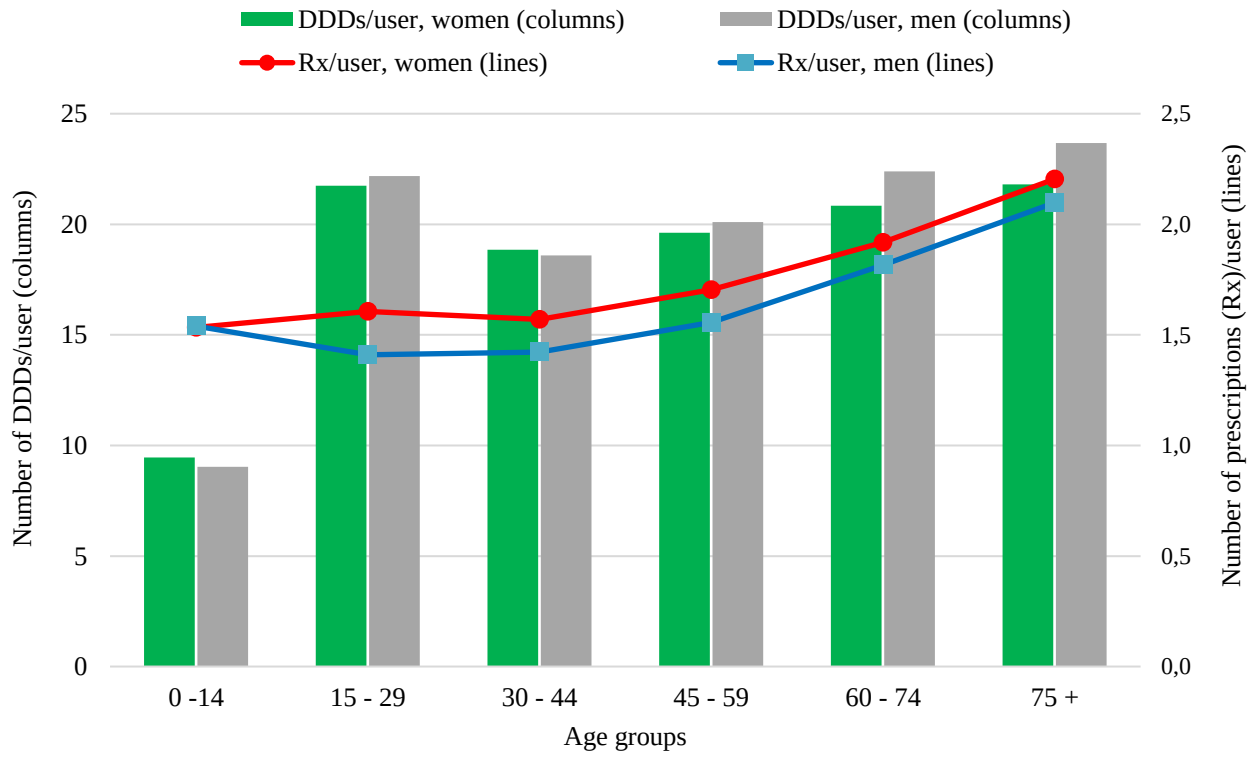


FIGURE 23. Mean number of prescriptions (Rx) per person and mean number of DDDs per person among users of antibacterials in ambulatory care by gender and age in Norway, 2019. Antibacterials included are antibacterials for systemic use (ATC group J01, excl. methenamine).

Antibiotic use in nursing homes

Use of antibiotics in Norwegian nursing homes (NHs) has previously not been included in the NORM report, and only scarcely investigated in descriptive studies apart from point prevalence surveys. Norway has more than 900 NHs with approximately 40,000 residents. A previous study by Blix *et al.* in 133 Norwegian NHs in 2003, found an average antibiotic use of 14.8 DDD/100 bed days (BDs) with a wide variation between the nursing homes (range 4.3-44.4).
In December 2015 the Norwegian Government launched the "National Action Plan against Antibiotic Resistance in the Health Services". As part of this plan, the Antibiotic Center for Primary Care has retrieved one-year antibiotic sales data for each participating NH in the quality improvement programme *RASK (**R**iktigere **A**ntibiotikabruk i **S**ykehjem/**K**ommunale helse-institusjoner*) aiming to improve antibiotic use in nursing homes/municipal acute care facilities. The programme was introduced county by county. In the period October 2015 - October 2019, 14 out of Norway`s 19 counties were covered, and 80-100% of all NHs in these counties have participated in the program.

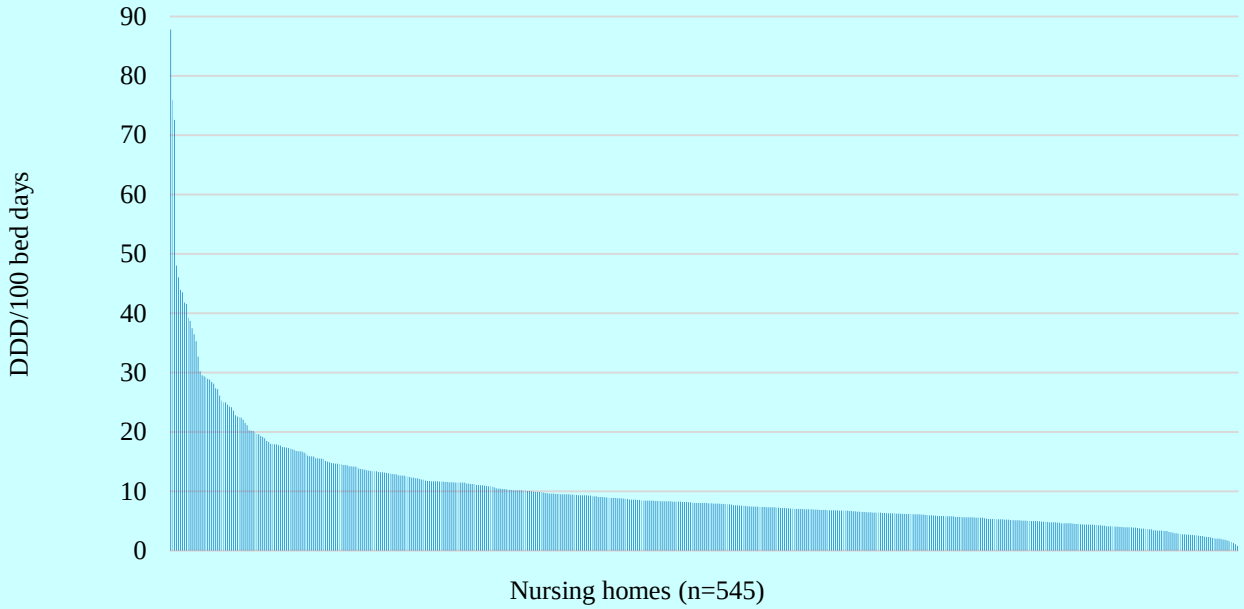


FIGURE 24. One-year total antibiotic consumption (J01 antibacterials excl. methenamine, A07AA09 oral vancomycin, P01AB01 oral metronidazole and J04AB02 rifampicin) expressed as DDD/100 bed days (BD) for each nursing home included in the RASK project in the period of October 2015 - December 2019 (n=545).

In Figure 24, nursing homes with a proportion of municipal acute care beds > 20 % of total beds were excluded (n=15), as patients admitted to municipal acute care units largely differ in characteristics from traditional nursing home residents. Moreover, three nursing homes were excluded as outliers. Median overall antibiotic use was 8.0 DDD/100 BDs, with a large variation ranging from 0.8 to 87.8 DDD/100 BDs. Based on data from RASK, the nursing home share of total antibiotic use for humans in Norway is estimated to be 6% of total antibiotic consumption, which is approximately the same as estimated in the study in 2003. Taking into consideration the high prevalence of bacterial infections in the NH population, and the observed wide variation between nursing homes there is a need for further quality improvement programs. Moreover, there is a need to develop systems for continuous, descriptive surveillance of antibiotic consumption in Norwegian NHs.

Nicolay Jonassen Harbin, Ruth Davey Eig, Sigurd Høye, Svein Gjelstad, Siri Jensen and Morten Lindbæk, The Antibiotic Centre for Primary Care (ASP), General Practice Medicine Unit, University of Oslo, and Hege Salvesen Blix, Department of Drug Statistics, Norwegian Institute of Public Health, Oslo, Norway.

RASK - Promoting appropriate use of antibiotics in municipal healthcare institutions

The Norwegian Government launched its "National Action Plan against Antibiotic Resistance in the Health Services" in 2015. As part of the plan, the Antibiotic Center for Primary Care (ASP) was asked to establish a quality improvement programme for physicians working in nursing homes and other community healthcare institutions, to promote appropriate antibiotic prescribing. RASK (Riktigere Antibiotikabruk i Sykehjem/Kommunale helseinstitusjoner – https://www.antibiotika.no/rask/) was launched in October 2016. So far 13 out of 19 counties have been included. Both long-term- and short-term care institutions as well as municipal acute care units (ØHD/KAD) are invited to join. The participation rate in the included counties has been between 80-100% of all invited institutions.

In RASK, physicians, nurses and other healthcare professionals are invited to a one-day conference with presentations and group discussions on infections and the use of antibiotics. Special attention is given to the diagnosis of urinary tract infections, as asymptomatic bacteriuria is common in the elderly population and may lead to unnecessary antibiotic treatment. Prior to the conference, all participating institutions receive a report presenting their own antibiotic consumption (based on sales statistics from the supplying pharmacy), compared to that of other participating institutions in the county. Participants are asked to organise educational activities on the same topic for their colleagues, and to set a goal for their institution during the one-year project period. New reports are issued after six and twelve months, and a follow-up conference is held after six to twelve months.

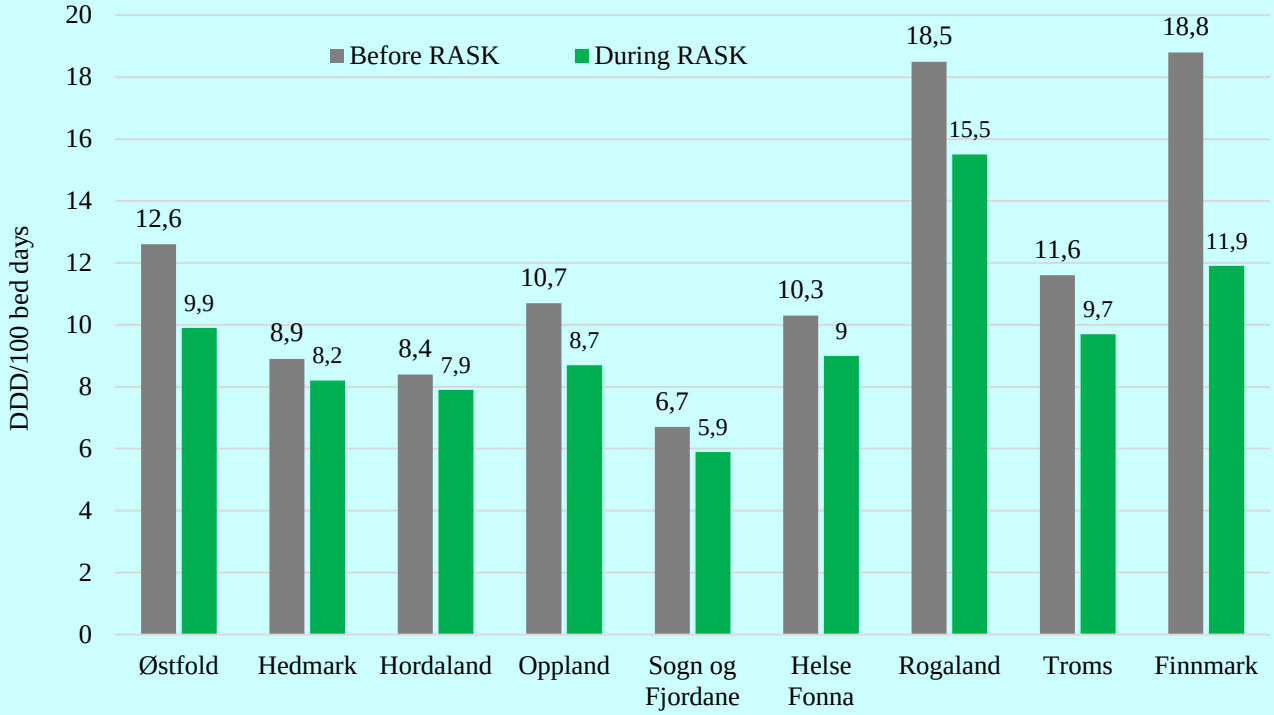


FIGURE 25. Mean antibiotic consumption (J01 antibacterials excl. methenamine, A07AA09 oral vancomycin, P01AB01 oral metronidazole and J04AB02 rifampicin) per participating institution per county, measured in DDD/100 bed days during 12 months prior to the RASK conference (grey bars) and during 12 month after (green bars). Only data for counties that have completed the programme are shown.

Results after one year participation in RASK show a marked reduction in the average antibiotic consumption in participating institutions, as shown in Figure 25, mean -16.4%, range [-6%,-36.7%].

Before the end of 2021, all counties will be included in the programme. ASP is working to establish RASK as a permanent quality improvement system for municipal healthcare institutions, and plans to offer each institution a yearly report.

Important collaborators in the work with RASK are, amongst others, the Norwegian Association of Old Age and Nursing Home Medicine, the Norwegian Advisory Unit for Antibiotic Use in Hospitals (KAS), and the Norwegian Institute of Public Health (FHI).

Ruth Davey Eig, Nicolay Jonassen Harbin, Svein Gjelstad, Sigurd Høye, Siri Jensen and Morten Lindbæk, The Antibiotic Centre for Primary Care (ASP), General Practice Medicine Unit, University of Oslo, and Hege Salvesen Blix, Department of Drug Statistics, Norwegian Institute of Public Health, Oslo, Norway.

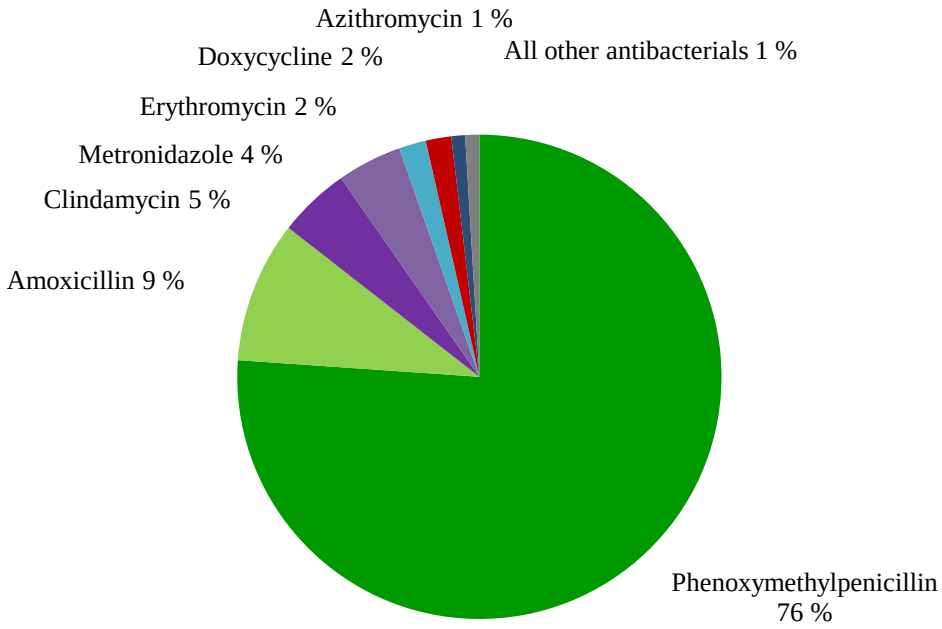


FIGURE 26. Relative amount of antibacterial agents for systemic use prescribed by dentists in 2019 as measured in Defined Daily Doses (DDD).

Antibiotic usage in hospital care

In 2019, the antibacterial sales (in DDDs) to hospitals represented around 8% of total sales of antibacterials for human use in the country. A slight decrease of 4% in DDD/1,000 inhibitants/day compared to 2012, but an increase of 3% since 2016 is observed (Figure 27). The last three years the total sales of antibiotics to hospitals have been stable with regard to DDD/1,000 inhabitants/day but a change in pattern of use has occurred – an increased use of narrow-spectrum antibiotics. The narrow-spectrum penicillins are highly utilised, for this group the theoretical value of DDDs is lower than the therapeutic doses most commonly prescribed in Norway. Furthermore, combination regimens with a narrow-spectrum penicillin plus an aminoglycoside accounts for more DDDs than if monotherapy with a cephalosporin or carbapenem is used. This implies that the total count of DDDs will show artificially higher values for volume.

The therapy pattern of antibacterials in hospitals does not change much from one year to another, however a decrease of 27% in use of selected broad-spectrum antibiotics has been observed since 2012. Broad-spectrum antibiotics (defined as J01_CR/DC/DD/DI/DF/DH/MA) accounted for 19% of total DDDs for hospitals in 2019 compared to 26% in 2012. The share of beta-lactamase sensitive penicillins is 19% of the total (Figure 27).

Penicillins (J01C) represent 47% of the use measured in DDDs in hospitals (J01CE 19%, J01CA 10%, J01CF 14% and J01CR 4%). The second largest group is the cephalosporins; 17% of all DDDs, the dominant subgroup being 3rd generation cephalosporins (J01DD). In 2019, six substances accounted for 53% of all DDDs used in hospitals. These are benzylpenicillin, cloxacillin, cefotaxime, cefazolin, gentamicin and doxycycline. Three single substances accounted for 34% of all antibacterial DDDs in hospitals; benzylpenicillin (15%), cloxacillin (12%) and cefotaxime (7%).

Figure 28 shows annual trends in national antibiotic use in hospitals by hospital activity data instead of population statistics. The two measurements (bed days and admissions) together show the interplay between shorter hospital stays and intensity of antibiotic treatment. The average length of stay (LOS) in Norwegian hospitals in the latest years is relatively stable according to national statistics, but the number of admissions and bed days are both going down. Data for antibiotic use in hospital care are usually presented as DDD/number of bed days or DDD/number of admissions to correct for activity, because that makes comparisons between hospitals possible. Reduced number of bed days in Norway the latest years does probably not reflect reduced hospital activity in the country as a whole, but a shift from in-patient treatment to day-care and out-patient treatment. Figur 29 visualises the impact of the reduction in bed days on antibiotic consumption statistics.

Seven selected groups that mainly are used in hospitals are shown in Figure 30. The use of piperacillin/tazobactam has been increasing for many years, but was markedly reduced in 2017 and 2018 due to a nationwide shortage. In 2019, there was no shortage, and a small increase compared to 2018 was observed. There was increased use of aminoglycosides, beta-lactamase resistant penicillins, sulfonamides and trimethoprim, and decreased use of 3rd and higher generation cephalosporins (not shown). This is probably due to implementation of antibiotic stewardship programs in Norwegian hospitals from 2016. The use of aminoglycosides increased by 39% from 2016 to 2019, whereas the use of quinolones has decreased by 31%. The use of carbapenems peaked in 2014 after many years of increasing use, and seems to have reached a stable level. Only parenteral formulations of 2nd, 3rd and higher generation cephalosporins as well as carbapenems are licensed in Norway. Figure 31 shows that the distribution between "Preferred antibiotics" (which largely reflects

standard treatment regimens in national guidelines) and resistance driving antibiotics for the different Norwegian hospitals. The proportion of preferred antibiotics varies from 30.3% to 69.7%.

There are large variations in volume of antibiotics used, measured in DDD/100 bed days, and in therapy profile between the hospitals. Figure 32 shows use of the five selected groups of broad-spectrum antibiotics targeted in the National Action Plan in all Norwegian hospitals/health trusts. The variations cannot be accounted for by differences in activity or patient composition alone.

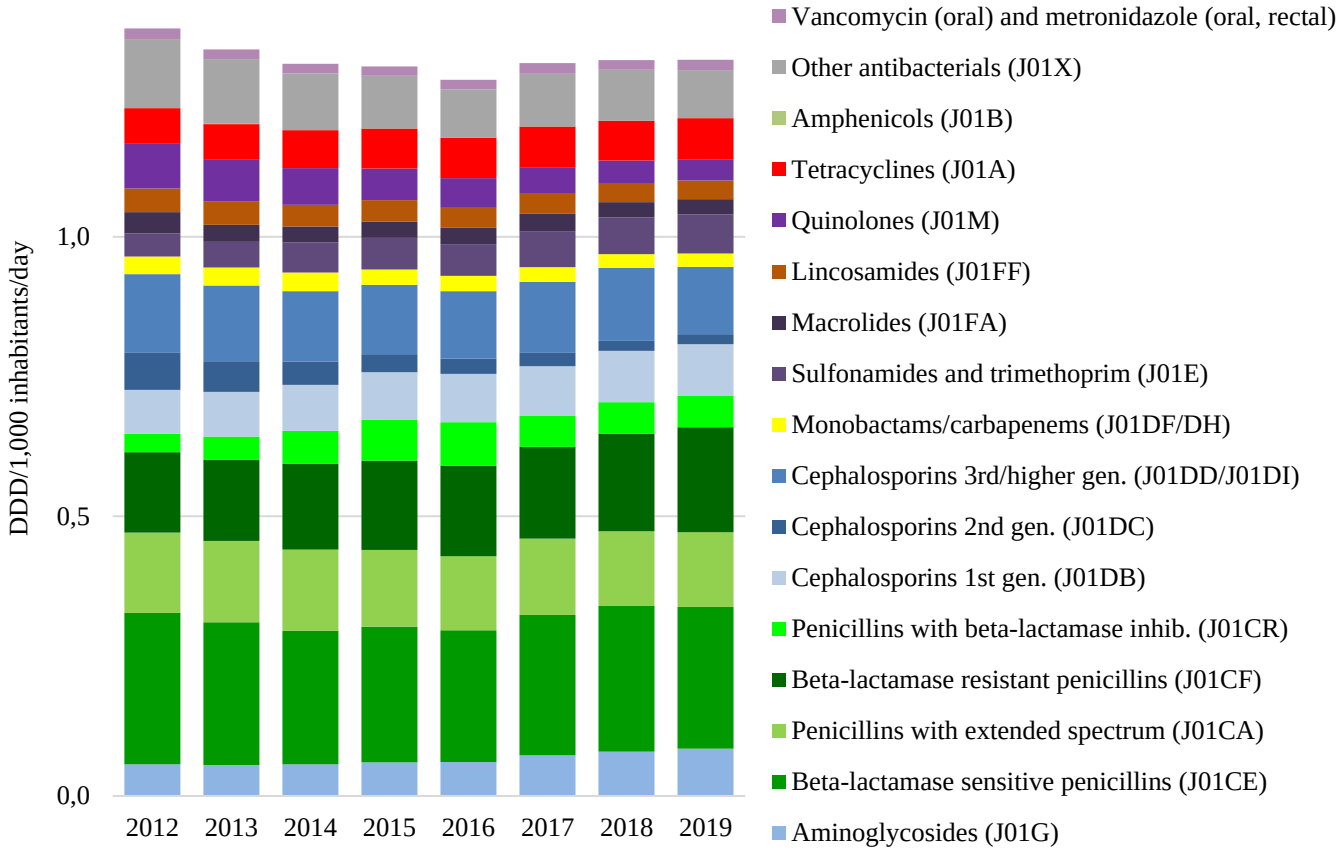


FIGURE 27. Proportions of antibacterial agents for systemic use (J01), vancomycin (A07AA09), and metronidazole (P01AB01) in Norwegian hospitals 2012-2019, measured in DDD/1,000 inhabitants/day.

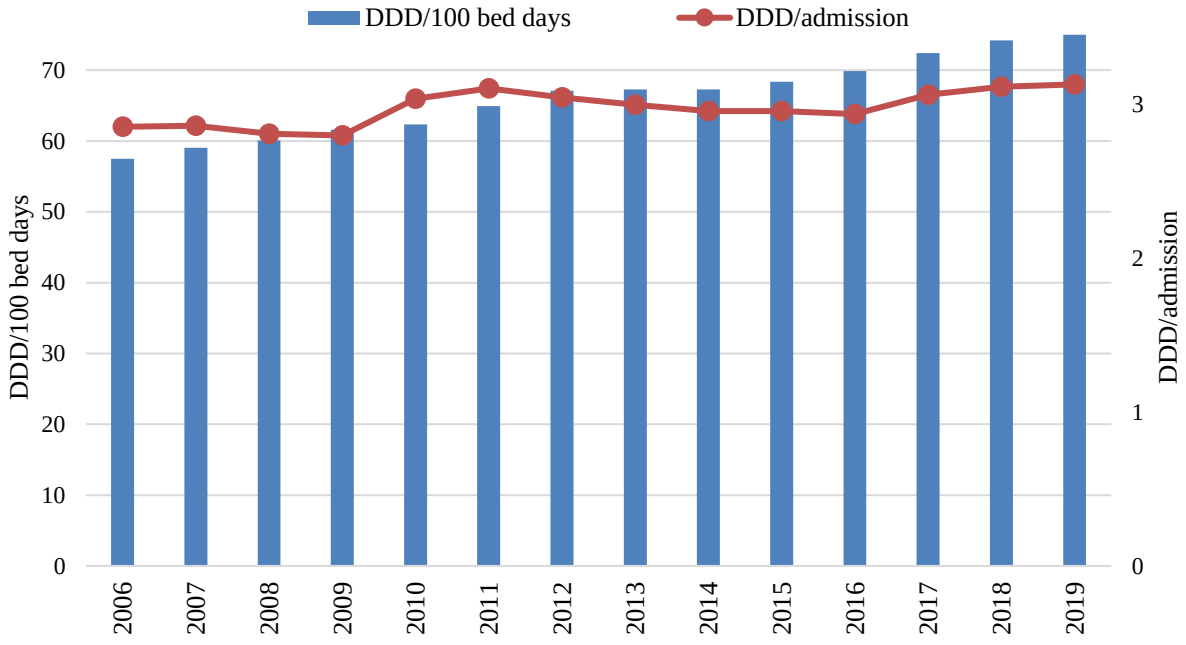


FIGURE 28. Total use of antibiotics in Norwegian hospitals (somatic) 2006-2019, measured in DDD/100 bed days (blue bars) and DDD/admission (red line). Antibiotics are defined as J01 antibacterials for systemic use, A07AA09 vancomycin (oral), A07AA12 fidaxomycin, and P01AB01 metronidazole (oral and rectal).

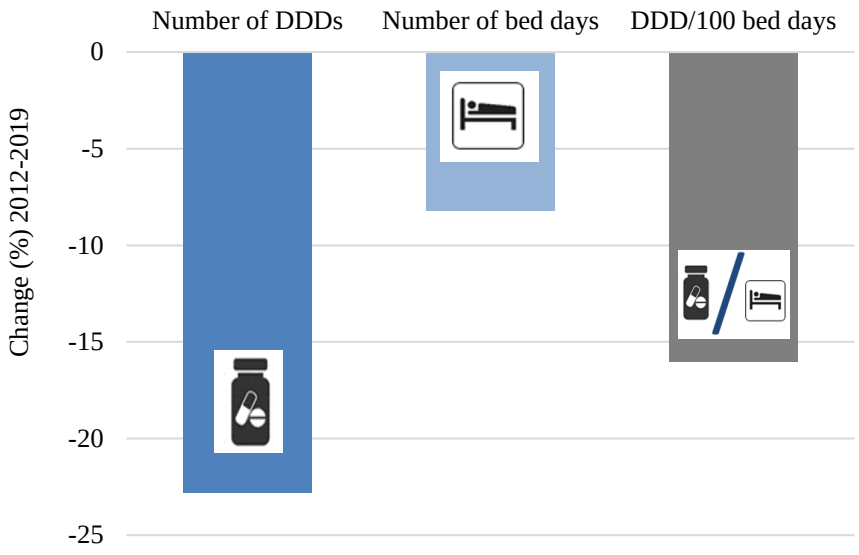


FIGURE 29. The figure shows the impact of change in denominator (i.e. bed days). Proportional change varies according to the measurments used (bed days, DDD or DDD related to the denominator; i.e. bed days). Antibiotic usage in hospitals is often presented in DDD/100 bed days, but total number of DDDs may also be used as a measure. The total number of bed days has been reduced by 8% since 2012. The figure visualises the impact of the reduction in bed days on antibiotic consumption statistics of broad-spectrum antibacterial agents for systemic use (ATC J01CR, J01DC, J01DD, J01DH, J01G, J01M and J01XA) in Norwegian hospitals 2012-2019, measured as % change either as change of total DDDs (23% reduction - grey bar) or change of DDD/100 bed days (16% reduction - blue bar).

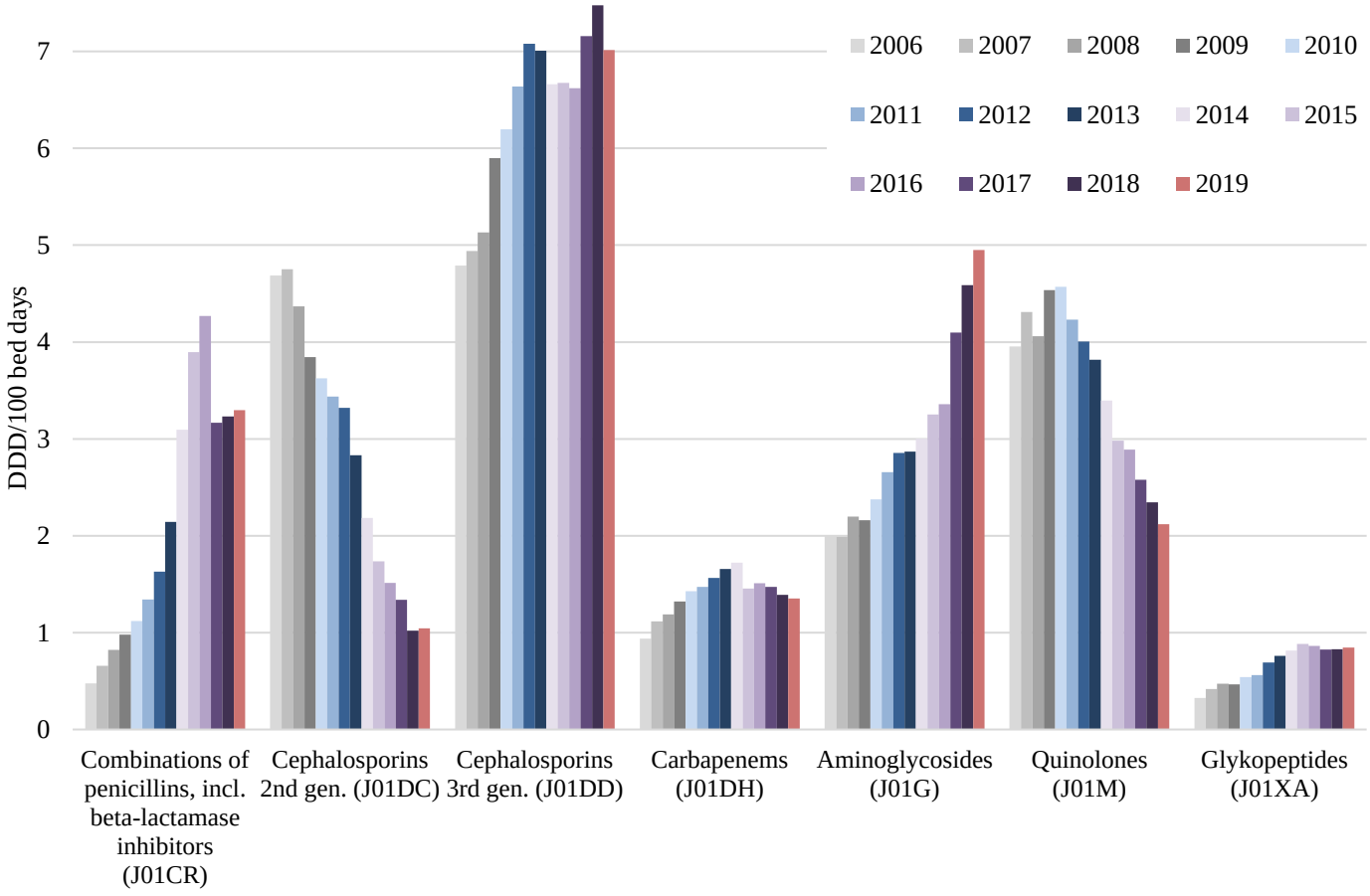


FIGURE 30. Changes in consumption of selected antibacterial agents for systemic use (ATC J01CR, ATC group J01DC, J01DD, J01DH, J01G, J01M and J01XA) in Norwegian hospitals 2006-2019, measured in DDD/100 bed days.

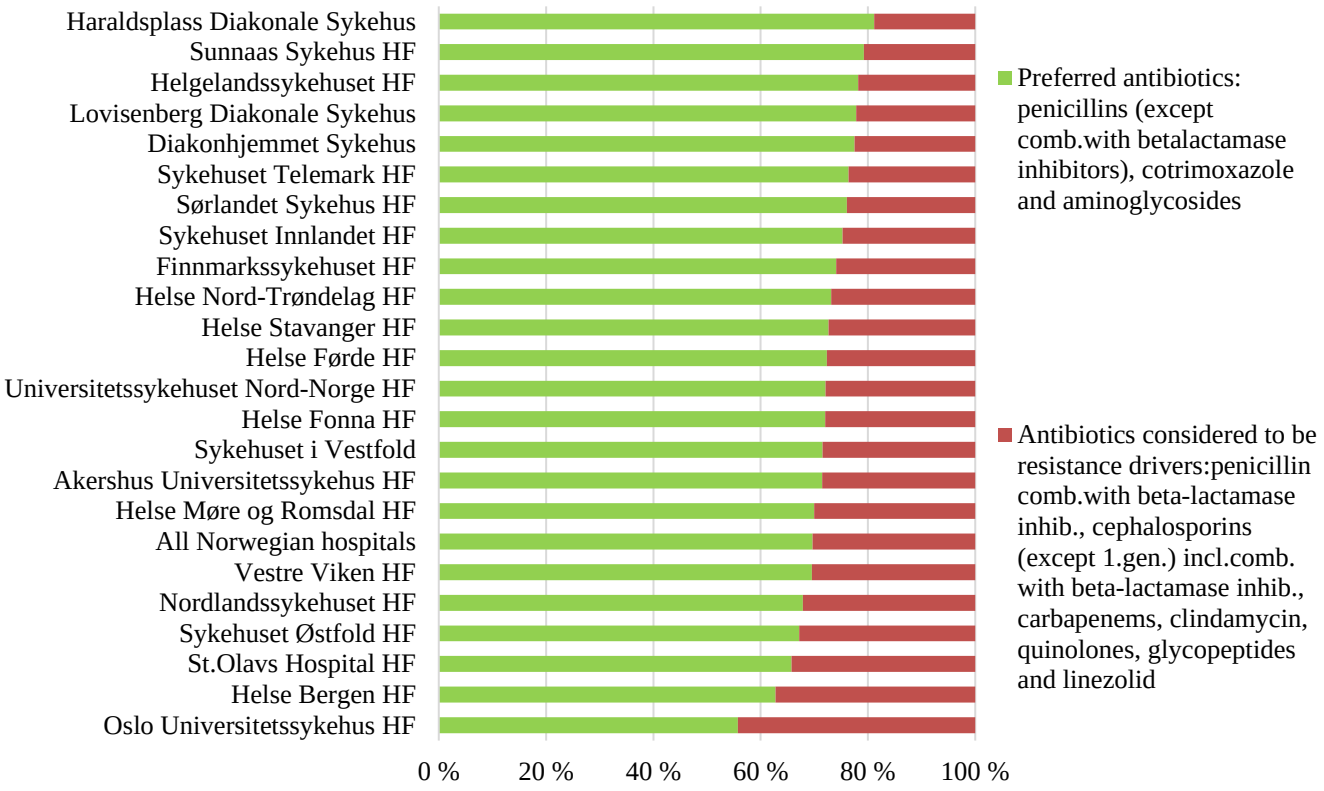


FIGURE 31. Proportions (% of total DDDs) of preferred antibiotics (green part of the column) and antibiotics that are considered to be drivers of antibiotic resistance (red part i.e. belonging to ATC groups J01CR, J01DC, J01DD, J01DE, J01DI, J01DH, J01M, J01XA and J01XX08) in Norway, presented per hospital/health trust in 2019. 1st generation cephalosporins and tetracyclines are not included as they in hospitals mainly are used for surgical prophylaxis. Metronidazole is also excluded from the figure because it does not readily fit either of the descriptions "preferred" or "resistance driver", and there are no alternative drugs mainly targeting anaerobic bacteria.

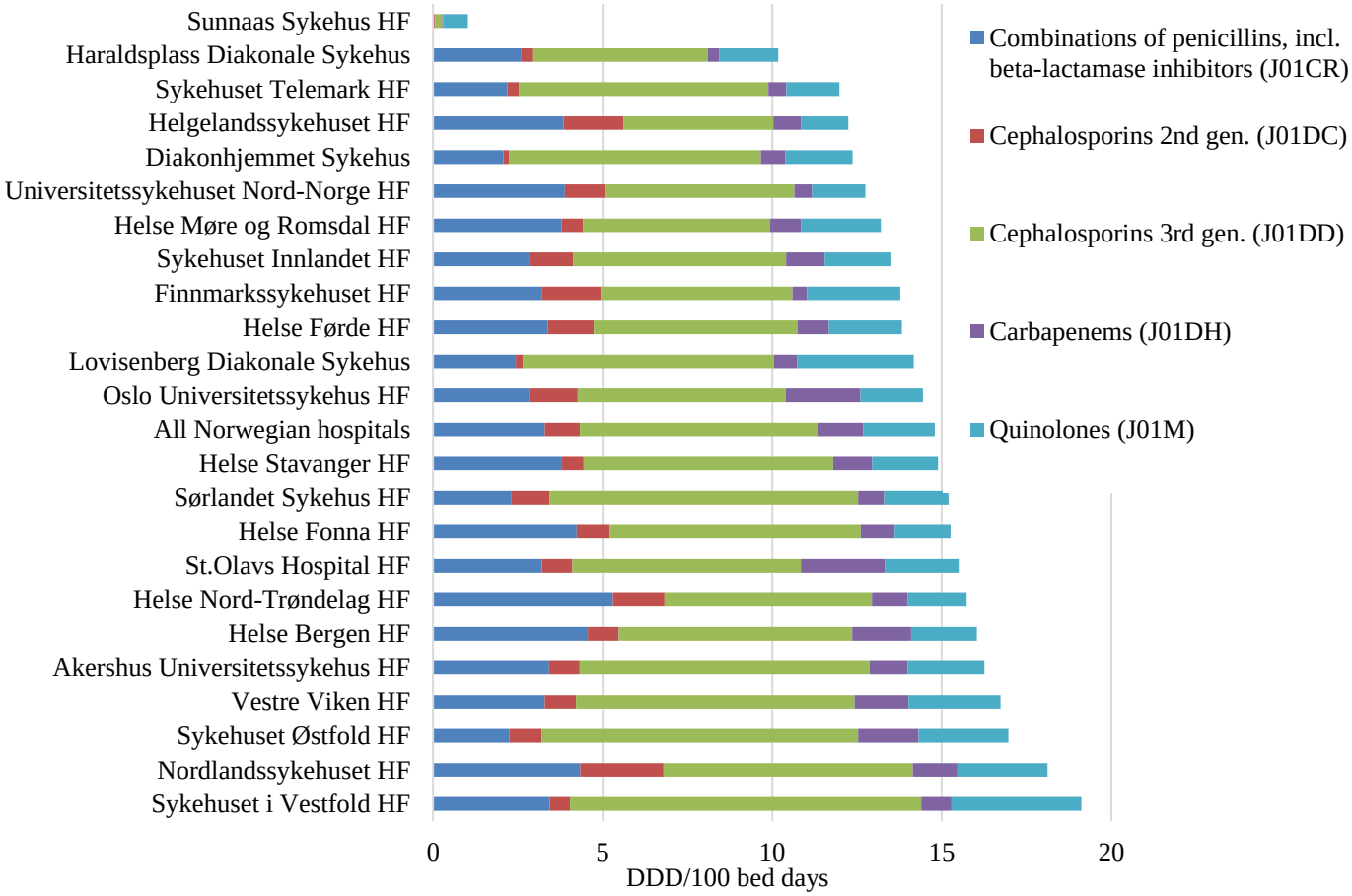


FIGURE 32. Proportions of selected antibacterial agents for systemic use (belonging to ATC groups J01CR, J01DC, J01DD, J01DH and J01M) in Norway, presented per hospital/health trust, in 2019, measured in DDD/100 bed days.

National Action Plan against Antibiotic Resistance in Healthcare – National Targets for Antibiotic Use and change according to targets

In 2015, a National Strategy against Antibiotic Resistance was agreed upon, aiming to reduce the total volume of antibiotic use by 30%, as compared to 2012, by the end of 2020. The Strategy was followed by a National Action Plan, issued January 2016, with suggested ways to reach the targets within 2020. The overall goal for total human consumption was reduction of DDDs by 30%. In addition, two sector specific goals in ambulatory care were introduced; reduction of average number of prescriptions (target; 250 precriptions per 1,000 inhabitants per year) and the reduction of antibiotics for respiratory tract infections by 20% (in DDD/1,000 inhabitants/day). Figure 33 shows total human use (J01) and use of antibiotics for respiratory tract infections in Norway since 2012 according to national targets. DDD/1,000 inhabitants/day for J01, excl. methenamine is reduced by 22% since 2012. There are county differences; some counties use more *Guidelines recommended antibiotics* (i.e. narrow-spectrum antibiotics), indicating a higher adherence rate to the national guidelines, Figure 34. The county differences in proportional use of *Guidelines recommended antibiotics* were smaller in 2019 compared to 2012, range 44-52% of total use in 2012 and 46-53% in 2019. This indicates that awareness of AMR as well as adherence to guidelines have increased in all counties in the period. Precriptions (Rx) per 1,000 inhabitants per year (J01, excl. methenamine) is reduced by 26% since 2012 from 444.4 to 331.0 Rx/1,000 inhabitants/year.

Since 2012, there has been a reduced prevalence of use in all age groups with the largest reduction in small children (0-9 years), around 33%, and the lowest reduction for elderly above 70 years, 15%. Moreover, the use in men is reduced more than in women; 27% reduction in prescriptions pr 1,000 in men vs. 23% in women. The highest reduction in prescriptions per 1,000 are observed in children 0-9 year old with approximately 38% less prescriptions pr 1,000 in 2019 compared to 2012.

For hospitals, the main target is 30% reduction in combined use of five selected groups of antibiotics. To reach this goal, the National Action Plan also made antibiotic stewardship programs mandatory in Norwegian hospitals. Figure 35 shows the annual variation of total hospital use of these groups in the years 2006-2019 according to the national target. Figure 36 shows how the use of these five groups has changed in the different Norwegian hospitals/health trusts in relation to the national target; a reduction by 30% is marked by a black dotted line in the figure. For all hospitals in Norway together there was 16% reduction in use of the five selected groups of broad-spectrum antibiotics from 2012 to 2019 when adjusted for activity (bed days). The number of bed days is going down every year and there is a large increase in outpatient consultations, therefore it is probably necessary to use more than one indicator of clinical activity in hospitals when assessing drug use data. Unadjusted sales data measured in DDDs shows a reduction of 23% for the same period (see also Figure 29).

Norway has two national advisory units for antibiotic use, one for primary care (established in 2006); the Antibiotics Center for Primary Health Care (ASP) and one for hospitals/specialist services (established in 2011); the National Centre for Antibiotic Use in Hospitals (KAS). These advisory units have been strenghtened and appointed key roles in the National Action plan. The Directorate of Health has in collaboration with the advisory units, issued National Antibiotic Treatment Guidelines for ambulatory care, nursing homes, dentists and hospitals.

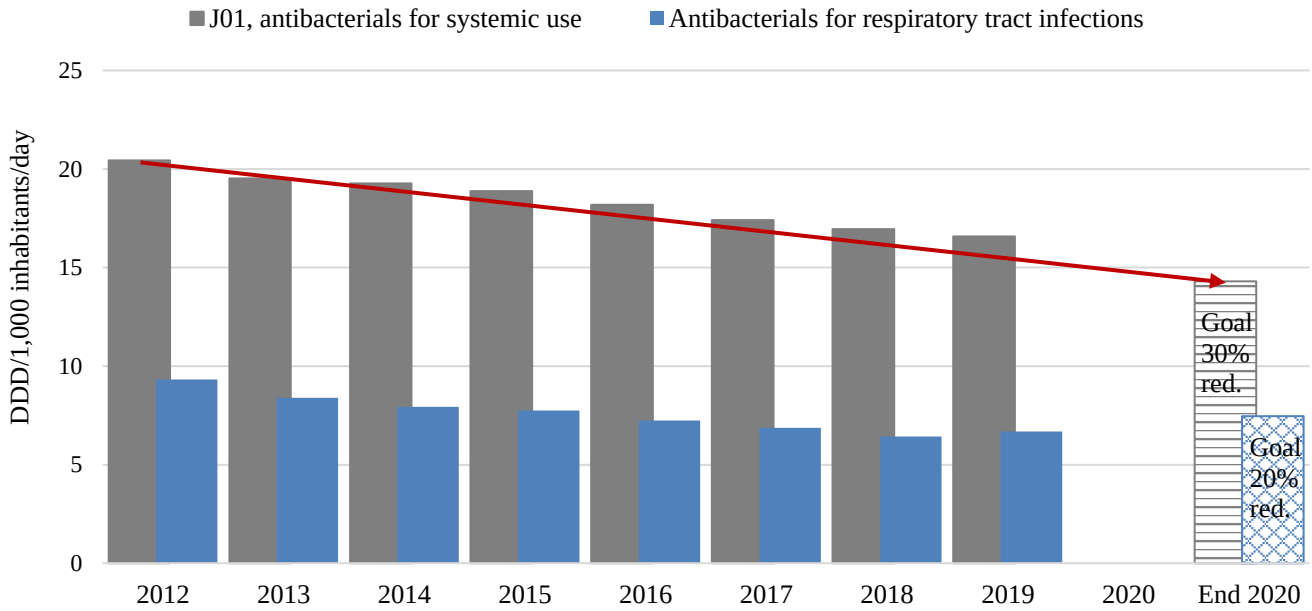


FIGURE 33. Total human sales of antibacterial agents for systemic use (ATC group J01) and sales of antibiotics for respiratory tract infections (amoxicillin, phenoxymethylpenicillin, macrolides and doxycycline) in Norway in 2012-2019 measured in DDD/1,000 inhabitants/day. According to the National Action Plan, the target for 2020 is 30% reduction, measured in DDDs. Bars show measured use 2012-2019 (grey; J01, blue; antibiotics for respiratory tract infections), red line and bars with pattern; targets set in the National Strategy against Antibiotic Resistance 2015-2020.

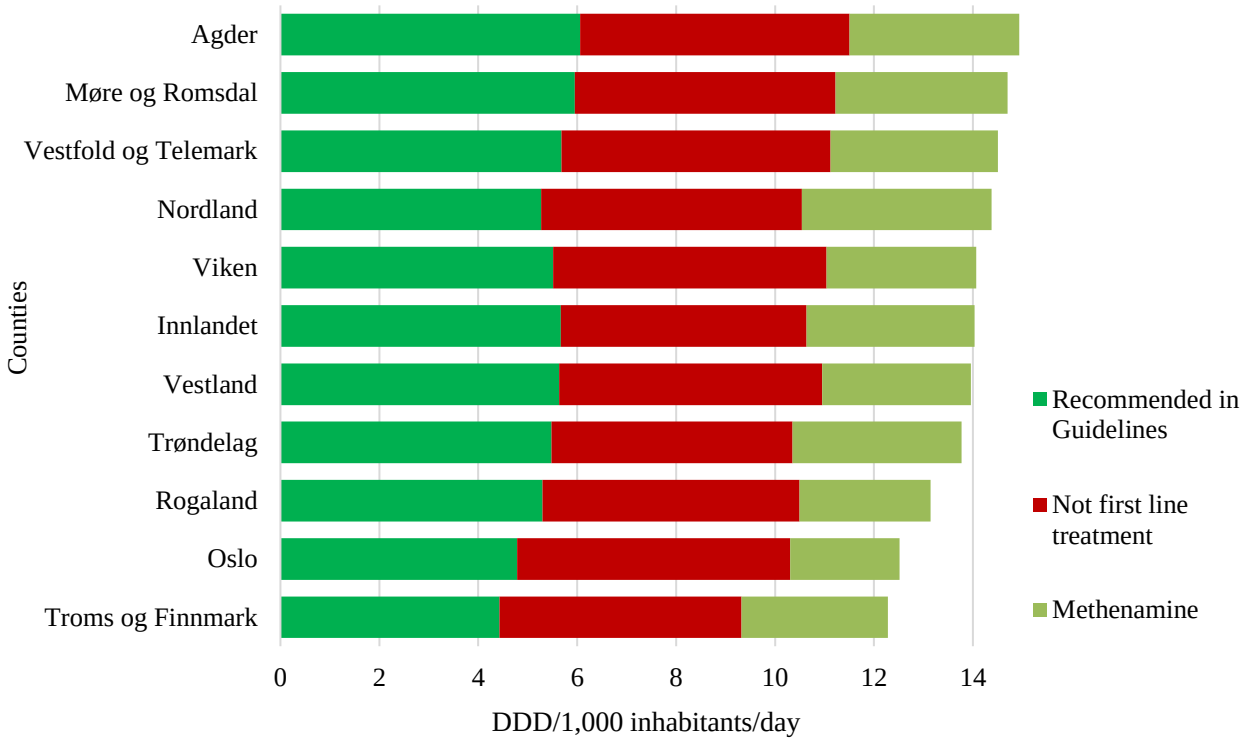


FIGURE 34. Consumption of antibacterial agents for systemic use (ATC group J01) in outpatients in the different counties of Norway in 2019. Aggregated in 3 groups; a) methenamine, b) recommended as first line treatment in the Guidelines for primary care (phenoxymethylpenicillin for respiratory tract infections, pivmecillinam, trimethoprim and nitrofurantoin for urinary tract infections and dicloxacillin for skin infections), c) not first line treatment includes all other antibiotics in J01. Measured as number of DDD/1,000 inhabitants/day. Data from NorPD (i.e. health institutions and sales to prescribers´ own practice not included).

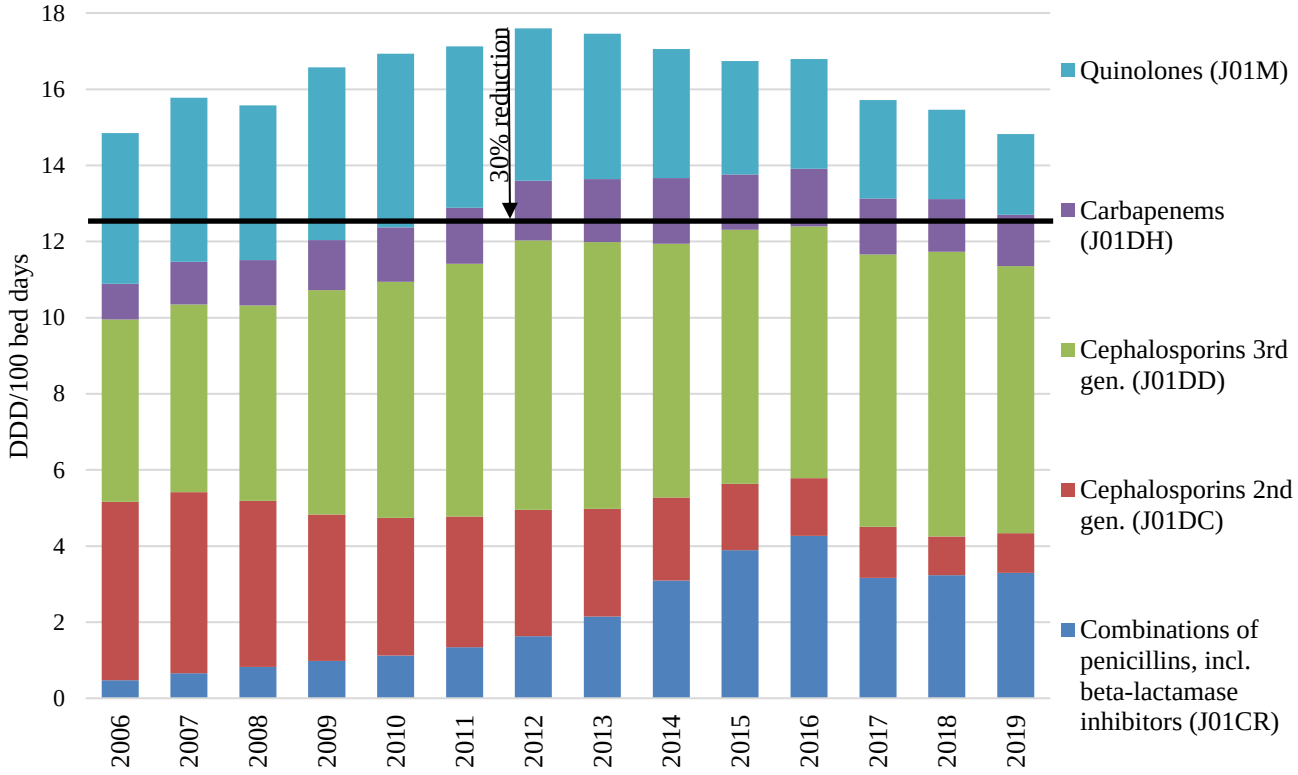


FIGURE 35. Consumption of selected antibacterial agents for systemic use (belonging to ATC-groups J01CR, J01DC, J01DD, J01DH and J01M) in Norwegian hospitals 2006-2019, measured in DDD/100 bed days.

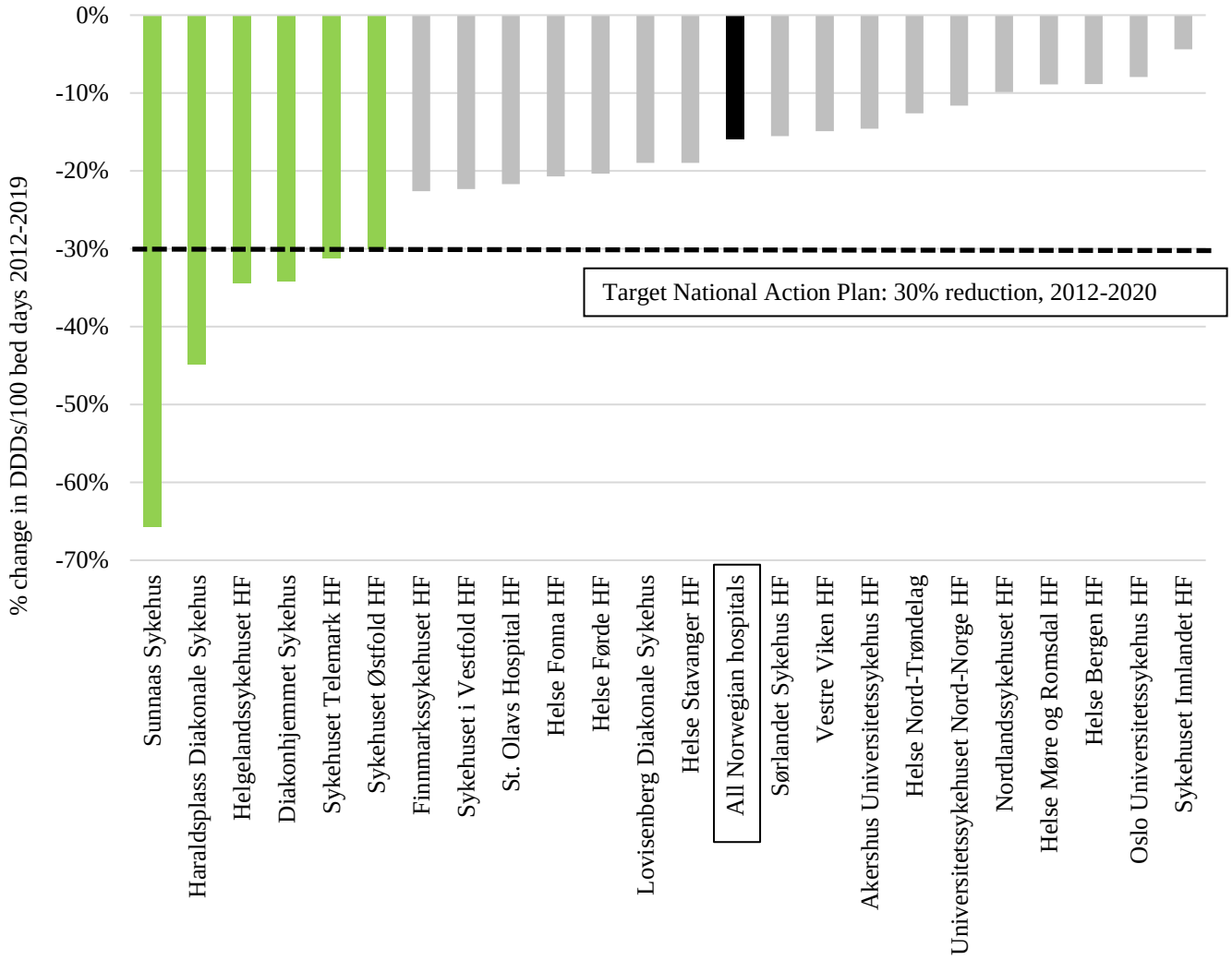


FIGURE 36. Change in consumption of selected antibacterials for systemic use (belonging to ATC-groups J01CR, J01DC, J01DD, J01DH and J01M) in Norway, 2012-2019. The data are presented per hospital/health trust as measured in DDD/100 bed days.

OCCURRENCE OF ANTIMICROBIAL RESISTANCE

ANIMAL CLINICAL ISOLATES

Madelaine Norström, Jannice Schau Slettemeås, Marianne Sunde and Anne Margrete Urdahl

The clinical isolates included in NORM-VET 2019 were *Escherichia coli*, *Staphylococcus pseudintermedius*, and *Streptococcus canis* from infections in dogs. One isolate per submission was susceptibility tested. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from dogs

A total of 132 isolates of *Escherichia coli* from clinical submissions in dogs, 89 from urinary tract infections (UTI) and 43 from other infections, were collected between 2016 and 2018. The results are presented in Table 12, Figures 37-38, and in the text.

TABLE 12. Antimicrobial resistance in *Escherichia coli* from clinical infections in dogs (n=132) divided by urinary tract infections (UTI, n=89) and infections in other organs (n=43) 2016-2018.

Substance	Sample	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	UTI	9.0	[4.0 – 16.9]								83.2	7.9				1.1	7.9		
	Other	14.0	[5.3 – 27.9]								81.4	4.7				4.7	9.3		
Tigecycline	UTI	0	[0.0 – 4.1]					98.9	1.1										
	Other	0	[0.0 – 0.8]					97.7	2.3										
Chloramphenicol	UTI	2.3	[0.3 – 7.9]										87.6	10.1	2.3				
	Other	0	[0.0 – 0.8]										86.1	14.0					
Ampicillin	UTI	20.2	[12.4–30.1]								21.4	55.1	3.4		1.1	1.1	18.0		
	Other	46.5	[31.2–62.3]							2.3	16.3	30.2	4.7			2.3	44.2		
Cefotaxime	UTI	0	[0.0 – 4.1]					100											
	Other	7.0	[1.5 – 19.1					93.0		2.3	2.3		2.3						
Ceftazidime	(UTI	0	[0.0 – 4.1]						100										
	Other	7.0	[1.5 – 19.1						93.0	2.3			4.7						
Meropenem	UTI	0	[0.0 – 4.1]		98.9	1.1													
	Other	0	[0.0 – 0.8]		100														
Sulfamethoxazole	UTI	16.9	[9.8 – 26.3]										51.7	10.1	14.6	6.7	1.1		15.7
	Other	23.3	[11.8-38.6]										41.9	18.6	11.6	4.7			23.3
Trimethoprim	UTI	9.0	[4.0 – 16.9]					27.0	49.4	14.6						9.0			
	Other	23.3	[11.8-38.6]					39.5	32.6	4.7					2.3	20.9			
Azithromycin	UTI	ND	ND								9.0	50.6	33.7	3.4	2.3	1.1			
	Other	ND	ND								16.3	44.2	27.9	4.7	7.0				
Gentamicin	UTI	2.3	[0.3 – 7.9]						56.2	32.6	9.0	2.3							
	Other	4.7	[0.6 – 15.8]						39.5	53.5	2.3				4.7				
Ciprofloxacin	UTI	4.5	[1.2 – 11.1]	48.3	46.1	1.1	1.1	2.3	1.1										
	Other	16.3	[6.8 – 30.7]	65.1	18.6			4.7	4.7					7.0					
Nalidixic acid	UTI	5.6	[1.8 - 12.6]									91.0	3.4	1.1		1.1	1.1	2.3	
	Other	16.3	[6.8 – 30.7]									83.7					4.7	11.6	
Colistin	UTI	0	[0.0 – 4.1]							100									
	Other	0	[0.0 – 0.8]							100									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. Clinical breakpoints (i.e. for human clinical isolates) are marked in blue dotted lines. In cases where clinical breakpoints are identical to ECOFF, ECOFF are shown. Clinical breakpoints are not defined for tetracycline, sulfamethoxazole, azithromycin, and nalidixic acid.

RESULTS AND COMMENTS

In total, 70.8% and 46.5% of the isolates originating from UTI and other infections, respectively, were susceptible to all antimicrobial classes included in the susceptibility testing. The following proportions of isolates were resistant to one or more antimicrobial classes: 13.5% and 20.9% (UTI/other) were resistant to one (mainly sulfonamides, trimethoprim and ampicillin), 5.6% and 9.3% (UTI/other) to two (mainly to sulfonamides and trimethoprim) and 10.1% and 23.3% (UTI/other) to three or more antimicrobial classes, respectively. In total, 16 of the 18 isolates displaying resistance to trimethoprim were also classified as resistant to sulfonamides. Resistance towards ampicillin, sulfamethoxazole, trimethoprim, tetracycline and quinolones were most common as shown in Table 12 and Figure 37.

Three isolates displayed reduced susceptibility to the extended-spectrum cephalosporins cefotaxime and ceftazidime (2.3% [95% CI: 0.5 – 6.5]). Two of the isolates displayed an AmpC beta-lactamase phenotype, and the resistance was due to mutations in the promoter and attenuator region in the chromosomally located *ampC* gene causing upregulation of this gene. The last isolate displayed an ESBL phenotype, and the $bla_{\text{CTX-M-15}}$ gene was detected. None of the *E. coli* isolates displayed resistance to the carbapenem meropenem.

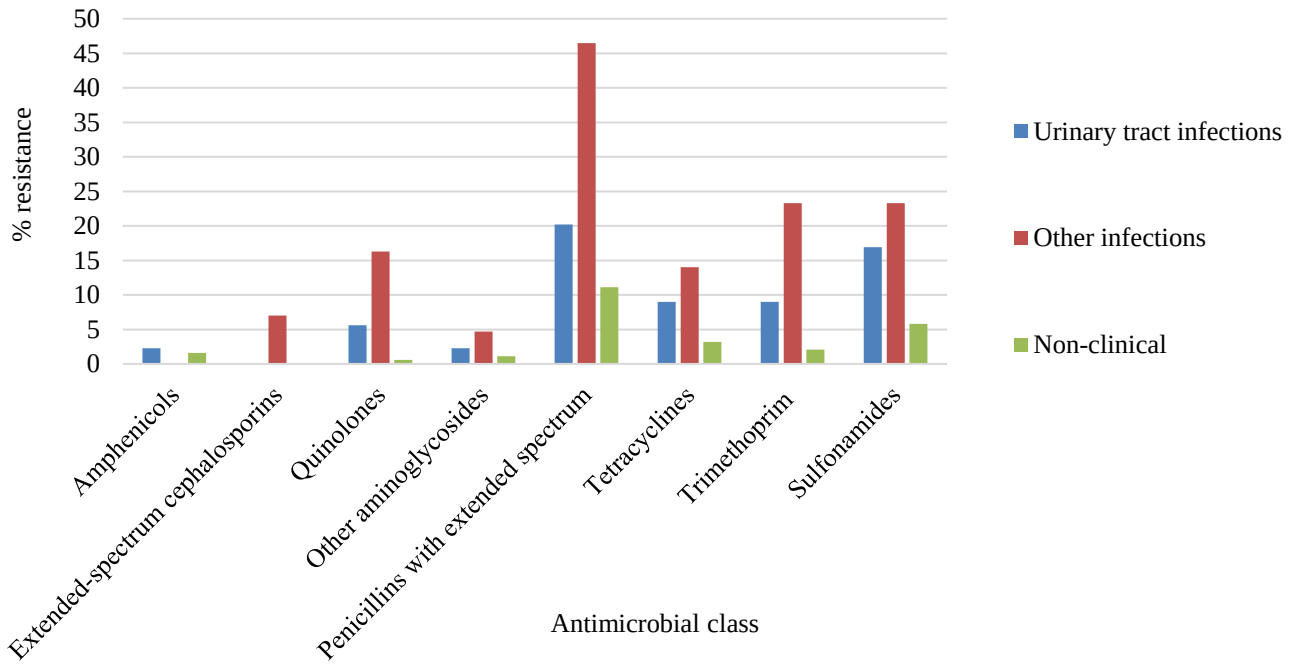


FIGURE 37. Prevalence of resistance to various antimicrobial classes in *Escherichia coli* from clinical (urinary tract and other infections sampled between 2016 and 2018) and non-clinical samples from dogs included in NORM-VET 2019 (page 65). The breakpoints used in NORM-VET 2019 were applied.

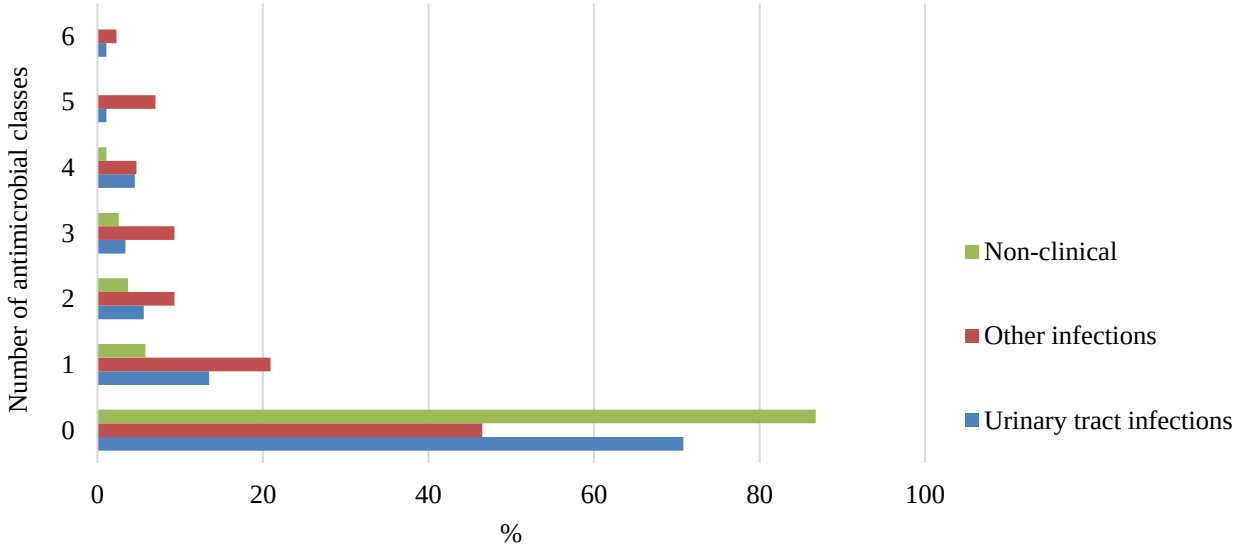


FIGURE 38. Antimicrobial resistance profiles for *Escherichia coli* from clinical (urinary tract and other infections sampled between 2016 and 2018) and non-clinical samples from dogs included in NORM-VET 2019 (page 65). Percentage of isolates susceptible to all (0), resistant to one (1), two (2), three (3), four (4), five (5) or six (6) antimicrobial classes are illustrated.

There is a higher proportion of overall antimicrobial resistance in these clinical *E. coli* isolates compared to antimicrobial resistance in indicator *E. coli* from dogs as presented in Figures 37-38 (see also page 65). The data also indicate a possible difference between the occurrence of resistance in isolates from other infections than in UTI, however this was only significant for ampicillin resistance. Epidemiological cut-off values (ECOFF) were used for the classification of resistance in these clinical *E. coli* isolates, facilitating comparison to surveillance results for indicator *E. coli*. Clinical breakpoints are shown in dotted blue lines in Table 12. However, these clinical breakpoints are defined in order to indicate if treatment of a specific pathogen is likely to succeed or not, and factors like dosage and formulations will affect the clinical result.

E. coli isolates from clinical submission from dogs have not been included in NORM-VET previously, and comparisons to previous years are therefore not possible.

Staphylococcus pseudintermedius from dogs

A total of 157 of *Staphylococcus pseudintermedius* isolates from clinical infections in dogs were included. The isolates were collected through the years 2017 and 2018.

The results are presented in Table 13, Figures 39-41, and in the text.

TABLE 13. Antimicrobial resistance in *Staphylococcus pseudintermedius* from clinical infections in dogs (n=157) in 2017-2018.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.016	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	26.8	[20.0 - 34.4]						73.2					0.6	26.1				
Chloramphenicol	6.4	[3.1 – 11.4]									70.1	23.6			6.4			
Benzylpenicillin[ψ]	59.9	[51.7 – 67.6]				40.1	6.4	4.5	2.5	1.9	44.6							
Cefoxitin	0.0	[0.0 - 2.3]						97.5	0.6	1.3	0.6							
Trimethoprim	8.9	[5.0 – 14.5]								52.2	38.2	0.6			8.9			
Sulfamethoxazole	56.1	[47.9 - 64.0]													41.4	2.6	15.3	40.8
Erythromycin	12.1	[7.5 – 18.3]					78.3	8.9	0.6	0.6			11.5					
Clindamycin	12.7	[8.0 – 19.0]				85.6	1.3	1.3			0.6	10.8						
Quinupristin/ dalfopristin	0.0	[0.0 - 2.3]						100										
Streptomycin	14.0	[9.0 – 14.0]									75.2	10.8			14.0			
Gentamicin	1.9	[0.4 - 5.5]							97.5				0.6	1.3				
Kanamycin	13.4	[8.5 - 19.7]									86.6				0.6	12.7		
Ciprofloxacin	1.3	[1.5 - 4.5]					92.4	3.8	2.5				1.3					
Vancomycin	0.0	[0.0 - 2.3]							98.7	1.3								
Fusidic acid	38.2	[30.6 – 46.3]						61.8		1.3	0.6	36.3						
Tiamulin	0.0	[0.0 - 2.3]						99.4	0.6									
Linezolid	0.0	[0.0 - 2.3]							96.2	3.8								
Mupirocin	0.0	[0.0 - 2.3]						100										
Rifampicin	0.0	[0.0 - 2.3]	98.7	1.3														

*Bold vertical lines denote epidemiological cut-off values (ECOFFs) for resistance. ECOFF for trimethoprim was set by the NRI method with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913, US Patent No. 7,465,559). ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. Clinical breakpoints (i.e. human clinical isolates) are marked in blue dotted lines. In cases where clinical breakpoint are identical to ECOFF, ECOFF are shown. Clinical breakpoints are not defined for sulfamethoxazole, streptomycin, kanamycin, tiamulin and mupirocin. [ψ]beta-lactamase production has not been investigated.

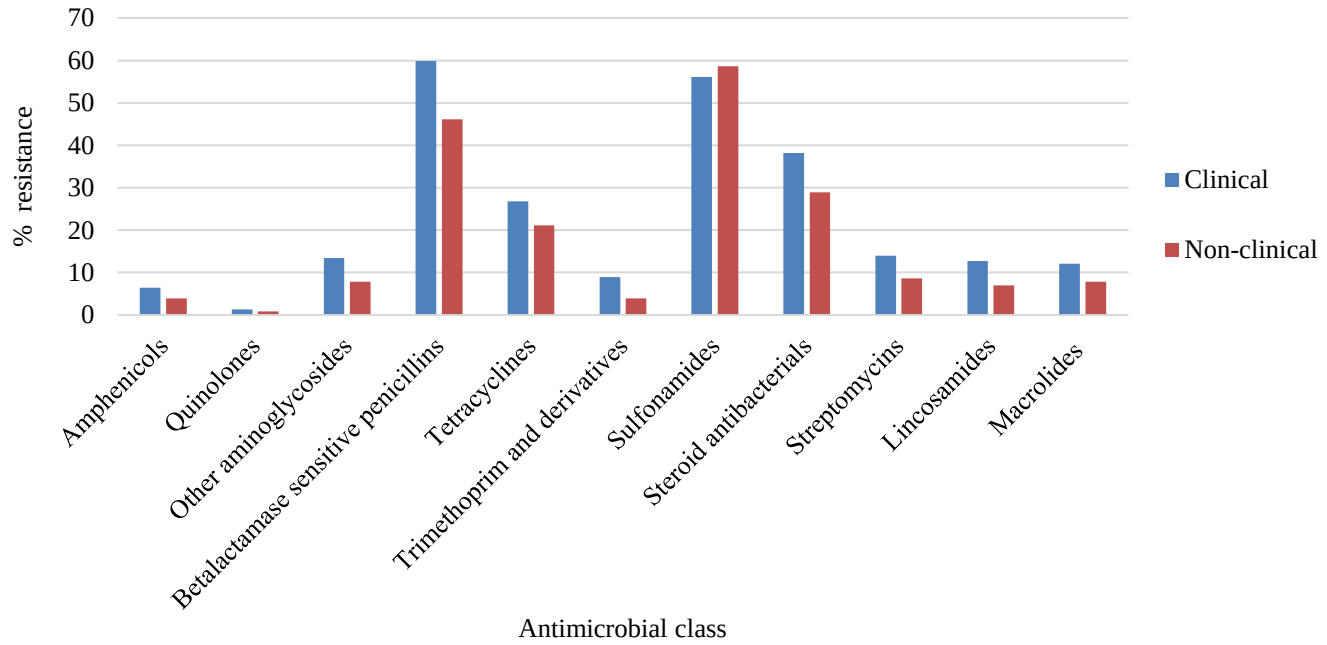


FIGURE 39. Prevalence of resistance to various antimicrobial classes in *Staphylococcus pseudintermedius* from clinical infections sampled between 2017 and 2018 and non-clinical samples from dogs included in NORM-VET 2019 (see page 65). The ECOFFs used in NORM-VET 2019 were applied.

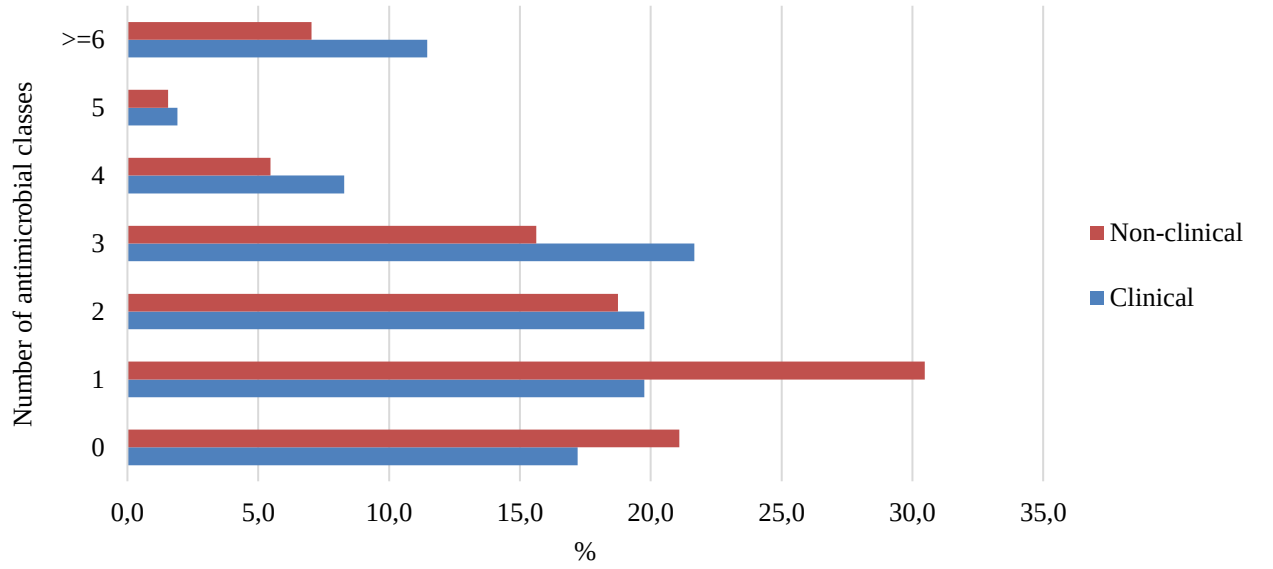


FIGURE 40. Antimicrobial resistance profile for *Staphylococcus pseudintermedius* from clinical infections sampled between 2017 and 2018 and non-clinical samples from dogs included in NORM-VET 2019 (see page 65). Percentage of isolates susceptible to all (0) or resistant to one (1), two (2), three (3), four (4), five (5) or six or more (≥ 6) antimicrobial classes are illustrated.

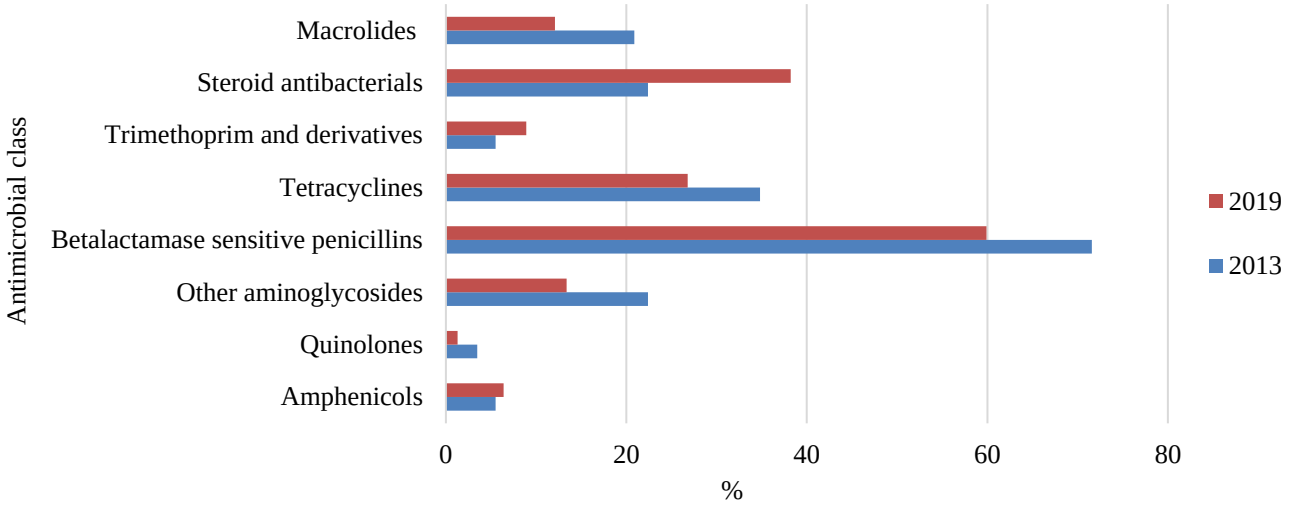


FIGURE 41. Prevalence of resistance to various antimicrobial classes in *Staphylococcus pseudintermedius* from infections in dog included in NORM-VET 2013 and in 2019. The ECOFFS used in NORM-VET 2019 were applied.

RESULTS AND COMMENTS

In total, 17.2% of the *S. pseudintermedius* clinical isolates were susceptible to all antimicrobial agents included in the susceptibility testing. The following proportions of isolates were resistant to one or more antimicrobial classes: 19.8% were resistant to one (sulfonamides or fusidic acid), 19.8% to two (mainly to beta-lactamase sensitive penicillins and sulfonamides) and 21.7% to three, 8.3% to four, 1.9% to five and 11.5% to six or more antimicrobial classes, respectively. Resistance towards benzylpenicillin, sulfamethoxazole, tetracycline and fusidic acid were the most common.

Seven isolates were identified as methicillin resistant *S. pseudintermedius* (MRSP) in the diagnostic laboratory, and *mecA* was identified by PCR. All these isolates had MIC for cefoxitin below ECOFF (i.e. 4 mg/L). For identifying MRSP by susceptibility testing, oxacillin is the preferred indicator and should have been included in the susceptibility panel. The panel used is designed for monitoring of staphylococcal isolates associated with both human and animals hosts. Some of the substances included may not be of relevance for clinical use in companion animals. The inclusion of all substances is relevant in a One Health perspective and to allow for evaluation of resistance development in the future.

Epidemiological cut-off values for *S. aureus* were used for the classification of resistance in these clinical isolates, facilitating comparison to the surveillance results for carriers, see page 69. Clinical breakpoints are shown in dotted blue lines in Table 13. However, these clinical breakpoints are defined in order to indicate if treatment of a specific pathogen is likely to succeed or not, and factors like dosage and formulations will affect the clinical result. Overall, there is a higher proportion of antimicrobial resistance in these clinical *S. pseudintermedius* isolates compared to antimicrobial resistance in *S. pseud-intermedius* from carrier dogs as shown in Figures 39-40 (see also page 69). Moreover, there is also a difference in antimicrobial resistance profile between these, as a higher proportion of the clinical isolates were resistant to two or more antimicrobial classes, while a higher proportion of the *S. pseudintermedius* from carrier dogs were resistant to only one antimicrobial class.

Susceptibility testing of clinical *S. pseudintermedius* was also included in 2013 (NORM-VET 2013). Comparisons have to take into consideration the changes made in the panel of antimiobial agents tested. Compared to the results from 2013, there is indications of a reduction in occurrence of resistance (Figure 41). However, this was only statistically significant for fusidic acid.

Notifiable antimicrobial resistant bacteria in animals – results from 2019

From 1st of June 2019, some antimicrobial resistant bacteria in animals became notifiable to the Norwegian Food Safety Authorities (NFSA). This was done for surveillance purposes, to gain more knowledge on occurrence of some specific resistance mechanisms in the Norwegian animal population. The resistances included in the provision were chosen in a One Health perspective.

Findings of the following resistant bacteria are included in the provision:

- Methicillin resistant *Staphylococcus aureus* (MRSA)
- Methicillin resistant *Staphylococcus pseudintermedius* (MRSP)
- *Enterobacteriaceae* resistant to extended-spectrum cephalosporins (ESC) (ESBL/AmpC-producing *Enterobacteriaceae*)
- Colistin resistant (COL-R) *Enterobacteriaceae*
- Fluoroquinolone resistant *Enterobacteriaceae* (QRE)
- Carbapenemase-producing *Enterobacteriaceae* (CPE)
- Linezolid resistant *Enterococcus faecium* and *E. faecalis*
- Vancomycin resistant *Enterococcus* spp. (VRE)

In 2019, a total of 33 findings of antimicrobial resistant bacteria were notified to the NFSA as shown in Table 14. From three reported cases, information on correct notifiable resistant bacterial species were missing. Findings from the national surveillance programmes, i.e. NORM-VET and MRSA in pigs, are not included. None of the other notifiable resistances were reported.

TABLE 14. Number of findings (n=30) of antimicrobial resistant bacteria in animals notified to the Norwegian Food Safety Authorities in Norway in 2019, where information regarding correct notifiable resistant bacterial species were included.

	No. animals					
Notifiable resistant bacteria	Cat	Dog	Broiler	Broiler breeders	ND	Total
ESC resistant *Enterobacteriaceae*						
ESC resistant *E. coli*		1	15	2	1	19
ESC resistant *Enterobacter cloacae*	1					1
Fluoroquinolone resistant *E. coli*		1	2			3
MRS*		1				1
MRSP	1	5				6

*Methicillin resistant *Staphylococcus*, bacterial species not defined.
ND = not defined

Anne Margrete Urdahl and Madelaine Norström, Norwegian Veterinary Institute, Kjell Hauge and Solfrid Åmdal, Norwegian Food Safety Authorities, Oslo, Norway.

Streptococcus canis from dogs

A total of 123 *Streptococcus canis* isolates from clinical infections in dogs were included. The isolates were collected through the years 2017-2018. The results are presented in Table 15 and in the text.

TABLE 15. Antimicrobial resistance in *Streptococcus canis* from clinical infections in dogs (n=123) in 2017-2018.

	Resistance (%)		Distribution (%) of MIC values (mg/L)*															
Substance		[95% CI]	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline**	ND	ND								2.4	31.7	24.4	41.5					
Tigecycline	0	[0.0-3.0]		2.4	69.1	28.4												
Chloramphenicol	0	[0.0-3.0]								2.4	93.5	4.1						
Benzylpenicillin	4.9	[1.8 – 10.4]		91.9	3.3	4.1	0.8											
Amoxicillin/ clavulanic acid	NA	NA								99.2		0.8						
Cefuroxime	NA	NA						98.4		1.6								
Cefotaxime	NA	NA				95.1	3.3					1.6						
Ceftriaxone	NA	NA				93.5	1.6	2.4	0.8		1.6							
Cefepime	NA	NA						98.4			1.6							
Meropenem	NA	NA					99.2			0.8								
Ertapenem	NA	NA						100										
Trimethoprim-sulfamethoxazole	1.6	[0.2-5.8]						95.9	2.4	0.8	0.8							
Erythromycin	16.3	[10.2 – 24.0]					82.9	0.8	2.4	5.7	8.1							
Azithromycin	13.0	[7.6 – 20.3]					83.7	3.3	0.8	4.1	8.1							
Clindamycin	15.4	[9.6 – 23.1]				79.7	4.9	0.8	2.4	12.2								
Levofloxacin	1.6	[0.2-5.8]						2.4	89.4	6.5	0.8	0.8						
Moxifloxacin	0.8	[0.0 – 4.4]							99.2		0.8							
Vancomycin	4.9	[1.8 – 10.4]						89.4	5.7	0.8		4.1						
Linezolid	0.8	[0.0 – 4.4]							17.1	80.5	1.6	0.8						
Daptomycin	4.1	[1.3 – 9.2]			8.1	80.5	7.3				4.1							

*Bold vertical lines denote epidemiological cut-off values for resistance as defined by the NRI method with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913, US Patent No. 7,465,559). ND = not defined. NA= Not applicable. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. Clinical breakpoints (i.e. for human clinical isolate) are marked in blue vertical lines. In cases where clinical breakpoints are identical to ECOFF, ECOFF are shown. **The range for tetracycline was too short to establish an ECOFF.

RESULTS AND COMMENTS

This was the first time *Streptococcus canis* was included in NORM-VET. At present there are no specific ECOFFs for *S. canis* established, and we therefore applied the NRI method with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913, US Patent No. 7,465,559) using the distributions obtained. The method can be used if the number of isolates is at least 100. However, for tetracycline the range tested was obviously not long enough to obtain an ECOFF, see also Appendix 6 for further information.

The results obtained need to be considered with care. Decreased susceptibility to erythromycin, clindamycin and azithromycin were the most commonly detected. The panel used is designed for monitoring of both human and animal isolates, and some of the substances included may not be of relevance in veterinary medicine. The inclusion of all substances is relevant in a One Health perspective, and to allow for evaluation of resistance development in the future.

The ECOFF may indicate emerging resistance in the bacterial populations, whereas clinical breakpoints, shown in dotted blue lines in Table 15, are defined in order to indicate if treatment of a specific pathogen is likely to succeed or not. Moreover, factors like dosage and formulations will affect the clinical result. Additional testing could be applied to assess whether an isolate is clinically resistant or not, which was beyond the scope of the current monitoring.

INDICATOR BACTERIA FROM ANIMALS

Madelaine Norström, Jannice Schau Slettemeås and Anne Margrete Urdahl

The prevalence of acquired antimicrobial resistance among certain bacteria of the normal enteric microflora can be used as an indicator of the selective pressure from use of antimicrobial agents in various populations. These bacteria may form a reservoir of transferable resistance genes from which antimicrobial resistance can be spread to other bacteria, including those responsible for infections in animals or humans. Thus, monitoring of resistance among indicator bacteria of the bacterial flora from healthy animals, as well as from feed and food (see separate chapters on feed and food), is important to get an overview of the prevalence of antimicrobial resistance, detect trends and evaluate effects of interventions.

NORM-VET is following the requirements set in Commission implementing decision of 12. Nov 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU). *Escherichia coli* and *Enterococcus* spp. are used as indicator bacteria, i.e. susceptibility testing of *E. coli* and *Enterococcus* spp. is used as an indicator for occurrence of antimicrobial resistance in the bacterial population. In addition, antimicrobial testing of bacteria from other sources than those included in this decision, or investigation of presence of specific antimicrobial resistant bacteria/ resistance mechanisms by selective methods, are included. Selective methods are for instance used for detection of *E. coli* resistant to extended-spectrum cephalosporins (ESC), quinolone resistant *E. coli* (QREC), carbapenemase-producing *Enterobacteriaceae* (CPE), colistin resistant (COL-R) *E. coli*, vancomycin resistant *Enterococcus* spp. (VRE), methicillin resistant *Staphylococcus aureus* (MRSA), and *S. pseudintermedius* (MRSP). The use of selective methods is especially relevant for low prevalent sources, as it enables early detection of specific emerging resistance mechanisms such as for instance ESC resistant *E. coli* and CPE; thereby enabling these to be monitored. Some of these antimicrobials are defined by the WHO as critically important for treatment of human infections. Significant reservoirs of such resistant bacteria in animals and the food production chain are of concern, as they may interact with the human bacterial populations and thus have an impact on resistance development in these.

In 2019, animal samples included caecal samples from cattle < one year and fattening pigs, faecal samples from goats, as well as faecal swabs from dogs for isolation of indicator bacteria and some emerging resistant bacteria. In addition, nasal swabs and environmental cloths from goat herds were included for detection of MRSA, and swabs from oral/nasal mucosa and perineum of dogs for detection of both MRSA, *S. pseudintermedius* and MRSP. The results from the surveillance programme for MRSA in pigs are described as well (separate presentation).

The substances included in the antimicrobial test panels might not always be those used in veterinary medicine, but are included because of their importance for human health. Some of the cut-off values defining resistance applied in NORM-VET have been changed over the years. To facilitate comparisons in this report, data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2019. Sampling, laboratory methods and data processing are described in Appendix 3.

PRODUCTION ANIMALS

Escherichia coli from cattle, goats and pigs

Caecal samples from a total of 319 cattle < one year and 286 fattening pigs, as well as 63 faecal samples from goats, were examined. *E. coli* isolates were obtained from 314 (98.4%) of the cattle, 62 (98.4%) of the goat and 285 (99.6%) of the pig samples. One isolate per positive sample was susceptibility tested. The results are presented in Table 16 and Figures 42-44, and in the text.

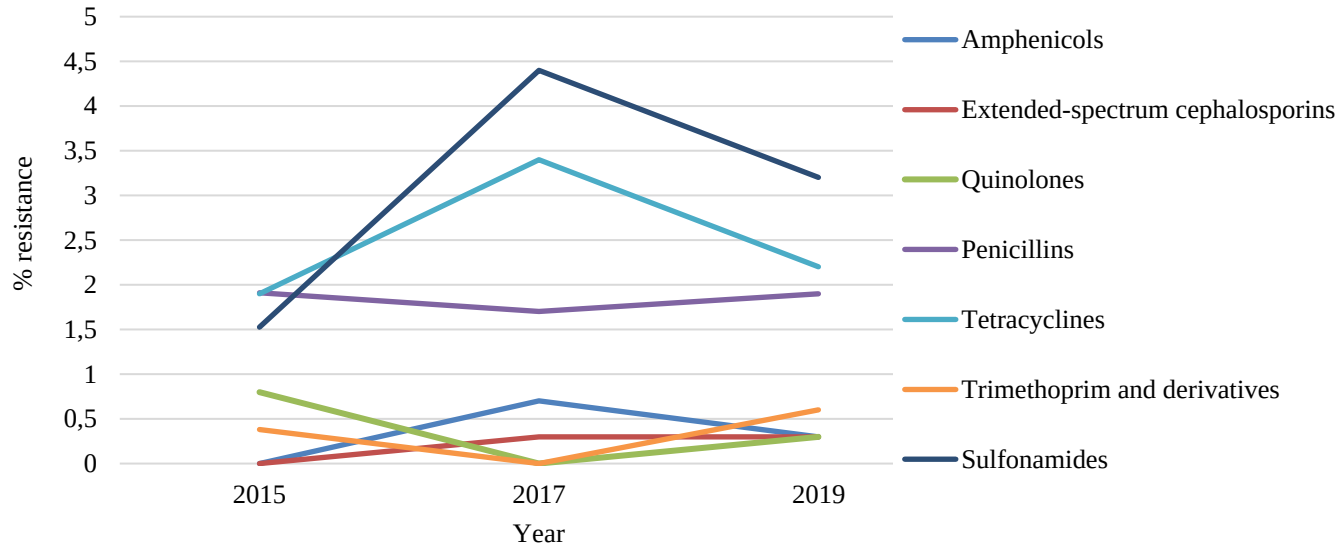


FIGURE 42. Prevalence of resistance to various antimicrobial agents in *Escherichia coli* from caecal samples from cattle < one year of age collected in 2015-2019. The breakpoints used in NORM-VET 2019 were applied.

TABLE 16. Antimicrobial resistance in *Escherichia coli* isolates from caecal samples of cattle < one year (n=314), fattening pigs (n=285), and faecal samples of goats (n=62) in 2019.

Substance	Sample	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*														
				0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	Cattle	2.2	[0.9 - 4.5]								91.1	6.1	0.6		0.3	1.9			
	Goat	0	[0.0 - 5.8]								96.8	3.2							
	Pig	2.5	[1.0 – 5.0]								93.0	4.2	0.4		0.4	1.8	0.4		
Tigecycline	Cattle	0	[0.0 - 1.2]					99	1.0										
	Goat	0	[0.0 - 5.8]					100											
	Pig	0	[0.0 - 1.3]					99.6	0.4										
Chloramphenicol	Cattle	0.3	[0.0 - 1.8]										96.8	2.9				0.3	
	Goat	0	[0.0 - 5.8]										98.4	1.6					
	Pig	0.4	[0.0 - 1.9]										97.5	2.1				0.4	
Ampicillin	Cattle	2.2	[0.9 - 4.5]							2.9	33.1	59.6	2.2				2.2		
	Goat	0	[0.0 - 5.8]							6.5	16.1	77.4							
	Pig	4.2	[2.2 - 7.2]							6.7	41.8	45.3	2.1				4.2		
Cefotaxime	Cattle	0.3	[0.0 - 1.8]					99.7			0.3								
	Goat	0	[0.0 - 5.8]					100											
	Pig	0	[0.0 - 1.3]					100											
Ceftazidime	Cattle	0.3	[0.0 – 1.8]						99.7			0.3							
	Goat	0	[0.0 - 5.8]						100										
	Pig	0	[0.0 - 1.3]						100										
Meropenem	Cattle	0	[0.0 - 1.2]		100														
	Goat	0	[0.0 - 5.8]		100														
	Pig	0	[0.0 - 1.3]		100														
Sulfamethoxazole	Cattle	3.2	[1.5 - 5.8]										84.7	10.8	1.0	0.3			3.2
	Goat	0	[0.0 - 5.8]										90.3	9.7					
	Pig	4.9	[2.7 - 8.1]										79.6	13.3	1.8	0.4			5.0
Trimethoprim	Cattle	0.6	[0.1 - 2.3]					67.5	29.6	2.2						0.6			
	Goat	0	[0.0 - 5.8]					74.2	22.6	3.2									
	Pig	4.6	[2.5 - 7.7]					57.9	33.7	2.8	1.1	0.4				4.2			
Azithromycin	Cattle	0	[0.0 - 1.2]								8.0	60.2	30.6	1.0	0.3				
	Goat	0	[0.0 - 5.8]								16.1	58.1	25.8						
	Pig	0	[0.0 - 1.3]								10.5	53.7	33	2.8					
Gentamicin	Cattle	0.3	[0.0 - 1.8]						77.4	20.1	2.2	0.3							
	Goat	0	[0.0 - 5.8]						80.6	19.4									
	Pig	0.4	[0.0 - 1.9]						73.7	21.4	4.6	0.4							
Ciprofloxacin	Cattle	0	[0.0 - 1.2]	91.1	8.3	0.6													
	Goat	0	[0.0 - 5.8]	91.9	8.1														
	Pig	0	[0.0 - 1.3]	89.1	10.5	0.4													
Nalidixic acid	Cattle	0.3	[0.0 - 1.8]									99.4	0.3	0.3					
	Goat	0	[0.0 - 5.8]									98.4	1.6						
	Pig	0	[0.0 - 1.3]									98.9	1.1						
Colistin	Cattle	0	[0.0 - 1.2]							97.8	2.2								
	Goat	0	[0.0 - 5.8]							83.9	16.1								
	Pig	0	[0.0 - 1.3]							98.9	1.1								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

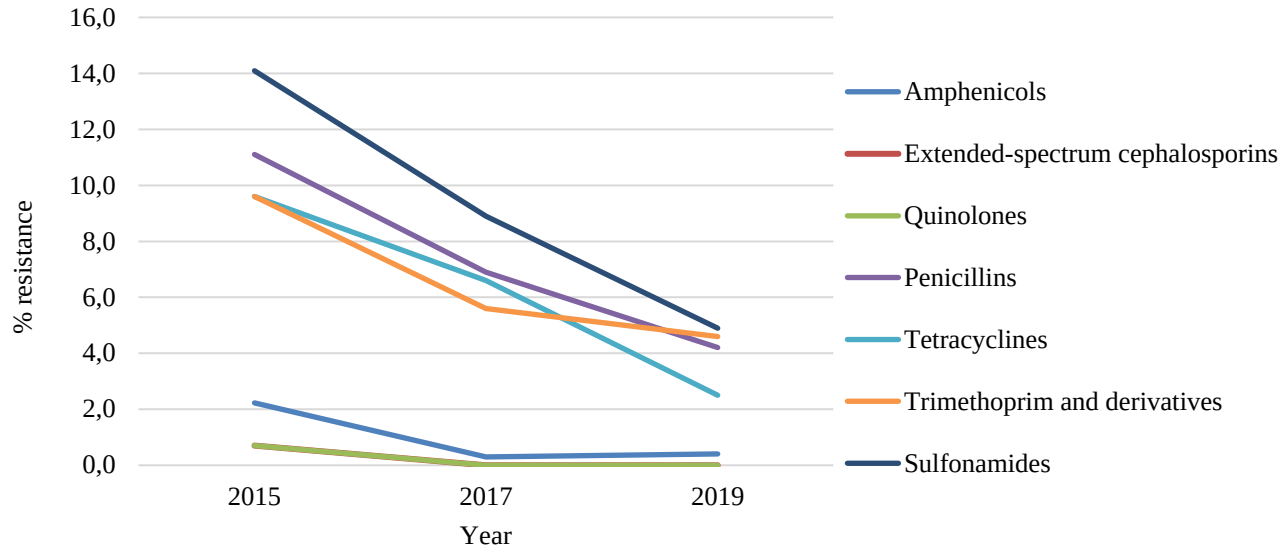


FIGURE 43. Prevalence of resistance to various antimicrobial classes in *Escherichia coli* from caecal samples from pig collected in 2015-2019. The breakpoints used in NORM-VET 2019 were applied.

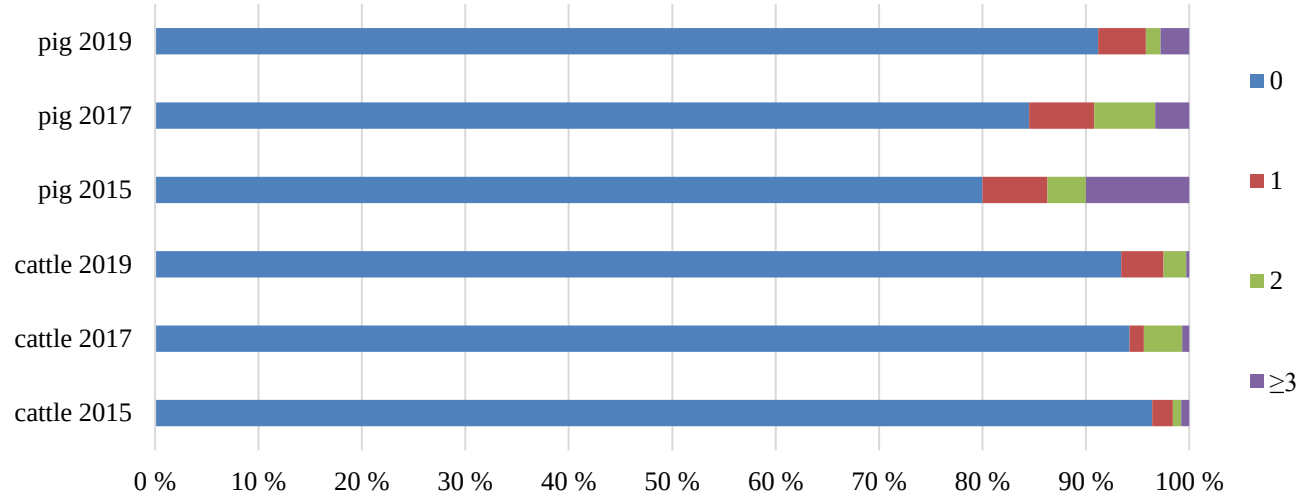


FIGURE 44. Antimicrobial resistance profile for *Escherichia coli* from caecal samples from fattening pigs and cattle < one year collected in 2015-2019. Percentage of isolates susceptible to all (0) or resistant to one (1), two (2), and three or more (≥3) antimicrobial classes are illustrated.

CATTLE

A total of 93.3% of the *E. coli* isolates from cattle caecal samples were susceptible to all antimicrobial classes included in the test panel, indicating a low occurrence of resistance among *E. coli* from cattle caecal samples according to the EFSA classification described in Appendix 6. The low occurrence is in concordance with the 2015 and 2017 results. Resistance to sulfamethoxazole, tetracycline and ampicillin were the most frequently identified resistance phenotypes. Resistance to one antimicrobial class occurred in 4.1% of the isolates, while resistance to two and three antimicrobial classes occurred in 2.2% and 0.3% of the isolates, respectively. One of the isolates displayed resistance to the extended-spectrum cephalosporins (ESC) cefotaxime and ceftazidime (0.3% [95% CI: 0.1 – 1.8]). This isolate had an AmpC beta-lactamase phenotype, and the resistance was due to mutations in the promoter and attenuator region of the chromosomally located *ampC* gene causing an upregulation. In addition, selective methods were applied on the same sample material to investigate the occurrence of *E. coli* resistance to ESC in cattle (see next page). None of the isolates displayed any resistance to ciprofloxacin, though one isolate displayed reduced susceptibility towards nalidixic acid. This is in concordance with results from previous years. In a European perspective, the occurrence of resistance among *E. coli* from cattle < one year in Norway is among the lowest of the countries reporting to EFSA (EFSA and ECDC Summary Report 2017). This situation corresponds to the limited use of antibiotics in the Norwegian cattle production.

GOAT

All the 62 *E. coli* isolates from goat faecal samples were susceptible to all antimicrobial agents included in the test panel [95% CI: 94.2 – 100.0]. The samples were also investigated by selective methods for detection of isolates resistant to ESC, quinolones and carbapenems as described in Appendix 3. Samples from goats have not been included in NORM-VET previously, and comparison to previous years is therefore not possible.

PIG

A total of 91.2% of the *E. coli* isolates from pig caecal samples were susceptible to all antimicrobial classes tested, indicating a low occurrence of resistance among *E. coli* from caecal samples of fattening pigs according to the EFSA classification described in Appendix 6. Resistance to sulfamethoxazole, trimethoprim, ampicillin and tetracycline were the most frequently identified resistance phenotypes. Altogether, 4.6% of the isolates were resistant to one antimicrobial class, 1.4% to two, and 2.9% to three or more antimicrobial classes. The proportion of isolates being fully susceptible has increased from 78.9% [95% CI: 73.5 – 83.6] in 2015, 83.6% [95% CI: 78.9 – 87.5] in 2017, to 90.9% [95% CI: 86.9 – 94.0] in 2019 (Figure 44). This is due to a corresponding increase in susceptibility for sulfamethoxazole, trimethoprim, ampicillin and tetracycline as indicated in Figure 43. Comparisons to data from years before 2015 have to take into consideration changes made in the panel of antimicrobial agents tested. Resistance to streptomycin, which is no longer part of the panel, has traditionally been most frequently identified in isolates from pig with 17.2% resistant isolates in 2011 (NORM/NORM-VET 2011). After the changes in the panel, the most frequently identified antimicrobial agent has been sulfamethoxazole, previously the second most frequently identified.

None of the isolates displayed reduced susceptibility to ESC (i.e. cefotaxime or ceftazidime) or quinolones (i.e. ciprofloxacin and/or nalidixic acid). This is in concordance with results from previous years. In addition, selective methods were applied on the same sample material to investigate the occurrence of *E. coli* resistant to ESC in fattening pigs (see below). In a European perspective, the occurrence of resistance among *E. coli* from fattening pigs in Norway is among the lowest (EFSA and ECDC Summary Report 2018). The occurrence varies markedly between countries reporting to EFSA, ranging from very few susceptible isolates and up to nearly 80% fully susceptible, with the levels of full susceptibility decreasing in a north to south gradient. This favourable Norwegian situation corresponds to the limited use of antibiotics in the Norwegian pig production.

Extended-spectrum cephalosporin resistant *Escherichia coli* from cattle, goats and pigs

A total of 319 cattle, 65 goat and 287 pig samples were investigated for the presence of *E. coli* resistant to extended-spectrum cephalosporins (ESC) by selective methods. One isolate per positive sample was susceptibility tested. Results are presented in Table 17, Figure 45, and in the text.

TABLE 17. Antimicrobial resistance in *Escherichia coli* isolates resistant to extended-spectrum cephalosporins from caecal samples of cattle < one year (n=14) and fattening pigs (n=54) in 2019.

Substance	Sample	n (resistance)	Distribution (n) of MIC values (mg/L)*															
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	Cattle	2								11	1				1	1		
	Pig	7								44	3				4	3		
Tigecycline	Cattle	0					14											
	Pig	0					54											
Chloramphenicol	Cattle	4										7	3		3	1		
	Pig	0										52	2					
Ampicillin	Cattle	13										1				13		
	Pig	54													1	53		
Cefotaxime	Cattle	13					1		1	7	1	4						
	Pig	54							1	44	3	6						
Ceftazidime	Cattle	14							1	3	5	5						
	Pig	54								1	43	7	3					
Meropenem	Cattle	0		14														
	Pig	0		54														
Sulfamethoxazole	Cattle	2										10	2					2
	Pig	5										43	6					5
Trimethoprim	Cattle	1					6	5	2						1			
	Pig	9					31	13	1				1		8			
Azithromycin	Cattle	ND								1	5	8						
	Pig	ND									21	32	1					
Gentamicin	Cattle	0						9	5									
	Pig	0						48	5	1								
Ciprofloxacin	Cattle	4	8	2				3	1									
	Pig	7	42	4	1		6	1										
Nalidixic acid	Cattle	4									10		4					
	Pig	2									49	3				2		
Colistin	Cattle	0							14									
	Pig	0							54									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

ESC resistant *E. coli* were detected from 14 of the cattle (4.4% [95% CI: 2.4 - 7.3]), none of the goat [95% CI: 0.0 - 5.5] and 54 of the pig (18.9% [95% CI: 14.5 - 23.8]) samples.

Of the 14 ESC resistant *E. coli* isolates from cattle caecal samples, seven isolates were resistant to three or more antimicrobial classes and thereby considered multi-drug resistant (MDR) isolates. Among the 54 ESC resistant *E. coli* isolates from pig caecal samples, 15 were resistant to three or more antimicrobial classes and thereby considered MDR isolates. None of the isolates showed decreased susceptibility to meropenem, the preferred carbapenem used for detection of carbapenemase-production. An overview of what antimicrobial agents the isolates showed decreased susceptibility to, is shown in Figure 45 together with the resistance genes responsible for the ESC resistance.

Nine of the 14 isolates from cattle caecal samples displayed an AmpC beta-lactamase phenotype. For these isolates the resistance was due to mutations in the promoter and attenuator region of the chromosomally located *ampC* gene causing an upregulation. Four of the isolates from cattle caecal samples displayed an ESBL phenotype and were genotyped as $bla_{\text{CTX-M-55}}$. These four additionally carried the *qnrS1* gene encoding quinolone resistance. The final isolate was only resistant to ceftazidime and susceptible to ampicillin, cefotaxime and cefoxitin. No genotype was detected after whole genome sequencing. According to EFSA, such isolates is regarded to have "other phenotype", and not AmpC beta-lactamase or ESBL phenotype (EFSA and ECDC Summary Report 2017).

Of the 54 isolates from pig caecal samples, 49 displayed an AmpC beta-lactamase phenotype due to mutations in the promoter and attenuator region of the chromosomally located *ampC* gene causing an upregulation. One of the isolates additionally carried the $bla_{\text{TEM-1B}}$ gene. The last five isolates displayed an ESBL phenotype and were genotyped as $bla_{\text{CTX-M-15}}$, two of these also harboured $bla_{\text{TEM-1b}}$. Four of these isolates additionally carried the *qnrS1* gene encoding quinolone resistance.

The overall occurrence of *E. coli* resistant to ESC when including all genotypes (i.e. both those displaying a AmpC beta-lactamase phenotype and an ESBL phenotype), both in cattle < one year and in pigs, is in concordance with the results from 2017. Compared to the results from 2015, there has been an increase in overall occurrence of ESC resistant *E. coli* in cattle. In 2015, *E. coli* resistant to ESC were detected from 0.4% [95% CI: 0.0 - 2.1] of the samples, while the detection rate increased to 5.3% [95% CI: 3.0 - 8.4] and 4.4% [95% CI: 2.4 - 7.3] in 2017 and 2019, respectively (NORM-VET 2015, NORM-VET 2017). There is a slightly increasing trend in the overall occurrence of ESC resistant *E. coli* in pigs as well during these years. However, this increase is not statistically significant and further monitoring is necessary to see whether this trend continues. This overall occurrence of *E. coli* resistant to ESC in cattle and pigs is mainly due to isolates with mutations in the chromosomal *ampC* gene. However, there has been a change regarding *E. coli* displaying an ESBL phenotype due to plasmid encoded genes. Though the prevalence is low, the variation in genes detected is increasing, indicating a dissemination of these genes within the cattle and pig populations. The first detection of plasmid encoded resistance in *E. coli* resistant to ESC from pigs was in 2011, an isolate containing a $bla_{\text{TEM-52}}$ gene (NORM-VET 2011). The source of introduction of plasmid encoded resistance in *E. coli* to cattle and pigs in Norway, as well as their ability to disseminate further, is currently unknown. There is negligible numbers of import of live cattle and pigs to Norway, which is a preventive measure for importing *E. coli* resistant to ESC from areas/countries with higher prevalence.

In a European perspective, the occurrence of *E. coli* resistant to ESC in cattle < one year and fattening pigs in Norway is among the lowest, though the occurrence varies markedly between countries reporting to EFSA (EFSA and ECDC Summary Report 2018). A continued awareness of animal bacterial reservoirs resistant to ESC is of importance to be able to implement control measures if needed.

Quinolone resistant *Escherichia coli* from goats

Selective method for detection of quinolone resistant *E. coli* (QREC) was performed on 60 faecal samples from goats. QREC was detected from one of 60 samples (1.7% [95% CI: 0.04 – 8.9]). This isolate displayed reduced susceptibility only to quinolones.

Carbapenemase-producing *Enterobacteriaceae* from cattle, goats and pigs

Selective method for detection of carbapenemase-producing *Enterobacteriaceae* (CPE) was performed on a total of 319 samples from cattle < one year, 56 samples from goats and 286 samples from pigs. No CPE was detected ([95% CI cattle: 0.0 – 1.1], [95% CI goats: 0.0 – 6.4], [95% CI pigs: 0.0 – 1.3]). Carbapenems are not approved for use in food-producing animals in the EU and EEA countries. Nevertheless, resistance to these critically important antimicrobial agents has sporadically been reported from animals in some of the EU/EEA countries, and further monitoring is recommended to follow the situation in the years to come.

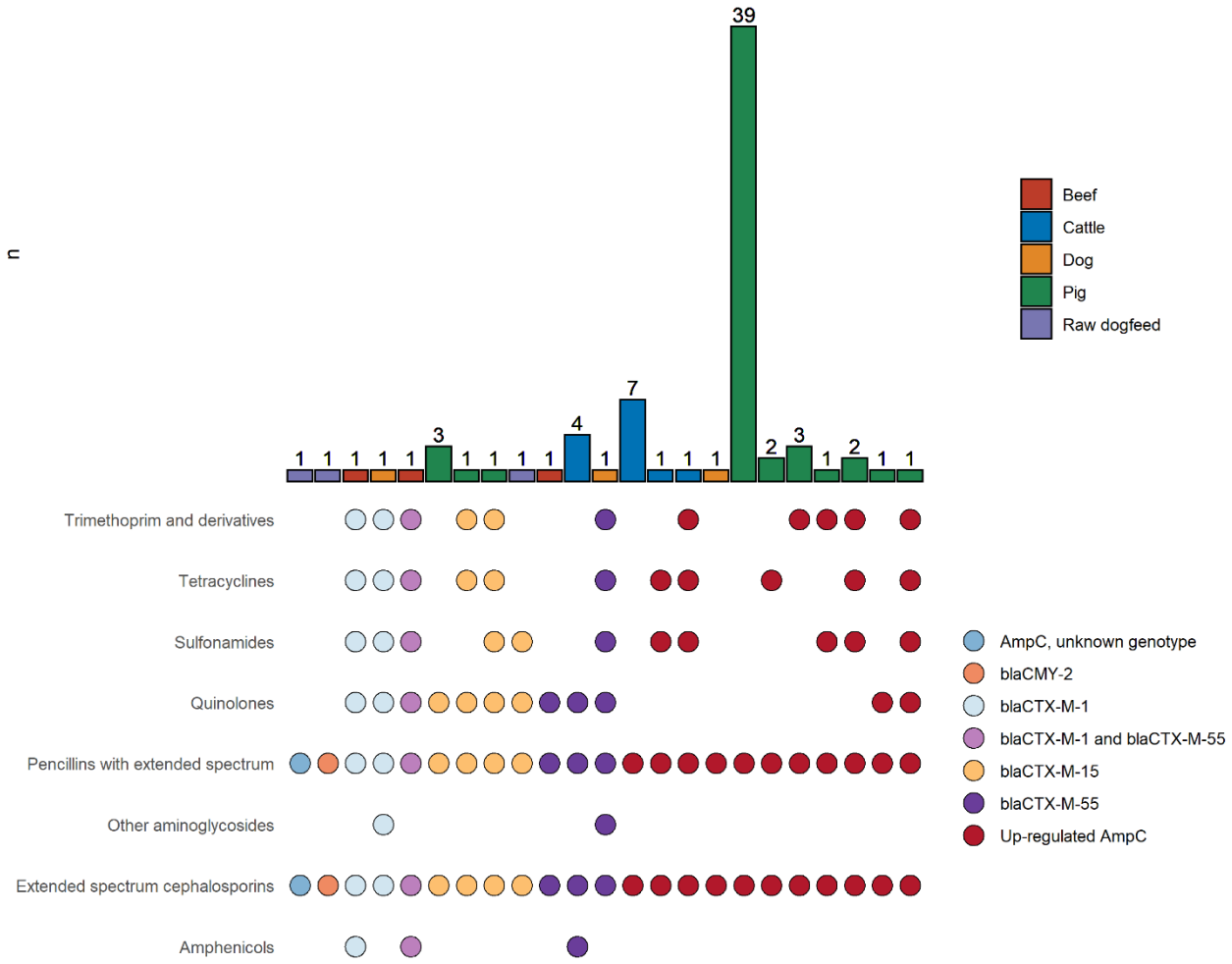


FIGURE 45. Overview of *Escherichia coli* resistant to extended-spectrum cephalosporins identified in NORM-VET 2019, their genotype, antimicrobial resistant patterns and source of origin. Histogram shows number of isolates.

Enterococcus spp. from cattle and pigs

Caecal samples from a total of 319 cattle < one year and 286 pigs were examined. *E. faecalis* was obtained from 12 (3.8%) and *E. faecium* from 20 (6.3%) of the cattle samples. From pigs, *E. faecalis* was obtained from 46 (16.1%) and *E. faecium* from 106 (37.1%) of the samples. One isolate of *E. faecalis* and/or *E. faecium* per positive sample was susceptibility tested. The results are presented in Tables 18-19, and in the text.

TABLE 18. Antimicrobial resistance in *Enterococcus faecalis* from caecal samples from cattle < one year (n=12) and fattening pigs (n=46) in 2019.

Substance	Sample	n (resistance)	Distribution (n) of MIC values (mg/L)*														
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	Cattle	3						9						3			
	Pig	29						17				1	12	14	2		
Tigecycline	Cattle	0		1	8	3											
	Pig	0		1	32	13											
Chloramphenicol	Cattle	0								4	8						
	Pig	0								2	41	3					
Ampicillin	Cattle	0						11	1								
	Pig	0						41	5								
Erythromycin	Cattle	0						8	4								
	Pig	0						18	25	3							
Quinupristin - dalfopristin	Cattle	ND							1		9	2					
	Pig	ND									36	10					
Gentamicin	Cattle	0									3	9					
	Pig	0									3	33	10				
Ciprofloxacin	Cattle	0					1	8	3								
	Pig	0					3	38	5								
Vancomycin	Cattle	0						5	7								
	Pig	0						35	11								
Teicoplanin	Cattle	0					12										
	Pig	0					46										
Linezolid	Cattle	0							12								
	Pig	0							45	1							
Daptomycin	Cattle	0						3	6	3							
	Pig	0					1	9	28	8							

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

TABLE 19. Antimicrobial resistance in *Enterococcus faecium* (n=106) from caecal samples from fattening pigs in 2019.

Substance	Resistance (%)		Distribution (%) of MIC values (mg/L)*														
	[95% CI]		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	22.6	[15.1 - 31.8]						77.4					0.9	19.8	1.9		
Tigecycline	0	[0.0 - 3.4]	3.8	31.1	58.5	6.6											
Chloramphenicol	0	[0.0 - 3.4]								17.9	71.7	10.4					
Ampicillin	6.6	[2.7 - 13.1]					11.3	17.9	24.5	39.6	6.6						
Erythromycin	3.8	[1.0 - 9.4]						23.6	53.8	18.9	0.9					2.8	
Quinupristin – dalfopristin	0	[0.0 - 3.4]					38.7	6.6	5.7	49.1							
Gentamicin	0	[0.0 - 3.4]									90.6	8.5	0.9				
Ciprofloxacin	1.9	[0.2 - 6.6]				0.9	31.1	18.9	19.8	19.8	7.5	0.9	0.9				
Vancomycin	0	[0.0 - 3.4]						93.4	6.6								
Teicoplanin	0	[0.0 - 3.4]					99.1	0.9									
Linezolid	0	[0.0 - 3.4]							67.9	32.1							
Daptomycin	0	[0.0 - 3.4]				4.7	11.3	18.9	22.6	38.7	3.8						

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

CATTLE

The 2019 data showed that nine of the 12 *E. faecalis* and 16 of the 20 *E. faecium* isolates from cattle caecal samples were susceptible to all antimicrobial agents included in the test panel. The remaining three *E. faecalis* and four *E. faecium* isolates were only resistant to tetracycline. Isolation of *Enterococcus* spp. has not been conducted on samples from cattle since 2003. Comparison is difficult due to few available isolates and differences in methodology.

PIG

The 2019 data showed that 17 of the 46 *E. faecalis* and 71.7% of the *E. faecium* isolates from pig caecal samples were susceptible to all antimicrobial agents included in the test panel. The remaining 29 *E. faecalis* isolates were only resistant to tetracycline. Among the *E. faecium* isolates, 21.7% were resistant to one antimicrobial class and 6.6% were resistant to two antimicrobial classes. Resistance to tetracycline was the most frequently identified resistance determinant, followed by resistance to ampicillin, erythromycin and ciprofloxacin. Isolation of *Enterococcus* spp. from pig samples has not been conducted since 2008.

Due to changes in methodology, comparison is difficult. However, the data indicate that there has been a decrease in resistance to erythromycin in *E. faecium* from 18.5% [95% CI: 10.3 – 30.5] in 2008 to 3.8% [95% CI: 1.0 – 9.4] in 2019. Resistance to tetracycline was among the most common resistance determinants both in 2008 and 2019.

Methicillin resistant *Staphylococcus aureus* (MRSA) from goats

Samples from a total of 94 goat herds were investigated for the presence of methicillin resistant *Staphylococcus aureus* (MRSA). MRSA was not detected from any of the herds [95% CI: 0.0 – 3.8].

Surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) in pig herds in Norway in 2019

There are several varieties of methicillin resistant *Staphylococcus aureus* (MRSA), some of which are associated with animals (especially pigs), and are collectively referred to as LA-MRSA (livestock-associated MRSA). Within a few years, LA-MRSA has become widespread in pig populations around the world, thereby representing a risk for dissemination to the human population. LA-MRSA in European pigs has mainly been attributed to clonal complex (CC) 398. As the only country in the world, Norway implemented a control strategy from 2013 including measures to eradicate MRSA in pigs as described in Grøntvedt *et al.* 2016 (1). The rationale behind this strategy was to prevent the pig population from becoming a domestic reservoir of MRSA with the potential of zoonotic transmission, as MRSA is not a significant cause of disease in pigs.

A yearly surveillance programme on MRSA in the pig population was implemented from 2014. The first year, all sow herds with more than ten sows were examined (n=986 herds) and a single positive herd with MRSA CC398, t011 was identified (2). In 2015, a total of 821 herds were included, of which 86 were nucleus or multiplier herds and 735 were finishing herds (3). LA-MRSA was identified in four herds; three finishing herds and one multiplier herd. The isolates from two finishing herds were typed as CC1, t177 and further outbreak tracing showed that the two herds belonged to the same cluster of positive herds. The last two herds were not linked, but both were positive for MRSA CC398, t034 (3). In 2016, a total of 872 herds were investigated, of which 87 genetic nucleus or multiplier herds, 12 sow pool herds and 773 herds with more than 10 sows (4). MRSA was not detected in any of the genetic nucleus, multiplier or sow pool herds. LA-MRSA CC398, t034 was, however, identified in one herd that had recently converted to a specialised finisher herd. Follow-up testing of contact herds, revealed two other herds positive for the same CC and *spa*-type, and eradication was initiated. No MRSA CC398 was detected among the 85 genetic nucleus or multiplier herds, 12 sow pool herds, or the 729 herds with more than 10 sows included in the 2017 surveillance programme. However, MRSA CC7, and CC130 and CC425 were detected in one multiplier herd and in two farrow to finish herds, respectively (5). MRSA was not detected in samples from any of the total 716 herds included in the 2018 surveillance (6).

The surveillance programme in 2019 detected one pig herd with MRSA. In total, 722 herds were included in the survey, of which 79 were genetic nucleus or multiplier herds, 12 herds were central units of the sow pool herds, 22 were of the largest farrow to grower or farrow to finish herds, and the remaining 609 were herds with more than 10 sows. Additional samples from six farrow to finish herds were received, though not included in the 2019 surveillance, and MRSA was detected from one of these as well. Further details of the surveillance can be found in the report "The surveillance programme for methicillin resistant *Staphylococcus aureus* in pigs in Norway 2019" (7).

TABLE 20. Summary of surveillance and surveys of MRSA in the Norwegian pig population 2008-2019.

Year	Survey / material	No. herds tested	MRSA positive herds	Type of MRSA
2008	EU baseline / dust	252	1	
2008	National study / abattoir	200	1	CC398
2011	National study / nasal swabs, abattoir	207	6 (from one abattoir)	CC398
2012	National study / 10 skin swabs at farm	175	1	CC398
2014	MRSA surveillance / sow farms	986	1	CC398
2015	MRSA surveillance / breeder and finisher farms	821	4	CC398 (2), CC1 (2)
2016	MRSA surveillance / sow farms	872	1	CC398
2017	MRSA surveillance / sow farms	826	3	CC7, CC130, CC425
2018	MRSA surveillance / breeder and finisher farms	716	0	
2019	MRSA surveillance / sow farms	722	1	CC398

References:

1. Grøntvedt. C.A., Elstrøm. P., Stegger. M., Skov. R.L., Skytt Andersen. P., Larssen. K.W., Urdahl. A.M., Angen. Ø., Larsen. J., Åmdal. S., Løtvedt. S.M., Sunde. M., Bjørnholt. J.V. Methicillin-Resistant *Staphylococcus aureus* CC398 in Humans and Pig in Norway: A "One Health" Perspective on Introduction and Transmission. Clin Infect Dis. 2016 Dec 1;63(11):1431-1438.
2. Urdahl AM, Bergsjø B, Hofshagen M, Norström M, Lium B. The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2014. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2014*. Oslo: Norwegian Veterinary Institute 2015.
3. Urdahl AM, Bergsjø B, Norström M, Grøntvedt CA, The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2015. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2015*. Oslo: Norwegian Veterinary Institute 2016.
4. Urdahl AM, Norström M, Bergsjø B, Grøntvedt CA. The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2016. *Surveillance programmes for terrestrial and aquatic animals in Norway. A'nnual report 2016*. Oslo: Norwegian Veterinary Institute 2017.
5. Urdahl AM, Norström M, Bergsjø B, Grøntvedt CA. The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2017. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2017*. Oslo: Norwegian Veterinary Institute 2018.
6. Urdahl AM, Norström M, Bergsjø B, Grøntvedt CA. The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2018. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2018*. Oslo: Norwegian Veterinary Institute 2019.
7. Urdahl AM, Norström M, Bergsjø B, Grøntvedt CA. The surveillance programme for methicillin resistant *Staphylococcus aureus* in pig in Norway 2019. *Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2019*. Oslo: Norwegian Veterinary Institute 2020.

Anne Margrete Urdahl, Madelaine Norström, Hilde Welde, Bjarne Bergsjø and Carl Andreas Grøntvedt. Norwegian Veterinary Institute,Oslo, Norway.

SPORTS AND FAMILY ANIMALS

Escherichia coli from dogs

Faecal swab samples from a total of 205 dogs were examined, and *E. coli* isolates were obtained from 190 (92.7%) of these. One isolate per positive sample was susceptibility tested. The results are presented in the text and in Table 21.

TABLE 21. Antimicrobial resistance in *Escherichia coli* isolates (n=190) from faecal samples from dogs in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	3.2	[1.2 - 6.7]								90	6.8				1.1	2.1		
Tigecycline	0	[0.0 - 1.9]					97.9	2.1										
Chloramphenicol	1.6	[0.3 - 4.5]										94.7	3.7	0.5			1.1	
Ampicillin	11.1	[7.0 - 16.4]							0.5	23.7	58.9	5.8			0.5	10.5		
Cefotaxime	0	[0.0 - 1.9]					100											
Ceftazidime	0	[0.0 - 1.9]						100										
Meropenem	0	[0.0 - 1.9]		100														
Sulfamethoxazole	5.8	[2.9 - 10.1]										83.7	9.5	0.5	0.5			5.8
Trimethoprim	2.1	[0.6 - 5.3]					33.2	56.8	7.4	0.5					2.1			
Azithromycin	0	[0.0 - 1.9]								13.7	60	25.8	0.5					
Gentamicin	1.1	[0.1 - 3.8]						73.2	21.6	4.2	0.5	0.5						
Ciprofloxacin	0.5	[0.0 - 2.9]	83.7	15.3	0.5		0.5											
Nalidixic acid	0	[0.0 - 1.9]									98.9	1.1						
Colistin	0	[0.0 - 1.9]							96.3	3.7								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

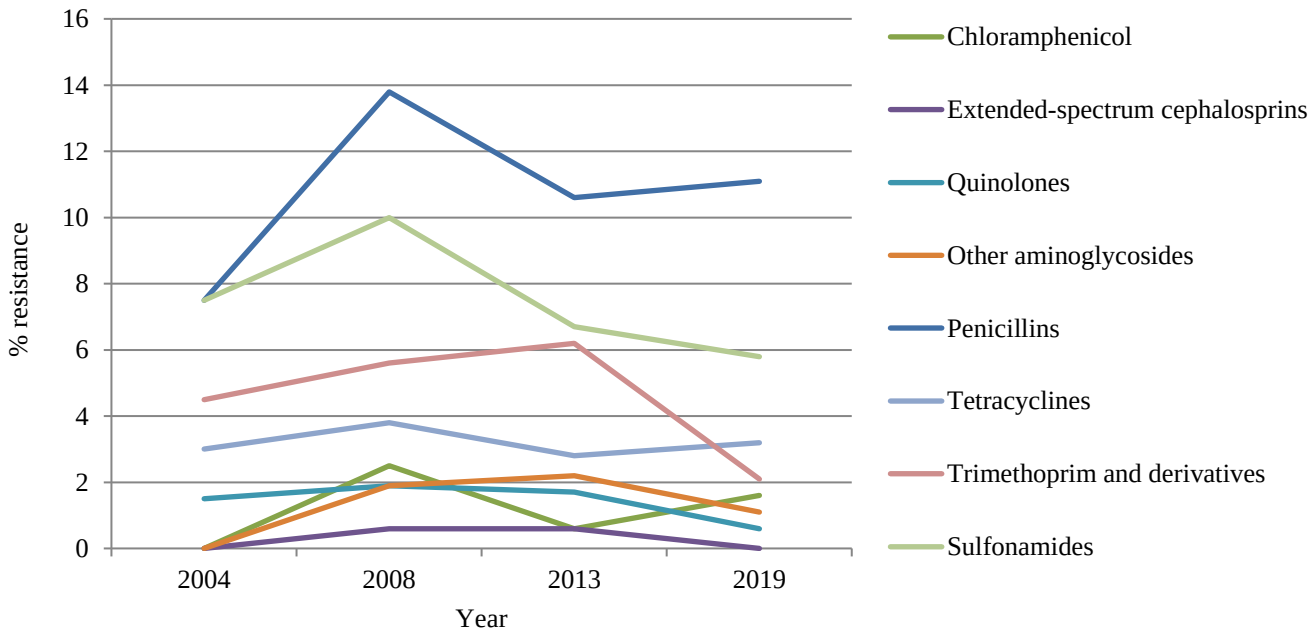


FIGURE 46. Prevalence of resistance to various antimicrobial classes in *Escherichia coli* from dog faecal samples collected in 2004-2019. The breakpoints used in NORM-VET 2019 were applied. Oxytetracycline was used instead of tetracycline before 2005. Note irregular time intervals on the x-axis.

RESULTS AND COMMENTS

A total of 86.8% of the isolates were susceptible to all antimicrobial agents included. In total, 13.2% of the isolates were resistant to at least one of the tested antimicrobial agents, indicating a moderate occurrence of resistance among *E. coli* from faecal samples of dogs according to the EFSA classification described in Appendix 6.

Altogether, 5.8% of the isolates were resistant to one antimicrobial class, 3.7% to two, 2.6% to three, and 1.1% to four antimicrobial classes. Resistance to ampicillin was the most frequently identified resistance determinant, followed by resistance to sulfamethoxazole and tetracycline.

None of the isolates displayed any resistance to the extended-spectrum cephalosporins cefotaxime or ceftazidime, while one isolate showed decreased susceptibility to the quinolone ciprofloxacin. Selective methods were also used on the same sample material to investigate the occurrence of these substances with more sensitive methods.

Samples from dogs have previously been included in NORM-VET in 2004, 2008 and 2013. Since 2013 there have been changes in the panel of antimicrobial agents tested for, and therefore comparison of overall resistance is difficult. Streptomycin is for instance no longer part of the test panel. In 2013, 8.4% of the tested isolates displayed reduced sensitivity towards streptomycin (NORM-VET 2013). However, no significant differences were seen in resistance for those antimicrobial agents present in both the 2019 and the 2013 panels. Resistance to ampicillin was the most frequently identified resistance determinant in 2013 as well as in 2019. This corresponds well to the usage data showing that penicillins are the most commonly used antimicrobial product in companion animals (Figure 9, page 23).

There is a lower proportion of overall antimicrobial resistance in these indicator *E. coli* isolates compared to the results for *E. coli* from infections in dogs as presented in Figure 37, page 52.

Extended-spectrum cephalosporin resistant *Escherichia coli* from dogs

A total of 231 faecal swab samples from dogs were investigated by selective methods for detection of *E. coli* resistant to extended-spectrum cephalosporins (ESC). *E. coli* resistant to ESC were found in three (1.3% [95% CI: 0.2-3.7]) of the samples. This is in concordance with previous results from 2013 (NORM-VET 2013). An overview of the isolates´ antimicrobial resistance patterns and their cephalosporin resistant genotypes are shown in Figure 45, page 62. One isolate displayed an AmpC beta-lactamase phenotype, and the resistance was due to mutations in the promoter and attenuator region of the chromosomally located *ampC* gene causing an upregulation. The two other isolates displayed an ESBL phenotype, and the first isolate harboured the $bla_{\text{CTX-M-1}}$ and $bla_{\text{OXA-1}}$ genes together with the *aac(6')-Ib-cr* gene encoding quinolone resistance, while the second isolate harboured the $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-1B}}$ genes.

Quinolone resistant *Escherichia coli* from dogs

Faecal samples from 231 dogs were investigated for the presence of quinolone resistant *E. coli* (QREC) by selective methods. QREC, *E. coli* resistant to ciprofloxacin and/or nalidixic acid, were found in 20 (8.7% [95% CI: 5.4-13.1]) of the samples. One isolate per positive sample was susceptibility tested. The results are presented in the text and in Table 22.

TABLE 22. Antimicrobial resistance in quinolone resistant *Escherichia coli* isolates (n=20) from faecal samples from dogs in 2019.

Substance	n (resistance)	Distribution (n) of MIC values (mg/L)*															
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	7								12	1				1	6		
Tigecycline	0					20											
Chloramphenicol	1										17	2	1				
Ampicillin	9								1	10					9		
Cefotaxime	1					19					1						
Ceftazidime	1						19			1							
Meropenem	0		20														
Sulfamethoxazole	6										14						6
Trimethoprim	8					3	6	3						8			
Azithromycin	ND								1	10	8				1		
Gentamicin	1						17	2					1				
Ciprofloxacin	19			1	3	10	3	1			1	1					
Nalidixic acid	16									1	3	1	1	1	6	7	
Colistin	0							20									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

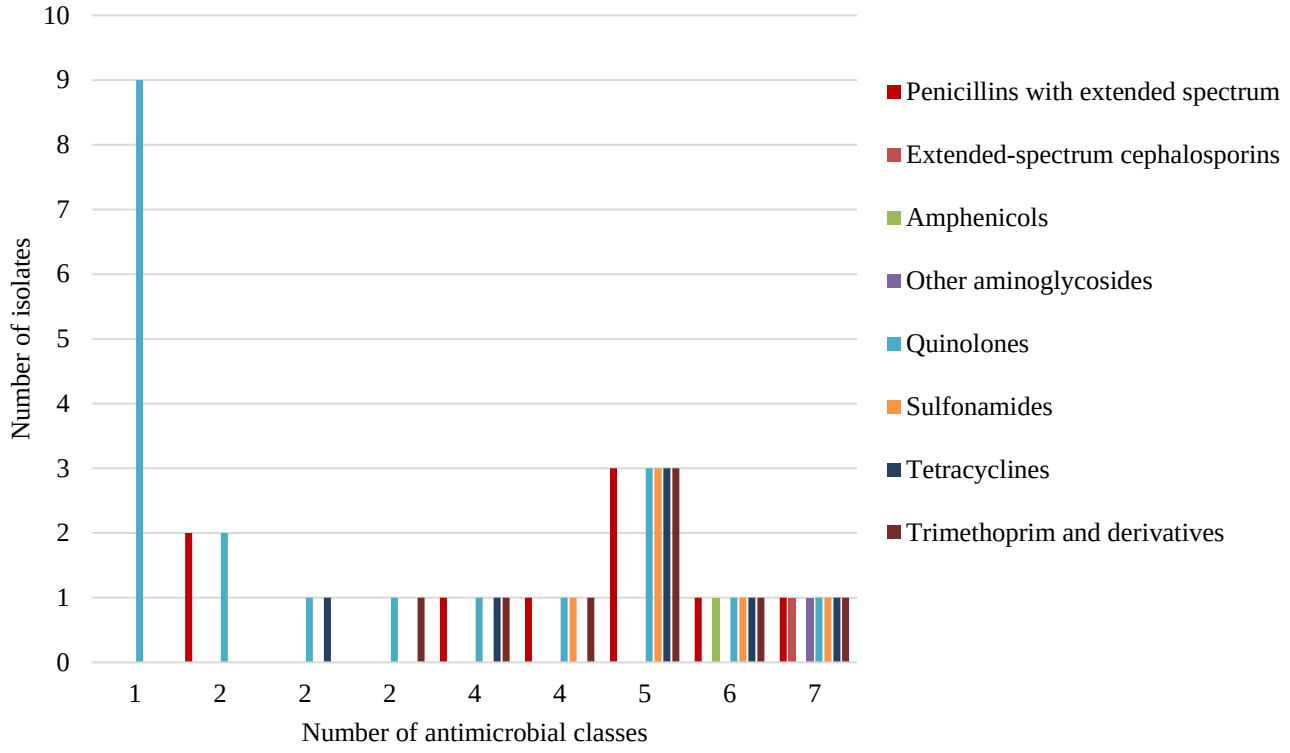


FIGURE 47. Resistance pattern among quinolone resistant *Escherichia coli* isolates (n=20) from faecal samples from dogs in 2019.

RESULTS AND COMMENTS

Nine of the 20 QREC showed decreased susceptibility only to quinolones (ciprofloxacin and/or nalidixic acid). Four isolates were additionally resistant to one antimicrobial class, two isolates to three antimicrobial classes, three isolates to four antimicrobial classes, one isolate to five antimicrobial classes, and one to seven antimicrobial classes (Figure 47).

One isolate showed decreased susceptibility to the ESC cefotaxime and ceftazidime, and displayed an ESBL phenotype. Whole genome sequencing detected the $bla_{\text{CTX-M-55}}$ and $bla_{\text{TEM-1B}}$ genes as the cause for the decreased susceptibility. The resistance to quinolones in this isolate was expressed due to mutations in the *gyrA* (S83L, D87N) and *parC* (S80I) genes.

Selective methods for isolation of QREC have not been performed on faecal dog samples previously, and comparisons to previous years are therefore not possible. The 2019 results do show that there are some very MDR *E. coli* present in dogs. These cannot be explained by the usage of antimicrobials to dogs in Norway. However, the Norwegian dog population must be regarded as an open population with contact to dog populations in other countries through import and travelling, thereby facilitating dissmination of antimicrobial resistance across the border. Transmission of MDR bacteria may also occur through feeding of raw dog feed (see chapter on feed page 77).

Carbapenemase-producing *Enterobacteriaceae* from dogs

A total of 231 samples from dogs were investigated for the presence of carbapenemase-producing *Enterobacteriaceae* (CPE) by selective methods.

No CPE isolates were detected [95% CI: 0.0 - 1.6]. Selective methods for isolation of CPE have not been performed on dog samples previously.

Enterococcus spp. from dogs

Faecal swab samples from a total of 218 dogs were examined. *E. faecalis* and *E. faecium* isolates were obtained from 129 (59.2%) and 34 (15.6%) of these, respectively. One isolate of both *E. faecalis* and *E. faecium* per positive sample was susceptibility tested. The results are presented in the text and in Tables 23-24.

TABLE 23. Antimicrobial resistance in *Enterococcus faecalis* (n=129) from dog faecal samples in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*														
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	38	[29.6 - 46.9]						61.2	0.8			0.8	2.3	31	3.9		
Tigecycline	0	[0.0 - 2.8]	3.9	13.2	69.8	13.2											
Chloramphenicol	3.1	[0.9 - 7.7]								23.3	70.5	3.1		3.1			
Ampicillin	0	[0.0 - 2.8]					0.8	85.3	14								
Erythromycin	5.4	[2.2 - 10.9]						47.3	41.1	6.2	0.8		0.8			3.9	
Quinupristin – dalfopristin	ND	ND								0.8	87.6	11.6					
Gentamicin	2.3	[0.5 - 6.6]									20.2	69.8	7.8				2.3
Ciprofloxacin	0.8	[0.0 - 4.2]					5.4	76	17.8				0.8				
Vancomycin	0	[0.0 - 2.8]						79.8	19.4	0.8							
Teicoplanin	0	[0.0 - 2.8]					100										
Linezolid	0	[0.0 - 2.8]						3.1	93.8	3.1							
Daptomycin	1.6	[0.2 - 5.5]				3.1	1.6	28.7	60.5	4.7	1.6						

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

TABLE 24. Antimicrobial resistance in *Enterococcus faecium* (n=34) from dog faecal samples in 2019.

Substance	n (resistance)	Distribution (n) of MIC values (mg/L)*														
		0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	3						31						2	1		
Tigecycline	0	6	14	13	1											
Chloramphenicol	0								6	24	4					
Ampicillin	0					6	16	8	4							
Erythromycin	3						12	11	8	1	1				1	
Quinupristin – dalfopristin	ND					6	6	5	15	1	1					
Gentamicin	0									27	5	2				
Ciprofloxacin	0				1	1	8	9	13	2						
Vancomycin	0						28	6								
Teicoplanin	0					34										
Linezolid	0						1	14	19							
Daptomycin	0						2	6	22	4						

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

The 2019 data showed that 59.7% of the *E. faecalis* and 29 of the 34 *E. faecium* isolates from dogs were susceptible to all antimicrobial agents included in the test panel.

Altogether, 31.8% of the *E. faecalis* isolates were resistant to one antimicrobial class, 7% to two, 0.8% to three and 0.8% to four antimicrobial classes. In total, 37.2% of the *E. faecalis* isolates were resistant to at least one antimicrobial agent. Resistance to tetracycline was the most frequently identified resistance determinant, followed by resistance to erythromycin, chloramphenicol and gentamicin. Among the 34 *E. faecium* isolates, four were resistant to one and one to two antimicrobial classes, respectively. Resistance to tetracycline and erythromycin was the only identified resistance determinant among these isolates.

Isolation of *Enterococcus* spp. from dog faecal samples has only been conducted once before, in 2004 (NORM-VET 2004). Since then there has been changes in the panel of antimicrobial agents tested for, and therefore comparison of overall resistance is difficult.

Staphylococcus pseudintermedius from dogs

A total of 207 samples from dogs were investigated for the presence of *Staphylococcus pseudintermedius*. *S. pseudintermedius* was detected from 128 of these (62.8 %). One isolate per positive sample was susceptibility tested. The results are presented in Table 25 and in the text.

TABLE 25. Antimicrobial resistance in *Staphylococcus pseudintermedius* isolates (n=128) from samples from dogs in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.016	0.032	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	21.1	[14.4 – 29.2]						78.9			0.8			20.3				
Chloramphenicol	3.9	[1.3 - 8.9]									91.4	4.7			3.9			
Benzylpenicillin[ψ]	46.1	[0.0 - 2.8]				53.9	13.3	4.7	10.2	7.8	10.2							
Cefoxitin	0.0	[0.0 - 2.8]						96.1	2.3	0.8	0.8							
Trimethoprim	3.9	[1.3 – 8.9]								85.9	9.4	0.8			3.9			
Sulfamethoxazole	58.6	[49.5 - 67.2]													39.8	1.6	10.9	47.6
Erythromycin	7.8	[3.8 - 13.9]					92.2						7.8					
Clindamycin	7.0	[3.3 - 12.9]				93.0		0.8	0.8			5.5						
Quinupristin/ dalfopristin	0.0	[0.0 - 2.8]						100										
Streptomycin	8.6	[4.3 – 14.9]									90.6		0.8		8.6			
Gentamicin	1.6	[0.2 – 4.3]							98.4			0.8	0.8					
Kanamycin	7.8	[3.8- 13.9]									91.4	0.8		0.8		7.0		
Ciprofloxacin	0.8	[0.2 - 4.3]					98.4	0.8					0.8					
Vancomycin	0.0	[0.0 - 2.8]							99.2	0.8								
Fusidic acid	28.9	[21.2 - 37.6]						71.1			0.8	28.1						
Tiamulin	0.0	[0.0 - 2.8]						100										
Linezolid	0.0	[0.0 - 2.8]							99.2	0.8								
Mupirocin	0.0	[0.0 - 2.8]						98.4	1.6									
Rifampicin	0.0	[0.0 - 2.8]	100															

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested. [ψ]Beta-lactamase production has not been investigated.

RESULTS AND COMMENTS

A total of 21.1% of the *S. pseudintermedius* isolates were susceptible to all antimicrobial agents included. Altogether, 30.5% of the isolates were resistant to one antimicrobial class, 18.8% to two, and 26.7% to three or more antimicrobial classes. Resistance to sulfamethoxazole was the most frequently identified resistance determinant, followed by resistance to benzylpenicillin, fusidic acid and tetracycline.

Isolation and susceptibility testing of *S. pseudintermedius* has not previously been performed in NORM-VET on samples from healthy dogs. However, susceptibility testing of *S. pseudintermedius* from clinical samples has been included several times (see clinical *S. pseudintermedius*, page 53). Overall, there is a higher proportion of antimicrobial resistance in the clinical *S. pseudintermedius* isolates compared to antimicrobial resistance in the *S. pseudintermedius* from carrier dogs as shown in Figures 39-40 on pages 53-54. There is also a difference in antimicrobial resistance profile between these, as a higher proportion of the clinical isolates was resistant to two or more antimicrobial classes, while a higher proportion of the *S. pseudintermedius* from carrier dogs was resistant to only one antimicrobial class.

Methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus pseudintermedius* (MRSP) from dogs

A total of 230 samples from dogs were investigated for the presence of methicillin resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus pseudintermedius* (MRSP). Neither MRSA nor MRSP were detected from any of the samples [95% CI: 0.0 - 1.6%].
Selective methods for detection of MRSA and MRSP from dog samples have not been included in NORM-VET previously. The results are, however, in concordance with a study performed in Norway in 2016-2017, performing selective isolation of both MRSA and MRSP on samples from healthy dogs (see separate presentation, text box below). Both MRSA and MRSP have, though, been detected among clinical isolates from dogs in Norway from 2008, indicating that these are present in the Norwegian dog population. Some MRSP isolates were also among the clinical *Staphylococcus pseudintermedius* included in 2019 (see page 53).
Emerging reservoirs in dogs of MRSA, and especially MRSP, constitute a challenge to infection management. Moreover, as dogs live in close contact with humans, zoonotic transmission between dogs and humans may occur.

Low occurrence of antimicrobial resistant bacteria in healthy dogs in Norway

Introduction
Healthy non-symptomatic carriers of antimicrobial resistant bacteria may act as a reservoir and contribute to further dissemination to other dogs, owners and the environment. Knowledge about carriage of resistant bacteria in the healthy dog population is therefore of importance. The aim of the present study was to investigate the occurrence of methicillin resistant *Staphylococci* (*S. pseudintermedius* and *S. aureus*) (MRS) and *E. coli* resistant to extended-spectrum cephalosporins (ESC), fluoroquinolones and carbapenems in healthy dogs from different regions of Norway.

Materials and methods
The dogs were recruited during routine consultations at six veterinary clinics in different parts of Norway in 2016-2017. Pooled samples from mouth and perineum were investigated for presence of MRS, and faecal swabs were investigated for the presence of *E. coli* resistant to ESC, carbapenems and fluoroquinolones.

The samples were investigated with selective methods as described in Appendix 3. All retrieved isolates were subjected to antimicrobial susceptibility testing using the broth microdilution method following the protocol described in Appendix 3. Isolates were classified as susceptible or resistant based on epidemiological cut-off values (ECOFFs) recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org). *E. coli* displaying resistance towards ESC were subjected to PCR for detection of specific resistance genes as described in Appendix 3.

Results
In total, 226 dogs were included in the study. Faecal swabs from two dogs were missing. An overview of the results from the investigations of MRS and *E. coli* resistant to ESC, fluoroquinolones and carbapenems, is shown in Table 26.

TABLE 26. The occurrence of MRS in pooled samples from mouth and perineum and *E. coli* resistant to extended-spectrum cephalosporins (ESC), fluoroquinolones and carbapenems in faecal swabs from healthy dogs sampled in 2016-2017.

No. dogs (%) positive for	No. dogs (%) positive for *E. coli* resistant to		
MRS (n= 226)	ESC (n=224)	carbapenems (n=224)	fluoroquinolones (n=224)
0 (0%)	11 (4.9%)	0 (0%)	33 (14.7%)
[95% CI: 0.0 – 1.6]	[95% CI: 2.5 – 8.6]	[95% CI: 0.0 – 1.6]	[95% CI: 10.4 – 20.1]

MRS and *E. coli* resistant to carbapenems were not detected. Fluoroquinolone resistant *E. coli* was most common with 14.7% of the dogs positive. In total, eleven (4.5%) of the dogs were carrying ESC resistant *E. coli*. Isolates from seven of these, expressed an AmpC beta-lactamase phenotype. The plasmid mediated bla_{CMY-2} gene was detected in five isolates, while the last two isolates expressed the AmpC beta-lactamase phenotype due to overexpression of the chromosomally encoded *ampC* gene. The remaining four isolates expressed an ESBL phenotype, and the genotypes detected in these were $bla_{CTX\text{-}M\text{-}27}$, $bla_{CTX\text{-}M\text{-}27}$/$bla_{TEM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$/$bla_{TEM\text{-}1}$, and $bla_{CTX\text{-}M\text{-}1}$/$bla_{TEM\text{-}1}$, respectively. The results from this study are in concordance with the NORM-VET 2019 results from dog samples presented in the current report on page 65.

Anne Nordstoga, Madelaine Norström, Jannice Schau Slettemeås, and Anne Margrete Urdahl, Norwegian Veterinary Institute, Oslo, Norway.

Antimicrobial resistance in dogs imported to Norway

Introduction

The National Strategy against Antibiotic Resistance 2015–2020[1], as well as the action plan from the Ministry of Agriculture and Food, state the importance of gathering knowledge on occurrence of antimicrobial resistance (AMR) in relevant animal populations, including dogs. Moreover, import of dogs and dogs travelling are highlighted as a risk factor for increasing the dissemination of AMR in an assessment of transfer of AMR between pets and humans performed by the Norwegian Scientific Committee for Food Safety (VKM) in 2015[2].

To specifically address import and travelling of dogs as a risk factor for importing emerging AMR bacteria, the Norwegian University of Life Sciences and the Norwegian Veterinary Institute initiated two studies in 2016 and 2017, respectively. The aims of these studies were to describe the occurrence of *Escherichia coli* resistant to extended-spectrum cephalosporins (ESC), carbapenems, fluoroquinolones and colistin in:

- dogs entering Norway through the veterinary border inspection post at Gardermoen, Oslo airport
- dogs imported for the first time to Norway from Southern- or Eastern Europe or "third countries"

Material and methods

Faecal swab samples were taken from dogs entering Norway through the veterinary border inspection post at Gardermoen, Oslo airport, and from dogs included in the surveillance programme for imported dogs in Norway in 2017, i.e. from dogs at least 6 months of age and imported for the first time to Norway from Southern - or Eastern Europe or "third countries"[3]. The samples were investigated for presence of *E. coli* resistant to ESC, carbapenems, fluoroquinolones and colistin with selective methods as described in Appendix 3. All retrieved isolates were subjected to antimicrobial susceptibility testing using the broth microdilution method following the protocol described in Appendix 3. Isolates were classified as susceptible or resistant based on epidemiological cut-off values (ECOFFs) recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, www.eucast.org). *E. coli* displaying resistance towards ESC were subjected to PCR for detection of specific resistance genes as described in Appendix 3.

Results

In total, 142 samples were retrieved. The dogs were of 38 different breeds, though the majority were of mixed breeds. The majority of the dogs had been in Spain, though a total of 27 different countries were reported as export/visiting countries. An overview of the results from the investigations of *E. coli* resistant to ESC, carbapenems, fluoroquinolones and colistin is shown in Table 27.

TABLE 27. The occurrence of *E. coli* resistant to extended-spectrum cephalosporins (ESC), carbapenems, fluoroquinolones and colistin in faecal swab samples from imported/travelling dogs sampled in 2016-2017.

No. dogs (%) positive for *E. coli* resistant to			
ESC (n=142)	carbapenems (n=130)	fluoroquinolones (n=142)	colistin (n=142)
13 (9.2%)	0 (0%)	30 (21.1%)	0 (0%)
[95% CI 4.9 – 15.1])	[95% CI 0.0 – 2.8])	[95% CI 14.7 – 28.8])	[95% CI 0.0 – 2.6])

Six of the 13 ESC *E. coli* isolates expressed an AmpC beta-lactamase phenotype and the plasmid-mediated bla_{CMY}-gene was detected in five of these. The last isolate expressed the AmpC beta-lactamase phenotype due to overexpression of the chromosomally encoded *ampC* gene. The remaining seven isolates expressed an ESBL phenotype. In three isolates, the bla_{SHV-12} gene encoded the resistance. Three isolates encoded the $bla_{CTX-M-15}$ gene (two also encoding the bla_{TEM-1}) and the last encoded the $bla_{CTX-M-27}$ (group 9).

There is a higher occurrence of ESC resistant *E. coli* and fluoroquinolone resistant *E. coli* in samples from these imported/travelling dogs compared to the occurrence in samples from dogs sampled under the auspices of NORM-VET in 2019 (see page 65). This supports that imported/travelling of dogs might be a risk factor for dissemination of emerging AMR bacteria.

References

1. Nasjonal strategi mot antibiotikaresistens 2015-2020. In: omsorgsdepartementet Ho, ed. Oslo: Helse og omsorgsdepartementet; 2015.
2. VKM. *Assessment of the transfer of antimicrobial resistance between pets and humans in Norway.* Oslo, Norway: Norwegian Scientific Committee for Food and Environment; 2015.
3. Jørgensen HJH, I.S.; Nordstoga, A.B.; Klevar, S. *The surveillance programme for imported dogs in Norway 2017* Norwegian Veterinary Institute: Norwegian Veterinary Institute; 2018.

Anne Margrete Urdahl, Jannice Schau Slettemeås, Marianne Sunde and Madelaine Norström, Norwegian Veterinary Institute, Oslo, Norway. Nina Askim Vatne, Faculty of Veterinary Medicine, Norwegian University of Life Sciences

INDICATOR BACTERIA FROM FOOD

Gro Johannessen, Madelaine Norström, Jannice Schau Slettemeås, and Anne Margrete Urdahl

NORM-VET is following the requirements set in Commission implementing decision of 12. Nov 2013 on the monitoring and reporting of antimicrobial resistant bacteria in food (2013/652/EU). In addition, antimicrobial testing of bacteria from other food sources than those included in this decision, or investigation of presence of specific antimicrobial resistant bacteria by selective methods, are included. Selective methods are for instance used for detection of *E. coli* resistant to extended-spectrum cephalosporins (ESC), quinolone resistant *E. coli* (QREC), carbapenemase-producing *Enterobacteriaceae* (CPE), and colistin resistant (COL-R) *E. coli*. The use of selective methods are especially relevant for low prevalent sources, as it enables early detection of specific emerging resistance mechanisms such as for instance ESC resistant *E. coli* and CPE; thereby enabling these to be monitored. Some of these antimicrobials are defined by the WHO as critically important for treatment of human infections. A significant reservoir of such resistant bacteria in the food production chain is of concern, as they may interact with the human bacterial populations and thus have an impact on resistance development in these.

In 2019, food samples included beef and pork, as well as leafy greens and leafy herbs. One isolate of interest per positive sample was susceptibility tested. Some of the cut-off values defining resistance applied in NORM-VET have changed over the years. To facilitate comparisons in this report, data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2019. Sampling, laboratory methods and data processing are described in Appendix 3.

MEAT

Extended-spectrum cephalosporin resistant *Escherichia coli* from beef and pork

In total, 349 beef and 352 pork samples were investigated for the presence of *E. coli* resistant to extended-spectrum cephalosporins (ESC), i.e. cefotaxime and/or ceftazidime, with selective methods. Results are presented in the text and in Figure 45, page 62.

RESULTS AND COMMENTS

ESC resistant *E. coli* was not detected in any of the pork samples [95% CI: 0.0 – 1.0]. From the beef samples, *E. coli* resistant to ESC were found in three of the samples (0.9% [95% CI: 0.2 – 2.5]). This is in concordance with previous NORM-VET results from 2017 where *E. coli* resistant to ESC were detected in only 0.3% of the pork samples and not detected at all from the beef samples.

Three *E. coli* isolates displayed an ESBL phenotype, and the resistance genes responsible were $bla_{\text{CTX-M-55}}$, $bla_{\text{CTX-M-1}}/bla_{\text{CTX-M-36}}/bla_{\text{TEM-1b}}$ and $bla_{\text{CTX-M-1}}/bla_{\text{TEM-1b}}$, respectively. The resistance genes responsible are also shown in Figure 45, page 62, together with an overview of what other antimicrobial agents the isolates showed decreased susceptibility to.

In a European perspective the occurrences of *E. coli* resistant to ESC in Norwegian beef and pork are among the lowest reported to EFSA (EFSA and ECDC Summary report 2018). The reported occurrences vary between the countries, where the south-eastern, south-central and south-western countries seem to have a higher occurrence of *E. coli* resistant to ESC than the Nordic and the Western countries.

Transmission of bacteria, including *E. coli* resistant to ESC, between food-producing animals and meat thereof to humans may occur. However, several studies indicate that there is only a small proportion of bacteria resistant to ESC in humans that may have animals and meat thereof as a source of infection (Day et al. 2019, Dorado-Garcia et al. 2018). Such studies reflect the situation at the time of the study, and prevalence changes in animals may lead to an increase in this proportion in humans. A continued awareness of animal/food bacterial reservoirs resistant to ESC is therefore of importance in order to be able to implement control measures if needed.

Carbapenemase-producing *Enterobacteriaceae* from beef and pork

A total of 349 beef and 352 pork samples were investigated for the presence of carbapenemase-producing *Enterobacteriaceae* (CPE). No CPE were detected (beef: [95% CI: 0.0 – 1.1] and pork: [95% CI: 0.0 – 1.0]). Carbapenems are not approved for use in food-producing animals in the EU and EEA countries. Nevertheless, resistance to these antimicrobial agents has sporadically been reported from animals in some of the EU/EEA countries. Carbapenems are defined by the WHO as critically important for treatment of human infections, and a possible development of a significant reservoir of carbapenem resistant bacteria in animals and food is therefore of concern. Further monitoring is recommended to follow the situation in the years to come.

VEGETABLES

Escherichia coli from leafy greens and leafy herbs

A total of 198 samples; i.e. 147 samples of leafy greens of which 62 were of domestic and 85 were of imported origin, and 51 samples of leafy herbs (all imported) were investigated for the presence of indicator *E. coli* after enrichment. *E. coli* was detected from a total of 33 of the leafy green and leafy herb samples. The results are presented in Table 28 and in the text.

TABLE 28. Antimicrobial resistance in *Escherichia coli* isolates (n=33) from leafy greens and leafy herbs in 2019.

Substance	Resistance (n)	Distribution (n) of MIC values (mg/L)*															
		0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	3								27	3				1	2		
Tigecycline	0					33											
Chloramphenicol	0										30	3					
Ampicillin	7								7	19				3	4		
Cefotaxime	0					33											
Ceftazidime	0						33										
Meropenem	0		32	1													
Sulfamethoxazole	2										27	4					2
Trimethoprim	1					16	13	3						1			
Azithromycin	ND								5	10	14	3	1				
Gentamicin	1						26	5	1					1			
Ciprofloxacin	2	30	1		1	1											
Nalidixic acid	2									31				1	1		
Colistin	0							31	2								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENT

In total, 24 of the 33 *E. coli* isolates from leafy greens and leafy herbs were susceptible to all antimicrobial agents included in the test panel. Altogether, six of the isolates were resistant to one antimicrobial class, two isolates to two antimicrobial classes, and one isolate to a total of six antimicrobial classes. None of the isolates displayed any resistance to the ESC cefotaxime or ceftazidime, nor to carbapenems or colistin. Selective methods were also used on the same sample material to investigate the occurrence of these substances with more sensitive methods.

Leafy herbs were also investigated in NORM-VET in 2017 and 2018, while leafy greens were investigated in 2015, 2017 and 2018. Comparisons between these years are difficult due to the limited number of isolates and variety of sampled products each year. A summary of the results from the period 2017-2019 is described in the text box, page 75.

Leafy greens and leafy herbs can become contaminated with antimicrobial resistant bacteria from animal and human sources during primary production and harvesting. As these products typically are consumed raw and without any heat treatment, presence of antimicrobial resistant bacteria may be of concern, especially plasmid encoded resistance due to its dissemination potential. Further monitoring is recommended to acquire more knowledge and to follow the situation on the occurrence of antimicrobial resistant bacteria in vegetables in general and especially in those consumed raw such as leafy greens and leafy herbs.

Extended-spectrum cephalosporin resistant *Escherichia coli* from leafy greens and leafy herbs

A total of 198 samples were investigated for the presence of *E. coli* resistant to extended-spectrum cephalosporins (ESC) with selective methods. ESC resistant *E. coli* was not detected in any of the 147 leafy green samples [95% CI: 0.0-2.5], nor in the 51 leafy herb samples [95% CI: 0.0-7.0]. The investigations in 2015 did not detect any *E. coli* resistant to ESC in leafy greens, while in 2017 it was detected from one sample. In 2018, ESC resistant *E. coli* was detected in one sample of leafy greens and three samples of leafy herbs. Comparison to the previous years should be done with caution due to sample variability. Results from 2017-2019 are further summarised in the text box, page 75.

There is a lack of data on occurrence of emerging antimicrobial resistant bacteria such as *E. coli* resistant to ESC in leafy greens and leafy herbs. Further monitoring is recommended to follow the situation in the years to come.

Quinolone resistant *Escherichia coli* from leafy greens and leafy herbs

Selective methods for isolation of quinolone resistant *E. coli* (QREC) were performed on a total of 198 samples. QREC was detected in a total of five (2.5% [95% CI: 0.8-5.8]) of the samples, all leafy greens. In addition to being resistant to quinolones, two isolates were resistant to one antimicrobial class, one isolate to two antimicrobial classes, one isolate to five antimicrobial classes, and one isolate to a total of six antimicrobial classes. None of the QREC isolates displayed any resistance to the ESC cefotaxime or ceftazidime, nor to carbapenems or colistin.

The survey performed in 2015 detected QREC in two of the investigated 243 samples of leafy greens. QREC was detected in three out of 187 samples in 2017, and in 12 out of 194 samples in 2018. One of the 2017 isolates and two of the 2018 isolates also displayed an ESBL phenotype. Comparison to previous years should be done with caution due to sample variability. Results from 2017-2019 are further summarised in the text box on page 75.

Colistin resistant *Escherichia coli* from leafy greens and leafy herbs

A total of 198 samples were investigated for the presence of colistin resistant (COL-R) *E. coli*. COL-R *E. coli* were not detected in any of the 147 leafy green samples [95% CI: 0.0-2.5], nor in the 51 leafy herb samples [95% CI: 0.0-7.0]. This is in concordance with previous years. However, comparison to previous years should be done with caution due to sample variability. In 2018 one isolate detected using the selective method for QREC showed decreased susceptibility to colistin and the plasmid mediated *mcr-1* gene encoding colistin resistance was detected. This was not captured by the selective colistin resistance method, indicating that the selective method in use could have been more sensitive. COL-R *E. coli* have been detected in imported herbs in several studies (Zurfluh *et al.* 2016, Manageiro *et al.* 2020). The occurrence of such emerging antimicrobial resistant bacteria in products like leafy greens and leafy herbs is of special concern as they are normally consumed raw. Results from 2017-2019 are further summarised in the text box on page 75.

Carbapenemase-producing *Enterobacteriaceae* from leafy greens and leafy herbs

A total of 198 samples were investigated for the presence of carbapenemase-producing *Enterobacteriaceae* (CPE). No CPE were detected in any of the 147 leafy green samples [95% CI: 0.0-2.5], nor in the 51 leafy herb samples [95% CI: 0.0-7.0].

The results from 2019 are in concordance with the results from the previous years. There is a lack of data on occurrence in leafy greens and leafy herbs of emerging antimicrobial resistant bacteria such as *E. coli* resistant to CPE. Further monitoring is recommended to follow the situation in the years to come.

Antimicrobial resistance in bacteria from leafy greens and leafy herbs – a summary of the 2017 - 2019 surveys

There is a lack of knowledge of antimicrobial resistant bacteria in fresh produce. Samples of leafy greens and leafy herbs have therefore been included in NORM-VET the last three years. The samples have been made available through a surveillance programme investigating *Escherichia coli* and *Salmonella* in leafy greens and leafy herbs. A total of 150 samples of leafy greens of both imported and domestic origin and 50 samples of imported leafy herbs were to be collected annually. The samples were collected by the Norwegian Food Safety Authority and sent overnight to the Norwegian Veterinary Institute. The samples were analysed for indicator *E. coli*, and by selective methods for *E. coli* resistant to extended-spectrum cephalosporins (ESC), quinolone resistant *E. coli* (QREC), colistin resistant (COL-R) *E. coli*, and carbapenemase-producing *Enterobacteriaceae* (CPE) as described in Appendix 3 in the NORM/NORM-VET report for the respective years.

In total, 580 samples of domestic and imported leafy greens and imported leafy herbs were analysed in the period 2017-2019, with 178, 249 and 153 samples in each category, respectively. A total of 93 indicator *E. coli* were susceptibility tested over the period (Figure 48). The majority (78.5%) of the isolates were susceptible to all antimicrobial agents included. Altogether, 10.8% of the isolates were resistant to one antimicrobial class, 4.3% to two, 1.1% to three, 2.1% to four and five antimicrobial classes, respectively, and 1.1% of the isolates were resistant to six antimicrobial classes.

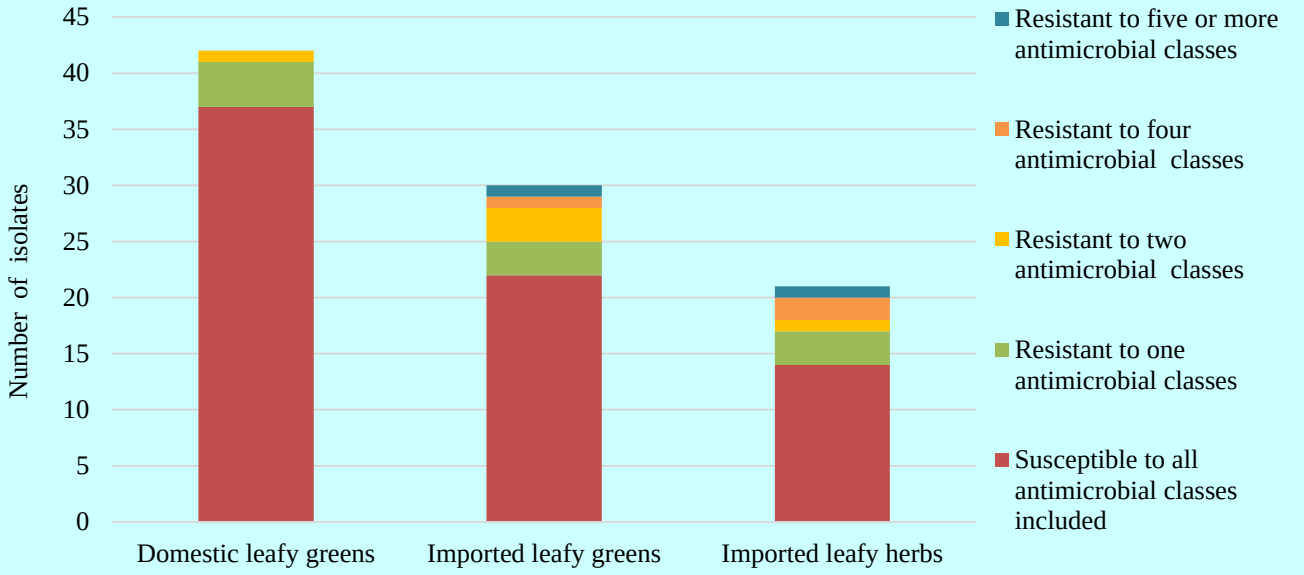


FIGURE 48. Antimicrobial resistance profile in 93 *E. coli* isolated from domestic and imported leafy greens and imported leafy herbs 2017-2019. Proportions of isolates fully susceptible, resistant to one, two, four, or five or more antimicrobial classes are shown.

The results from the selective methods for the years 2017-2019 are summarised in Table 29. Altogether five *E. coli* isolates resistant to ESC were obtained, all displaying an extended-spectrum beta-lactamase (ESBL) phenotype (for genotypes, see Table 30). All five isolates were detected in imported products. The resistance in the three isolates from leafy herbs was encoded by *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-55}}$, and *bla*$_{\text{CTX-M-65}}$, respectively. For the two isolates from leafy greens the resistance mechanism was encoded by *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{SHV-12}}$, respectively. Three isolates also harboured plasmid-encoded quinolone resistance (*qnrS1* or *qnrB19*) and one isolate harboured plasmid-encoded colistin resistance (*mcr-1*). QREC were isolated from a total of 20 samples. COL-R *E. coli* and CPE were not isolated from any of the samples by selective methods. However, as mentioned above, one of the isolates that displayed an ESBL phenotype (with additional quinolone resistance due to chromosomal mutations), also harboured the *mcr-1* gene as identified by whole genome sequencing.

Comparisons between the different categories of leafy greens and leafy herbs are difficult due to the low number of samples and isolates retrieved. Caution should therefore be taken when interpreting the results. However, the results show that imported leafy greens and leafy herbs can be contaminated with some emerging resistant bacteria carrying genes that are not commonly identified among production animals in Norway, nor from domestically produced food.

TABLE 29. A summary of the results from the NORM-VET analyses of samples from domestic and imported leafy greens and imported leafy herbs for the years 2017-2019.

No. samples	Sample type	No. samples	Selective method			
			No. extended-spectrum cephalosporin resistant *E. coli*	No. quinolone resistant *E. coli*	No. colistin resistant *E. coli*	No. carbapenemase-producing *Enterobacteriaceae*
580	Domestic leafy greens	178	0	4	0	0
	Imported leafy greens	249	2	11	0	0
	Imported leafy herbs	153	3*	5	0	0

*One of the isolates was additionally resistant to colistin and carried the *mcr-1* gene. Two isolates were additionally resistant to quinolones and were both detected by the corresponding selective methods.

TABLE 30. Genotypes and resistance profile of the five *E. coli* isolates resistant to third generation cephalosporins.

Sample type	Isolate ID	Genotype	Resistance to			
			3rd and 4th generation cephalosporins	Ampicillin	Quinolones	Colistin
Leafy herbs	A	*bla*$_{\text{CTX-M-55}}$	x			
		bla$_{\text{TEM-1B}}$		x		
		qnrS1			x	
	B	*bla*$_{\text{CTX-M-15}}$	x			
		qnrS1			x	
	C	*bla*$_{\text{CTX-M-65}}$	x			
		bla$_{\text{TEM-1B}}$		x		
		mcr-1				x
		point mutation *gyrA* S83L			x	
		point mutation *gyrA* D87N			x	
Leafy green	D	*bla*$_{\text{SHV-12}}$	x			
		point mutation S83L			x	
		point mutation D87N			x	
Leafy green	E	*bla*$_{\text{CTX-M-14}}$	x			
		bla$_{\text{TEM-1C}}$		x		
		qnrB19			x	

Leafy greens and leafy herbs are exposed to contamination from the production environment, and can become contaminated with antimicrobial resistant bacteria from both animal and human sources through primary production and harvesting. Such products are to a large extent consumed without prior heat treatment, and thus, the presence of antimicrobial resistant bacteria in such products is of concern. Moreover, import comprises a large proportion of the market of such products, facilitating import of antimicrobial resistant bacteria from countries with an overall higher level of antimicrobial resistance than Norway.

There is a gap of knowledge on occurrence of antimicrobial resistance in fresh produce, including the occurrence of emerging antimicrobial resistant bacteria. Further monitoring of both leafy greens and leafy herbs, and other products eaten raw, is recommended to obtain better data for assessing human exposure from these products.

Gro S. Johannessen, Madelaine Norström, Jannice Schau Slettemeås and Anne Margrete Urdahl, Norwegian Veterinary Institute, Oslo, Norway.

INDICATOR BACTERIA FROM FEED

Gro Johannessen, Madelaine Norström, Jannice Schau Slettemeås and Anne Margrete Urdahl

In NORM-VET, antimicrobial testing of bacteria from feed is included some years. In 2019, samples of raw dog feed were included for isolation of *E. coli*, *E. coli* resistant to extended-spectrum cephalosporins (ESC), quinolone resistant *E. coli* (QREC), colistin resistant (COL-R) *E. coli*, carbapenemase-producing *Enterobacteriaceae* (CPE), *Enterococcus* spp., and vancomycin resistant *Enterococcus* spp. (VRE).

One isolate per positive sample was susceptibility tested. Some of the cut-off values defining resistance applied in NORM-VET have changed over the years. To facilitate comparisons in this report, data on prevalence of resistance presented in earlier reports have been recalculated using the cut-off values applied in 2019. Sampling, laboratory methods and data processing are described in Appendix 3.

Escherichia coli from dog feed

A total of 73 samples of raw dog feed were included. *E. coli* were obtained from 65 (89.0%) of the samples. The results are presented in Table 31 and in the text.

TABLE 31. Antimicrobial resistance in isolates of *Escherichia coli* from samples of raw dog feed (n=65) in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*															
			0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	10.8	[4.4 - 20.9]								87.7	1.5				6.2	4.6		
Tigecycline	0	[0.0 - 5.5]					100											
Chloramphenicol	0	[0.0 - 5.5]										100						
Ampicillin	9.2	[3.5 - 19]							6.2	35.4	47.7	1.5				9.2		
Cefotaxime	0	[0.0 - 5.5]					100											
Ceftazidime	0	[0.0 - 5.5]						100										
Meropenem	0	[0.0 - 5.5]		100														
Sulfamethoxazole	10.8	[4.4 - 20.9]										81.5	3.1	4.6				10.8
Trimethoprim	9.2	[3.5 - 19]					36.9	49.2	4.6						9.2			
Azithromycin	0	[0.0 - 5.5]								10.8	32.3	56.9						
Gentamicin	0	[0.0 - 5.5]						84.6	13.8	1.5								
Ciprofloxacin	7.7	[2.5 - 17]	90.8	1.5			6.2					1.5						
Nalidixic acid	6.2	[1.7 - 15]									93.8				1.5	3.1	1.5	
Colistin	0	[0.0 - 5.5]							89.2	10.8								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENT

In total, 53 of the 65 (81.5% [95% CI: 70.0-90.1]) *E. coli* isolates from raw dog feed were susceptible to all antimicrobial agents tested for, two isolates were resistant to one antimicrobial class, six isolates to two antimicrobial classes, one isolate to three antimicrobial classes, one isolate to four antimicrobial classes and two isolates to five antimicrobial classes. Resistance to tetracycline and sulfamethoxazole were the most frequently identified resistance determinants, followed by resistance to ampicillin and trimethoprim, ciprofloxacin and nalidixic acid. None of the isolates displayed any resistance to colistin, nor to the ESC cefotaxime and ceftazidime. These results are in concordance with the results from 2016 where 76 samples of raw dog feed were investigated. Comparisons should, however, be done with caution as raw dog feed is a composite category with different raw materials.

Extended-spectrum cephalosporin resistant *Escherichia coli* from dog feed

A total of 73 raw dog feed samples were investigated by selective methods for the occurrence of *E. coli* resistant to extended-spectrum cephalosporins (ESC). In total, *E. coli* resistant to ESC were detected from three (4.1% [95% CI: 0.9-11.5]) samples. Two of the isolates were resistant only to beta-lactams, i.e. ampicillin and the ESC cefotaxime and ceftazidime. One isolate was MDR, with additional resistance to quinolones and sulfamethoxazole. The resistance genes responsible are shown in Figure 45, page 62, together with an overview of what other antimicrobial agents the isolate showed decreased susceptibility to. Two isolates had a cephalosporin resistance profile corresponding to an AmpC beta-lactamase phenotype. Genotyping showed that one of the isolates contained the $bla_{\text{CMY-2}}$ gene. In the other isolate, whole genome sequencing did not detect any genes causing beta-lactamase production, and the genetic basis for the beta-lactamase resistance needs further investigations. The last isolate had a cephalosporin resistance profile corresponding to an ESBL phenotype. Genotyping showed that this isolate contained the $bla_{\text{CTX-M-15}}$ gene. In 2016, *E. coli* resistant to ESC was detected in 15 out of 68 samples. All the isolates had a cephalosporin resistance profile corresponding to an AmpC beta-lactamase phenotype, and the $bla_{\text{CMY-2}}$ gene was the main underlying mechanism in 13 of the 15 isolates. Comparisons should, however, be done with caution as raw dog feed is a composite category with different raw materials. In addition, the number of samples per year has been limited.

Quinolone resistant *Escherichia coli* from dog feed

A total of 73 raw dog feed samples were investigated by selective methods for the occurrence of *E. coli* resistant to quinolones (QREC). QREC were found in 17 (23.3% [95% CI: 14.2-34.6]) of the samples. The results are presented in Table 32, Figure 49, and in the text.

TABLE 32. Antimicrobial resistance in quinolone resistant *Escherichia coli* from samples of raw dog feed (n=17) in 2019.

Substance	n resistant	Distribution (%) of MIC values (mg/L)*														
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	6								11					6			
Tigecycline	0					17											
Chloramphenicol	2										15				2		
Ampicillin	6								6	5					6		
Cefotaxime	0					17											
Ceftazidime	0						17										
Meropenem	0		17														
Sulfamethoxazole	6										10	1					6
Trimethoprim	8					2	3	4					8				
Azithromycin	ND								3	8	5	1					
Gentamicin	1						13	3					1				
Ciprofloxacin	17				2	10	3				2						
Nalidixic acid	15									2		1	3	6	5		
Colistin	0							14	3								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

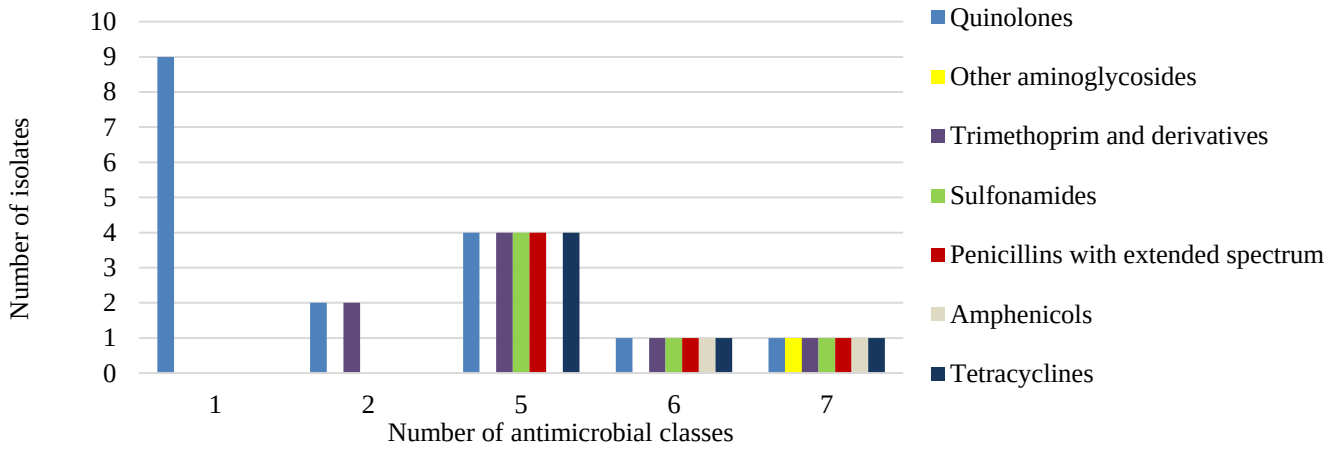


FIGURE 49. The resistance pattern for quinolone resistant *E. coli* (n = 17) from raw dog feed sampled in 2019. Number of isolates resistant from one to seven antimicrobial classes, respectively, are shown.

RESULTS AND COMMENTS

Nine of the QREC isolates from raw dog feed showed decreased susceptibility only to quinolones (ciprofloxacin and/or nalidixic acid) and two QREC isolates were additionally resistant to trimethoprim, whereas the remaining isolastes were MDR as shown in Figure 49. Additional resistance to trimethoprim, ampicillin, sulfamethoxazole and tetracycline were most commonly detected. None of the isolates were resistant to the carbapenem meropenem. This is in concordance with previous results from 2016. Selective methods for detection of QREC from raw dog feed have been performed only once before, in 2016, with QREC being detected from a total of 51.8% of 76 samples. Comparisons should, however, be done with caution as raw dog feed is a composite category with different raw materials.

Colistin resistant *Escherichia coli* from dog feed

A total of 73 raw dog feed samples were investigated by selective methods for the occurrence of colistin resistant (COL-R) *E. coli*. No COL-R *E. coli* were detected [95% CI: 0.0-4.9]. Selective methods for detection of COL-R *E. coli* have not been performed on raw dog feed samples previously, and comparison to results from previous years are therefore not possible. However, a COL-R *E. coli* isolate carrying the plasmid-mediated *mcr-1* gene was detected through the selective method for detection of quinolone resistance in 2016.

Carbapenemase-producing *Enterobacteriaceae* from dog feed

A total of 73 raw dog feed samples were investigated by selective methods for the occurrence of carbapenemase-producing *Enterobacteriaceae* (CPE). No CPE were detected [95% CI: 0.0-4.9]. This is in concordance with the results from 2016.

Enterococcus spp. from dog feed

A total of 72 raw dog feed samples were examined. *E. faecalis* was obtained from 56 (77.8%) and *E. faecium* from 61 (83.6%) of the samples. The results are presented in Tables 33-34, and in the text.

TABLE 33. Antimicrobial resistance in *Enterococcus faecalis* from raw dog feed samples (n=56) in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*														
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	30.4	[18.8 - 44.1]						66.1		3.6			7.1	14.3	8.9		
Tigecycline	0	[0.0 - 6.4]	5.4	16.1	57.1	19.6	1.8										
Chloramphenicol	0	[0.0 - 6.4]								28.6	69.6		1.8				
Ampicillin	0	[0.0 - 6.4]					3.6	85.7	8.9	1.8							
Erythromycin	5.4	[1.1 - 14.9]						35.7	55.4	3.6				1.8		3.6	
Quinupristin - dalfopristin	ND	ND					1.8			3.6	78.6	16.1					
Gentamicin	0	[0.0 - 6.4]									19.6	51.8	28.6				
Ciprofloxacin	1.8	[0.0 - 9.6]					8.9	62.5	26.8				1.8				
Vancomycin	0	[0.0 - 6.4]						87.5	12.5								
Teicoplanin	0	[0.0 - 6.4]					98.2	1.8									
Linezolid	1.8	[0.0 - 9.6]						7.1	89.3	1.8	1.8						
Daptomycin	0	[0.0 - 6.4]					5.4	21.4	62.5	10.7							

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

TABLE 34. Antimicrobial resistance in *Enterococcus faecium* from raw dog feed samples (n=61) in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*														
			0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥512
Tetracycline	4.9	[1.0 - 13.5]						91.8		3.3			1.6		1.6	1.6	
Tigecycline	0	[0.0 - 8.7]	31.1	24.6	26.2	9.8											
Chloramphenicol	0	[0.0 - 5.8]								54.1	41.0	4.9					
Ampicillin	0	[0.0 - 5.8]					27.9	29.5	34.4	1.6							
Erythromycin	4.9	[1.0 - 13.5]						23.0	29.5	42.6	4.9						
Quinupristin - dalfopristin	ND	ND					31.1	19.7	14.8	34.4							
Gentamicin	0	[0.0 - 5.8]									85.2	6.6	8.2				
Ciprofloxacin	4.9	[1.0 - 13.5]			3.3	1.6	16.4	23.0	21.3	26.2	3.3	1.6	3.3				
Vancomycin	0	[0.0 - 5.8]						85.2	14.8								
Teicoplanin	0	[0.0 - 5.8]					98.4	1.6									
Linezolid	0	[0.0 - 5.8]					1.6		73.8	24.6							
Daptomycin	0	[0.0 - 5.8]				1.6		8.2	29.5	55.7	4.9						

*Bold vertical lines denote microbiological cut-off values for resistance. ND = not defined. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

The 2019 data showed that 69.6% of the *E. faecalis* and 83.6% of the *E. faecium* isolates from raw dog feed samples were susceptible to all antimicrobial classes included in the test panel. Altogether, 25.0% of the *E. faecalis* isolates were resistant to one antimicrobial class, 3.6% to two antimicrobial classes, and 1.8% to four antimicrobial classes. Among the *E. faecium* isolates, 16.4% were resistant to one antimicrobial class. For *E. faecalis*, resistance to tetracycline was most commonly detected, followed by resistance to erythromycin. For *E. faecium*, resistance to tetracycline, erythromycin and ciprofloxacin were most commonly detected. In total, 30.4% of the *E. faecalis* isolates and 16.4% of the *E. faecium* isolates were resistant to at least one antimicrobial class, indicating a high occurrence of resistance, respectively, according to the EFSA classification described in Appendix 6.

Detection of *E. faecalis* and *E. faecium* has not been performed on raw dog feed samples previously and comparisons to results from previous years are therefore not possible.

Vancomycin resistant *Enterococcus* spp. from dog feed

A total of 73 raw dog feed samples were investigated for the presence of vancomycin resistant *Enterococcus* spp. (VRE). No VRE were detected [95% CI: 0.0-4.9].

ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

Umaer Naseer, Madelaine Norström, Jannice Schau Slettemeås and Anne Margrete Urdahl

Zoonotic and non-zoonotic enteropathogenic bacteria represent a considerable public health problem. Furthermore, the occurrence of acquired antimicrobial resistance in such bacteria represents a major public health concern. Therefore, it is of great importance to monitor the occurrence of antimicrobial resistance in zoonotic and other enteropathogenic bacteria at relevant stages in the farm-to-fork continuum.

Salmonella isolates from control programmes on animals and food products, as well as diagnostic samples from animals are monitored for antimicrobial resistance annually. In 2019, susceptibility testing of *Salmonella* spp. isolated from raw dog feed, *Campylobacter coli* from fattening pigs and pathogenic *Yersinia enterocolitica* from minced pork meat were included as well. One bacterial isolate per positive sample was susceptibility tested. From human clinical samples; *Salmonella*, *Shigella* and *Yersinia enterocolitica* isolates, as well as a representative number of *Campylobacter* isolates were monitored. Sampling, laboratory methods and data processing are described in Appendix 4.

SALMONELLA SPP.

Salmonella from animals

The occurrence of *Salmonella* spp. in food-producing animals in Norway is very favourable as such animal populations are considered virtually free from *Salmonella* spp. To document and maintain this situation, Norway runs an extensive surveillance programme that covers both live animals (cattle, pigs and poultry) and meat. The *Salmonella* isolates examined in NORM-VET include those that are detected in this programme, as well as those detected by clinical submissions to the Norwegian Veterinary Institute. The data are presented in Table 35 and in the text.

TABLE 35. Antimicrobial resistance in *Salmonella* spp. (n=7) from animals (one turkey, one poultry, one dog, three cats and one pig); *S.* Typhimurium (n=4), other *Salmonella* spp. (n=3) in 2019.

Substance	n (resistance)	Distribution (n) of MIC values (mg/L)*																
		0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512	≥1024
Tetracycline	0								7									
Tigecycline	0					7												
Chloramphenicol	0										7							
Ampicillin	0							5	2									
Cefotaxime	0					7												
Ceftazidime	0						7											
Meropenem	0		7															
Sulfamethoxazole	0										2	5						
Trimethoprim	0					7												
Azithromycin	ND									6	1							
Gentamicin	0						6	1										
Ciprofloxacin	0	4	3															
Nalidixic acid	0									7								
Colistin	3							1	3	1	2							

*Bold vertical lines denote microbiological cut-off values. ND = not defined. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

RESULTS AND COMMENTS

In 2019, seven *Salmonella* spp. isolates from animals were susceptibility tested. The seven isolates included one each from turkey, poultry, dog, and pig, and three from cats. Four isolates were of *S.* Typhimurium, while three isolates were of *S.* Entertitidis (n=1), *S.* Give (n=1), and *S.* Agona (n=1), respectively. With the exception of colistin, the isolates were fully susceptible to all tested antimicrobial agents included in the panel. For colistin, however, the ECOFFs suggested by EFSA have been shown to be placed within the distribution of some *Salmonella* spp. (Agersø *et al.* 2012), indicating that the suggested ECOFF may be less suitable for some *Salmonella* spp. The three isolates showing MIC-values to colistin above the ECOFFs were investigated by WGS, and no genes or mutations causing colistin resistance were detected.

Salmonella from dog feed

In 2019, two isolates of *Salmonella* spp., one *S.* Derby and one *S.* Typhimurium, from raw dog feed were susceptibility tested. The isolates were obtained through a survey investigating a total of 73 samples of raw dog feed. The isolates were fully susceptible to all substances included in the panel, except for *S.* Derby which showed resistance to sulfamethoxazole with an MIC ≥ 1024.

Salmonella from human clinical specimens

In 2019, 1,094 human cases of nontyphoidal salmonellosis and 13 cases of typhoid fever were notified to the Norwegian Surveillance System for Communicable Disease (MSIS). The majority of these cases were infected abroad (59%). In 2019, the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) received 1,015 *Salmonella* isolates from primary diagnostic laboratories in Norway. Antimicrobial susceptibility testing was performed on all *Salmonella* Typhimurium, *Salmonella* Typhi and *Salamonella* Paratyphi A isolates, and based on information at the point of reception, all non-travel associated *Salmonella* Enteritidis isolates. In addition, antimicrobial susceptibility was performed on all *Salmonella* isolates recovered from blood cultures. Information on place of acquisition was completed and updated for all isolates by data from MSIS. A total of 401 unique *Salmonella* spp. isolates were tested (Table 36). All isolates were susceptibility tested against four different antibiotic groups: beta-lactams (ampicillin, cefotaxime, ceftazidime and meropenem), fluoroquinolones (ciprofloxacin/pefloxacin), tetracycline and chloramphenicol.

TABLE 36. Number of antimicrobial susceptibility tested *Salmonella* isolates recoved from human clinical specimens in Norway 2019, by serovar and place of acquisition.

Salmonella serovars	No. of isolates tested in 2019	Place of acquistion		
		Norway	Abroad	Unknown
S. Typhimurium	101	49	43	9
S. Typhimurium monophasic (4,[5],12:i-)	59	22	32	5
S. Enteritidis	191	91	70	30
S. Typhi	13	0	9	4
S. Paratyphi A	17	0	9	8
Other *Salmonella*	20	1	10	9
Total	401	163	173	65

A total of 62 isolates were recovered form blood cultures representing 6% of all *Salmonella* infections, including 10 of the 13 *S.* Typhi (77%), 13 of the 17 *S.* Paratyphi (76%), 15 of the 190 *S.* Enteritidis (8%), 4 of the 101 *S.* Typhimurium (4%), and the rest from other *Salmonella* species (n=20). Among the other *Salmonella* species, 5 of the 15 *S.* Chester (33%) and 6 of the 28 *S.* Paratyphi B (21%) isolates submitted to NRL were recovered from blood cultures.

The results from the antimicrobial susceptibility testing for *Salmonella* isolates are presented in Tables 37-42, Figures 50-55, and in the connected text.

TABLE 37. Percentage distributions of antimicrobial susceptibility categories of domestically acquired *Salmonella* Typhimurium (n=49) from human clinical specimens in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	85.7	-	14.3
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Pefloxacin[1]	≥ 24 mm	< 24 mm	87.8	-	12.2
Tetracycline[2]	≥ 17 mm	< 17 mm	81.6	-	18.4
Chloramphenicol	≤ 8	> 8	91.8	-	8.2

[1]Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).[2]Breakpoints according to national zone distributions.

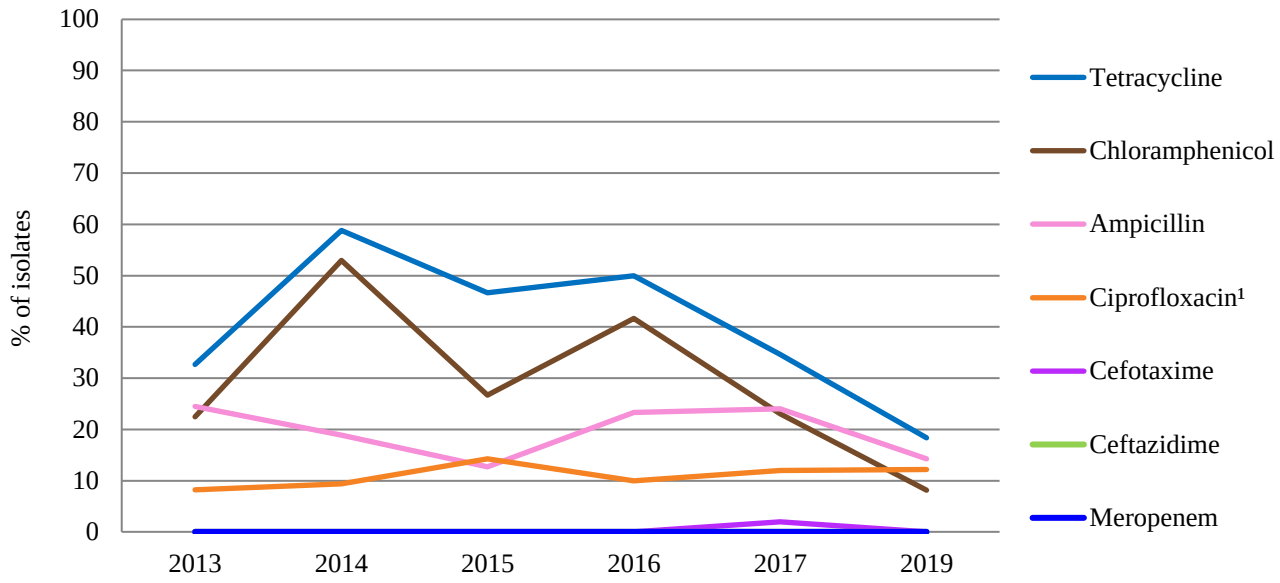


FIGURE 50. Percentage of domestically acquired *Salmonella* Typhimurium resistant to selected antimicrobial agents in Norway, trend 2013-2019. [1]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion 2016 onwards. Please, note that the x-axis is not to scale.

TABLE 38. Percentage distributions of antimicrobial susceptibility categories of travel-associated *Salmonella* Typhimurium (n=43) from human clinical specimens in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	72.1	-	27.9
Cefotaxime	≤ 1	> 2	95.3	0.0	4.7
Ceftazidime	≤ 1	> 4	95.3	0.0	4.7
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Pefloxacin[1]	≥ 24 mm	< 24 mm	74.4	-	25.6
Tetracycline[2]	≥ 17 mm	< 17 mm	65.1	-	34.9
Chloramphenicol	≤ 8	> 8	83.7	-	16.3

[1]Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).[2]Breakpoints according to national zone distributions.

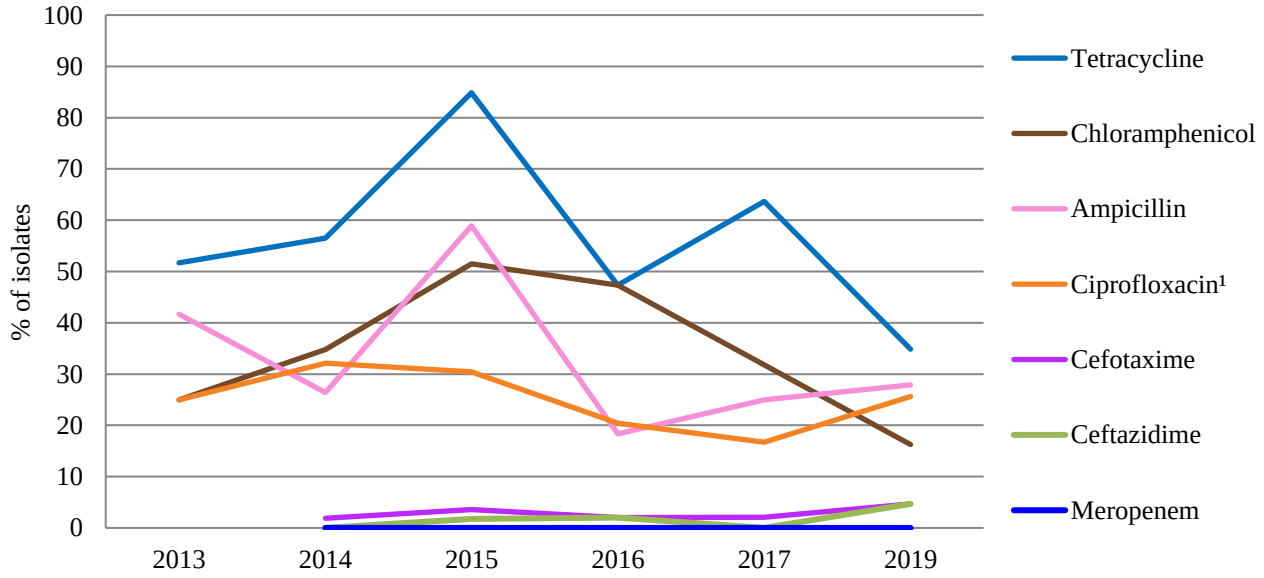


FIGURE 51. Percentage of travel associated *Salmonella* Typhimurium resistant to selected antimicrobial agents in Norway, trend 2013-2019. [1]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion 2016 onwards. Please, note that the x-axis is not to scale.

ANTIMICROBIAL RESISTANCE IN MONOPHASIC SALMONELLA TYPHIMURIUM

TABLE 39. Percentage distributions of antimicrobial susceptibility categories of domestically acquired monophasic *Salmonella* Typhimurium (n=22) from human clinical specimens in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	13.6	-	86.4
Cefotaxime	≤ 1	> 2	95.5	0.0	4.5
Ceftazidime	≤ 1	> 4	95.5	0.0	4.5
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Pefloxacin[1]	≥ 24 mm	< 24 mm	100.0	-	0.0
Tetracycline[2]	≥ 17 mm	< 17 mm	4.5	-	95.5
Chloramphenicol	≤ 8	> 8	95.5	-	4.5

[1]Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).[2]Breakpoints according to national zone distributions.

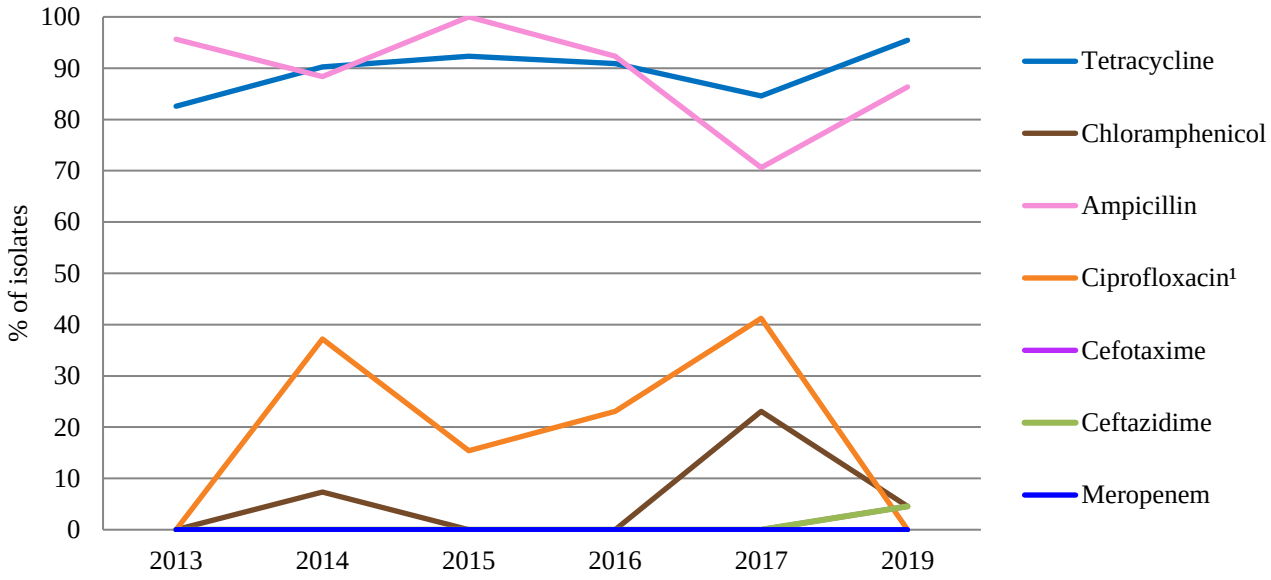


FIGURE 52. Percentage of domestically acquired monophasic *Salmonella* Typhimurium resistant to selected antimicrobial agents in Norway, trend 2013-2019. [1]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion 2016 onwards. Please, note that the x-axis is not to scale.

TABLE 40. Percentage distributions of antimicrobial susceptibility categories of *Salmonella* Enteritidis (n=191) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	96.9	-	3.1
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	99.5	0.5	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Pefloxacin[1]	≥ 24 mm	< 24 mm	78.0	-	22.0
Tetracycline[2]	≥ 17 mm	< 17 mm	95.3	-	4.7
Chloramphenicol	≤ 8	> 8	99.0	-	1.0

[1]Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).[2]Breakpoints according to national zone distributions.

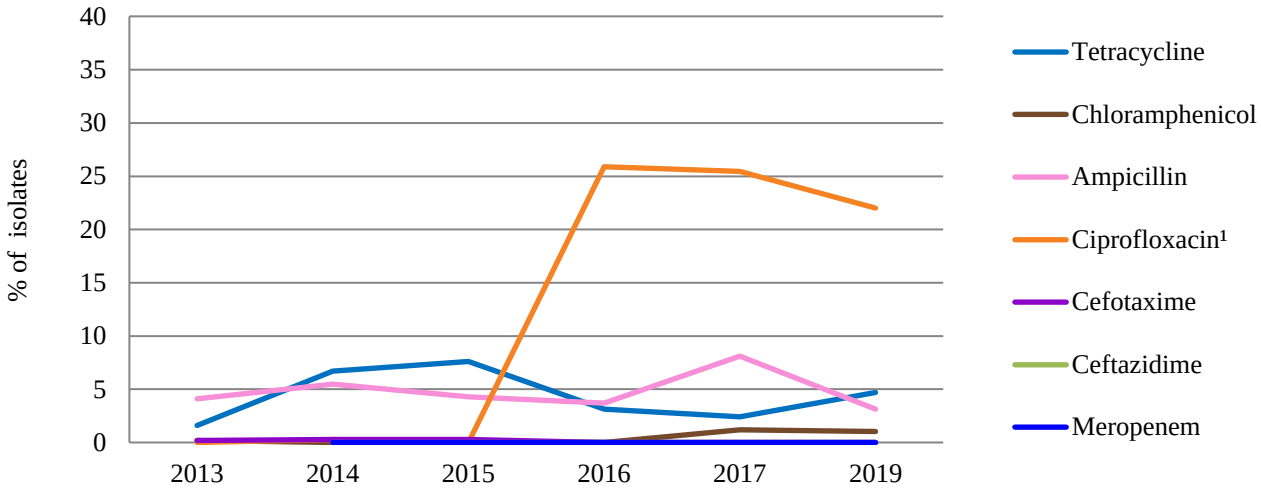


FIGURE 53. Percentage of *Salmonella* Enteritidis resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2013-2019. [1]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion 2016 onwards.

ANTIMICROBIAL RESISTANCE IN SALMONELLA TYPHI

TABLE 41. Percentage distributions of antimicrobial susceptibility categories of *Salmonella* Typhi (n=13) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	46.2	-	53.8
Cefotaxime	≤ 1	> 2	84.6	0.0	15.4
Ceftazidime	≤ 1	> 4	84.6	0.0	15.4
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Pefloxacin[1]	≥ 24 mm	< 24 mm	0.0	-	100.0
Tetracycline[2]	≥ 17 mm	< 17 mm	100.0	-	0.0
Chloramphenicol	≤ 8	> 8	46.2	-	53.8

[1]Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).[2]Breakpoints according to national zone distributions.

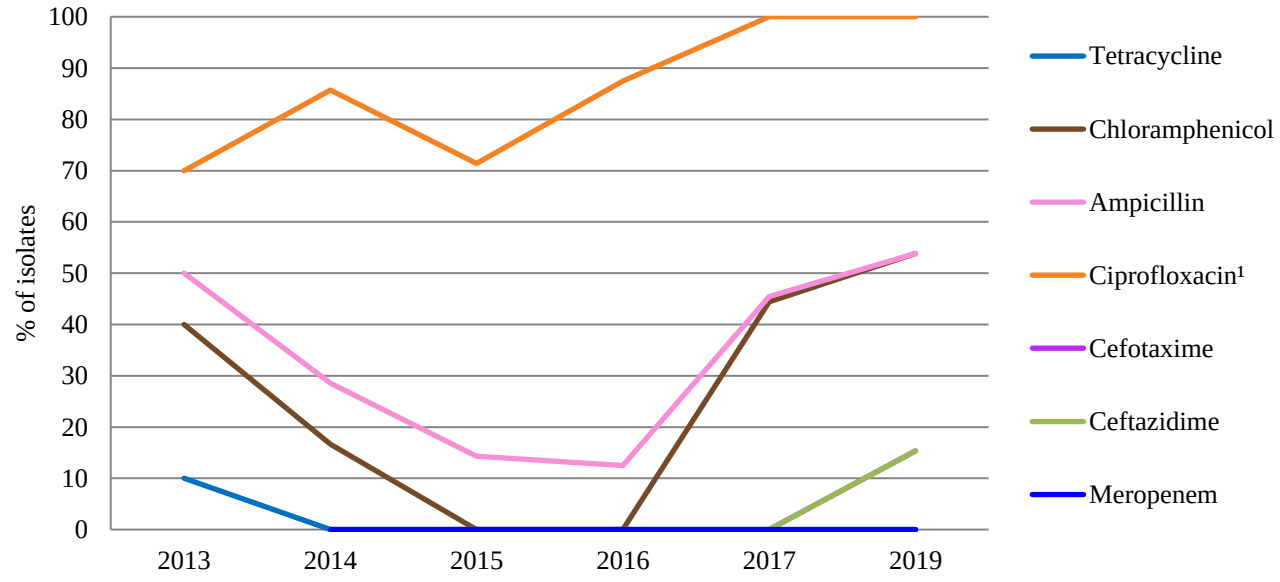


FIGURE 54. Percentage of *Salmonella* Typhi resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2013-2019. [1]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion 2016 onwards. Please, note that the x-axis is not to scale.

MULTI-DRUG RESISTANT SALMONELLA

TABLE 42. Number of multi-drug resistant *Salmonella* isolates identified in Norway 2019, stratified according to serovar and resistance to antibiotic categories.

		Antibiotic[1]	Total	*Salmonella* Typhimurium	*Salmonella* Typhimurium (monophasic)	*Salmonella* Enteritidis	*Salmonella* Typhi	*Salmonella* Infantis
Multi-drug resistant		NS ≥ 3 categories	33	14	8	3	7	1
Antibiotic categories	Pencillins	AMP	33	14	8	3	7	1
	Extended-spectrum cephalosporins	CTX/CTZ	6	2	1	0	2	1
	Carapenems	MEM	0	0	0	0	0	0
	Fluoroquinolones	CIP	28	10	7	3	7	1
	Tetracyclines	TET	26	14	8	3	0	1
	Phenicols	CAM	23	11	4	1	7	0

[1]NS: non-suscetibility, AMP: Ampicillin, CTX: Cefotaxime, CTZ: Ceftazidime, MEM: Meropenem, CIP: Ciprofloxacin (inferred from pefloxacin), TET: Tetracycline, CAM: Chloramphenicol.

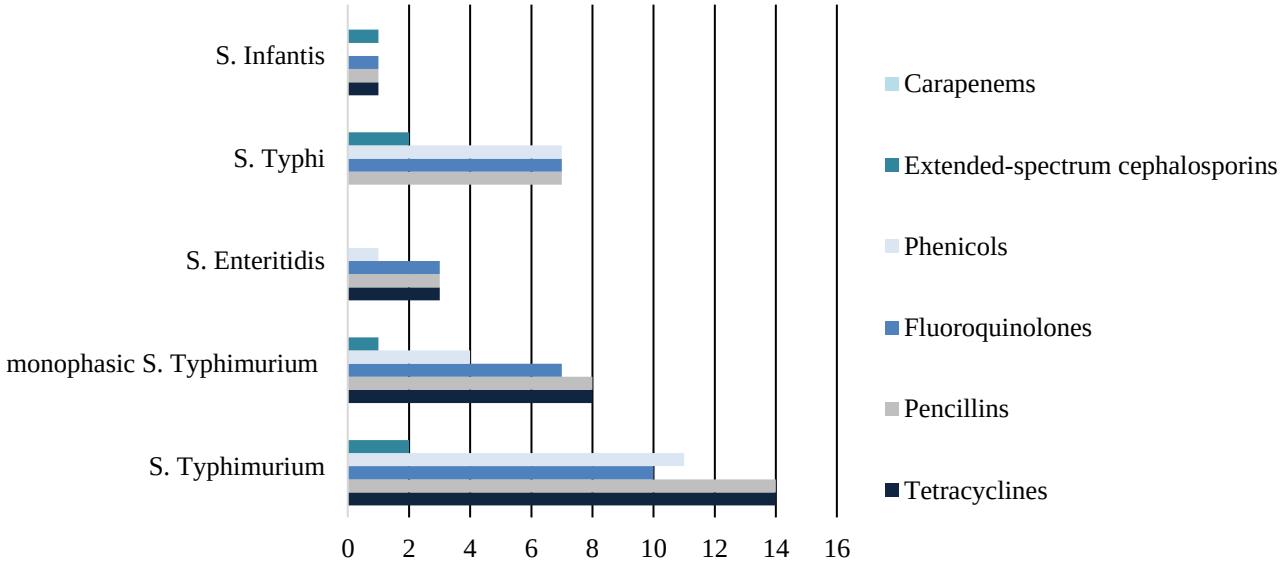


FIGURE 55. Multi-drug resistant *Salmonella* isolates identified in Norway 2019, stratified according to serovar and resistance to antibiotic categories.

RESULTS AND COMMENTS

Following the reorganisation of the NRL for Enteropathogenic Bacteria at the NIPH and paused antimicrobial susceptibility testing for 2018, the NRL resumed antimicrobial susceptibility testing for a selection of the received *Salmonella* isolates in 2019. Selection criteria were set to ensure inclusion of important *Salmonella* serovars and antibiotics for the monitoring of emergence and dissemination of antimicrobial resistance in Norway.

For *S.* Typhimurium isolates, overall resistance levels were higher for travel-associated strains when compared to domestically acquired strains. A continued descending trend in resistance to tetracycline and chloramphenicol, irrespective of place of acquisition, was observed. Several European countries have reported similar trends, which also include a declining trend in ampicillin resistance.

For the monophasic variant of *S.* Typhimurium, overall resistance level is higher than for *S.* Typhimurium. We observed stable resistance levels over the last five years for the tested antibiotics, although high for both ampicillin and tetracycline, in both domestically acquired and travel-associated strains.

Antibiotic resistance in *S.* Enteritidis is generally low, and has been reported low over a long period. An apparent sudden emergence of ciprofloxacin resistance in 2016 was linked to the change in antibiotic used for screening fluoroquinolone resistance (pefloxacin). Antibiotic

susceptibility was not performed for all travel-associated *S.* Enteritidis.

The overall level of antibiotic resistance in *S.* Typhi is high, with an observed rising trend for resistance against ciprofloxacin, tetracycline, chloramphenicol, and extended-spectrum cephalosporins. Multi-drug resistance (MDR) was also a characteristic feature of a considerable proportion of the *S.* Typhi isolates (54%). The MDR phenotype in *S.* Typhi was largely attributed to resistance towards ampicillins, fluoroquinolones and phenicols. Comparatively, an MDR phenotype accounted for 14% of the *S.* Typhimurium and monophasic *S.* Typhimurium isolates, and was largely attributed to resistance towards tetracycline, ampicillins and fluoroquinolones.

Antibiotic susceptibility testing was not performed for other *Salmonella* species unless recovered from blood cultures. Six isolates were resistant to extended-spectrum cephalosporins, *S.* Typhi (n=2), *S.* Typhimurium (n=2), monophasic *S.* Typhimurium (n=1), and *S.* Infantis (n=1). Five where classified as $ESBL_A$, encoding different $bla_{CTX\text{-}M}$ genes, while one was classified as $ESBL_{M,}$ encoding the $bla_{CMY\text{-}2}$ gene. Four of these isolates also carried the acquired ciprofloxacin resistance gene *qnrS*, in addition to the phenicol resistance gene *catA1*.

CAMPYLOBACTER SPP.

Campylobacter coli from fattening pigs

Caecal samples from a total of 288 fattening pigs were examined. *C. coli* isolates were obtained from 249 of these (86.5%). The results are presented in Table 43, Figure 56, and in the text.

TABLE 43. Antimicrobial resistance in *Campylobacter coli* isolates (n=249) from caecal samples of fattening pigs in 2019.

Substance	Resistance (%) [95% CI]		Distribution (%) of MIC values (mg/L)*												
			0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Tetracycline	0.4	[0.0 - 2.2]				97.6	2.0						0.4		
Erythromycin	0	[0.0 - 1.5]					99.6		0.4						
Streptomycin	41	[34.8 - 47.4]						2.4	56.6	10.4		30.5			
Gentamicin	0	[0.0 - 1.5]			1.2	14.5	83.5	0.8							
Ciprofloxacin	15.7	[11.4 - 20.8]		83.9	0.4		0.4	0.8	6.0	8.0	0.4				
Nalidixic acid	15.7	[11.4 - 20.8]					0.4		73.9	10.0		0.8	4.8	10.0	

*Bold vertical lines denote microbiological cut-off values. CI = confidence interval. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested are given as the lowest MIC value above the range. MIC values equal to or lower than the lowest concentration tested are given as the lowest concentration tested.

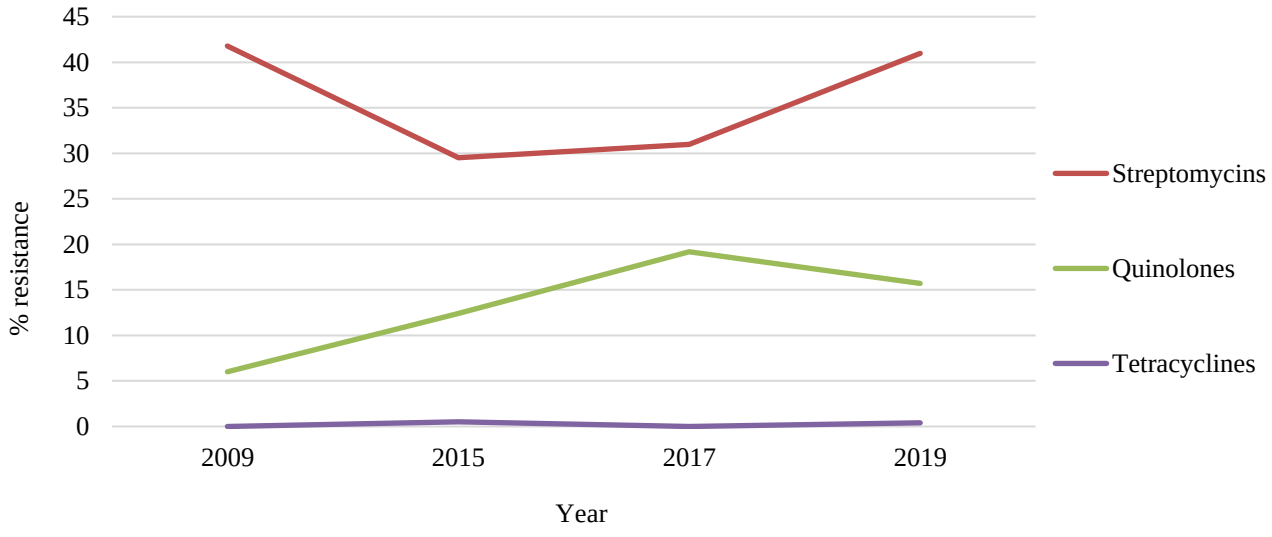


FIGURE 56. Prevalence of resistance to various antimicrobial classes in *Campylobacter coli* from pig faecal or caecal samples isolated between 2009-2019. The breakpoints used in NORM-VET 2019 were applied.

RESULTS AND COMMENTS

A total of 53.4% of the *C. coli* isolates from fattening pigs were susceptible to all antimicrobial agents tested. Altogether, 36.1% were resistant to one antimicrobial class and 10.4% to two of the antimicrobial classes tested. Resistance to streptomycin was the most frequently identified resistance determinant (41.0%), followed by resistance to ciprofloxacin and nalidixic acid (15.7%). Reduced susceptibility to erythromycin and gentamicin was not detected. None of the isolates were multi-drug resistant (i.e. resistant to ≥ three antimicrobial classes). Overall, 46.6% of the isolates were resistant to at least one antimicrobial agent, indicating a high occurrence of resistance among *C. coli* from pigs according to the EFSA classification described in Appendix 6. The occurrence of streptomycin resistance at 41% accounts for a large proportion of this. Streptomycin is rarely used in

Norwegian pig production, and the observed streptomycin resistance in *C. coli* is therefore difficult to explain.
C. coli has previously been investigated in 2009, 2015 and 2017. There has been an increase in resistance to quinolones these years; from 4.5% [95% CI: 1.2–13.4] for ciprofloxacin and 6% [95% CI: 1.9 15.4] for nalidixic acid in 2009, to 18.9% [95% CI: 14.2 – 24.2] for ciprofloxacin and 19.2% [95% CI: 14.6 – 24.6] for nalidixic acid in 2017, and 15.7% [95% CI: 11.4 - 20.8] for both ciprofloxacin and nalidixic acid in 2019. This increasing trend is also reported by others, both for human and animal *C. coli* isolates.
In the EFSA and ECDC Summary report from 2017, resistance to ciprofloxacin among *C. coli* is reported to be > 60% in human isolates and ~ 52% in isolates from pigs. The occurrence in fattening pigs varies, however, markedly between the reporting countries (EFSA and ECDC Summary report 2018). The results from Norway are still among the lowest reported. This situation is most likely due to the rather limited use of antibiotics in the Norwegian pig production. The causes for increasing quinolone resistance, despite the limited use of antibiotics, are unknown.
None of the isolates showed reduced susceptibility to erythromycin. The occurrence of *Campylobacter* spp. isolates displaying combined resistance to ciprofloxacin and erythromycin is of great importance to public health, since both compounds are recognised as critically important antimicrobials for the treatment of *Campylobacter* infections in humans (WHO, 2019).

Campylobacter spp. from human clinical cases

In 2019, 4,154 human campylobacteriosis cases were notified to MSIS. The largets proportion of cases were infected abroad (45%). Surveillance data suggested that the vast majority of cases were sporadic, although a large waterborne outbreak was reported from Western Norway. The first five *Campylobacter* isolates each month from five sentinel regional laboratories were submitted to the NRL for Enteropathogenic Bacteria at the NIPH. In addition, isolates recovered from blood cultures, and isolates that were part of an outbreak investigation were submitted to the NRL for surveillance purposes (Table 44).
Antimicrobial susceptibility testing was performed on a total of 497 *Campylobacter jejuni* and *Campylobacter coli* isolates against four different antibiotic groups: macrolides (erythromycin), aminoglycosides (gentamicin), fluoro-quinolones (ciprofloxacin), and tetracycline. The results from the antimicrobial susceptibility testing are presented in Tables 44-47, Figures 57-59, and in the text.

TABLE 44. Number of antimicrobial susceptibility tested *Campylobacter* spp. isolates recoved from human clinical specimens in Norway 2019, by species and place of acquisition.

Campylobacter spp.	No. of isolates tested in 2019	Place of acquistion		
		Norway	Abroad	Unknown
Campylobacter jejuni	470	196	249	25
Campylobacter coli	27	4	20	3
Total	497	200	269	28

ANTIMICROBIAL RESISTANCE IN CAMPYLOBACTER JEJUNI

TABLE 45. Percentage distributions of antimicrobial susceptibility categories of domestically acquired *Campylobacter jejuni* (n=196) from human clinical specimens in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	S	I	R
Tetracycline	≤ 2	> 2	80.1	-	19.9
Erythromycin	≤ 4	> 4	99.0	-	1.0
Gentamicin[1]	≤ 2	> 2	99.5	-	0.5
Ciprofloxacin	≤ 0.5	> 0.5	69.9	-	30.1

[1]Breakpoints according to national zone distributions.

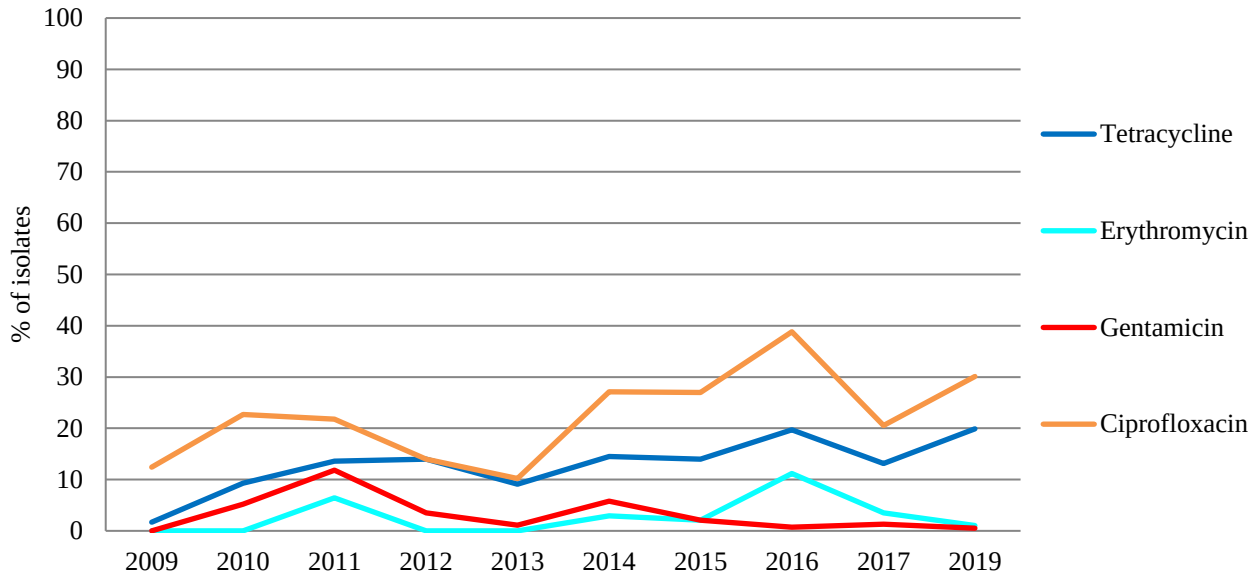


FIGURE 57. Percentage of domestically acquired *Campylobacter jejuni* resistant to selected antimicrobial agents in Norway, trend 2009-2019.

TABLE 46. Percentage distributions of antimicrobial susceptibility categories of travel-associated *Campylobacter jejuni* (n=249) from human clinical specimens in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	S	I	R
Tetracycline	≤ 2	> 2	41.4	-	58.6
Erythromycin	≤ 4	> 4	95.6	-	4.4
Gentamicin[1]	≤ 2	> 2	98.8	-	1.2
Ciprofloxacin	≤ 0.5	> 0.5	15.7	-	84.3

[1]Breakpoints according to national zone distributions.

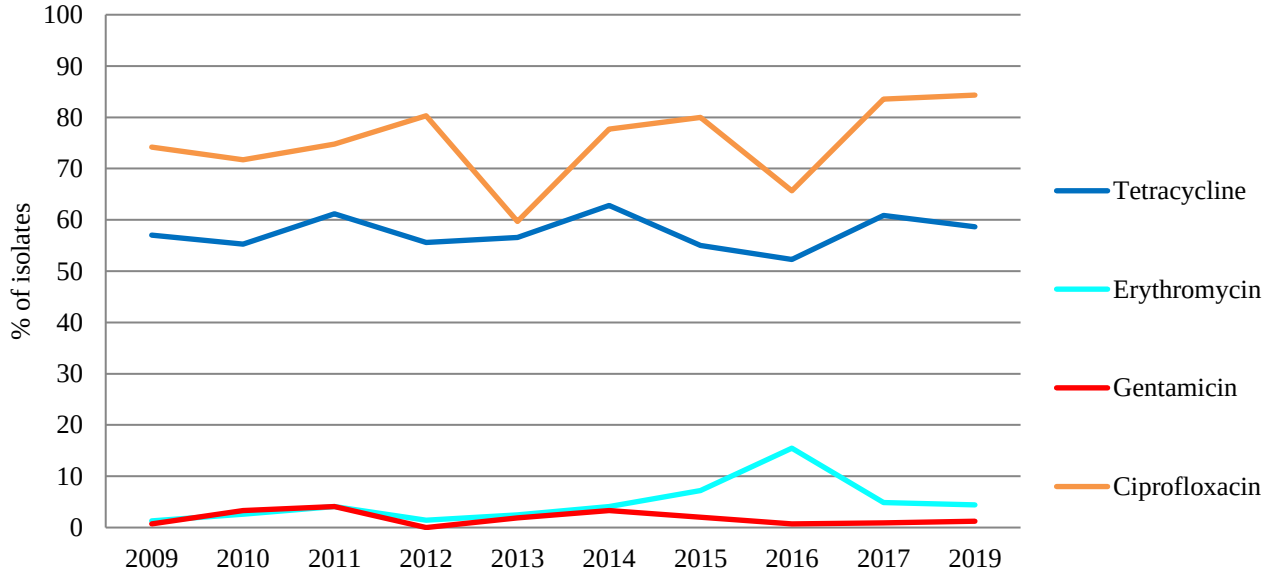


FIGURE 58. Percentage of travel associated *Campylobacter jejuni* resistant to selected antimicrobial agents in Norway, trend 2009-2019.

ANTIMICROBIAL RESISTANCE IN CAMPYLOBACTER COLI

TABLE 47. Percentage distributions of antimicrobial susceptibility categories of *Campylobacter coli* (n=27) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	S	I	R
Tetracycline	≤ 2	> 2	33.3	-	66.7
Erythromycin	≤ 8	> 8	81.5	-	18.5
Gentamicin[1]	≤ 2	> 2	92.6	-	7.4
Ciprofloxacin	≤ 0.5	> 0.5	11.1	-	88.9

[1]Breakpoints according to national zone distributions.

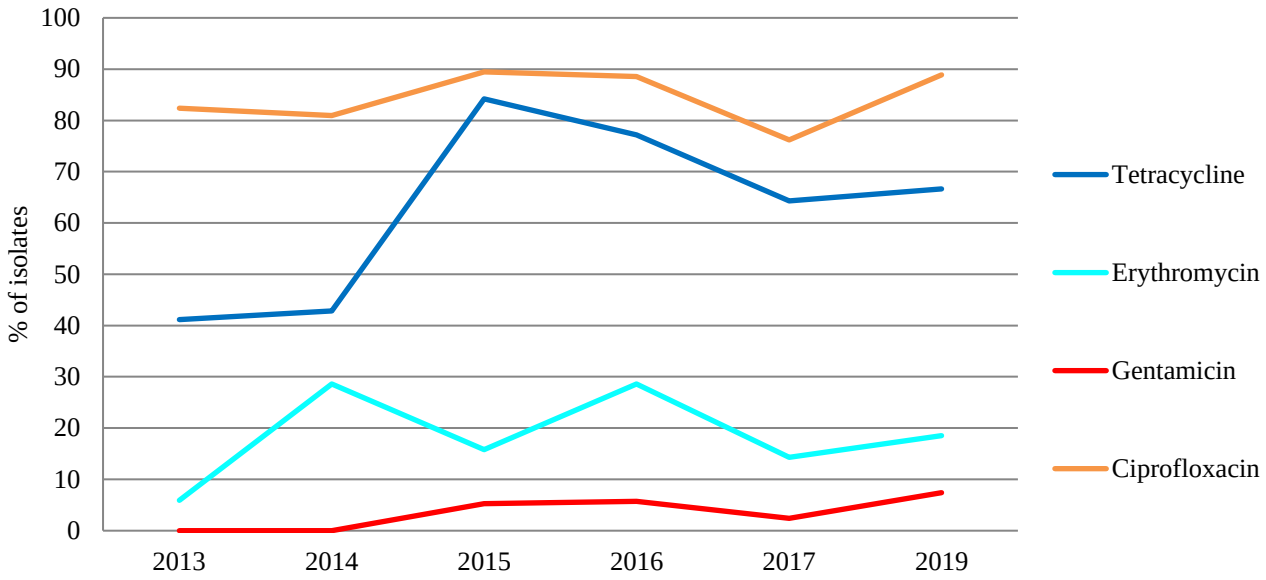


FIGURE 59. Percentage of *Campylobacter coli* resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2013-2019.

RESULTS AND COMMENTS

Following the reorganisation of the NRL for Entero-pathogenic Bacteria at the NIPH and paused antimicrobial susceptibility testing for 2018, the NRL resumed antimicrobial susceptibility testing for all *C. jeuni* and *C. coli* isolates received in 2019.

For the *C. jejuni* isolates, overall resistance levels against ciprofloxacin and tetracycline were higher for travel-associated strains when compared to domestically acquired strains. A continued rising trend in resistance to ciprofloxacin and tetracycline for domestically acquired strains was observed. An increase in resistance to fluoroquinolones has also been reported from several European countries and is a major cause of concern.

Resistance in *C. coli* follows similar patterns as for *C. jejuni*, although *C. coli* are observed to be more resistant to erythromycin.

An MDR phenotype was observed in 14 isolates, 10 *C. jejuni* and 4 *C. coli*. All, but one isolate were associated to travel. MDR was recorded against fluoroquinolones, tetracycline and macrolides for 12 isolates, and additionally to gentamicin for the remaining two isolates.

Pathogenic *Yersinia enterocolitica* from minced pork meat

Nine pathogenic *Y. enterocolitica* isolates were obtained from a survey of minced pork meat investigating a total of 152 samples. All nine isolates were fully susceptible to all antimicrobial agents included in the test panel.

Yersinia enterocolitica from human clinical specimens

In 2019, 85 human yersiniosis cases were notified to MSIS. The majority of cases were domestically acquired (55%). A total of 70 unique isolates of pathogenic *Yersinia enterocolitica* were antimicrobial susceptibility tested in 2019. Fifty-six belonged to serotype 3, ten to serotype 9 and three to other serotypes (Table 48). All isolates were susceptibility tested against four different antibiotic groups: beta-lactams (ampicillin, cefotaxime, ceftazidime and meropenem), fluoroquinolone (ciprofloxacin), tetracycline and chloramphenicol. The results are presented in Table 49 and Figure 60.

TABLE 48. Number of antimicrobial susceptibility tested *Yersinia enterocolitica* isolates recoved from human clinical specimens in Norway 2019, by serotype and place of acquisition.

Yersinia enterocolitica	No. of isolates tested in 2019	Place of acquistion		
		Norway	Abroad	Unknown
Y. enterocolitica O:3	56	30	17	9
Y. enterocolitica O:9	11	7	2	2
Y. entericolitica (other serotypes)	3	1	0	2
Total	70	38	19	13

ANTIMICROBIAL RESISTANCE IN YERSINIA ENTEROCOLITICA SEROTYPE O:3 AND O:9

TABLE 49. Percentage distributions of antimicrobial susceptibility categories of *Yersinia enterocolitica* O:3 and O:9 (n=67) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	3.0	-	97.0
Cefotaxime	≤ 1	> 2	98.5	0.0	1.5
Ceftazidime	≤ 1	> 4	98.5	0.0	1.5
Meropenem	≤ 2	> 8	98.5	1.5	0.0
Ciprofloxacin	≤ 0.25	> 0.5	94.0	3.0	3.0
Tetracycline[1]	≥ 17 mm	< 17 mm	94.0	-	6.0
Chloramphenicol	≤ 8	> 8	77.6	-	22.4

[1]Breakpoints according to national zone distributions.

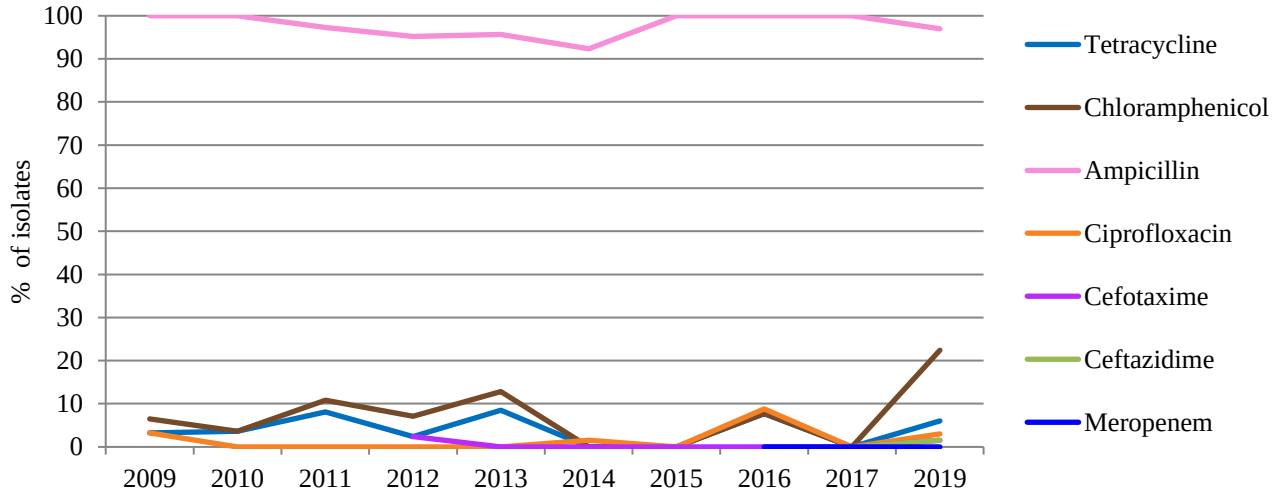


FIGURE 60. Percentage of *Yersinia enterocolitica* O:3 and O:9 resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2009-2019.

RESULTS AND COMMENTS

Following the reorganisation of the NRL for Enteropathogenic Bacteria at the NIPH and paused antimicrobial susceptibility testing for 2018, the NRL resumed antimicrobial susceptibility testing for human pathogenic *Yersinia enterocolitica* in 2019.

Antimicrobial resistance for *Yersinia enterocolitica* serotypes O:3 and O:9 have been combined and presented without differentiation of place of acquisition. All isolates of pathogenic *Y. enterocolitica* expressed intrinsic resistance to ampicillin, with little or no resistance to other antibiotic groups. A singe isolate of *Y. enterocolitica* O:3 was resistant to extended-spectrum cephalosporins and displayed reduced susceptibility to meropenem (susceptible to increased exposure). Molecular analysis did not reveal any ESBL or carbapenemase encoding gene. The phenotype was assumed to be a combinatory effect of enhanced expression of the intrinsic chromosomal penicillinase (*blaA*) and reduced permeability. No further analysis was performed to confirm this hypothesis.

Shigella spp. from human clinical specimens

In 2019, 133 human cases of shigellosis were notified to MSIS. The majority of cases were infected abroad (64%). The NRL for Enteropathogenic Bacteria at the NIPH received 111 *Shigella* spp. isolates from the primary diagnostic laboratories in Norway. Antimicrobial susceptibility testing was performed on all *Shigella sonnei*, *Shigella flexneri* and S*higella boydii* isolates (Table 50).

All isolates were susceptibility tested against four different antibiotic groups: beta-lactams (ampicillin, cefotaxime, ceftazidime and meropenem), fluoroquinolone (ciprofloxacin), tetracycline and chloramphenicol. The results are presented in Tables 51-52 and Figures 61-62.

TABLE 50. Number of antimicrobial susceptibility tested *Shigella* spp. isolates recoved from human clinical specimens in Norway 2019, by species and place of acquisition.

Shigella spp.	No. of isolates tested in 2019	Place of acquistion		
		Norway	Abroad	Unknown
S. sonnei	71	14	49	8
S. flexneri	33	9	22	2
S. boydii	7	1	5	1
Total	111	24	76	11

ANTIMICROBIAL RESISTANCE IN SHIGELLA SONNEI

TABLE 51. Percentage distributions of antimicrobial susceptibility categories of *Shigella sonnei* (n=71) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	59.2	-	40.8
Cefotaxime	≤ 1	> 2	78.9	0.0	21.1
Ceftazidime	≤ 1	> 4	97.2	1.4	1.4
Meropenem	≤ 2	> 8	100.0	0.0	-
Ciprofloxacin	≤ 0.25	> 0.5	76.1	4.2	19.7
Tetracycline[1]	≥ 17 mm	< 17 mm	22.5	-	77.5
Chloramphenicol	≤ 8	> 8	95.8	-	4.2

[1]Breakpoints according to national zone distributions.

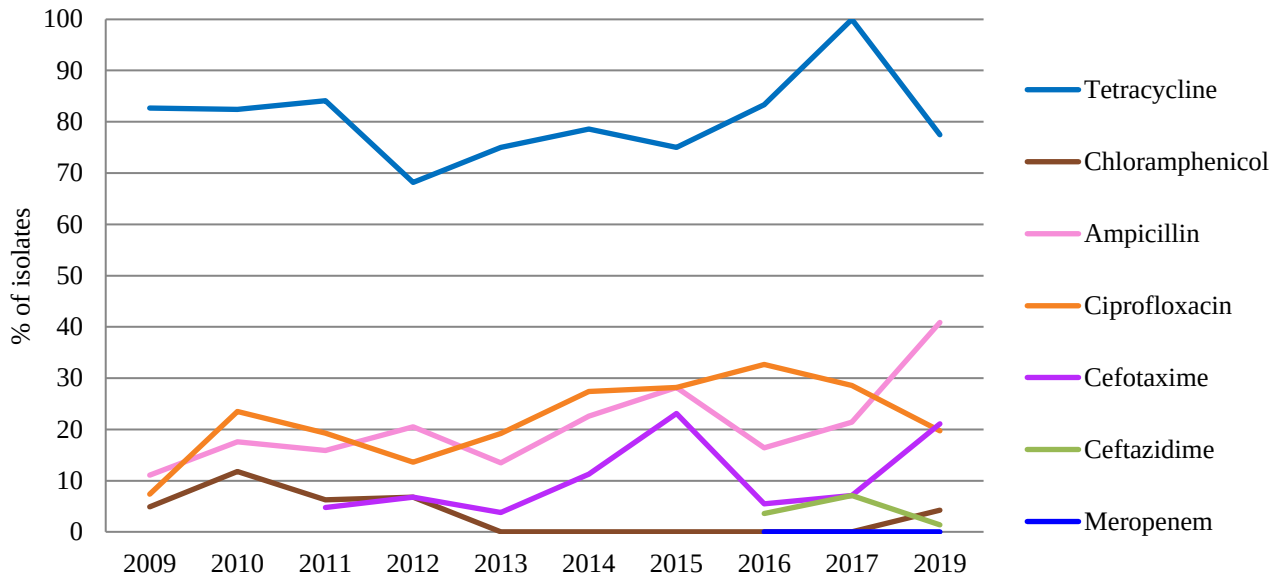


FIGURE 61. Percentage of *Shigella sonnei* resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2009-2019.

ANTIMICROBIAL RESISTANCE IN SHIGELLA FLEXNERI

TABLE 52. Percentage distributions of antimicrobial susceptibility categories of *Shigella flexneri* (n=33) from human clinical specimens irrespective of place of acquisition in Norway 2019.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	24.2	-	75.8
Cefotaxime	≤ 1	> 2	94.0	3.0	3.0
Ceftazidime	≤ 1	> 4	100.0	0.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin	≤ 0.25	> 0.5	81.8	0.0	18.2
Tetracycline[1]	≥ 17 mm	< 17 mm	18.2	-	81.8
Chloramphenicol	≤ 8	> 8	33.3	-	66.7

[1] Breakpoints according to national zone distributions.

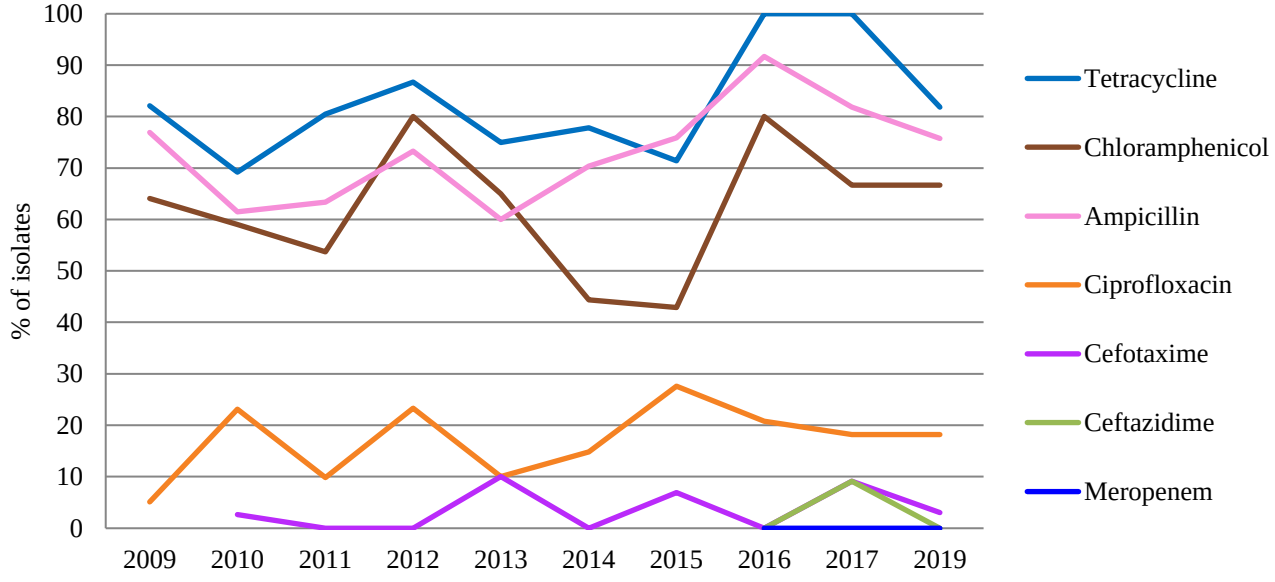


FIGURE 62. Percentage of *Shigella flexneri* resistant to selected antimicrobial agents irrespective of place of acquisition in Norway, trend 2009-2019.

RESULTS AND COMMENTS

Following the reorganisation of the NRL for Enteropathogenic Bacteria at the NIPH and paused antimicrobial susceptibility testing for 2018, the NRL resumed antimicrobial susceptibility testing for all *Shigella* spp. in 2019. Antimicrobial resistance profiles and trends are only presented for *S. sonnei* and *S. flexneri*.

A stable and high proportion (77.5%) of *S. sonnei* was observed to be resistant to tetracycline over the last decade. In addition, an increasing trend of resistance towards extended-spectrum cephalosporins and ciprofloxacin was observed. Fluoroquinolones are among the first-choice antimicrobial drugs for treatment of *Shigella* spp. infections, and an increasing trend of ciprofloxacin resistance is a cause of concern.

Also in *S. flexneri*, a stable and high proportion of isolates has been observed to be resistant to tetracycline over the last decade (81.8%). In addition, a stable and high proportion of *S. flexneri* isolates are resistant to chloramphenicol and ampicillin.

Fourteen *S. sonnei* and two *S. flexneri* displayed reduced susceptibility to extended-spectrum cephalosporins. All were classified as $ESBL_A$, encoding different $bla_{CTX\text{-}M}$ genes (n=13) and $bla_{SHV\text{-}12}$ (n=1).

HUMAN CLINICAL ISOLATES

Gunnar Skov Simonsen, Cecilie Torp Andersen, Dominique Caugant, Petter Elstrøm, Hege Enger, Frode Width Gran, Einar Heldal and Aleksandra Jakovljev

Distribution of bacterial species in blood cultures

Surveillance of antimicrobial resistance is usually based on prevalences of reduced susceptibility and resistance to certain combinations of antimicrobials and bacterial species in clinical samples. The NORM surveillance programme generally follows this approach. However, there is a serious limitation to the model because a transition in the distribution from susceptible bacterial species to inherently more resistant ones will represent de facto emergence of resistance which is not easily detected. In order to complement the surveillance of individual species, NORM collects data on all positive blood cultures from the laboratory information systems of the participating institutions. A patient with a given microbial isolate was excluded from registration with a new isolate of the same species within a month from the first entry. This rule was applied irrespectively of changes in the organism´s susceptibility pattern. Isolates of a different species from the same patient were included in the surveillance. It proved difficult to systematically evaluate the clinical significance of species which are commonly part of the normal skin flora. In Table 53, proportions are therefore estimated for all isolates and for all isolates excluding species considered to be common skin contaminants such as coagulase negative staphylococci, *Micrococcus* spp., *Corynebacterium* spp., *Bacillus* spp. and *Cutibacterium* spp. This does not imply that such isolates cannot cause infections, but only that it was not possible to enforce a standardised protocol for inclusion of the clinically significant isolates. Similarly, all isolates were registered as individual findings although polymicrobial bloodstream infections are regularly detected in some patients. Limitations in the data extraction procedure prohibited in-depth analysis of these parameters.

TABLE 53. Number of blood culture isolates in 2019, proportion of all isolates, and proportion of isolates excluding possible skin contaminants (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Cutibacterium* spp.) 2015-2019. The table is based on data from the information systems of all laboratories in Norway.

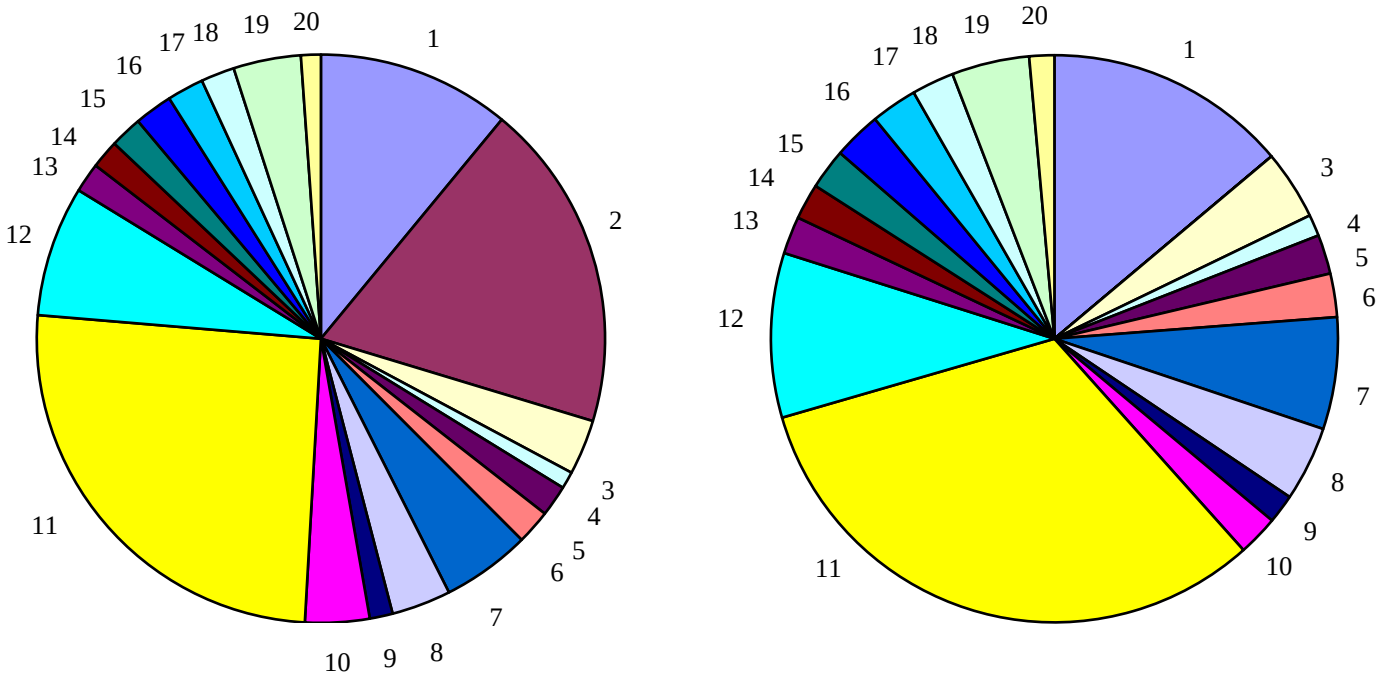
Species	No. of isolates 2019	% of all isolates					% of all isolates excluding skin flora				
		2015	2016	2017	2018	2019	2015	2016	2017	2018	2019
Staphylococcus aureus	2,117	11.1	10.5	10.1	11.1	11.0	14.4	13.6	13.1	14.2	13.9
Coagulase negative staphylococci	3,603	21.1	20.7	20.9	19.5	18.7	-	-	-	-	-
Streptococcus pneumoniae	604	3.2	3.4	2.7	3.3	3.1	4.2	4.4	3.6	4.2	4.0
Streptococcus pyogenes	187	1.3	1.1	1.2	1.2	1.0	1.7	1.4	1.5	1.5	1.2
Streptococcus agalactiae	341	1.7	1.6	1.4	1.5	1.8	2.2	2.1	1.8	1.9	2.2
Beta-haemolytic streptococci group C and G	378	1.5	1.3	1.5	2.0	2.0	2.0	1.7	2.0	2.5	2.5
Viridans- and non-haemolytic streptococci	972	4.6	5.0	5.5	5.1	5.0	6.0	6.5	7.2	6.4	6.4
Enterococcus faecalis	651	3.1	3.6	3.6	3.4	3.4	4.0	4.6	4.7	4.4	4.3
Enterococcus faecium	252	1.4	1.4	1.4	1.2	1.3	1.8	1.9	1.9	1.5	1.7
Other Gram-positive aerobic and facultative anaerobic bacteria	705	3.6	3.3	3.5	3.1	3.7	2.3	2.3	2.2	2.0	2.3
Escherichia coli	4,900	24.8	24.9	24.9	25.5	25.4	32.4	32.2	32.2	32.6	32.2
Klebsiella spp.	1,430	6.9	7.1	7.0	6.8	7.4	9.1	9.2	9.1	8.7	9.4
Enterobacter spp.	325	1.7	1.7	1.9	1.9	1.7	2.3	2.2	2.4	2.4	2.1
Proteus spp.	311	1.6	1.6	1.5	1.6	1.6	2.1	2.1	2.0	2.0	2.0
Other *Enterobacteriaceae*	417	1.8	1.8	2.3	3.4	2.2	2.3	2.3	3.0	4.3	2.7
Pseudomonas spp.	352	1.7	1.6	1.4	1.7	1.8	2.2	2.0	1.8	2.1	2.3
Other Gram-negative aerobic and facultative anaerobic bacteria	402	2.1	2.4	2.0	1.0	2.1	2.7	3.0	2.6	1.3	2.6
Bacteroides spp.	368	2.2	1.9	2.3	1.9	1.9	2.8	2.4	2.9	2.4	2.4
Other anaerobic bacteria	740	3.2	3.8	3.7	3.7	3.8	3.7	4.4	4.4	4.2	4.4
Yeasts	217	1.4	1.3	1.2	1.1	1.1	1.8	1.7	1.6	1.4	1.4
Total	**19,272**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

As seen in Table 53 and Figure 63, aerobic and facultative Gram-positive and Gram-negative bacteria represented 51.0% and 42.2% of all isolates, respectively. The predominance of Gram-positives among all isolates was at the same level as in previous years. The most common Gram-positive species were coagulase negative staphylococci, which represented 18.7%. This is a decrease from 19.5% in 2018, but minor fluctuations may result from inconsistent reporting from the laboratories. The difference between aerobic Gram-positives and Gram-negatives was reversed when species of the skin flora (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Cutibacterium* spp.) were excluded with 38.5% aerobic Gram-positives and 53.3% aerobic Gram-negatives.

Among aerobic Gram-positives, the prevalence of *S. pneumoniae* has steadily declined from 12.1% in 2005 to 3.6% in 2017 (skin contaminants excluded), following the introduction of the conjugate pneumococcal vaccine in the national childhood immunisation programme in June 2006. The proportion has now stabilised at 4.2% in 2018 and 4.0% in 2019, corresponding to 604 cases in both years. The proportions of other aerobic Gram-positives have remained stable over many years.

E. coli (32.2%) and other *Enterobacteriaceae* (16.2%) accounted for the vast majority of aerobic Gram-negative isolates, but the proportions have remained relatively unchanged over the years. *Pseudomonas* spp. (2.3%) has been fairly stable after a peak in 2005 (2.8%), all figures excluding skin flora.

Anaerobic bacteria and yeasts were less prevalent. Anaerobes accounted for 5.7% (6.8% excluding skin flora). Yeasts accounted for 1.1% (1.4% excluding skin flora) which is unchanged from earlier years. The major pathogens among anaerobes were members of *Bacteroides* spp. (1.9%/2.4%) and among yeasts *Candida albicans* (0.7%/0.9%). However, a multitude of other species were also represented.



1. *Staphylococcus aureus*
2. Coagulase negative staphylococci
3. *Streptococcus pneumoniae*
4. *Streptococcus pyogenes*
5. *Streptococcus agalactiae*
6. Betahaemolytic streptococci group C and G
7. Non-haemolytic and viridans streptococci
8. *Enterococcus faecalis*
9. *Enterococcus faecium*
10. Other Gram-positive bacteria
11. *Escherichia coli*
12. *Klebsiella* spp.
13. *Enterobacter* spp.
14. *Proteus* spp.
15. Other *Enterobacteriaceae*
16. *Pseudomonas* spp.
17. Other Gram-negative bacteria
18. *Bacteroides* spp.
19. Other anaerobic bacteria
20. Yeasts

FIGURE 63. Distribution of all blood culture isolates (left, n=19,272) and blood culture isolates excluding common skin contaminants (right, n=15,251) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Cutibacterium* spp. Data for 2019 were retrieved from the information systems of all Norwegian laboratories.

Escherichia coli in blood cultures

TABLE 54. *Escherichia coli* blood culture isolates in 2019 (n=2,350). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	57.7	-	43.3
Amoxicillin-clavulanic acid*	≤ 8	> 8	74.6	-	25.4
Piperacillin-tazobactam	≤ 8	> 16	96.3	2.2	1.5
Cefuroxime	≤ 0.001	> 8	0.0	89.5	10.5
Cefotaxime	≤ 1	> 2	92.5	0.3	7.2
Ceftazidime	≤ 1	> 4	92.6	1.4	6.0
Cefepime	≤ 1	> 4	92.8	0.9	6.3
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	94.1	-	5.9
Ciprofloxacin	≤ 0.25	> 0.5	85.7	3.0	11.3
Tigecycline	≤ 0.5	> 0.5	99.6	-	0.4
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	75.0	0.4	24.6
ESBL	Negative	Positive	92.9	-	7.1

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for infections other than uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

NORM results are interpreted according to NordicAST/ EUCAST clinical breakpoints at the time of analysis. The isolates are categorised as susceptible with standard exposure (S), susceptible with increased exposure (I), or resistant (R). The vast majority of isolates were susceptible (S or I) to broad-spectrum antimicrobial agents such as cefotaxime (92.5%), ceftazidime (92.6%), gentamicin (94.1%), cefepime (92.8%), piperacillin-tazobactam (96.3%), tigecycline (99.6%) and meropenem (100.0%) (Table 54). There were no significant changes in the prevalence of resistance to these agents from 2018.

The prevalence of resistance to gentamicin remained stable at 5.9% in 2019 compared to 5.4% in 2018 (Figure 64). The data were interpreted according to the breakpoints for systemic urinary tract infections, although NordicAST/ EUCAST no longer considers gentamicin sufficient for monotherapy in infections originating from other sources. The prevalence of gentamicin resistance is approximately six times higher than at the turn of the century. A high proportion of gentamicin resistant isolates (44/138, 31.9%) also produced ESBL enzymes. The prevalence at individual laboratories varied due to relatively small numbers. When aggregated by region there were only minor geographical differences (North 6.2%, South-East 6.0%, Middle 5.8% and West 5.4%).

The prevalence of resistance to ciprofloxacin was 11.3% in 2019, compared to 15.2% in 2017 and 11.7% in 2018. The breakpoint for ciprofloxacin resistance has been changed many times over the years, most recently in 2017 with a reduction from R > 1 mg/L to R > 0.5 mg/L and from S ≤ 0.5 mg/L to S ≤ 0.25 mg/L. The long-term trend for ciprofloxacin resistance cannot be precisely determined due to changes in susceptibility test methodology, but it appears that the increase seen 2006-2017 has now stabilised when using the present breakpoint. The temporal association between ciprofloxacin resistance and ciprofloxacin usage is depicted in Figure 65. A similar association between quinolone use and resistance in systemic *E. coli* isolates is also reported internationally. Further surveillance is needed to ascertain whether reduced ciprofloxacin usage will lead to a reduction of quinolone resistance rates. The resistance rates for ampicillin (43.2% in 2018, 43.3% in 2019) and trimethoprim-sulfamethoxazole (25.1% in 2018, 24.6% in 2019) are relatively stable.

Detection of extended-spectrum beta-lactamases (ESBL) was based on reduced zone diameters for cefotaxime and/or ceftazidime. All isolates with reduced susceptibility were further characterised by combination MIC gradient tests. A total of 167 isolates (7.1%) were reported as ESBL positive, which is a slight increase from 2018 (6.5%) (Figure 67). The isolates originated from laboratories across the country, but estimates at local level are uncertain due to small numbers. When aggregated at regional level there was some geographical variation in the prevalence of ESBL production; South-East (8.1%), North (7.3%), West (6.3%) and Middle (4.4%). Most of the ESBL isolates were resistant to cefuroxime (n=167), cefotaxime (n=162), cefepime (n=136) and ceftazidime (n=131). Many isolates were susceptible to piperacillin-tazobactam at standard (n=140) or increased (n=11) exposure. Seventy-five isolates were susceptible to amoxicillin-clavulanic acid using breakpoints for non-urinary tract infections, whereas 92 were resistant. The ESBL isolates displayed high rates of co-resistance to ciprofloxacin (n=110), trimethoprim-sulfamethoxazole (n=110) and/or gentamicin (n=44). All isolates were fully susceptible to meropenem according to both clinical and screening breakpoints, thus no carbapenemase-producing isolates were detected.

E. coli blood culture isolates from 2019 with suspected ESBL production were subjected to whole-genome sequencing. The analysis is presented in a separate text box on page 101.

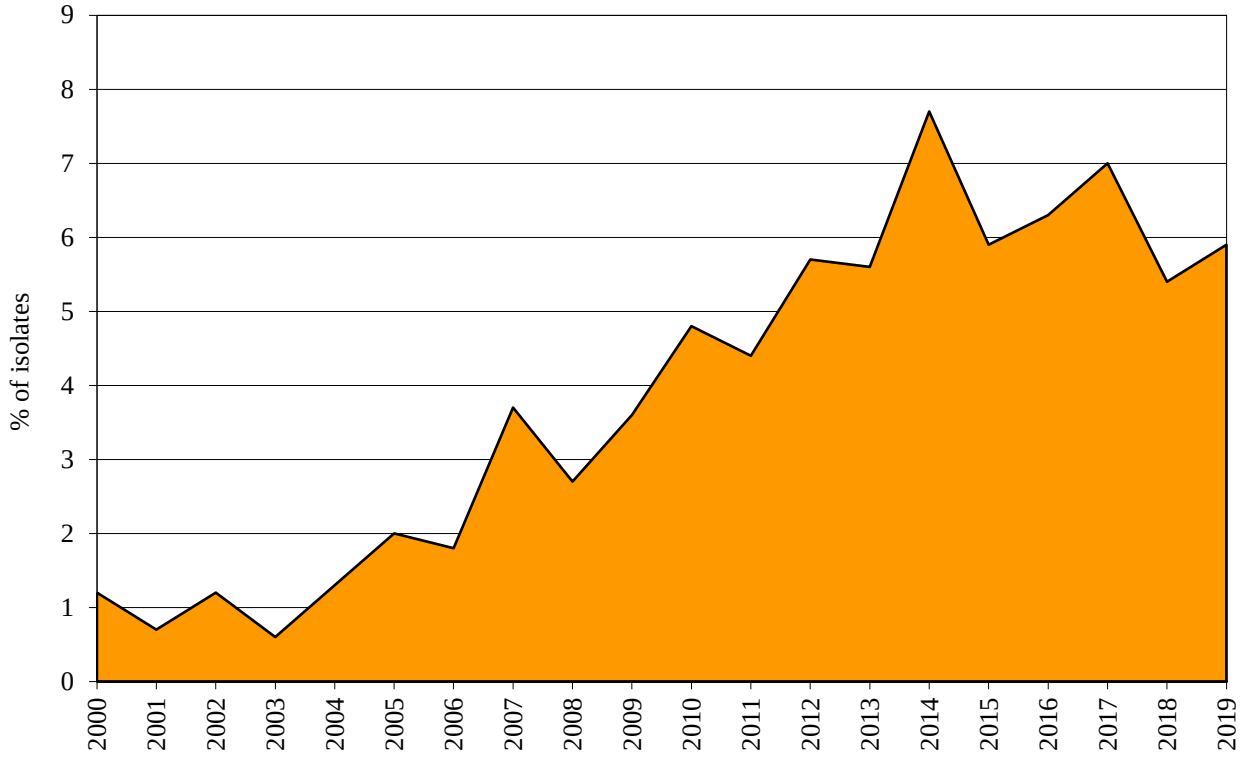


FIGURE 64. Prevalence of resistance to gentamicin in *Escherichia coli* blood culture isolates 2000-2019.

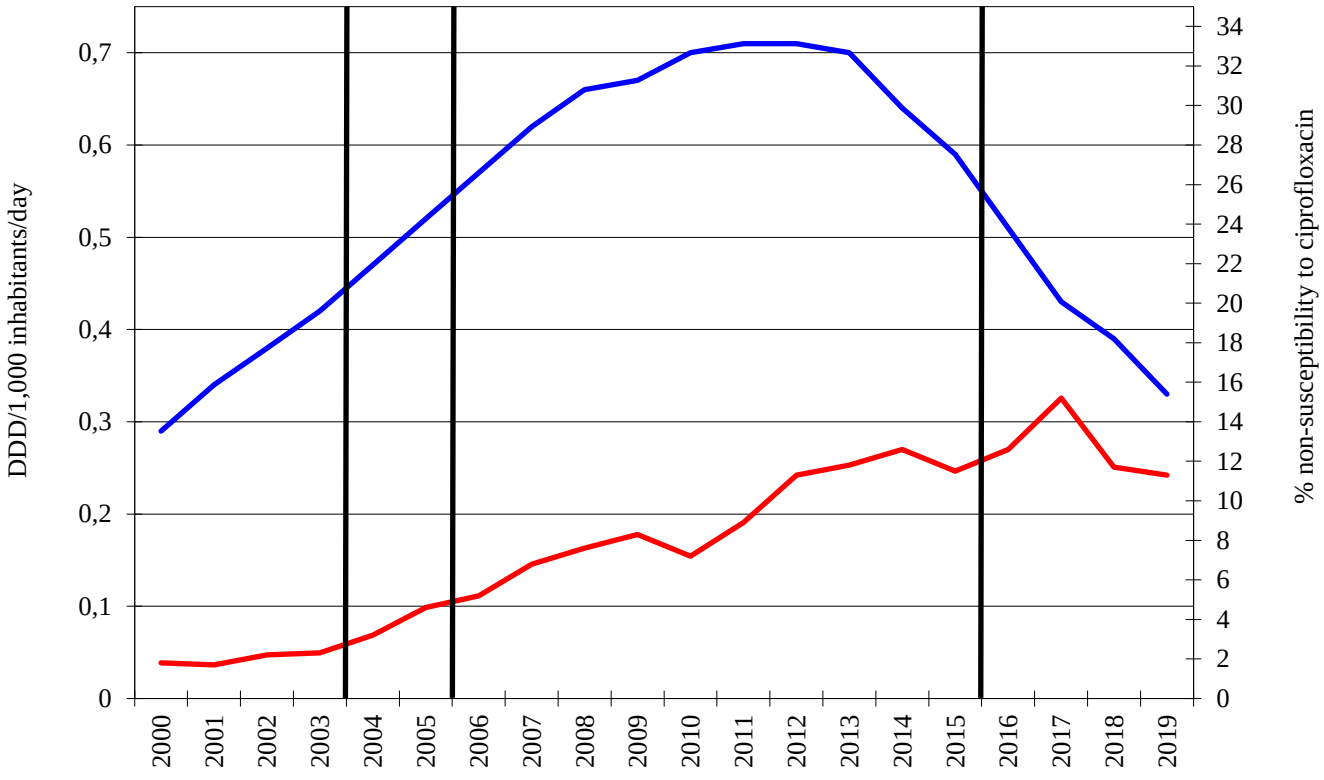


FIGURE 65. Usage of ciprofloxacin (blue) and prevalence of ciprofloxacin resistance in *Escherichia coli* blood culture isolates (red) as defined by MIC > 4 mg/L (2000-2003), MIC > 2 mg/L (2004-2005), MIC > 1 mg/L (2006-2015), and MIC > 0.5 mg/L (2016-2019). The breakpoint cannot be calibrated over the entire time period due to changes in susceptibility test methodology.

Escherichia coli in urine

TABLE 55. *Escherichia coli* urinary tract isolates in 2019 (n=1,501). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 8	> 8	65.4	-	34.6
Mecillinam	≤ 8	> 8	96.1	-	3.9
Amoxicillin-clavulanic acid*	≤ 32	> 32	93.6	-	6.4
Cefotaxime	≤ 1	> 2	96.6	0.2	3.2
Ceftazidime	≤ 1	> 4	96.8	0.9	2.3
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	95.6	-	4.4
Ciprofloxacin	≤ 0.25	> 0.5	89.1	2.6	8.3
Nitrofurantoin	≤ 64	> 64	99.1	-	0.9
Fosfomycin	≤ 32	> 32	96.5	-	3.5
Trimethoprim	≤ 4	> 4	76.2	-	23.8
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	78.6	0.9	20.5
ESBL	Negative	Positive	97.0	-	3.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Urinary tract isolates of *E. coli* have been included in the surveillance programme every year since NORM was established in 2000. The prevalence of resistance for 2019 is shown in Table 55 and the rates of resistance for 2000-2019 are shown in Figure 66.

The prevalence of resistance among urinary tract isolates has remained relatively stable over the last ten years, but is slowly increasing for most antibiotics. The prevalence of resistance to ampicillin has gradually increased from approximately 25% to 35%. Resistance to trimethoprim and trimethoprim-sulfamethoxazole has remained stable around 20-25%. The prevalence of resistance to mecillinam was 3.9% in 2019 compared to 6.0% in 2017 and 4.4% in 2018. Ciprofloxacin is used as a second line agent for urinary tract infections in Norway. When adjusting for changes in breakpoint (see text Figure 65), the prevalence of resistance has remained stable around 8-9% over the last five years. In 2019, 8.3% of the isolates were resistant to ciprofloxacin in addition to 2.6% that were only susceptible to increased exposure through adjustment of dosage or higher concentration at the site of infection. The corresponding rates for blood culture isolates were 11.3% resistance and 3.0% susceptibility to increased exposure. The persistent discrepancy between urinary tract isolates and isolates from bloodstream infections suggests that systemic infections are caused by selected pathogenic lineages with increased virulence and accumulation of mutations in gyrase and/or topoisomerase genes, whereas urinary tract isolates may be more representative of the wild-type normal flora.

The prevalence of resistance to amoxicillin-clavulanic acid was 6.4% in 2019 compared to 7.3% in 2017 and 4.4% in 2018. The breakpoint used (R > 32 mg/L) is only valid for uncomplicated urinary tract infections. Almost all isolates (99.1%) remained susceptible to nitrofurantoin. Fosfomycin has been included in NORM since 2017. The vast majority of isolates were categorised as susceptible (96.5%), but the analysis may be technically challenging for inexperienced personnel and the results should be interpreted with caution.

Fourty-five isolates (3.0%) were reported as ESBL producers. This is at small decrease from 2018 (3.7%), but at the same level as previous years. As seen in Figure 67, the prevalence of *E. coli* ESBL is still lower in urine than in blood culture isolates (7.1%). The ESBL positive strains were isolated at 15 different laboratories in all parts of the country. Thirty isolates were retrieved from samples submitted by general practitioners, while the others were found in hospitalised patients (n=6) or patients in nursing homes (n=3) or outpatient clinics (n=6). The ESBL isolates were all resistant to ampicillin, and the majority were also resistant to cefotaxime (44/45) and ceftazidime (32/45). All isolates were registered as *in vitro* susceptible to mecillinam. Recent data suggest that this may be a viable treatment option provided a dosage of 400 mg x 3. Many of the ESBL isolates were resistant to ciprofloxacin (29/45), trimethoprim (35/45) and trimethoprim-sulfamethoxazole (30/45), but remained susceptible to nitrofurantoin (43/45), fosfomycin (39/45) and gentamicin (30/45). All ESBL isolates were clinically susceptible to carbapenems, and no carbapenemase-producers were detected by phenotypical screening.

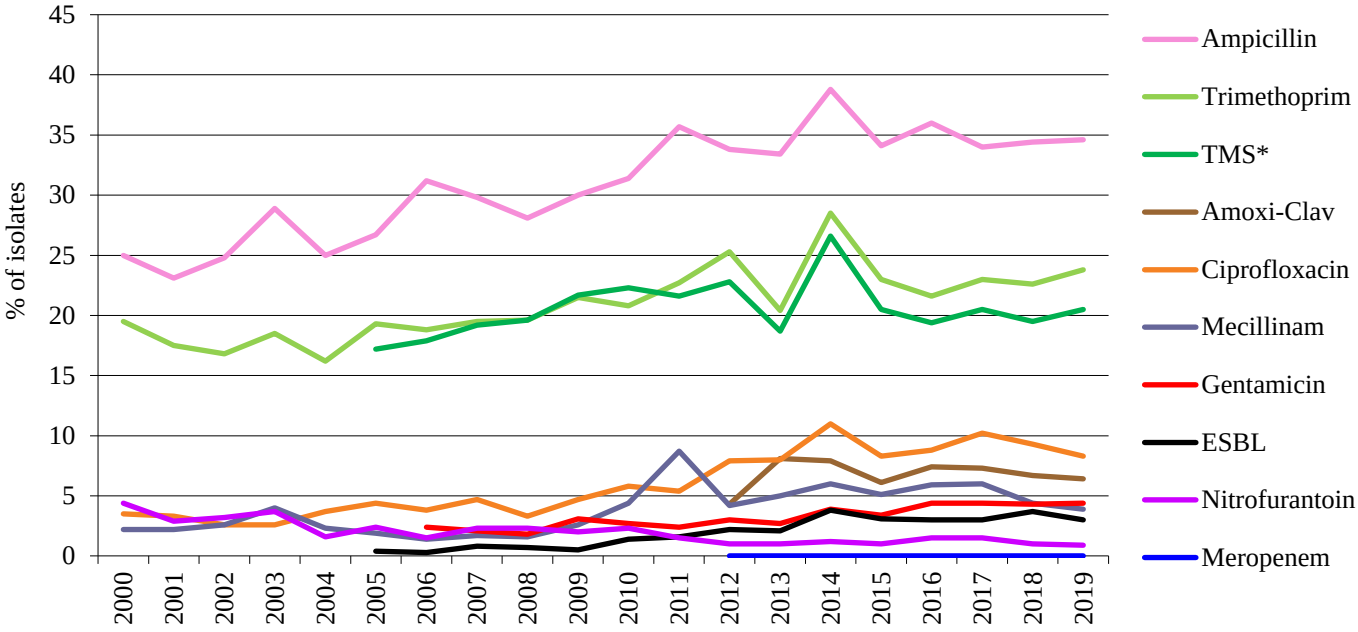


FIGURE 66. Prevalence of resistance to various antimicrobial agents in urinary tract *Escherichia coli* isolates 2000-2019. Isolates are categorised according to the breakpoints at the time of analysis for each year.
*TMS=Trimethoprim-sulfamethoxazole.

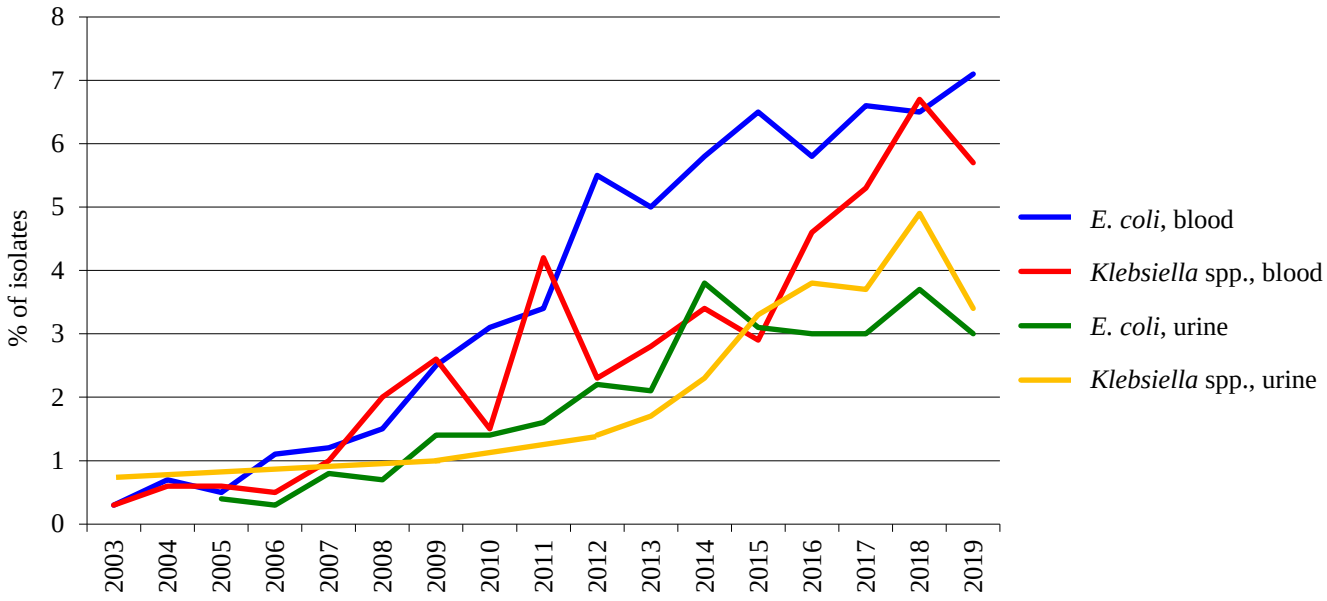


FIGURE 67. Prevalence of ESBL production among *Escherichia coli* and *Klebsiella* spp. isolates from blood and urine 2003-2019.

Whole-genome sequencing of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae*

Background

The Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res) has previously genotyped ESBL-producing isolates with respect to ESBL-genes as part of the NORM surveillance. However, this offers a limited resolution and no information about the genetic background of the isolates. Thus, whole-genome sequencing (WGS) has been performed on all ESBL-producing isolates in NORM 2019. WGS offers a higher resolution required to follow trends in the spread of specific high-risk lineages and resistance genes. For ESBL-producing *E. coli* and *K. pneumoniae* studies show that the global spread is associated with widely dispersed clones such as *E. coli* sequence type (ST)131 and *K. pneumoniae* ST307 (1,2). WGS also provides the opportunity to identify possible cases of transmission.

Materials and methods

Two hundred and twenty five blood culture isolates were reported as ESBL-producing in NORM and 219 isolates were received for WGS. Six isolates were lacking, because the strains had not been stored or due to double registration in the NORM database. Two CTX-M positive *Klebsiella oxytoca* isolates of different STs were also not included in the further analyses. In total, 163 *E. coli* and 54 *K. pneumoniae* were sequenced. DNA was extracted using NucliSENS easyMAG (bioMerieux) or PureLink Microbiome DNA Purification Kit (Invitrogen). Library preparation and WGS were performed at the Genomics Support Centre Tromsø using Illumina technology. WGS data for five isolates were obtained from Stavanger University Hospital. WGS data were analysed using the multilocus sequence typing (MLST) schemes for *E. coli* (https://enterobase.warwick.ac.uk/species/index/ecoli) and *K. pneumoniae* (https://bigsdb.pasteur.fr/klebsiella/klebsiella.html). Analysis for resistance genes was based on the NCBI bacterial antimicrobial resistance reference gene database (BioProject PRJNA313047). Phylogenetic analysis was done with the Ridom SeqSphere+ software using default settings. Six isolates were excluded for further analysis since they did not pass the WGS quality control score of more than 30X coverage and less than 500 contigs. Twenty isolates were negative for known ESBL-genes based on the WGS data. This included *E. coli* and *K. pneumoniae* with plasmid-mediated AmpC (e.g. DHA or CMY, n=14), *E. coli* with no acquired β-lactamases (n=3), *K. pneumoniae* with only the intrinsic SHV-1 β-lactamase (n=2) or other non-ESBL SHV-genes (n=2). Furthermore, one isolate received as *E. coli* turned out to be *Enterobacter cloacae*. One *K. pneumoniae* isolate harbouring the SHV-12 ESBL was also not included for further phylogenetic analysis

ESBL-producing *E. coli*

56.7% (80/141) of the ESBL-producing *E. coli* belonged to ST131 (Figure 68). Other prevalent STs (≥ 5 isolates) included ST38 (5.0%, 7/141), ST10 (3.5%, 5/141), ST648 (3.5%, 5/141) and ST1193 (3.5%, 5/141). Four isolates belonged to a novel ST.

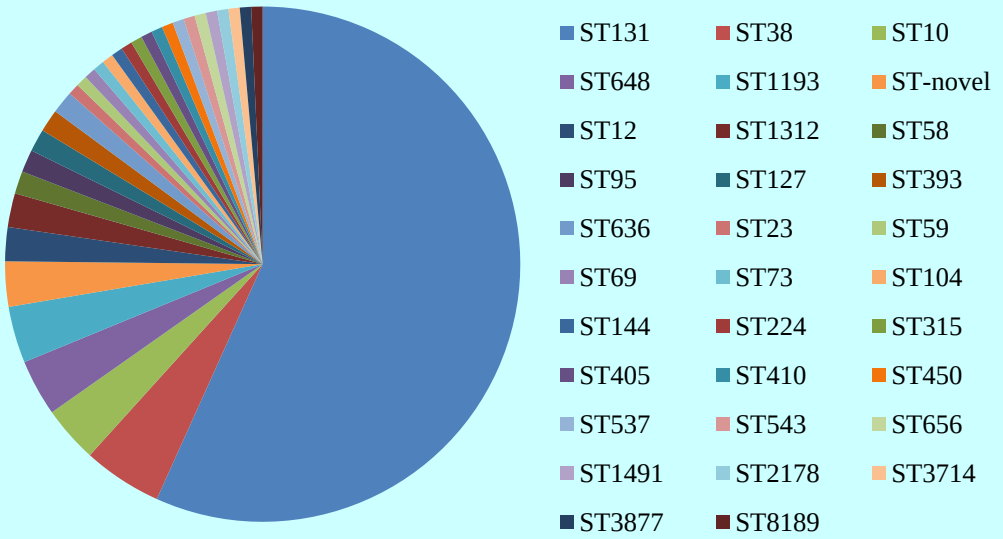


FIGURE 68. ST distribution of CTX-M-producing *E. coli*

All ESBL-producing *E. coli* isolates harboured CTX-M with CTX-M-15 identified in 57.4% (81/141), followed by CTX-M-27 (22.0%, 31/141), CTX-M-14 (7.8%, 11/141), CTX-M-3 (5.7%, 8/141) and CTX-M-1 (3.5%, 5/141). Single isolates with CTX-M-9, CTX-M-15+CTX-M-27, CTX-M-32, CTX-M-55 and CTX-M-102/-121 were also identified. No carbapenemase genes were identified.

Phylogenetic analysis based on core genome MLST revealed several likely cases of nosocomial transmission. Using a cluster threshold of ≤ 10 single nucleotide polymorphisms (SNP), 11 clusters of closely related isolates were identified among ST131 isolates (Figure 69). Three clusters included three isolates, while the remaining eight clusters consisted of two isolates each. Seven clusters included isolates from the same laboratory. Three clusters included isolates from different laboratories but within the same health region. One cluster included isolates from different health regions. Seven of the clusters included ST131 with CTX-M-15. Three and one clusters included ST131 with CTX-M-27 and CTX-M-1, respectively.

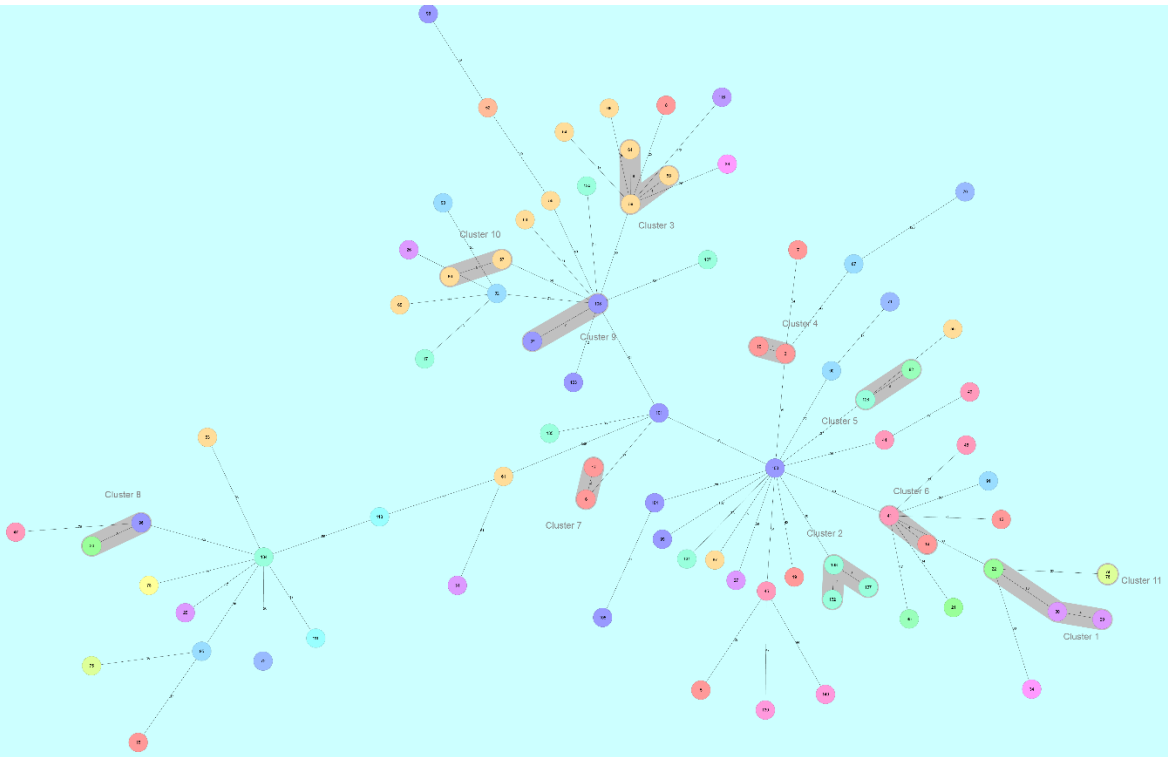


FIGURE 69. Minimum spanning tree of ESBL-producing *E. coli* ST131 based on the core genome allele profiles using SeqSphere and *E. coli* K12 as the reference strain and default settings. The isolates are coloured according to the laboratory. Closely related isolates (≤ 10 SNPs) are highlighted with grey shading.

Two clusters were identified among the other STs. One cluster consisted of three ST1312 isolates with CTX-M-15 from two different laboratories, but within the same health region. One cluster consisted of two isolates with CTX-M-3 identified in the same laboratory.

ESBL-producing *K. pneumoniae*

In total, 20 different STs were observed with ST307 (29.2%, 14/48) as the dominant ST (Figure 70). The other STs included one to four isolates. CTX-M-15 was identified in 93.8% (45/48) of the isolates. This included one isolate (ST147) with CTX-M-15 and the carbapenemase NDM-1. The remaining included CTX-M-3, CTX-M-14 and CTX-M-55 in single isolates.

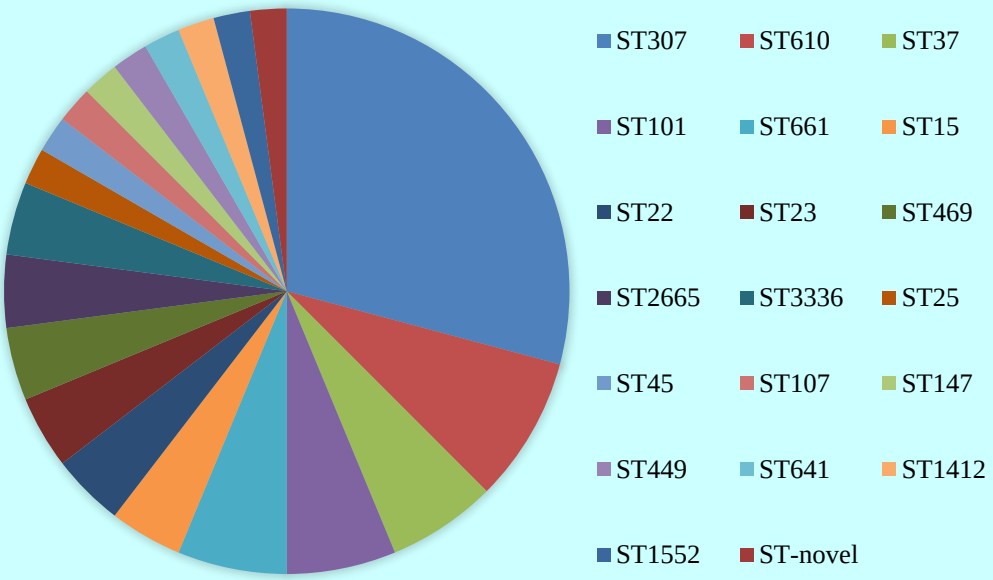


FIGURE 70. ST distribution of CTX-M-producing *K. pneumoniae*

Phylogenetic analysis based on core genome MLST and a cluster threshold of ≤ 15 SNP revealed nine clusters representing possible cases of nosocomial transmission (Figure 71). Within ST307, ten isolates were closely related (Figure 72). These were identified at eight different laboratories and in all four health regions. The other clusters included (Figure 71): (i) ST610 (n=4) from four laboratories in three health regions, (ii) ST101 (n=3) from three laboratories in two health regions, (iii) ST661 (n=3) identified at the same laboratory, and (iv) five clusters of two isolates in each including ST22, ST23, ST469, ST2665 and ST3336 identified at the same laboratory. All clusters consisted of CTX-M-15-producing *K. pneumoniae*.

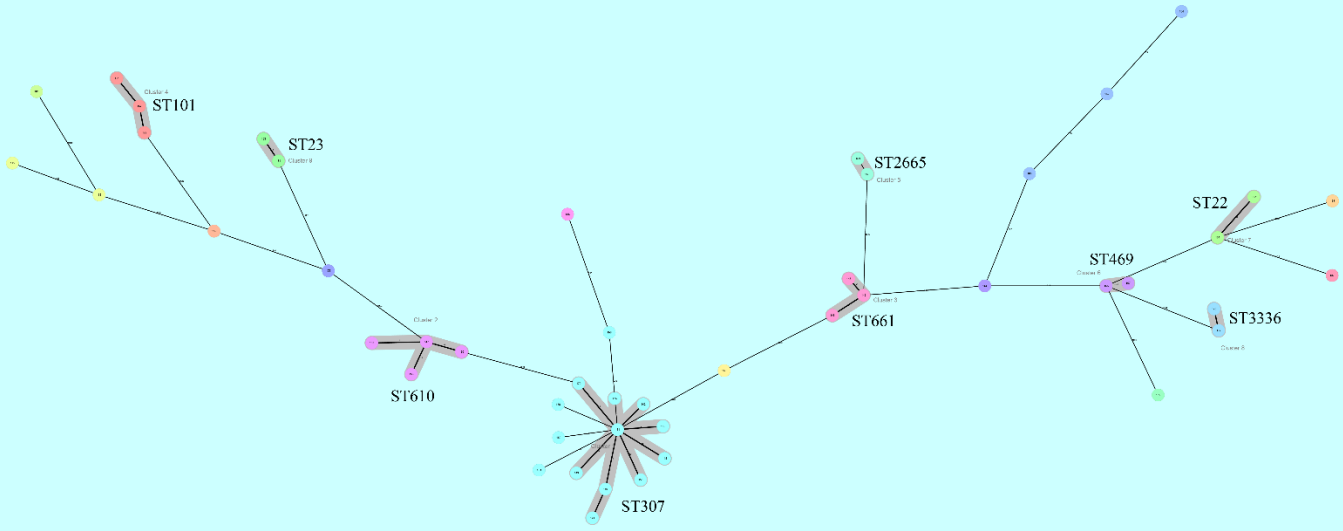


FIGURE 71. Minimum spanning tree of ESBL-producing *K. pneumoniae* based on the core genome allele profiles using SeqSphere and *K. pneumoniae* NTUH-K2044 as the reference strain and default settings. The isolates are coloured according to ST. Closely related isolates (≤ 15 SNP) are highlighted with grey shading.

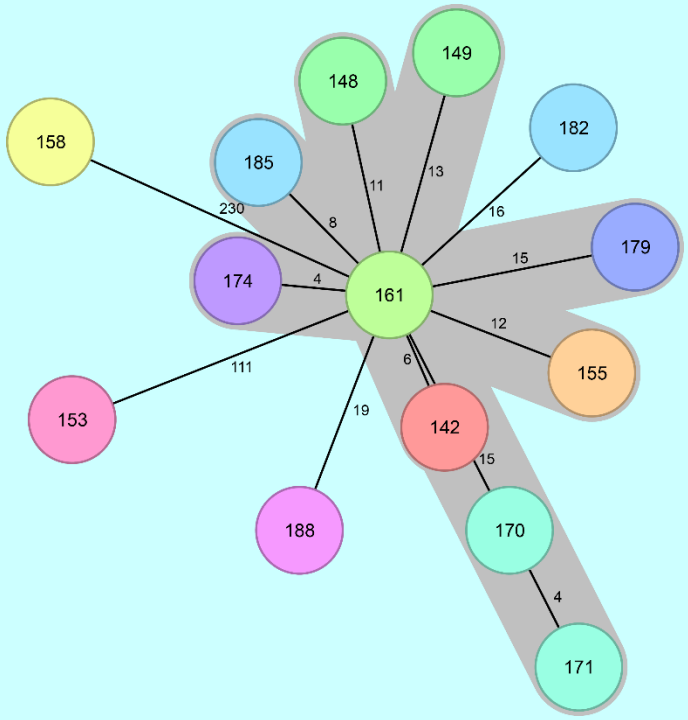


FIGURE 72. Minimum spanning tree of ST307 ESBL-producing *K. pneumoniae* based on the core genome allele profiles using SeqSphere and *K. pneumoniae* NTUH-K2044 as the reference strain. The isolates are coloured according to laboratory. Closely related isolates (≤ 15 SNP) are highlighted with grey shading.

Conclusions

The WGS data reveal that the increasing prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in Norway is mainly driven by the spread of globally distributed high-risk multi-drug resistant (MDR) clones, specifically *E. coli* ST131 (1) and *K. pneumoniae* ST307 (2). In addition, WGS shows multiple cases of possible transmission both within and possibly between health regions. A limitation of the analysis is the lack of associated epidemiological data required to resolve transmission chains. Strengthened infection control and implementation of real-time WGS analysis in connection with epidemiological data could contribute to rapid identification of high-risk MDR-clones to control and limit further spread.

References

1. Pitout JDD and Finn TJ. The evolutionary puzzle of *Escherichia coli* ST131. Infect. Gen. Evol. 2020;81:104265.
2. Wyres KL, Hawkey J, Hetland MAK, Fostervold A, Wick RR, Judd LM, Hamidian M, Howden BP, Löhr IH, Holt KE. Emergence and rapid global dissemination of CTX-M-15-associated *Klebsiella pneumoniae* strain ST307. J. Antimicrob. Chemother. 2019;74:577-581

Ørjan Samuelsen, Bjørg Haldorsen, Ellen Josefsen, Jessin Janice and Arnfinn Sundsfjord, Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway and UiT The Arctic University of Norway, Tromsø.

Klebsiella spp. in blood cultures

TABLE 56. *Klebsiella* spp. blood culture isolates in 2019 (n=1,017). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Amoxicillin-clavulanic acid*	≤ 8	> 8	85.3	-	14.7
Piperacillin-tazobactam	≤ 8	> 16	90.4	5.2	4.4
Cefuroxime	≤ 0.001	> 8	0.0	87.9	12.1
Cefotaxime	≤ 1	> 2	93.1	0.6	6.3
Ceftazidime	≤ 1	> 4	92.7	1.0	6.3
Cefepime	≤ 1	> 4	91.7	2.3	6.0
Meropenem	≤ 2	> 8	99.9	0.0	0.1
Gentamicin	≤ 2	> 2	95.6	-	4.4
Ciprofloxacin	≤ 0.25	> 0.5	89.9	4.0	6.1
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	88.6	0.1	11.3
ESBL	Negative	Positive	94.3	-	5.7

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for infections other than uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 57. *Klebsiella pneumoniae* blood culture isolates in 2019 (n=712). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Amoxicillin-clavulanic acid*	≤ 8	> 8	85.0	-	15.0
Piperacillin-tazobactam	≤ 8	> 16	90.8	6.5	2.7
Cefuroxime	≤ 0.001	> 8	0.0	87.4	12.6
Cefotaxime	≤ 1	> 2	92.7	0.0	7.3
Ceftazidime	≤ 1	> 4	91.5	1.1	7.4
Cefepime	≤ 1	> 4	91.0	2.0	7.0
Meropenem	≤ 2	> 8	99.9	0.0	0.1
Gentamicin	≤ 2	> 2	94.8	-	5.2
Ciprofloxacin	≤ 0.25	> 0.5	87.4	4.9	7.7
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	85.9	0.1	14.0
ESBL	Negative	Positive	92.7	-	7.3

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for infections other than uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 58. *Klebsiella oxytoca* blood culture isolates in 2019 (n=201). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Amoxicillin-clavulanic acid*	≤ 8	> 8	87.6	-	12.4
Piperacillin-tazobactam	≤ 8	> 16	87.6	2.0	10.4
Cefuroxime	≤ 0.001	> 8	0.0	88.6	11.4
Cefotaxime	≤ 1	> 2	95.5	3.0	1.5
Ceftazidime	≤ 1	> 4	99.0	0.0	1.0
Cefepime	≤ 1	> 4	92.5	4.5	3.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	98.5	-	1.5
Ciprofloxacin	≤ 0.25	> 0.5	98.0	0.5	1.5
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	96.5	0.0	3.5
ESBL	Negative	Positive	99.0	-	1.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for infections other than uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The surveillance of *Klebsiella* spp. in blood cultures included 712 *K. pneumoniae* (70.0%), 201 *K. oxytoca* (19.8%), and 104 (10.2%) isolates not identified to the species level, giving a total of 1,017 *Klebsiella* spp. isolates (Tables 56-58).

The majority of *Klebsiella* spp. isolates remained susceptible to aminoglycosides, and the prevalence of gentamicin resistance decreased from 5.2% in 2018 to 4.4% in 2019. *K. pneumoniae* isolates were more often resistant to aminoglycosides (5.2%) than *K. oxytoca* isolates (1.5%). Aminoglycoside resistance in common *Enterobacterales* species is a cause for great concern as these antimicrobials have traditionally been used in the empirical regimen for treatment of septicemia in Norway.

As for *E. coli*, the breakpoints for ciprofloxacin were reduced from R > 1 mg/L to R > 0.5 mg/L and from S ≤ 0.5 to S ≤ 0.25 in 2017. The prevalence of resistance to ciprofloxacin peaked at 11-12% in 2016-2017, but decreased to 8.1% in 2018 and 6.1% in 2019. The results should be interpreted with caution due to the repeated changes in breakpoints and test methodology over the last decade. Suscpetibility testing for quinolones may be technically challenging, and further surveillance is needed to determine the long-term trend for ciprofloxacin resistance in *Klebsiella* spp. Resistance to ciprofloxacin is much more common in *K. pneumoniae* (7.7%) than in *K. oxytoca* (1.5%). Resistance to trimethoprim-sulfamethoxazole decreased from 13.9% in 2018 to 11.3% in 2019. As for ciprofloxacin, the prevalence of resistance to trimethoprim-sulfamethoxazole was significantly lower in *K. oxytoca* (3.5%) than in *K. pneumoniae* (14.0%).

A comparison of resistance to beta-lactam antibiotics between *Klebsiella* species is complicated by the diagnostic challenges of the chromosomal K1 beta-lactamase in *K. oxytoca*. Most *Klebsiella* spp. isolates were susceptible (defined as S+I) to cefotaxime (93.7%), ceftazidime (93.7%) and the beta-lactam/beta-lactamase inhibitor combination piperacillin-tazobactam (95.6%), see Figure 73. The prevalence of resistance to third generation cephalosporins were essentially unchanged from previous years.

As for *E. coli*, the detection of extended-spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime disks. Isolates with reduced zone diameters were further characterised by combination MIC gradient tests. The prevalence of phenotypically confirmed ESBL isolates decreased from 6.6% in 2018 to 5.7% in 2018 (Figure 67). The 58 ESBL isolates originated from 15 different laboratories and were identified as *K. pneumoniae* (n=52, 7.3%), *K. oxytoca* (n=2, 1.0%) and *Klebsiella* spp. (n=4, 3.8%). The ESBL isolates were generally resistant to cefuroxime (57/58), cefotaxime (57/58), ceftazidime (56/58) and cefepime (54/58), and co-resistance was frequently seen for trimethoprim-sulfamethoxazole (53/58), ciprofloxacin (38/58) and gentamicin (35/59). Many isolates were susceptible to piperacillin-tazobactam at standard (34/58) or increased (15/58) exposure. Two isolates displayed a zone diameter below the EUCAST meropenem breakpoint for carbapenemase production. One isolate contained the NDM-1 carbapenemase gene, while the other isolate was negative for carbapenemase production.

Klebsiella spp. blood culture isolates from 2019 with suspected ESBL production were subjected to whole-genome sequencing. The analysis is presented in a separate text box on page 101.

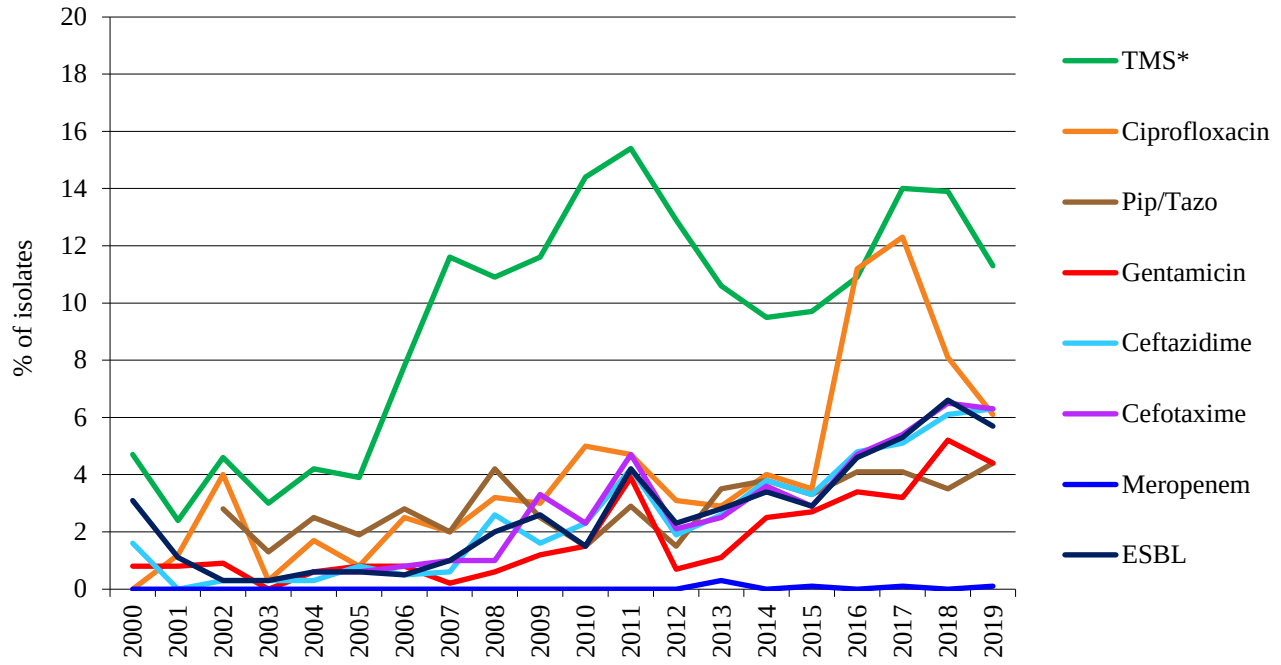


FIGURE 73. Prevalence of resistance to various antimicrobial agents in *Klebsiella* spp. blood culture isolates 2000-2019. Isolates are categorised according to the breakpoints at the time of analysis for each year.
*TMS=Trimethoprim-sulfamethoxazole.

Klebsiella spp. in urine

TABLE 59. *Klebsiella* spp. urinary tract isolates in 2019 (n=921). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Mecillinam	≤ 8	> 8	91.9	-	8.1
Amoxicillin-clavulanic acid*	≤ 32	> 32	92.9	-	7.1
Piperacillin-tazobactam	≤ 8	> 16	90.2	5.6	4.2
Cefotaxime	≤ 1	> 2	95.7	0.8	3.6
Ceftazidime	≤ 1	> 4	94.6	2.0	3.5
Cefepime	≤ 1	> 4	93.7	2.3	4.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	96.6	-	3.4
Ciprofloxacin	≤ 0.25	> 0.5	90.8	5.0	4.2
Trimethoprim	≤ 4	> 4	81.3	-	18.7
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	86.6	0.2	13.1
ESBL	Negative	Positive	96.6	-	3.4

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Klebsiella spp. urinary tract isolates have previously been included in the NORM surveillance programme in 2001, 2003, 2009 and 2012-2018. Due to methodological changes it is not possible to directly compare the results from 2001 and 2003 with the ones from later surveys. There are no *Klebsiella* spp. disk diffusion breakpoints for fosfomycin or nitrofurantoin.

The prevalence of resistance to urinary tract antibiotics was slightly lower in *Klebsiella* spp. than in *E. coli* isolates (Tables 59-61). The majority of isolates were susceptible (S+I) to gentamicin at 96.6% compared to 97.0% in 2018. Among urinary tract *E. coli*, 95.6% were susceptible to gentamicin in 2019. The rates of resistance to ciprofloxacin in *Klebsiella* spp. decreased from 7.9% in 2017 and 6.3% in 2018, to 4.2% in 2019. The comparable rate for urinary

tract *E. coli* in 2019 was 8.3%. Susceptibility to trimethoprim (82.8% in 2018, 81.3% in 2019) and trimethoprim-sulfamethoxazole (86.6% in 2018, 86.8% in 2019) was higher than in *E. coli* (76.2% and 79.5% in 2019, respectively). Our data may indicate that the *E. coli* breakpoints for fosfomycin are not suitable for *Klebsiella* (80.6% resistance).

All *Klebsiella* isolates are inherently resistant to ampicillin due to the chromosomal SHV beta-lactamase. As for *Klebsiella* spp. blood culture isolates, ESBL detection in urinary tract isolates was based on resistance to cefotaxime and/or ceftazidime and subsequent confirmatory ESBL MIC gradient tests. Thirty-one isolates were reported as ESBL positive, of which twenty-nine were *K. pneumoniae*, one was *K. oxytoca*, and one was not identified to the species level. The 31 ESBL isolates were retrieved from 15 different laboratories and originated from general practices (n=15), hospitals (n=11), outpatient clinics (n=2) and nursing homes (n=3). The 3.4% ESBL rate (4.2% in *K. pneumoniae*) was a decrease from 2018 (4.9% for all *Klebsiella*, 5.9% in *K. pneumoniae*). The 31 ESBL isolates were often resistant to trimethoprim (n=27), trimethoprim-sulfamethoxazole (n=26), ciprofloxacin (n=17) and gentamicin (n=15), but many remained susceptible to mecillinam (n=28) and piperacillin-tazobactam (n=28). All isolates were susceptible to meropenem according to the clinical breakpoints, and no carbapenemase-producing isolates were detected by the screening breakpoint.

TABLE 60. *Klebsiella pneumoniae* urinary tract isolates in 2019 (n=686). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Mecillinam	≤ 8	> 8	92.6	-	7.4
Amoxicillin-clavulanic acid*	≤ 32	> 32	95.0	-	5.0
Piperacillin-tazobactam	≤ 8	> 16	90.9	6.9	2.2
Cefotaxime	≤ 1	> 2	95.6	0.1	4.2
Ceftazidime	≤ 1	> 4	93.6	2.2	4.2
Cefepime	≤ 1	> 4	93.6	1.6	4.8
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	96.2	-	3.8
Ciprofloxacin	≤ 0.25	> 0.5	88.9	6.1	5.0
Trimethoprim	≤ 4	> 4	78.0	-	22.0
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	83.4	0.3	16.3
ESBL	Negative	Positive	95.8	-	4.2

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 61. *Klebsiella oxytoca* urinary tract isolates in 2019 (n=154). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Mecillinam	≤ 8	> 8	87.7	-	12.3
Amoxicillin-clavulanic acid*	≤ 32	> 32	85.1	-	14.9
Piperacillin-tazobactam	≤ 8	> 16	85.1	0.6	14.3
Cefotaxime	≤ 1	> 2	94.8	3.9	1.3
Ceftazidime	≤ 1	> 4	99.4	0.0	0.6
Cefepime	≤ 1	> 4	92.9	5.2	1.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 2	98.1	-	1.9
Ciprofloxacin	≤ 0.25	> 0.5	98.1	0.0	1.9
Trimethoprim	≤ 4	> 4	94.2	-	5.8
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	98.1	0.0	1.9
ESBL	Negative	Positive	99.4	-	0.6

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. ESBL=Extended-spectrum beta-lactamase. *Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Pseudomonas aeruginosa in blood cultures

TABLE 62. *Pseudomonas aeruginosa* blood culture isolates in 2019 (n=228). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 0.001	> 16	0.0	94.3	5.7
Ceftazidime	≤ 0.001	> 8	0.0	95.6	4.4
Aztreonam	≤ 0.001	> 16	0.0	96.1	3.9
Imipenem	≤ 0.001	> 4	0.0	90.8	9.2
Meropenem	≤ 2	> 8	89.0	5.3	5.7
Tobramycin	≤ 2	> 2	100.0	-	0.0
Amikacin	≤ 16	> 16	97.4	-	2.6
Ciprofloxacin	≤ 0.001	> 0.5	0.0	93.4	6.6

RESULTS AND COMMENTS

NORM has previously reported on *Pseudomonas aeruginosa* blood culture isolates in 2005, 2010 and 2015. The first two surveys were based on MIC determination, whereas the isolates from 2015 and 2019 were examined by disk diffusion. Moreover, NordicAST/EUCAST performed a major revision of clinical breakpoints in 2019 where wild-type strains were defined as susceptible only to increased exposure of many antibiotics including piperacillin-tazobactam, ceftazidime, aztreonam, imipenem and ciprofloxacin. Breakpoints for resistance were also adjusted as detailed in the legend to Figure 74. Comparison of data from different years should therefore be done with caution.

Most isolates were susceptible to all relevant anti-microbials, and very few displayed resistance to multiple classes as commonly seen in other countries (Table 62). The prevalence of resistance has increased over the last decade for all beta-lactam antibiotics as seen in Figure 74. Of special concern is the relatively high prevalence of meropenem resistance (3.7% in 2015, 5.7% in 2019) as this substance is often the drug of choice in invasive pseudomonal infections. Many of these isolates were concomitantly resistant to other beta-lactam antibiotics normally active against *P. aeruginosa*, including piperacillin-tazobactam (6/13), ceftazidime (5/13) and imipenem (10/13). Carbapenemase-producing isolates are reported in a separate text box on page 110. The prevalence of resistance to aminoglycosides is still very low, and resistance to ciprofloxacin has remained stable at around 4-6%.

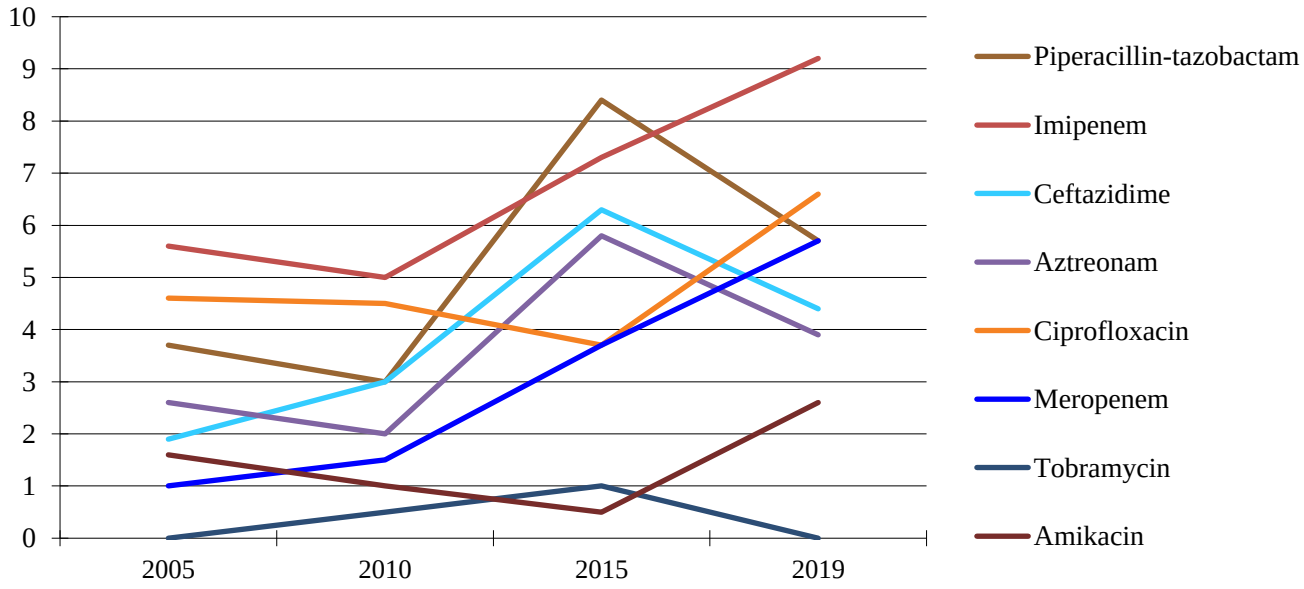


FIGURE 74. Prevalence of resistance to various antimicrobial agents in *Pseudomonas aeruginosa* blood culture isolates 2005-2019. The breakpoint for resistance to ciprofloxacin was reduced from R > 1 mg/L to R > 0.5 mg/L, for imipenem from R > 8 mg/L to R > 4 mg/L, and for tobramicin from R > 4 mg/L to R > 2 mg/L, all in 2019. Please note that the X axis is not to scale.

Pseudomonas aeruginosa in urine

TABLE 63. *Pseudomonas aeruginosa* urinary tract isolates in 2019 (n=144). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Piperacillin-tazobactam	≤ 0.001	> 16	0.0	96.5	3.5
Ceftazidime	≤ 0.001	> 8	0.0	96.5	3.5
Aztreonam	≤ 0.001	> 16	0.0	95.1	4.9
Imipenem	≤ 0.001	> 4	0.0	91.0	9.0
Meropenem	≤ 2	> 8	93.1	4.9	2.1
Tobramycin	≤ 2	> 2	99.3	-	0.7
Amikacin	≤ 16	> 16	99.3	-	0.7
Ciprofloxacin	≤ 0.001	> 0.5	0.0	89.6	10.4

RESULTS AND COMMENTS

Pseudomonas aeruginosa urinary tract isolates have never been included in NORM before. Only a very limited number of antimicrobials are clinically active against *P. aerugionsa*, and only ciprofloxacin is suitable for oral treatment of urinary tract infections. The protocol requires that isolates should be retrieved from uncomplicated cases, but *P. aeruginosa* is often associated with more complicated cases of recurrent or chronic infections. One may therefore suspect that some isolates have been exposed to various antibiotics before sampling.

In general, there were no major differences in the rates of resistance between blood culture and urinary tract isolates. However, ciprofloxacin resistance was seen in 10.4% of urinary tract isolates compared to 6.6% in blood cultures. This may be a consequence of previous exposure to fluoroquinolones or the enrichment of mutations in specific uropathogenic clones.

Carbapenemase-producing Gram-negative bacteria in Norway 2019

Colonisation or infections with carbapenemase-producing Gram-negative bacteria (*Enterobacterales*, *Pseudomonas* and *Acinetobacter*) are notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS) after confirmation at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res). Here we summarise the findings of carbapenemase-producing Gram-negative bacteria in 2019, including whole-genome sequencing data (WGS).

In 2019, 75 patients were identified with carbapenemase-producing *Enterobacterales* (CPE), an increase from 54 cases in 2018 (Figure 75).

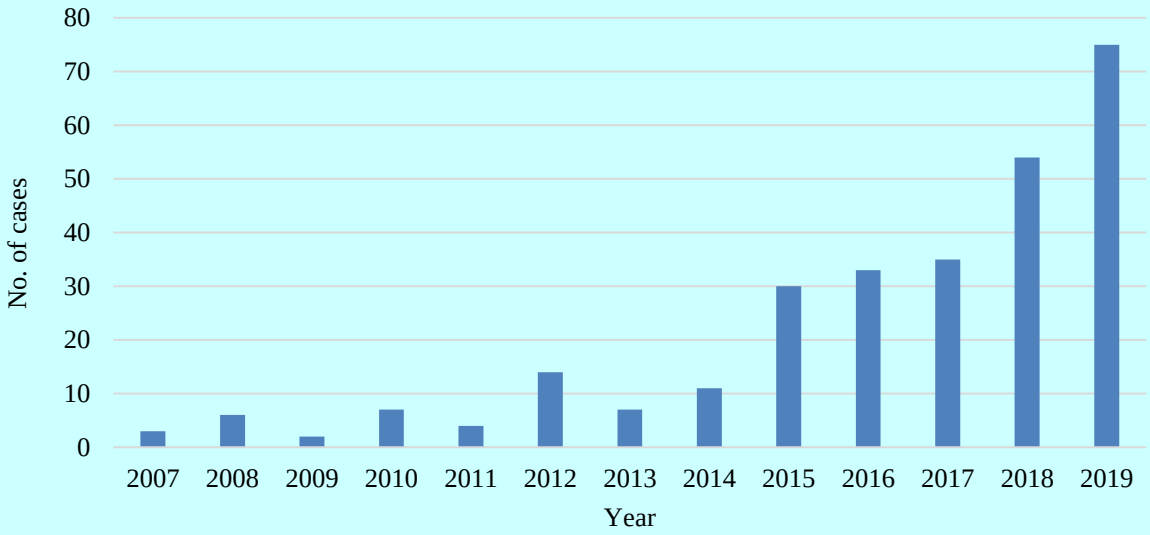


FIGURE 75. Number of cases with carbapenemase-producing *Enterobacterales* in Norway 2007-2019.

Eighty-six CPE isolates were identified (Figure 76). Six patients harboured more than one isolate of either different species and/or carbapenemase-variant. *Escherichia coli* was the dominant species (n=44) followed by *Klebsiella pneumoniae* (n=28). Overall *E. coli* represented the highest increase with a proportional increase from 44% in 2018 to 51% in 2019. The proportion of *K. pneumoniae* decreased from 44% in 2018 to 33% in 2019.

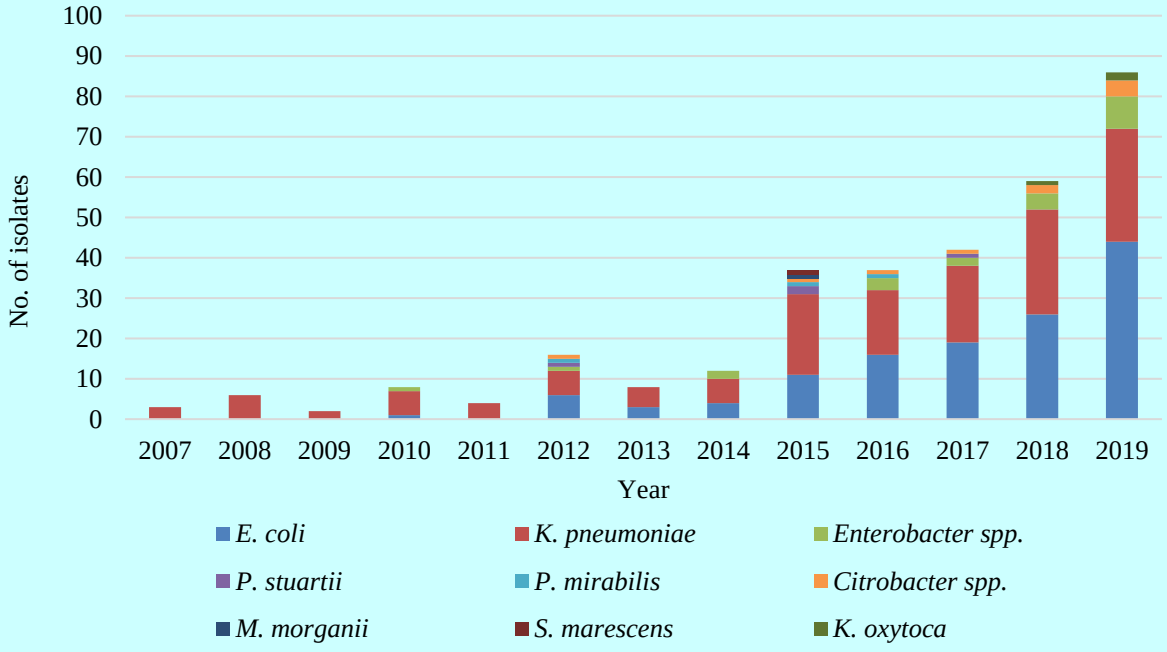


FIGURE 76. Number of carbapenemase-producing *Enterobacterales* isolates according to species.

With respect to carbapenemase-variants, a marked increase in NDM-positive isolates was observed from 14 in 2018 to 43 in 2019 (Figure 77). The number of isolates with OXA-48-variants remained stable. Three isolates were identified harbouring two carbapenemases, both NDM and OXA-48 (n=2) or NDM and IMP (n=1). KPC, VIM and IMI were identified in four, two and one isolates, respectively.

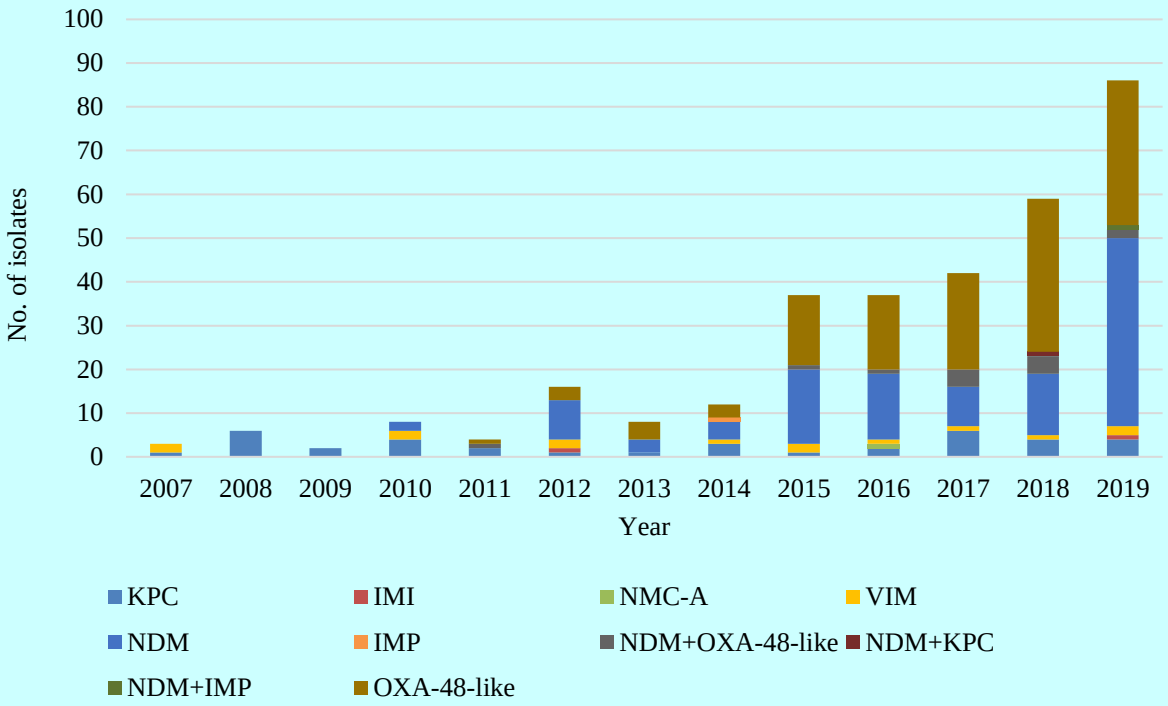


FIGURE 77. Number of carbapenemase-producing *Enterobacterales* according to carbapenemase variant.

WGS of *E. coli* and *K. pneumoniae* showed a relatively large genetic diversity with respect to sequence type (ST) and carbapenemase-variants. However, cases of closely related isolates were observed (Figures 78-79). Seventeen different STs were observed among the *E. coli* isolates. The dominant combination was ST167 with NDM-5, identified in eight patients of which the isolates from three cases were closely related (6-7 single nucleotide polymorphisms; SNPs) (Figure 78). The isolates were identified at three different laboratories within a nine-month period. Thus, no clear epidemiological connection was identified from the information acquired at the laboratory level. The same situation was also observed with two other clusters of closely related isolates. This included two cases of ST38 with OXA-244 (10 SNP differences) and two cases of ST648 with NDM-5 (five SNP differences). An increase of ST38-OXA-244 in Europe has recently been reported (1). Globally, widespread STs of known extra-intestinal pathogenic *E. coli*, including ST38, ST131, ST167, ST405, ST410 and ST648 were identified (2).

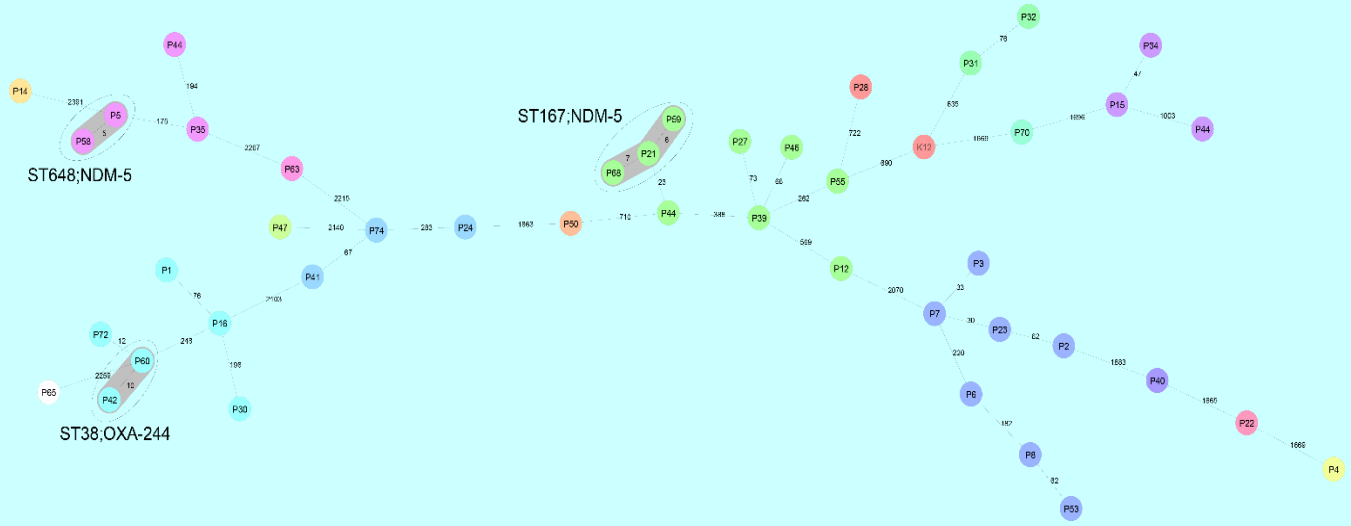


FIGURE 78. Minimum spanning tree based on the core genome allele profile of carbapenease-producing *E. coli* in Norway 2019, using SeqSphere and *E. coli* K12 as reference strain. The isolates are coloured according to ST. Closely related isolates (≤ 10 SNPs) are highlighted with grey shading.

Twelve different STs were observed among the 28 carbapenemase-producing *K. pneumoniae* isolates. The dominant combination was ST147 with NDM-1 identified in seven patients (Figure 79). Nosocomial transmission was confirmed for two cases where the isolates showed only one SNP difference. Two cases of ST392 with OXA-48 with 11 SNPs differences were observed, but the isolates were identified at two different laboratories within a three-month period and one case was associated with travel to Gran Canaria. No epidemiological connection was identified for the other ST-carbapenemase-variant combinations, but several of the ST variants observed (e.g. ST11, ST17, ST37, ST147 and ST307) are known, globally widespread clones associated with the spread of carbapenemases (3,4).

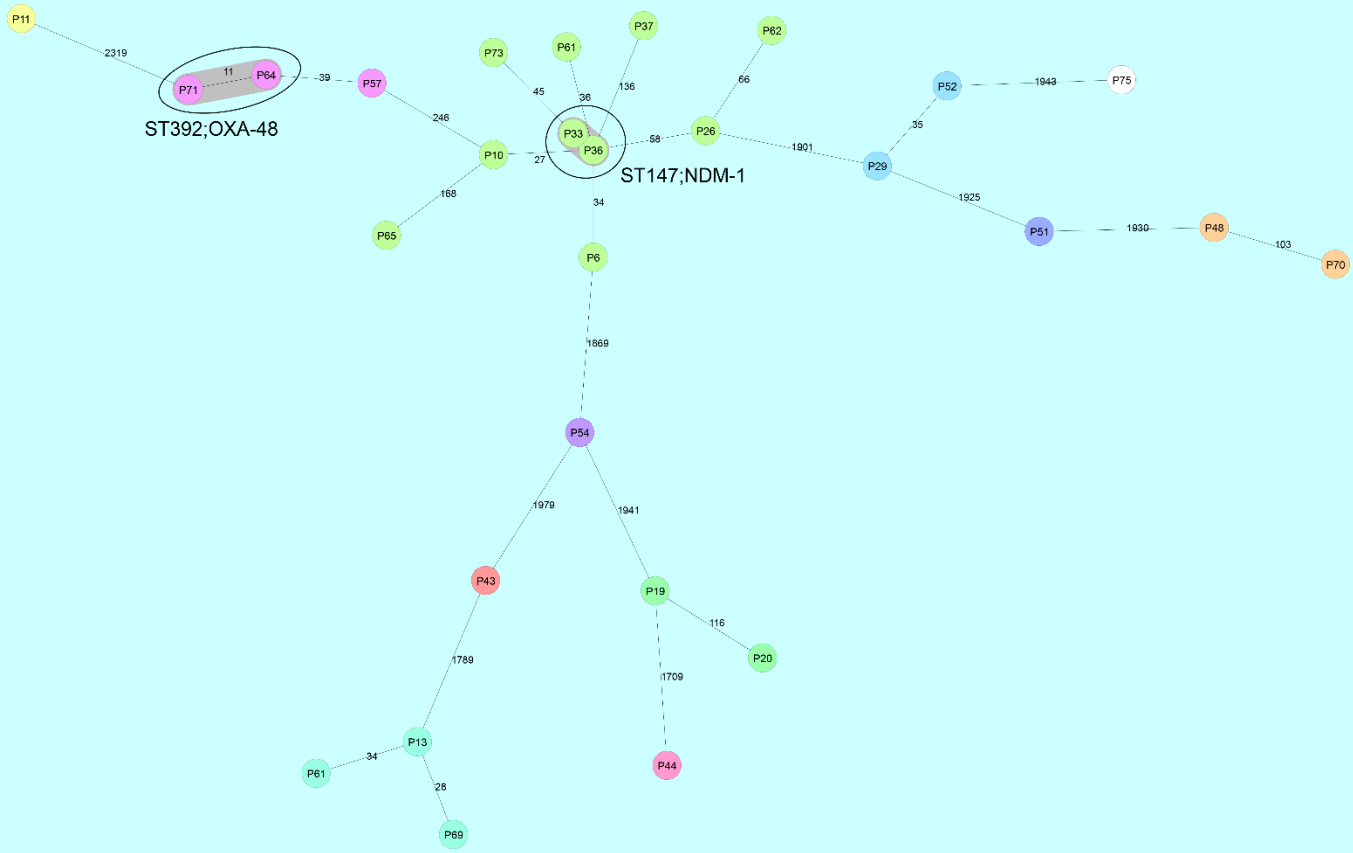


FIGURE 79. Minimum spanning tree based on the core genome allele profile of carbapenease-producing *K. pneumonia* in Norway 2019, using SeqSphere and *K. pneumoniae* NTUH-K2044 as reference strain. The isolates are coloured according to ST. Closely related isolates (≤15 SNP) are highlighted with grey shading.

WGS of the 14 cases of other carbapenemase-producing *Enterobacterales* observed in 2019 showed a diversity of ST-carbapenemase-variant combination (Table 64). One case of possible transmission was identified related to two cases of *Klebsiella oxytoca* ST46 with OXA-48.

TABLE 64. ST-carbapenemase-variant combinations identified in *Enterobacter* sp, *Citrobacter* sp. and *K. oxytoca.*

Species	ST-carbapenemase-variant combination
Enterobacter sp. (n=8)	ST78-NDM-1+IMP-4 (*n*=1); ST114-NDM-1 (*n*=1); ST121-VIM-1 (*n*=1); ST171-NDM-1 (*n*=1); ST171-NDM-4 (*n*=1); ST264-VIM-1 (*n*=1); ST412-IMI-1 (*n*=1); ST462-NDM-1 (*n*=1)
Citrobacter sp. (n=4)	ST96-OXA-48 (*n*=1); ST107-NDM-1 (*n*=1); ST112-NDM-1+OXA-181 (*n*=1); ST-novel-NDM-1 (*n*=1)
K. oxytoca (n=2)	ST46-OXA-48 (*n*=2)

Five cases of carbapenemase-producing *Pseudomonas aeruginosa* were identified in 2019 compared to three cases in 2018. Different ST-carbapenemase-variant combinations were observed in all cases (Table 65).

TABLE 65. ST-carbapenemase-variant combinations identified in *Pseudomonas aeruginosa.*

ST235-NDM-1 (*n*=1); ST308-NDM-1 (*n*=1); ST357-IMP-13 (*n*=1); ST654-VIM-2 (*n*=1); ST1047-VIM-2 (*n*=1)

We observed a minor increase in carbapenemase-producing *Acinetobacter* from 19 cases in 2018 to 23 cases in 2019. In total, 24 isolates were identified as two genetically unrelated *A. baumannii* with the same carbapenemase gene ($bla_{\text{OXA-72}}$) was identified in one patient. *A. baumannii* was the dominant species and OXA-23 the dominant carbapenemase gene (Table 66). *A. baumannii* ST2 is a globally widespread clone frequently associated with carbapenemase genes (5) which could explain the dominance. For the carbapenemase-producing *A. baumannii*, within-country transmission was not identified based on the WGS and epidemiological data. Four of the cases with NDM-producing non-*A. baumannii* species were identified at two laboratories in the same health region that also observed NDM-producing non-*A. baumannii* in 2018.

TABLE 66. ST-carbapenemase-variant combinations identified in *Acinetobacter* sp.

Species	ST-carbapenemase-variant combination
A. baumannii (n=18)	ST2-OXA-23 (n=6); ST2-NDM-1+OXA-23 (n=2); ST636-OXA-72 (n=2); ST1-OXA-23 (n=1); ST15-OXA-23 (n=1); ST78-OXA-72 (n=1); ST158-OXA-23 (n=1); ST160-OXA-23 (n=1); ST717-OXA-23 (n=1); ST-novel-NDM-1+OXA-23 (n=1); ST-novel-OXA-72 (n=1)
A. pittii (n=3)	NDM-1 (n=2); OXA-72 (n=1)
A. lwoffii (n=1)	NDM-1 (n=1)
A. nosocomialis (n=1)	NDM-1+OXA-58 (n=1)
A. soli (n=1)	NDM-1 (n=1)

Conclusion

The number of carbapenemase-producing Gram-negative bacteria is increasing in Norway. The number of patients identified with CPE increased from 54 in 2018 to 75 in 2019. A gradual, but smaller increase was observed for *Acinetobacter* and *Pseudomonas*. Phylogenetic analysis shows small clusters of closely related strains, but there is no clear evidence for interregional spread. In general, the spread is associated with a relatively high diversity of clones and carbapenemase genes. A marked increase in carbapenemase-producing *E. coli* between 2018-2019 was observed compared to other *Enterobacterales* species. Among the different carbapenemase genes the increase was highest for NDM-variants. For *E. coli*, *K. pneumoniae* and *A. baumannii*, globally disseminated clones associated with specific carbapenemase genes were observed.

References

1. European Centre for Disease Prevention and Control. OXA-244-producing *Escherichia coli* in the European Union/European Economic Area and the UK since 2013 – 18 February 2020. ECDC: Stockholm; 2020. https://www.ecdc.europa.eu/sites/default/files/documents/RRA-E-coli-OXA-244-producing-E-coli-EU-EEA-UK-since-2013.pdf
2. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev. 2019; 32(3):e00135-18. doi: 10.1128/CMR.00135-18.
3. Wyres KL, Lam MMC, Holt KE. Population genomics of *Klebsiella pneumoniae*. Nat Rev Microbiol.2020 Jun;18(6):344-359. doi: 10.1038/s41579-019-0315-1.
4. David S, Reuter S, Harris SR, Glasner C, Feltwell T, Argimon S, Abudahab K, Goater R, Giani T, Errico G, Aspbury M, Sjunnebo S; EuSCAPE Working Group; ESGEM Study Group, Feil EJ, Rossolini GM, Aanensen DM, Grundmann H. Epidemic of carbapenem-resistant *Klebsiella pneumoniae* in Europe is driven by nosocomial spread. Nat Microbiol. 2019 Nov;4(11):1919-1929. doi: 10.1038/s41564-019-0492-8.
5. Hamidian M, Nigro SJ. Emergence, molecular mechanisms and global spread of carbapenem-resistant *Acinetobacter baumannii*. Microb Genom. 2019 Oct;5(10):e000306. doi: 10.1099/mgen.0.000306.

Ørjan Samuelsen, Jessin Janice and Arnfinn Sundsfjord, Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Department of Microbiology and Infection Control, University Hospital of North Norway and UiT – The Arctic University of Norway, Tromsø, and Petter Elstrøm and Oliver Kacelnik, Department of Antibiotic Resistance and Infection Prevention, Norwegian Institute of Public Health, Oslo, Norway.

Neisseria meningitidis in blood cultures and cerebrospinal fluids

TABLE 67. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2019 (n=16). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Penicillin G*	≤ 0.06	> 0.25	87.5	12.5	0.0
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.03	> 0.03	100.0	-	0.0
Chloramphenicol	≤ 2	> 2	100.0	-	0.0
Rifampicin	≤ 0.25	> 0.25	100.0	-	0.0
Tetracycline	≤ 2	> 2	100.0	-	0.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *Penicillin G=Benzylpenicillin.

TABLE 68. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2019 (n=16). Distribution (n) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G*				3	11	1	1									
Ceftriaxone			16													
Ciprofloxacin	16															
Chloramph.						1	3	11	1							
Rifampicin	12	3	1													
Tetracycline			1		1	12		2								
Azithromycin						9	6	1								

Shaded areas in each row indicate susceptibility with standard exposure (light), susceptibility with increased exposure (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. *Penicillin G=Benzylpenicillin.

RESULTS AND COMMENTS

N. meningitidis from blood cultures and cerebrospinal fluids were first included in NORM in 2013. The Reference Laboratory at the Norwegian Institute of Public Health provides data on *N. meningitidis* on a yearly basis. The results are presented in Tables 67-68.

A total of 16 isolates were recovered from blood cultures (n=12), cerebrospinal fluids (n=3) and "other material" (n=1). All isolates were from unique patients and there were no known associations between the cases. The isolates belonged to serogroups B (n=5), C (n=1), Y (n=6) and W (n=4). The serogroup C and the serogroup W isolates belonged to the sequence type (ST) 11 clonal complex while the serogroup Y isolates belonged to the ST-23 clonal complex; thus these two complexes accounted for 69% of the cases. Serogroup W isolates belonging to ST-11 have recently been increasing elsewhere in Europe. Two of the serogroup B isolates with the same genotype were both isolated in Vestre Viken 8 months apart. One isolate displayed a penicillin G MIC of 0.25 mg/L and was thus only susceptible to increased exposure to this agent. The genetic basis for non-susceptibility was not determined, but was most likely caused by alterations in the penicillin-binding protein 2 (PBP2) encoded by *penA*. All isolates were fully susceptible to ceftriaxone, ciprofloxacin, chloramphenicol, and rifampicin. NordicAST/EUCAST has recently defined a breakpoint of 2 mg/L for tetracycline resistance as this agent can be used to predict sensitivity to minocycline prophylaxis against *N. meningitidis*. All isolates displayed MIC values below this value. Similarly, no clinical breakpoints have been established for azithromycin, but the MIC distribution does not indicate the presence of acquired macrolide resistance (Table 68).

Neisseria gonorrhoeae

TABLE 69. *Neisseria gonorrhoeae* from all specimen types in 2019 (n=623). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Penicillin G*	≤ 0.06	> 1	1.3	80.1	18.6
Ceftriaxone	≤ 0.125	> 0.125	99.7	-	0.3
Cefixime	≤ 0.125	> 0.125	98.9	-	1.1
Ciprofloxacin	≤ 0.03	> 0.06	40.9	0.0	59.1
Tetracycline	≤ 0.5	> 1	45.7	23.3	31.0
Spectinomycin	≤ 64	> 64	100.0	-	0.0
Beta-lactamase	Negative	Positive	84.6	-	15.4

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant.

TABLE 70. *Neisseria gonorrhoeae* from all specimen types in 2019 (n=623). Distribution (%) of MICs (mg/L).

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G*			0.5	0.8	2.1	12.5	23.8	27.8	14.0	5.6	1.8	1.0	1.8	8.5		
Ceftriaxone	45.4	24.7	18.5	4.8	4.2	2.1	0.2									
Cefixime			79.3	10.9	6.3	2.4	1.1									
Ciprofloxacin	28.9	6.3	4.2	1.6		0.6	1.8	5.3	7.4	10.0	8.5	6.1	2.7	16.7		
Tetracycline	0.2				1.8	10.9	13.8	19.1	23.3	10.4	1.1	3.0	11.9	3.7	0.5	0.3
Spectinomycin					0.2					0.2	1.8	22.8	70.8	4.3		
Azithromycin			0.2	0.8	4.8	16.5	31.3	18.1	8.8	13.8	4.8	0.5				0.3

Shaded areas in each row indicate susceptibility with standard exposure (light), susceptibility with increased exposure (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. *Penicillin G=Benzylpenicillin.

RESULTS AND COMMENTS

Neisseria gonorrhoeae was surveyed in NORM in 2003 and 2010, and then yearly since 2013. The results for 2019 are not directly comparable to 2018 as only samples submitted to Oslo University Hospital were included in the statistics in that year. Data were submitted to NORM by the reference laboratory at the Norwegian Institute of Public Health. Only a single isolate from each disease episode was included from each patient. The microbiological data could not be linked to information in the Norwegian Surveillance System for Communicable Diseases (MSIS).

In 2019, a total of 623 isolates were available for analysis. The isolates were reported to originate from urethra (n=249), cervix uteri (n=76), anus (n=177), throat (n=95), eye (n=3) or "others/unknown" (n=23). A total of 522 (83.8%) isolates originated from men, 96 (15.4%) from women and 5 (0.8%) from unknown gender. The geographical location where the infection was acquired was in most cases unknown to the laboratory. From MSIS it is reported that gonococcal infections frequently are acquired abroad with secondary transmission in sexual networks within Norway. There is an ongoing outbreak among men who have sex with men, but the strains linked to this outbreak could not be identified in the NORM protocol.

The results from susceptibility testing are presented in Tables 69-70. A majority of isolates were either susceptible to increased exposure (80.1%) or resistant (18.6%) to penicillin G. The corresponding figures for 2017 were 81.5% and 16.6%, respectively. Ninety-six isolates (15.4%) produced beta-lactamase and were phenotypically resistant to penicillin G, which is at the same level as in 2017 (15.3%). Most beta-lactamase positive isolates (89/96, 92.7%) were also resistant to ciprofloxacin. Fourty-one isolates (7.8%) were resistant, and 478 (90.7%) were only susceptible to increased exposure to penicillin G in spite of being beta-lactamase negative. This illustrates the alternative mechanisms for penicillin resistance, such as alterations in penicillin binding proteins (PBPs) and/or reduced permeability through the outer cell membrane.

A single isolate was categorised as resistant to ceftriaxone with an MIC of 0.25 mg/L. Ceftriaxone resistant isolates from Norway have previously been linked to treatment failure. Six additional isolates were susceptible to ceftriaxone, but resistant to cefixime. Cefixime is no longer recommended for empirical treatment in Norway. The results confirm the emergence of cephalosporin resistant gonococci in Norway, which is extremely alarming from both a clinical and a public health perspective.

The current European treatment guidelines recommend empirical combination treatment with ceftriaxone and azithromycin. It should be noted that 19.4% of the isolates displayed azithromycin MIC values above the EUCAST screening breakpoint for acquired resistance at 1 mg/L. The corresponding figure for 2017 was 4.7%.

Ciprofloxacin was previously used for empirical treatment of gonorrhoeae acquired outside South-East Asia. The prevalence of ciprofloxacin resistance persisted at a high level (59.1%) in 2019. Ciprofloxacin is consequently not a viable alternative except in cases where susceptibility has been documented. All strains were susceptible to the aminocyclitol spectinomycin.

Staphylococcus aureus in blood cultures

TABLE 71. *Staphylococcus aureus* blood culture isolates in 2019 (n=1,492). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Erythromycin	≤ 1	> 2	94.5	0.1	5.4
Clindamycin	≤ 0.25	> 0.5	98.5	0.2	1.3
Fusidic acid	≤ 1	> 1	96.4	-	3.6
Ciprofloxacin	≤ 0.001	> 1	0.0	95.4	4.6
Gentamicin	≤ 1	> 1	99.8	-	0.2
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.1	0.5	0.4
Tetracycline	≤ 1	> 2	97.4	0.5	2.1
Tigecycline	≤ 0.5	> 0.5	97.0	-	3.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.6	0.1	0.3
Beta-lactamase	Negative	Positive	29.4	-	70.6
Cefoxitin screen	≥ 22	< 22	99.2	-	0.8
MRSA** (*mecA*)	Negative	Positive	99.2	-	0.8

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. MRSA=methicillin resistant *Staphylococcus aureus*. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only. **MRSA=Methicillin resistant *Staphylococcus aureus*.

RESULTS AND COMMENTS

Twelve methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2019, corresponding to a prevalence of 0.8% (Table 71). This is at the same level as in 2017 and 2018 (both 0.8%). The resistance phenotype was confirmed by *mecA* PCR in all cases. The isolates originated from ten different hospitals. Laboratory screening for MRSA in NORM is performed using cefoxitin disks. A single MRSA isolate was repeatedly susceptible to cefoxitin (zone diameter 24 mm), but was detected by molecular methods in the routine laboratory. Some MRSA isolates were concomitantly resistant to erythromycin (6/12), ciprofloxacin (3/12), tetracycline (3/12), clindamycin (1/12), and/or fusidic acid (1/12). All MRSA isolates were susceptible to gentamicin, tigecycline, trimethoprim-sulfamethoxazole, linezolid and rifampicin. The results from susceptibility testing of all Norwegian MRSA isolates are presented in Table 76 on page 121. No methicillin susceptible *S. aureus* (MSSA) isolates were reported with cefoxitin zone diameters below the screening breakpoint. The NORM findings are at the same level as the reports from the databases of the participating laboratories where 19 out of 2,142 (0.9%) *S. aureus* blood culture isolates were MRSA. None of the 16 *S. aureus* isolates recovered from cerebrospinal fluids were methicillin resistant, thus bringing the total number of systemic MRSA isolates to 19/2,158 (0.9%). This is at the same level as in 2018 (0.8%).

Eighty *S. aureus* isolates (5.4%) were resistant to erythromycin. This is at the same level as in previous years (5.2% in 2016, 3.0% in 2017, and 5.3% in 2018). The macrolide resistance phenotypes of erythromycin resistant isolates were determined by the double disk diffusion (DDD) test. Nine isolates (11%) were constitutively MLS_B resistant, 52 (65%) were inducibly MLS_B resistant, and 19 (24%) displayed efflux mediated M-type resistance. These figures represent 0.6%, 3.5% and 1.3% of all *S. aureus* isolates from blood cultures, respectively. The distribution of MLS phenotypes was essentially unchanged from 2018 to 2019.

The prevalence of resistance to fusidic acid at 3.6% was at the same level as 4.1% in 2017 and 3.0% in 2018. The 4.6% prevalence of ciprofloxacin resistance was essentially unchanged from 4.4% in 2018, but below 6.9% in 2016. The breakpoint for susceptibility to ciprofloxacin was reduced from S ≤ 1 mg/L to S ≤ 0.001 mg/L in 2020, thus the wild-type population of *S. aureus* is now defined as susceptible only to increased exposure to this agent. There were no significant changes for gentamicin, rifampicin, tigecycline or trimethoprim-sulfamethoxazole. A single isolate was resistant to linezolid by disk diffusion (zone diameter 20 mm), but the MIC value remained in the susceptible range (4 mg/L). The general test panel for *S. aureus* did not include vancomycin in 2019.

Figure 80 shows the prevalence of resistance to various antimicrobials. A total of 70.6% of the isolates were beta-lactamase positive, which is at the same level as 70.3% in 2017 and 69.8% in 2018. There were only minor differences in the prevalence of resistance to non-beta-lactam antibiotics between beta-lactamase positive and negative isolates.

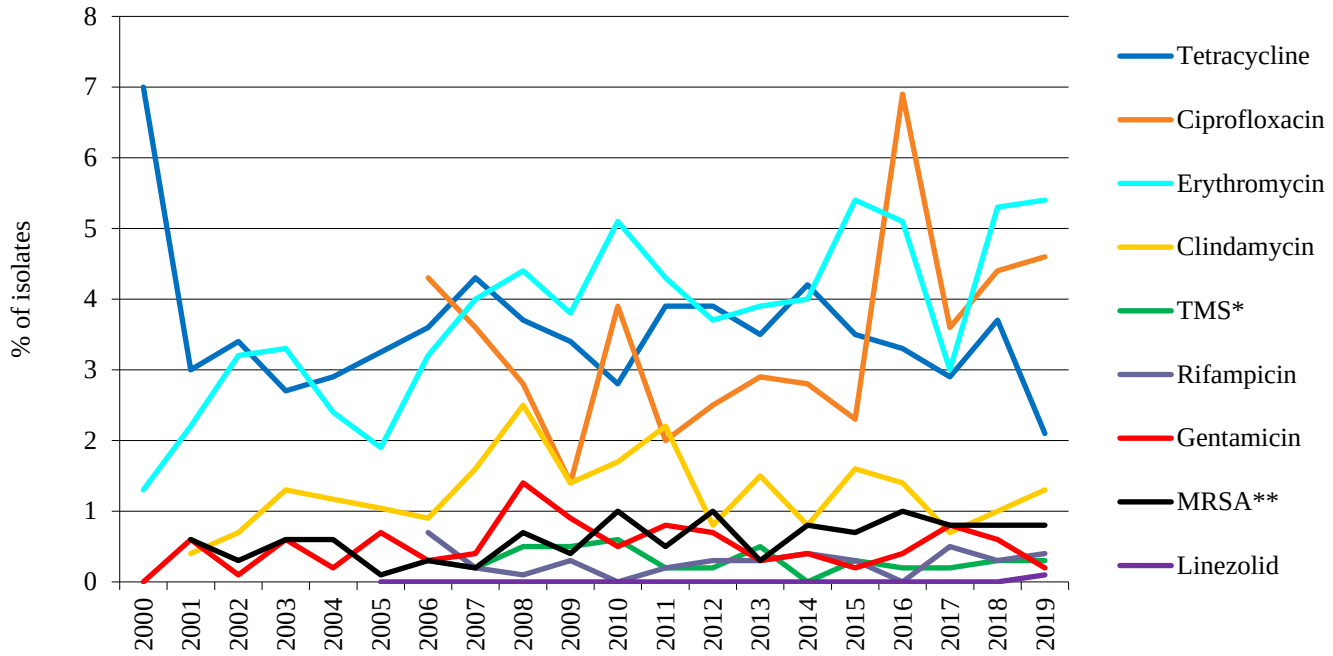


FIGURE 80. Prevalences of antimicrobial resistance among *Staphylococcus aureus* blood culture isolates 2000-2019. Doxycycline was replaced by tetracycline in 2006. Isolates are categorised according to the breakpoints at the time of analysis for each year. *TMS=Trimethoprim-sulfamethoxazole. **MRSA=Methicillin resistant *Staphylococcus aureus*.

Staphylococcus aureus in wound specimens

TABLE 72. *Staphylococcus aureus* isolates from wound specimens in 2019 (n=1,014). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Erythromycin	≤ 1	> 2	94.1	0.0	5.9
Clindamycin	≤ 0.25	> 0.5	99.3	0.2	0.5
Fusidic acid	≤ 1	> 1	94.7	-	5.3
Ciprofloxacin	≤ 0.001	> 1	0.0	97.2	2.8
Gentamicin	≤ 1	> 1	99.4	-	0.6
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.3	0.5	0.2
Tetracycline	≤ 1	> 2	94.4	0.6	4.0
Tigecycline	≤ 0.5	> 0.5	99.0	-	1.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.6	0.1	0.3
Beta-lactamase	Negative	Positive	25.0	-	75.0
Cefoxitin screen	≥ 22	< 22	98.7	-	1.3
MRSA** (*mecA*)	Negative	Positive	98.7	-	1.3

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only. **MRSA=Methicillin resistant *Staphylococcus aureus*.

RESULTS AND COMMENTS

S. aureus from wound specimens were screened for methicillin resistance by the cefoxitin disk method in the same way as blood culture isolates. Thirteen out of 1,014 (1.3%) isolates were confirmed as MRSA by *mecA* PCR. The prevalence was at the same level as in 2017 (1.2%) and 2018 (1.7%). The MRSA isolates originated from patients visiting general practitioners (n=7), hospital wards (n=4), an outpatient clinic (n=1), and a nursing home (n=1) in different parts of the country. Most MRSA isolates were co-resistant to erythromycin (7/13), tetracycline (2/13), ciprofloxacin (1/13), fusidic acid (1/13) and/or gentamicin (1/13) in different combinations. All MRSA isolates were susceptible to clindamycin, rifampicin, linezolid and trimethoprim-sulfamethoxazole. No isolates were reported with zone diameters below the cefoxitin screening breakpoint without being confirmed as MRSA by *mecA* PCR. This indicates high specificity of the cefoxitin screen as well as a low prevalence of *mecC* MRSA (see page 119).

The prevalence of resistance to fusidic acid in *S. aureus* wound isolates decreased from 5.9% in 2018 to 5.3% in 2019 (Table 72 and Figure 81). This confirms that the gradually declining prevalence of fusidic acid resistance has now levelled off after the epidemic which peaked at 25.0% in 2004. The prevalence of resistance to fusidic acid is still lower in blood culture isolates (3.6 %).

For other antimicrobial agents such as trimethoprim-sulfamethoxazole, gentamicin, rifampicin, and tetracycline there were only minor changes from 2018 to 2019, and the prevalence of resistance was in general similar for blood culture isolates and isolates from wound specimens. All isolates remained phenotypically susceptible to linezolid. Sixty (5.9%) isolates were resistant to erythromycin, which is a slight increase from 5.3% in 2017 and 5.5% in 2018. Fifty-nine of them were further examined for determination of resistance phenotype and the majority were either inducibly (43/59, 73% of erythromycin resistant isolates) or constitutively (2/59, 3% of erythromycin resistant isolates) resistant to clindamycin, thus representing the $iMLS_B$ and $cMLS_B$ phenotypes, respectively. A minor proportion of the isolates displayed low-level resistance to erythromycin only (14/59, 24% of erythromycin resistant isolates) compatible with efflux mediated M-type resistance. The findings are in accordance with the results from previous years.

A total of 75.0% of the isolates were beta-lactamase positive compared to 74.6% in 2017 and 72.6% in 2018. Beta-lactamase negative isolates were more likely to be resistant to erythromycin (7.5%) and ciprofloxacin (3.6%) compared to beta-lactamase positive isolates (5.4% and 2.5%, respectively). For the other antimicrobials there were only minor differences.

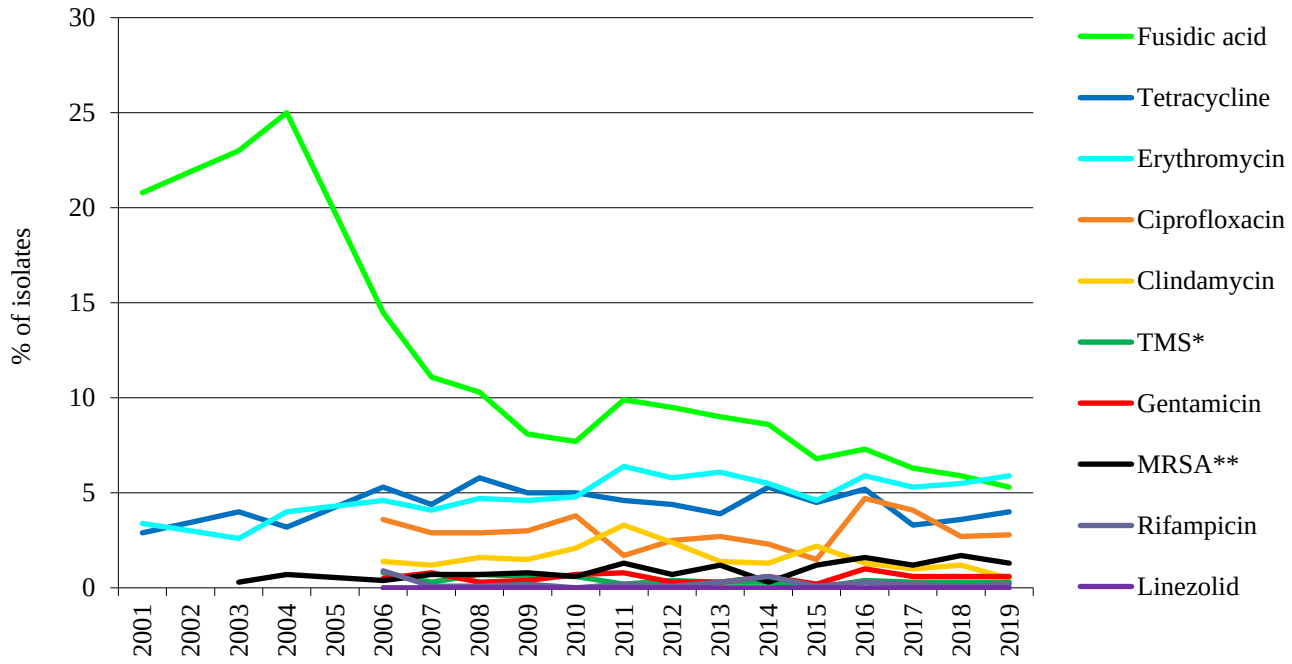


FIGURE 81. Prevalence of antimicrobial resistance among *Staphylococcus aureus* wound isolates 2001-2019. Doxycycline was replaced by tetracycline in 2006. Isolates are categorised according to the breakpoints at the time of analysis for each year. *TMS=Trimethoprim-sulfamethoxazole. **MRSA=Methicillin resistant *Staphylococcus aureus*.

Methicillin resistant *Staphylococcus aureus* (MRSA) infections in Norway 2019

The total number of people notified to MSIS with MRSA has not increased in the last three years (incidence rate ratio (IRR) 0.99; 95% CI 0.97 – 1.01). In all 2,474 notifications from 2,444 persons were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2019, giving an incidence rate of 46 persons per 100,000 person years. Of these, 945 (38%) patients were notified with clinical infections while 1,499 were colonised (Figure 82).

In the last five years, the annual number of infections has gradually risen (IRR 1.02; 95% CI 1.00 – 1.04). During the same period, the number of persons reported colonised reached a peak in 2017, and has been decreasing significantly in the last two years (IRR 0.93; 95% CI 0.90 – 0.97).

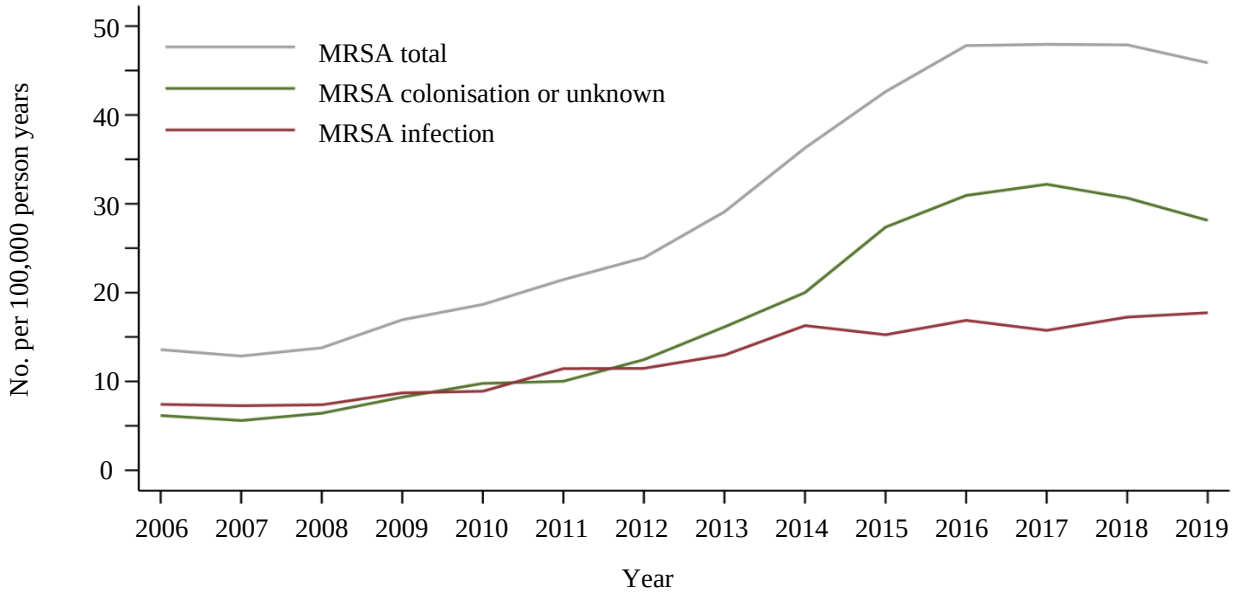


FIGURE 82. Number of persons notified with MRSA per 100,000 person years in Norway 2006-2019, by infection and colonisation.

The rise in MRSA infections is statistically significant among patients in hospitals, while the annual number of infections diagnosed in the community or in nursing homes has not changed in the last five years (Table 73).

TABLE 73. Number of notified MRSA infections in 2014 and 2019, percentage change and incidence rate ratio of annual infections in the period 2014-2019.

Place of diagnosis	No. of inf. 2014	No. of inf. 2019	% change	IRR	95% CI
Hospital	189	257	+ 36%	1.05	1.04 – 1.07
Nursing home	22	28	+ 27%	0.99	0.90 – 1.10
General practitioner	621	663	+ 7%	1.01	0.99 – 1.03

As seen in previous years, people infected in other countries make up a large part of the total number of people diagnosed with MRSA in Norway. In 2019, 39% of all people notified were reported or assessed as infected in other countries.

Less than five people were notified with livestock associated MRSA in 2019.

The Norwegian Reference Laboratory for Methicillin Resistant *Staphylococcus aureus* (MRSA) at St. Olavs Hospital, Trondheim University Hospital, received 2,697 MRSA isolates in 2019. 1,910 isolates were prioritised for genotyping staphylococcal protein A (*spa*) typing, 354 isolates were randomly selected for genotyping, and 5 isolates were genotyped by request from local microbiology laboratories. Among the genotyped isolates, 319 different *spa*-types were identified, 173 *spa*-types were reported as single events, and 111 *spa*-types were reported from two to ten times. Only 35 *spa*-types were reported more than 10 times. Table 74 shows the 10 most common *spa*-types in Norway, 2019.

TABLE 74. The 10 most common *spa*-types in Norway in 2019.

spa-type	CC	No. of isolates	% of isolates genotyped
t002	5	153	8.0
t127	1	129	6.8
t008	8	125	6.5
t304	6	123	6.4
t019	30	91	4.8
t223	22	82	4.3
t034	398	47	2.5
t105	5	42	2.2
t1476	8	41	2.1
t437	59	38	2.0

Based on *spa*-type, the isolates were assigned to multilocus sequence type (MLST) and clonal complex (CC) (Table 75). The 10 most prevalent CCs comprised 1,526 isolates (88.0%).

TABLE 75. The 10 most common clonal complexes (CC) in human clinical strains in Norway in 2019.

CC	*spa*-types grouped in CC*	No. of isolates	% of isolates genotyped
5	t002 (153), t105 (42), t688 (25), t003 (12), t3217 (13)	327	17.1
8	t008 (125), t1476 (41), t024 (17), t064 (8), t1767 (5), t4549 (5)	231	12.1
1	t127 (129), t657 (32), t386 (15), t345 (8), t177 (5)	217	11.3
22	t223 (82), t005 (32), t309 (11), t8934 (8), t2933 (8)	186	9.7
30	t019 (91), t021 (35), t665 (9), t363 (7), t318 (5)	185	9.7
6	t304 (123), t121 (5), t711 (4), t701 (3), t18538 (2), t5593 (2)	147	7.7
88	t690 (32), t786 (9), t1339 (7), t2526 (5), t186 (4)	96	5.0
45	t026 (14), t015 (13), t1081 (11), t004 (10), t550 (3)	75	3.9
398**	t034 (47), t011 (7), t1344 (1), t2123 (1)	56	2.9
59	t437 (38), t216 (8), t172 (2), t441 (3), t1950 (1)	54	2.8

*The five most common *spa*-types in each CC (n). **All isolates from patients with association to livestock are genotyped, in addition to other CC398 strains. This includes both PVL positive and negative strains.

The MRSA reference laboratory identified 21 Livestock Associated MRSA (LA-MRSA) (CC398, PVL (Panton-Valentine Leucocidin) negative) in humans, of *spa*-type t034 (n=13), t011 (n=7) and t1344 (n=1). Three isolates were positive for *mecC* (*spa*-type t843, CC130 (n=2) and t19020, ST2496 (belongs to no known CC)).

Antimicrobial susceptibility testing was performed by the local laboratories according to the EUCAST 2019 disk diffusion method and the NordicAST 2019 breakpoints (Table 76). The MRSA reference laboratory received 2,567 complete antibiograms. Among these strains, 1,073 (41.8%) were sensitive to all antibiotics tested except beta-lactams (cefoxitin). The highest proportion of resistance was found for erythromycin (33%), followed by tetracycline (25.9%) and ciprofloxacin (24.8%). The lowest rate of resistance was found for mupirocin (0.5%), rifampicin (0.6%) and trimethoprim-sulfamethoxazole (1.1%). No isolates showed decreased susceptibility to linezolid or vancomycin in 2019.

TABLE 76. MRSA isolates from human cases in 2019 (n=2,976). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Erythromycin	≤ 1	> 2	66.6	0.4	33.0
Clindamycin	≤ 0.25	> 0.5	90.2	2.1	7.8
Fusidic acid	≤ 1	> 1	88.5	-	11.5
Ciprofloxacin	≤ 0.001	> 1	0.0	75.2	24.2
Gentamicin	≤ 1	> 1	86.7	-	13.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	98.6	0.9	0.6
Tetracycline	≤ 1	> 2	73.6	0.5	25.9
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	97.2	1.7	1.1
Mupirocin	≤ 1	> 256	96.6	2.9	0.5
Vancomycin	≤ 2	> 2	100.0	-	0.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Whole genome sequencing (WGS) was performed on 190 isolates from samples received in 2019. This includes mainly MRSA strains (n=144), as well as a few MSSA (n=33) and other *Staphylococcus* species (n=47). Strains prioritised for WGS included outbreak investigations (n=40), blood cultures (n=7), unusual resistance phenotypes/profiles (n=11), genotyping of new *spa*- or sequence types (n=22), and selected projects (n=80).

The MRSA strains from blood cultures were of *spa*-type t008 (n=2), t1979 (n=1), t304 (n=1), t223 (n=1), t131 (n=1) and t121 (n=1). All of the strains were *mecA* positive, however only six of the strains were phenotypically cefoxitin resistant. Investigation of the genome sequence revealed that this strain had a premature stop codon in the *mecA* open reading frame, likely leading to a non-functional MecA protein. Other detected putative resistance genes include *blaZ* (n=6), *ant(6)-Ia* (n=3), *aph(3')-III* (n=3), *mphC* (n=2), *msrA* (n=2), *tetK* (n=1), and *fusB* (n=1).

Enterococcus spp. in blood cultures

TABLE 77. *Enterococcus* spp. blood culture isolates in 2019 (n=656). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 4	> 8	82.4	0.2	17.4
Imipenem	≤ 0.001	> 4	0.0	81.1	18.9
Gentamicin HLR*	≤ 128	> 128	82.9	-	17.1
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.25	95.3	-	4.7
Vancomycin (any genotype)	≤ 4	> 4	97.8	-	2.2
Vancomycin (*vanA* or *vanB*)	Negative	Positive	99.7	-	0.3

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *HLR=High Level Resistance.

TABLE 78. *Enterococcus faecalis* blood culture isolates in 2019 (n=470). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 4	> 8	100.0	0.0	0.0
Imipenem	≤ 0.001	> 4	0.0	99.6	0.4
Gentamicin HLR*	≤ 128	> 128	86.4	-	13.6
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.25	95.7	-	4.3
Vancomycin (*vanA* or *vanB*)	Negative	Positive	100.0	-	0.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *HLR=High Level Resistance.

TABLE 79. *Enterococcus faecium* blood culture isolates in 2019 (n=145). Sampling, laboratory methods, and data handling are described in Appendix 5. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Ampicillin	≤ 4	> 8	23.4	0.7	75.9
Imipenem	≤ 0.001	> 4	0.0	19.3	80.7
Gentamicin HLR*	≤ 128	> 128	67.6	-	32.4
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.25	93.8	-	6.2
Vancomycin (*vanA* or *vanB*)	Negative	Positive	98.6	-	1.4

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *HLR=High Level Resistance.

RESULTS AND COMMENTS

As in previous years, enterococci were analysed both as a genus and separately for *E. faecalis* and *E. faecium*. The results for each species are microbiologically more valid as resistance rates differ significantly between *E. faecalis* and *E. faecium*. However, overall rates of resistance are of interest when formulating empirical treatment strategies because they include the probability of systemic infections with each enterococcal species. The overall results for enterococci are presented in Table 77. The surveillance in NORM 2019 included 470 (71.6%) *E. faecalis* isolates (71.2% in 2018), 145 (22.1%) *E. faecium* isolates (23.5% in 2018), and 41 (6.3%) unspeciated enterococcal isolates (5.3% in 2018). The ratio of *E. faecalis* to *E. faecium* isolates has declined in many countries as the incidence of *E. faecium* bacteremia has increased. In Norway this ratio was 2.4 in 2017, 3.0 in 2018 and 3.2 in 2019, which is within the variation seen in previous years. The panel of antimicrobial agents examined remained unchanged from 2018 to 2019. The breakpoints for imipenem were changed from S ≤ 4 mg/L and R > 8 mg/L, to S ≤ 0.001 mg/L and R > 4 mg/L. The wild-type population was thus defined as only susceptible to increased exposure to this agent.

E. faecalis was universally susceptible to ampicillin (Table 78). The prevalence of resistance to ampicillin in *E. faecium* was 75.9% in 2019 compared to 72.9% in 2017 and 75.3% in 2018 (Table 79). As expected, the results for imipenem closely mirrored those for ampicillin. The prevalence of high-level gentamicin resistance (HLGR) in *E. faecalis* was 13.6%, which is a further decrease from 15.5% in 2017 and 14.1% in 2018 (Figure 83). The prevalence of HLGR in *E. faecium* was stable at 32.4% compared to 32.0% in 2018. Almost all (46/47) HLGR *E. faecium* isolates were concomitantly resistant to ampicillin and imipenem. Conversely, 46 of 110 (41.8%) ampicillin resistant *E. faecium* also displayed HLGR. High-level gentamicin resistance in enterococci is of great concern as it abolishes the bactericidal synergy between aminoglycosides and beta-lactams often used for treatment of severe enterococcal infections.

Transferable vancomycin resistance has not yet become endemically established in clinical enterococcal isolates in Norway, but recent outbreaks have occurred in different parts of the country. Fourteen blood culture isolates were reported as vancomycin resistant in NORM 2019 (2.2%), but only two of these were confirmed by PCR to harbour transferable vancomycin resistance (both *vanB E. faecium*). The two *vanB* isolates were isolated at different hospitals. The remaining twelve vancomycin resistant isolates were either *E. gallinarum* (n=11) or *E. casseliflavus* (n=1), which are inherently low-level resistant to vancomycin due to expression of the VanC ligase. All enterococcal isolates were susceptible to linezolid.

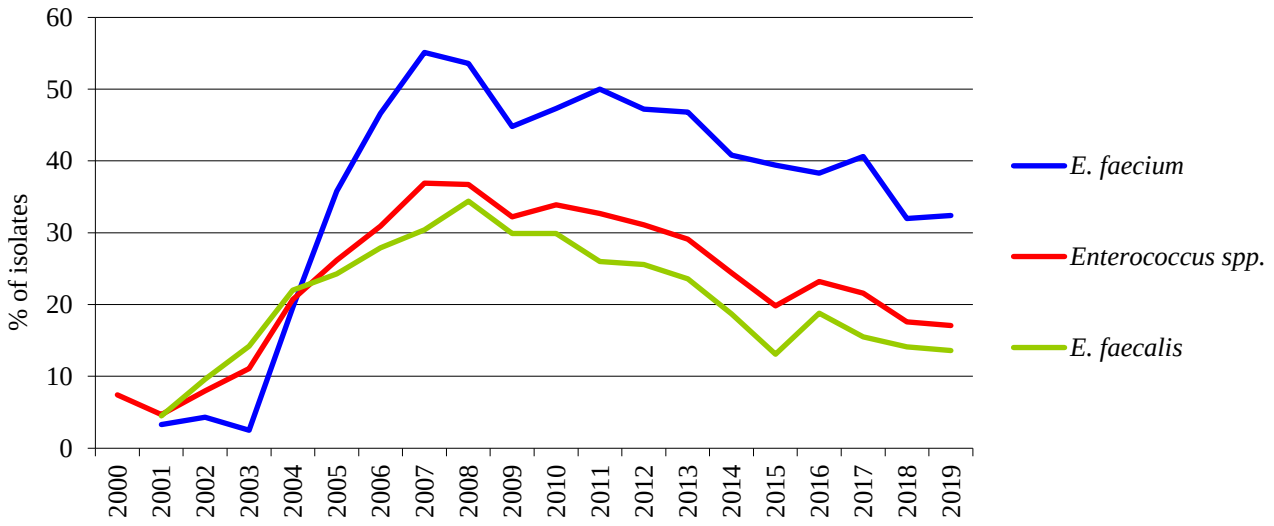


FIGURE 83. Prevalence of high-level resistance to gentamicin in blood culture isolates of *Enterococcus faecalis*, *E. faecium* and all enterococci combined during 2000-2019. The breakpoint was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

Vancomycin and linezolid resistant enterococci in Norway, 2019

Vancomycin resistant enterococci

Enterococci are the third most common bacterial cause of hospital associated infections in Europe (1) and the fifth most common bacterial genus in blood culture isolates in Norway (2). They are intrinsically resistant to many antimicrobial agents and readily acquire resistance towards new clinically important antimicrobials including vancomycin (3).

Vancomycin binds with high affinity to the peptidoglycan sidechain ends that are important for crosslinks in the peptidoglycan layer of the cell wall. Vancomycin resistance in enterococci is due to gene clusters contributing to changes in the sidechain ends leading to reduced affinity of vancomycin (4). Currently, nine vancomycin resistance encoding gene clusters (*vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG*, *vanL*, *vanM* and *vanN*) have been identified in enterococci, including *vanC* gene clusters that are intrinsic to *Enterococcus casseliflavus* and *Enterococcus gallinarum.* The other gene clusters are acquired and mostly occur in *Enterococcus faecalis* and/or *Enterococcus faecium*. The acquired gene clusters are associated with mobile genetic elements such as plasmids and integrative conjugative elements. The most common acquired gene clusters worldwide are *vanA* followed by *vanB* (5).

In Norway, vancomycin resistant enterococci (VRE) are notifiable to the Norwegian Surveillance System for Communicable Diseases (MSIS). The Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res) confirms the resistance phenotype with the reference method (microbroth dilution) and performs genetic characterisation with PCR and whole genome sequencing (WGS) on selected isolates to clarify resistance mechanisms and genetic relatedness between isolates monitoring regional/national spread.

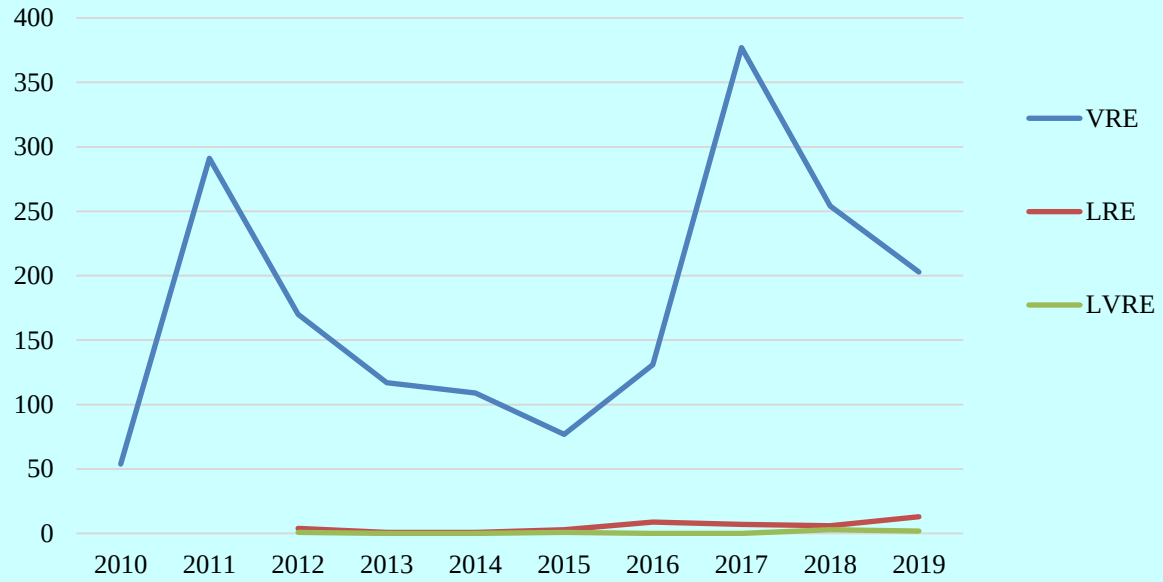


FIGURE 84. Number of vancomycin resistant (VRE), linezolid resistant (LRE), and both vancomycin and linezolid resistant (LVRE) enterococci in Norway 2010-2019. Combined data from MSIS.no and K-res.

In Europe, a worrying increase of vancomycin resistant *E. faecium* has been reported in the last three years (6), while in Norway the incidence of VRE has varied the last 10 years. A total of 204 VRE (including two LVRE) were reported in Norway in 2019 (Figure 84). K-res has received isolates and/or WGS data for 90 of these 204 (44%). This is not a complete overview of the VRE situation in Norway, however, trends can be observed. The distribution of VRE (including LVRE) by health regions and the number of WGS-characterised VRE are given in Table 80.

TABLE 80. Numbers of reported VRE and WGS-characterised VRE distributed by Health regions in Norway for 2019.

Health region	VRE	VRE with WGS data
South-Eastern	99	23
Western	95	61
Central	1	0
Northern	8	6
Unknown	1	0

The majority of the VRE from 2019 are *vanB E. faecium* (n=67) and *vanA E. faecium* (n=19). Particular findings include a single *E. faecium* isolate containing both *vanA* and *vanB* as well as *E. faecalis* with *vanE* (Figure 85). Worldwide, vancomycin resistant *E. faecium* is also much more prevalent than vancomycin resistant *E. faecalis* (7,8). In some European countries, as well as Australia, the prevalence of *vanB* has in periods been higher than of *vanA* (5). In Norway, *vanB E. faecium* is to a large extent associated with outbreaks in the Western and South-Eastern regions (Figure 86).

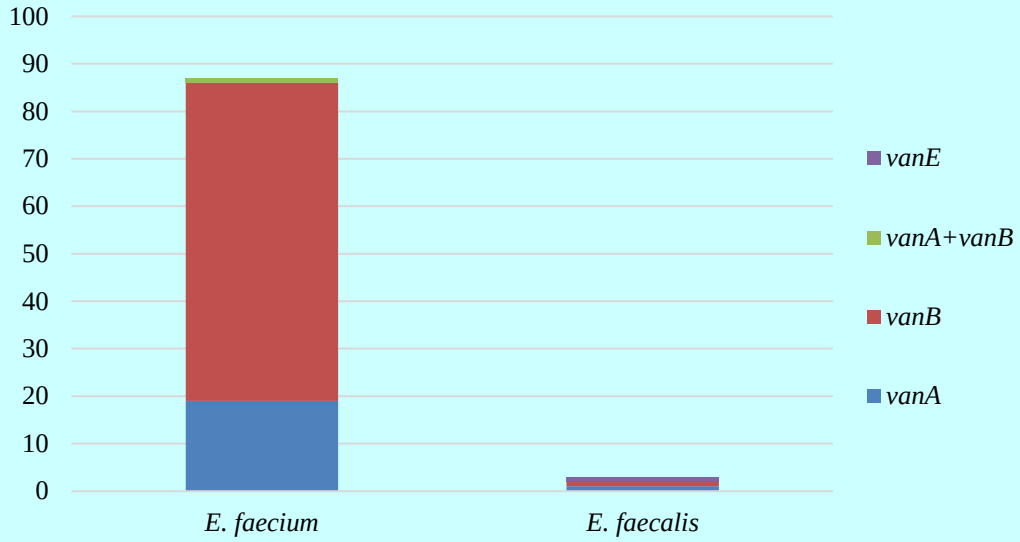


FIGURE 85. Distribution of VRE based on species and genotype of the 90 Norwegian VRE isolates that K-res has WGS data on.

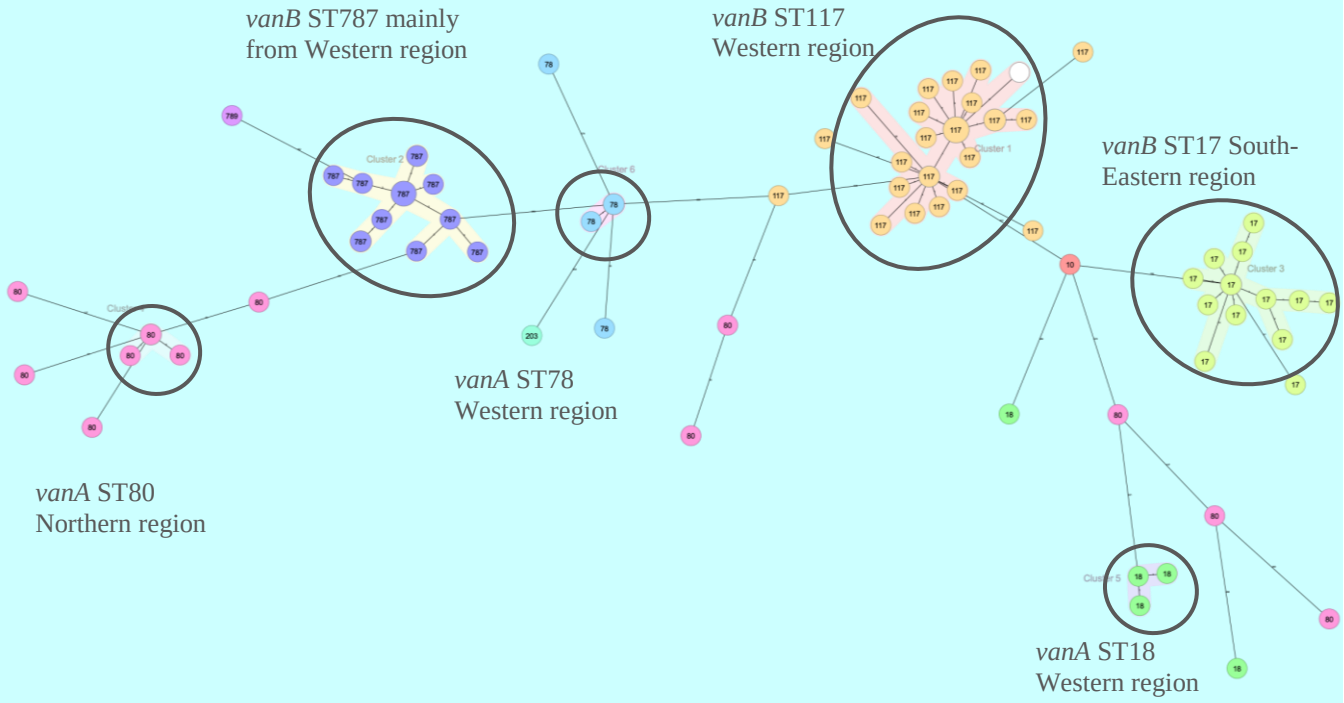


FIGURE 86. Minimum spanning tree based on the core genome allelic profiles of 87 VRE *E. faecium* isolates in Norway 2019, using the SeqSphere+ software and Aus0004 ST17 as reference strain. The isolates are coloured according to ST. Six genetically related clusters are encircled.

We observed eight different sequence types (STs) of *E. faecium* VRE in 2019 (Figure 87). All these belong to known hospital adapted clones that are also reported in many other countries. The most prevalent STs of *E. faecium* are linked to ST117 *vanB* and ST787 *vanB* clusters in the Western region, ST17 *vanB* in the South-Eastern region and ST80 *vanA* in the Northern region. Two smaller clusters of ST18 and ST78 with *vanA* were found in the Western region (Figure 86). We have also registered two different STs of *E. faecalis*, which have been linked previously to clinical isolates and hospitals (Figure 87).

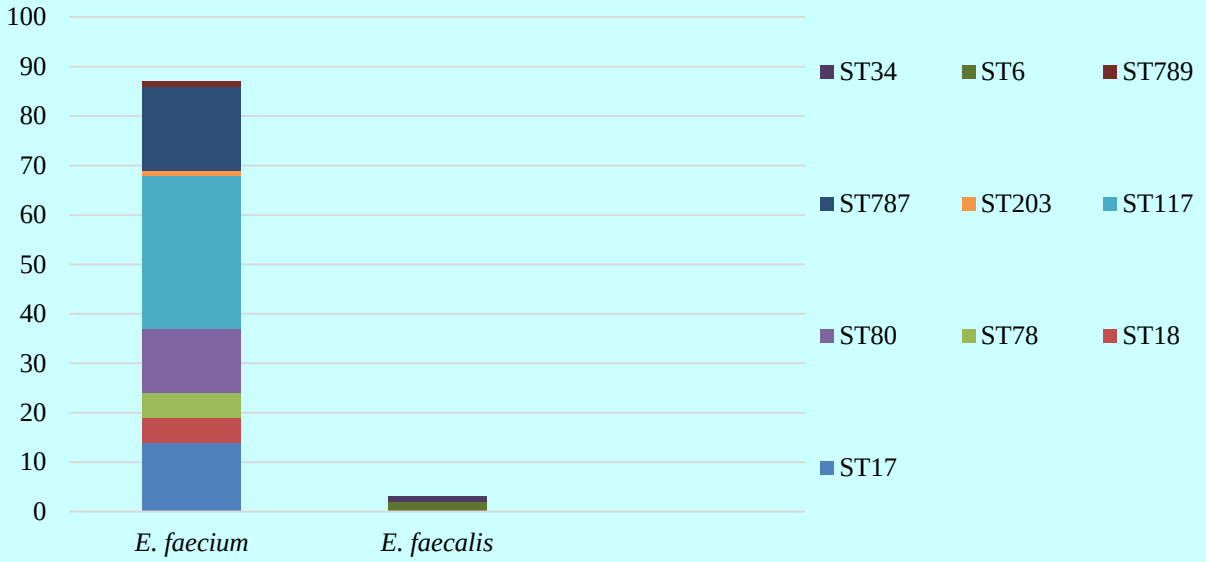


FIGURE 87. ST-distribution of VRE (n=90) by species based on WGS-analyses at K-res, in Norway 2019.

Conclusion
204 isolates of VRE were identified in Norway in 2019. In this report, we present WGS-data for 90 of these. The majority of VRE were *E. faecium* with *vanB* genotype. These were mainly associated to outbreaks in the Western and South-Eastern health regions. Smaller outbreaks and clusters with *vanA E. faecium* were also registered in the Northern and Western region. All VRE *E. faecium* belong to dispersed hospital adapted clones identified in several other countries.

Linezolid resistant enterococci

Linezolid is considered a last resort treatment in infections caused by multi-resistant enterococci, in particular VRE. The prevalence of linezolid resistant enterococci (LRE) is still low (< 1%) worldwide (9) but is increasing in many countries also in Europe (10,11).

Linezolid binds to the ribosome and inhibits bacterial protein synthesis. Acquired resistance to linezolid may be due to structural changes in the ribosome based on mutations in the ribosomal RNA and/or ribosomal proteins as well as through gene products that chemically modify (methylate) the ribosome (*cfr*). Another resistance mechanism is proteins (encoded by *optrA* and *poxtA*) that protect the ribosome against the binding of linezolid. The *cfr*, *optrA* and *poxtA* genes can all be localised on mobile genetic elements (10,12,13).

In Norway, LRE are notifiable to MSIS including confirmation at the National Reference Laboratory for LRE, K-res. K-res confirms the resistance phenotype with the reference method (microbroth dilution) and performs genetic characterisation with PCR and WGS to find resistance mechanisms and monitor genetic relatedness between the isolates. Susceptibility testing of enterococci for linezolid is not performed routinely in the diagnostic laboratories. Thus, the actual prevalence of linezolid resistance in enterococci is at present not known.

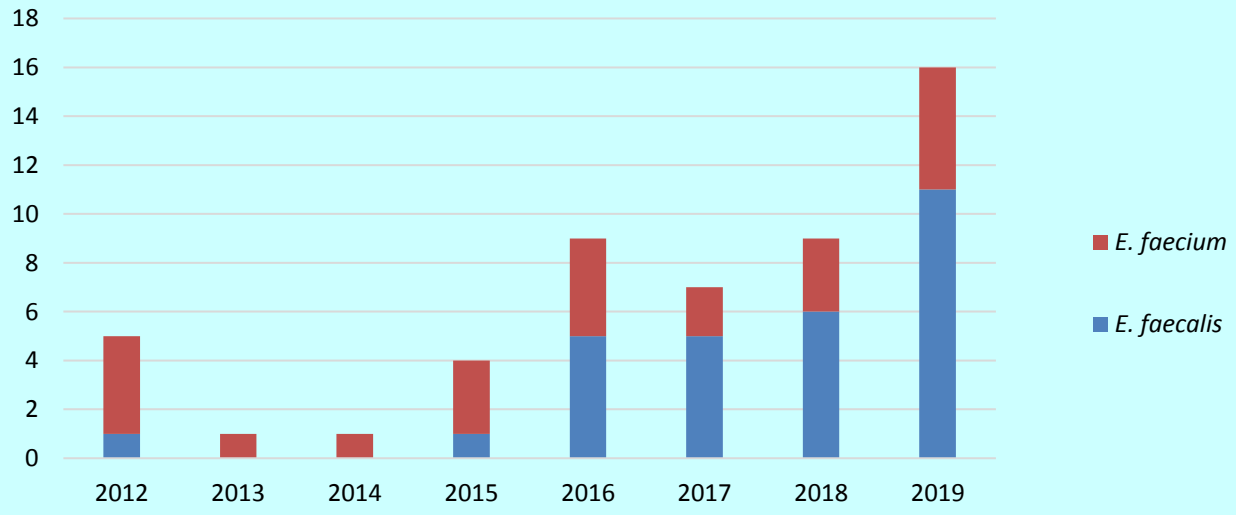


FIGURE 88. Number of linezolid resistant *E. faecium* and *E. faecalis* in Norway 2012-2019. This overview also includes LRE that are vancomycin resistant.

Sixteen cases of LRE were detected in Norway in 2019 compared to nine cases in 2018. Phylogenetic analyses of isolates with the same ST have not revealed any close genetic relatedness between strains. There has been an increase in LRE per year as of 2016 and simultaneously the species distribution changed from a dominance of *E. faecium* to *E. faecalis* (Figure 88). The observed increase in *E. faecalis* LRE in Norway from 2016 (total n=27) is due to non-clonal spread of isolates with *optrA* (n=25) (Figure 89).

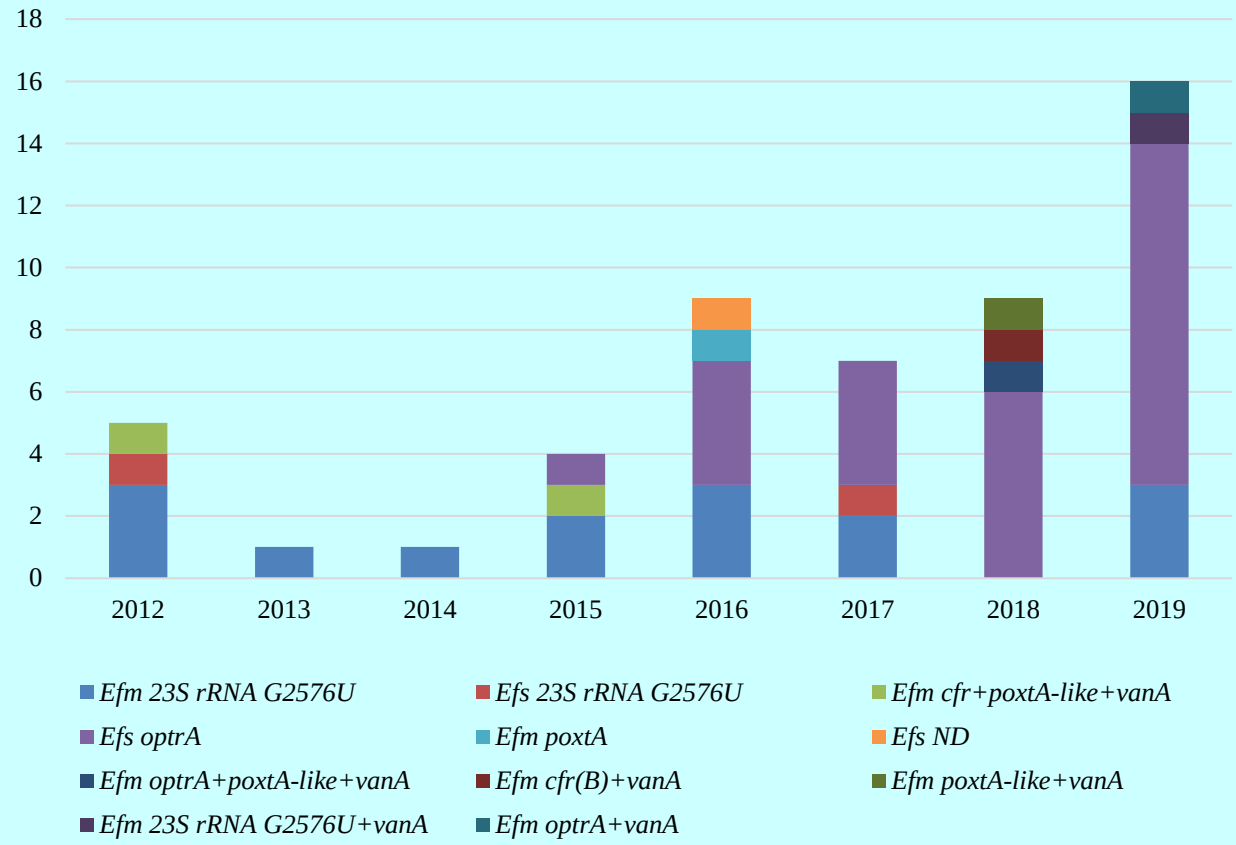


FIGURE 89. Number of LRE according to resistance mechanisms per year. *Efm=E. faecium. Efs=E. faecalis*. ND=not determined genotype. This isolate was not sent to K-res or archived at the primary laboratory.

Linezolid resistance in enterococci has traditionally been mediated by point mutations in the 23S rRNA region, mainly the G2576U mutation. Mutations are known to occur after long-term exposure to linezolid (14). In 2019 only four isolates with mutational based linezolid resistance were reported. The remaining isolates (n=12) had *optrA* (Figure 89). Ten isolates were from infections and nine of these had *optrA*. The rest were carrier isolates. Four isolates were found in patients who were probably infected abroad. For ten isolates information about place of acquisition is lacking. The *E. faecium* isolates (n=5) belonged to two hospital associated sequence types (ST203 n=3 and ST80 n=2). All *E. faecalis* isolates (n=11) had *optrA* but belonged to nine different STs with ST16 (n=3) being the most common (Table 81). Internationally, *E. faecalis* ST16 has also been reported to be the most prevalent ST type associated with *optrA* (15).

TABLE 81. Species, resistance mechanism and sequence type among LRE in Norway 2018.

Species	Resistance mechanism	ST
E. faecalis (n=11)	*optrA* (n=11)	ST16 (n=3); ST69 (n=1); ST192 (n=1); ST394 (n=1); ST476 (n=1); ST480 (n=1); ST585 (n=1); novel STs (n=2)
E. faecium (n=5)	23S rRNA G2576U mutasjon (n=4); *optrA* (n=1)	ST80 (n=2); ST203 (n=3)

Conclusion

The number of new LRE cases per year is increasing, but still low in Norway. Since 2016 there has been a change from *E. faecium* with mutation-based linezolid resistance to LRE with transferable resistance mechanisms dominated by *E. faecalis* with *optrA*. The majority of LRE isolates from 2019 were clinical isolates. There is no evidence of domestic spread of LRE in Norway.

References

1. European Centre for Disease Prevention and Control. Point Prevalence Survey of Healthcare-Associated Infections and Antimicrobial Use in European Acute Care Hospitals 2011-2012. 2013. ISBN 978-92-9193-485-0. doi 10.2900/86011.
2. NORM/NORM-VET 2018. Usage of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromsø/Oslo 2019. ISSN: 1502-2307 (print)/1890-9965 (electronic).
3. García-Solache M, Rice LB. The *Enterococcus*: a model of adaptability to its environment. Clin Microbiol Rev. 2019;32(2). doi: 10.1128/CMR.00058-18.
4. Courvalin P. Vancomycin resistance in Gram-positive cocci. Clin Infect Dis. 2006 Jan 1;42 Suppl 1:S25-34.
5. Hegstad K, Samuelsen Ø, Hegstad J, Sundsfjord A. 2015. Molecular methods for detection of antibacterial resistance genes: rationale and applications, p. 408-49. *In* D. Amsterdam (ed.) Antibiotics in Laboratory Medicine, 6th Edition. Wolters Kluwer. ISBN-13: 978-1-4511-7675-9.
6. European Centre for Disease Prevention and Control. Surveillance of antimicrobial resistance in Europe 2018. Stockholm: ECDC; 2019. DOI 10.2900/22212.
7. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019. http://dx.doi.org/10.15620/ cdc:82532.
8. European Antimicrobial Resistance Surveillance Network (EARS-Net). 2018. Surveillance Atlas of Infectious diseases. https://atlas.ecdc.europa.eu/public/index.aspx?Dataset=27&HealthTopic=4.
9. Mendes RE, Hogan PA, Jones RN, Sader HS, Flamm RK. Surveillance for linezolid resistance via the Zyvox(R) Annual Appraisal of Potency and Spectrum (ZAAPS) programme (2014): evolving resistance mechanisms with stable susceptibility rates. J Antimicrob Chemother. 2016;71:1860-5. doi: 10.1093/jac/dkw052.
10. Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, Hammerum AM, Schaffer K, Burns K, Murchan S, Novais C, Freitas AR, Peixe L, Del Grosso M, Pantosti A, Werner G. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resist Updat. 2018;40:25-39. doi: 10.1016/j.drup.2018.10.002.
11. Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, Mischnik A, Werner G. Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. J Glob Antimicrob Resist. 2015;3:128-31. doi: 10.1016/j.jgar.2015.02.007.
12. Sadowy E. Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci. Plasmid. 2018;99:89-98. doi: 10.1016/j.plasmid.2018.09.011.
13. Brenciani A, Fioriti S, Morroni G, Cucco L, Morelli A, Pezzotti G, Paniccià M, Antonelli A, Magistrali CF, Rossolini GM, Giovanetti E. Detection in Italy of a porcine *Enterococcus faecium* isolate carrying the novel phenicol-oxazolidinone-tetracycline resistance gene *poxtA*. J Antimicrob Chemother. 2019;74:817-8. doi: 10.1093/jac/dky505.
14. Pai MP, Rodvold KA, Schreckenberger PC, Gonzales RD, Petrolatti JM, Quinn JP. Risk factors associated with the development of infection with linezolid- and vancomycin-resistant *Enterococcus faecium*. Clin Infect Dis. 2002;35:1269-72.
15. Chen M, Pan H, Lou Y, Wu Z, Zhang J, Huang Y, Yu W, Qiu Y. Epidemiological characteristics and genetic structure of linezolid-resistant *Enterococcus faecalis*. Infect Drug Resist. 2018;11:2397-2409. doi: 10.2147/IDR.S181339.

Kristin Hegstad, Jessin Janice and Arnfinn Sundsfjord, Norwegian National Advisory Unit on Detection of Antimicrobial Resistance, Dept. of Microbiology and Infection Control, University Hospital of North Norway and UiT – The Arctic University of Norway, Tromsø, Christoffer Lindemann, Department of Microbiology, Haukeland University Hospital, Bergen, Iren Høyland Löhr, Department of Medical Microbiology, Stavanger University Hospital, Stavanger, and Petter Elstrøm and Oliver Kacelnik, Section for Antibiotic Resistance and Infection Prevention, Norwegian Institute of Public Health, Oslo, Norway.

Streptococcus pyogenes in specimens from the respiratory tract and wounds

TABLE 82. *Streptococcus pyogenes* in respiratory tract specimens in 2019 (n=293). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	94.9	1.0	4.1
Clindamycin	≤ 0.5	> 0.5	97.3	-	2.7
Tetracycline	≤ 1	> 2	94.2	0.0	5.8
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	100.0	0.0	0.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 83. *Streptococcus pyogenes* in respiratory tract specimens in 2019 (n=293). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	0.7	24.2	64.2	9.9	0.7	0.3										
Erythromycin			0.3		3.4	47.4	43.7	1.0		0.7	0.3	0.3			0.3	2.4
Clindamycin			0.3	0.3	14.3	63.8	18.4			0.3						2.4
Tetracycline			0.3	1.0	28.0	56.3	8.5					0.3	2.4	2.7	0.3	
TMS*			1.4	3.8	16.7	40.3	20.8	14.7	2.4							

Shaded areas in each row indicate susceptibility with standard exposure (light), susceptibility with increased exposure (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. *TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 84. *Streptococcus pyogenes* in wound specimens in 2019 (n=229). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	93.9	2.2	3.9
Clindamycin	≤ 0.5	> 0.5	97.8	-	2.2
Tetracycline	≤ 1	> 2	85.2	0.0	14.8
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	98.2	0.9	0.9

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant. *Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 85. *Streptococcus pyogenes* in wound specimens in 2019 (n=229). Distribution (%) of MICs (mg/L) and zone diameters for oxacillin (mm).

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	0.9	18.8	73.8	6.1		0.4										
Erythromycin		0.4		0.4	5.2	45.0	42.8	2.2		0.4				0.4	0.4	2.6
Clindamycin				0.4	16.2	65.1	16.2		0.4							1.7
Tetracycline			0.4		29.7	48.5	6.6				0.4	0.9	7.4	5.7		0.4
TMS*			1.7	5.2	16.6	32.3	14.4	24.0	3.9	0.9				0.9		

Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method. Non-shaded rows indicate that breakpoints have not been defined. *TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Streptococcus pyogenes (beta-haemolytic group A streptococci - GAS) from wounds and respiratory tract specimens have previously been surveyed in NORM in 2002, 2004, 2006, 2008 and 2013. The results from 2019 are presented in Tables 82-85 and the trends during 2002-2019 in Figure 80. The relevant breakpoints have remained unchanged over many years, and the results for all years are interpreted according to the 2020 NordicAST/ EUCAST protocol. Susceptibility data for systemic isolates were not reported by the reference laboratory at Norwegian Institute of Public Health in 2019.

Penicillin G non-susceptibility has never been detected in group A streptococci, and the highest MIC value in this study was 0.125 mg/L, which is well below the breakpoint for susceptibility. Most isolates displayed MICs of 0.008-0.016 mg/L.

Macrolide resistant group A streptococci have been a problem in many European countries. In NORM, the prevalence of erythromycin resistance remained relatively stable in 2019 with 4.1% resistance in respiratory tract samples (5.4% in 2013) and 3.9% in wound samples (4.1% in 2013), respectively. The prevalence of resistance to clindamycin was also essentially unchanged at 2.7% in 2019 compared to 3.1% in 2013, and at 2.2% in 2019 compared to 1.6% in 2013 for respiratory tract and wound isolates, respectively. In total, 21 non-systemic isolates were erythromycin resistant and were classified as either inducibly (7/21, 1.3% of all isolates) or constitutively (6/21, 1.1% of all isolates) MLS_B resistant. In addition, eight isolates displayed a phenotype compatible with *mef*-encoded efflux (1.5% of all isolates). Two isolates had clindamycin MICs of 1 and 2 mg/L, but were susceptible to erythromycin (MICs 0.125 and 0.25 mg/L). This could be caused by alterations in ribosomal proteins but was not further explored.

As seen in Figur 80, the prevalence of resistance to tertacycline in isolates from wound specimens was 14.8%. This is significantly higher than in respiratory tract isolates (5.8%) and consistent with previous findings in NORM. Similar differences in resistance rates by sample source are not seen for trimethoprim-sulfamethoxazole or erythromycin. One may speculate that differences in resistance rates between isolates from different clinical conditions are caused by clonal variation, but further studies are needed to support this hypothesis.

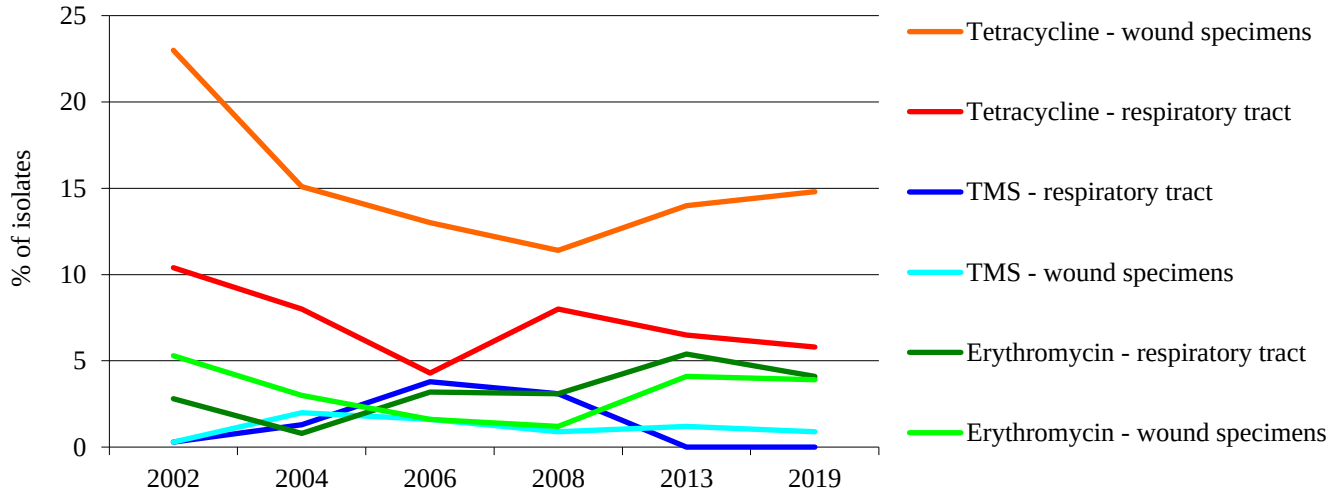


FIGURE 80. Prevalences of resistance to various antimicrobial agents in *Streptococcus pyogenes* in specimens from the respiratory tract and wounds 2002-2019. Doxycycline used in 2002 and 2006 was replaced by tetracycline in 2008. All data are categorised according to the 2020 NordicAST/EUCAST breakpoint protocol. Please note that the x-axis is not to scale. TMS=Trimethoprim-sulfamethoxazole.

Streptococcus agalactiae in blood cultures and cerebrospinal fluids

TABLE 86. *Streptococcus agalactiae* isolates in blood cultures and cerebrospinal fluids in 2019 (n=310). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	S	R	S	I	R
Penicillin G	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	74.5	0.0	25.5
Clindamycin	≤ 0.5	> 0.5	86.8	-	13.2
Tetracycline	≤ 1	> 2	22.0	0.3	77.7
Vancomycin	≤ 2	> 2	100.0	-	0.0

S=Susceptible with standard exposure, I=Susceptible with increased exposure, R=Resistant.

TABLE 87. *Streptococcus agalactiae* isolates in blood cultures and cerebrospinal fluids in 2019 (n=310). Distribution (%) of MICs (mg/L).

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	≥ 128
Penicillin G	0.3	0.6	2.9	39.0	57.1											
Erythromycin				5.2	25.5	30.6	13.2		0.6	3.9	4.8	5.5	1.3			9.4
Clindamycin				1.3	22.9	55.5	1.6	5.5	3.9			0.3				9.0
Tetracycline				1.9	14.5	2.3	1.0	1.3	1.0	0.3	1.0	14.5	44.2	17.7	0.3	
Vancomycin				1.9	5.2	46.1	46.5	0.3								
Gentamicin											0.6	2.2	11.2	42.6	41.6	1.6

Shaded areas in each row indicate susceptibility with standard exposure (light), susceptibility with increased exposure (medium) and resistance (dark). Non-shaded cells represent MIC values that are not covered by the standard test method.

RESULTS AND COMMENTS

All systemic isolates of *Streptococcus agalactiae* (beta-haemolytic group B streptococci) in Norway are referred to the National Reference Laboratory at St. Olavs Hospital, Trondheim University Hospital, where confirmatory identification and susceptibility testing are performed. Since 2014, the reference laboratory has provided resistance data for invasive *S. agalactiae* isolates to NORM on a yearly basis. Relevant breakpoints have remained unchanged since 2009.

A total of 310 isolates were retrieved from invasive infections (bacteremia and cerebrospinal infections) in 2019. Thirty isolates originated from neonates and small children < 1 year of age. Most isolates (99.7%) were recovered from blood cultures, but there were also five isolates from cerebrospinal fluids.

As seen in Tables 86-87 there were no isolates with reduced susceptibility to penicillin G or vancomycin. Seventy-nine isolates (25.5%) were resistant to erythromycin compared to 22.6% in 2018. Seventy-seven erythromycin resistant isolates were analysed by double disk diffusion for MLS_B resistance phenotype. Constitutive MLS_B resistance was found in 50 isolates (65%), while inducible MLS_B resistance was detected in 21 isolates (27%). The remaining six isolates (8%) had results in accordance with the efflux-mediated M phenotype encoded by *mef* genes. A single isolate was recorded as clindamycin resistant (MIC 1 mg/L) in spite of being susceptible to erythromycin (MIC 0.064 mg/L).

There are no clinical breakpoints for aminoglycosides in *S. agalactiae*, but combination therapy with a beta-lactam is often used in clinical practice for treatment of sepsis of unknown origin. High-level resistance to gentamicin (MIC ≥ 128 mg/L) was detected in 1.6% of the isolates. The prevalence of resistance to tetracycline (77.7%) was at the same level as in 2018 (75.4%) with the majority of isolates displaying MIC values of 32-64 mg/L (Table 87).

Mycobacterium tuberculosis

In 2019, 165 persons were reported with tuberculosis disease (TB) to the Norwegian Surveillance System for Communicable Diseases (MSIS). Of these, 18 were born in Norway. 122 had TB for the first time, of which three had received preventive treatment. Twelve had had previous TB, of which 11 had been treated with anti-TB drugs previously. The rest, 28 cases, were categorised as uncertain if they had received TB treatment previously.

126 cases were confirmed with *M. tuberculosis*-complex (MTBC) by culture, of these one identified as *M. africanum* and one as *M. bovis*, the rest were *M. tuberculosis*. All isolates except one had phenotypic DST (drug susceptibility test) results to rifampicin, isoniazid, ethambutol and pyrazinamide. The isolate without phenotypic DST (due to contamination with NTM) were sensitive to rifampicin and isoniazid by molecular DST (no *rpoB, katG* or *inhA* mutations). The results are presented in Table 88. There were two MDR-TB cases. One of them was resistant to moxifloxacin and levofloxacin, but not to amikacin or capreomycin, and it were consequently not classified as XDR-TB. Both cases had co-resistance to ethambutol, one also to pyrazinamide. Both were sensitive to prothionamide, bedaquilin and linezolid. One of the cases had been treated for TB previously.
In addition to the two MDR cases, eight cases had strains resistant to isoniazid, but three of them only with low-level resistance. Five patients with culture result negative or without culture result, had result of molecular/genotypic test showing MTBC and sensitivity to rifampicin (no *rpoB* mutation).

TABLE 88. Antimicrobial susceptibility of 125 isolates of *M. tuberculosis*-complex (MTBC, not including *M. bovis* BCG) from human infections in 2019. Figures from 2018 in parentheses.

Origin of birth	No. of cases	No. of isolates	Resistance to antimicrobial agents (No. of isolates)				
			Isoniazid n=125	Rifampicin n=125	Ethambutol n=125	Pyrazinamid n=125	MDR-TB n=125
Norway	18 (29)	8 (21)	0 (2)	0 (0)	0 (0)	0 (1)	0 (0)
Europe excl. Norway	24 (25)	20 (22)	2 (3)	0 (3)	0 (2)	1 (2)	0 (2)
Asia	59 (79)	45 (65)	3 (5)	2 (2)	2 (1)	5 (3)	2 (2)
Africa	61 (76)	51 (55)	5 (5)	0 (1)	0 (0)	2 (0)	0 (0)
America	1 ()	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oseania	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	2 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	165 (209)	126 (167)	10 (15)	2 (6)	2 (3)	8* (6)	2 (4)
Proportion resistant isolates (%)			8.0 (9.0)	1.6 (3.6)	1.6 (1.8)	6.4 (3.6)	1.6 (2.4)

*Of these, one *M. bovis* isolate in 2019 with inherent resistance to pyrazinamide. MDR-TB: Multi-drug resistant tuberculosis, resistant to at least rifampicin and isoniazid XDR-TB: Extensively drug-resistant tuberculosis, resistant to at least rifampicin and isoniazid plus any fluoroquinolone and at least one of three injectable second line drugs (i.e., amikacin, kanamycin, or capreomycin).

Candida spp. in blood cultures

TABLE 89. Antimicrobial susceptibility of *Candida albicans* blood culture isolates in 2019 (n=116). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)*		
	S	R	S	I	R
Amphotericin B	≤ 1	> 1	100.0	-	0.0
Fluconazole	≤ 2	> 4	100.0	0.0	0.0
Voriconazole	≤ 0.064	> 0.25	100.0	0.0	0.0
Anidulafungin**	≤ 0.032	> 0.032	100.0	-	0.0
Micafungin**/***	≤ 0.016	> 0.016	99.1	-	0.9

*S=Susceptible, I=Intermediately susceptible, R=Resistant. Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST 2019. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin and micafungin are considered susceptible. ***With EUCAST revised BP 2020-02-04, micafungin MIC 0.03 mg/L is defined as ATU and the resistant isolate is regarded susceptible.

TABLE 90. *Candida albicans* blood culture isolates in 2019 (n=116). Distribution (%) of MICs (mg/L).*

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B			0.9	0,9	3.4	14.7	65.5	14.7									
Fluconazole						5.2	55.2	37.1	1.7	0.9							
Voriconazole	15.5	72.4	10.3	1.7													
Anidulafungin	69.0	28.4	2.6														
Micafungin**	2.6	50.9	45.7	0.9													
Caspofungin***			5.2	22.4	48.3	19.8	4.3										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **With EUCAST revised BP 2020-02-04, micafungin MIC 0.03 mg/L is defined as ATU and the resistant isolat is regarded susceptible. ***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.

TABLE 91. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates in 2019 (n=29). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)*		
	S	R	S	I	R
Amphotericin B	≤ 1	> 1	100.0	-	0.0
Fluconazole	≤ 0.002	> 32	0.0	69.0	31.0
Anidulafungin**	≤ 0.064	> 0.064	100.0	-	0.0
Micafungin**	≤ 0.032	> 0.032	100.0	-	0.0

*S=Susceptible, I=Intermediately susceptible, R=Resistant. Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST 2019. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. There is insufficient evidence that *C. glabrata* is a good target for therapy with voriconazole and no clinical breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported. **There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin and micafungin are considered susceptible.

TABLE 92. *Candida glabrata* blood culture isolates in 2019 (N=29). Distribution (%) of MICs (mg/L)*.

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B					3.4	13.8	6.9	72.4	3.4								
Fluconazole									3.4	0.0	20.7	34.5	6.9	3.4	6.9	10.3	13.8
Voriconazole**				3.4	6.9	34.5	20.7	6.9	10.3	3.4	3.4	3.4	6.9				
Anidulafungin	6.9	44.8	414		6.9												
Micafungin		55.2	44.8														
Caspofungin***					10.3	44.8	41.4	3.4									

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **There is insufficient evidence that *C. glabrata* is a good target for therapy with voriconazole and no clinical breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported. ***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin as well as micafungin are considered susceptible.

TABLE 93. Antimicrobial susceptibility of *Candida parapsillosis* blood culture isolates in 2019 (n=22). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)*		
	S	R	S	I	R
Amphotericin B	≤ 1	> 1	100.0	-	0.0
Fluconazole	≤ 2	> 4	85.7	14.3	0.0
Voriconazole	≤ 0.125	> 0.25	100.0	0.0	0.0
Anidulafungin**	≤ 0.002	> 4	0.0	100.0	0.0
Micafungin**	≤ 0.002	> 2	0.0	95.5	4.5

*S=Susceptible, I=Intermediately susceptible, R=Resistant. Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST 2019. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **There are no European breakpoints for caspofungin. Strains intermediate to anidulafungin and micafungin are considered intermediate to caspofungin. From 2020 the wild-type population of *C. parapsilosis* is regarded suscepible to the echinocandins.

TABLE 94. *Candida parapsilosis* blood culture isolates in 2019 (N=22). Distribution (%) of MICs (mg/l)*.

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							22.7	50.0	27.3								
Fluconazole						9.1	4.5	54.5	27.3								4.5
Voriconazole	9.1	18.2	36.4	31.8					4.5								
Anidulafungin							4.5	18.2	13.6	40.9	22.7						
Micafungin**							13.6	59.1	22.7		4.5						
Caspofungin**							4.5	81.8	9.1	4.5							

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **There are no European breakpoints for caspofungin. Isolates intermediate to anidulafungin and micafungin are considered intermediate to caspofungin. From 2020 the wild-type population of *C. parapsilosis* is regarded suscepible to the echinocandins.

TABLE 95. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates in 2019 (n=17).Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)*		
	S	R	S	I	R
Amphotericin B	≤ 1	> 1	100.0	-	0.0
Fluconazole	≤ 2	> 4	94.4	0.0	5.6
Voriconazole	≤ 0.125	> 0.25	94.4	0.0	5.6
Anidulafungin**	≤ 0.064	> 0.064	100.0	-	0.0

*S=Susceptible, I=Intermediately susceptible, R=Resistant. Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST 2019. The revised breakpoints and necessary changes to match the new EUCAST definitions of S, I and R were released in February 2020 and were not used in 2019. **There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin are considered susceptible to caspofungin.

TABLE 96. *Candida tropicalis* blood culture isolates in 2019 (N=17). Distribution (%) of MICs (mg/L)*.

	≤0.004	0.008	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥ 256
Ampho. B							17.6	52.9	29.4								
Fluconazole							5.9	58.8	29.4						5.9		
Voriconazole		5.9	23.5	47.1	11.8	5.9			5.9								
Anidulafungin	5.9	41.2	52.9														
Micafungin**	11.8	64.7	23.5														
Caspofungin***					35.3	47.1	17.6										

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). The EUCAST AFST subcommittee is currently reviewing breakpoint tables to introduce necessary changes to match the new EUCAST definitions of S, I and R. Non-shaded cells represent MIC values that are not covered by the standard test method. **There is insufficient evidence whether the wild-type population of *C.tropicalis* can be considered susceptible to micafungin. ***There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin are considered susceptible to caspofungin

RESULTS AND COMMENTS

In 2019 the National Mycology Reference Laboratory received 199 isolates from unique candidemias in 185 patients. Seven infections were regarded as persistent infections or reinfections with the same species more than four weeks apart, in five patients, of whom one patient had candidemias with different species, mixed infections and reinfections over a period of three months. In six patients we observed seven mixed yeast infections. The combination *Candida glabrata* and *Candida dubliniensis* was found in three patients and *Candida albicans* and *Candida tropicalis* in another. In two candidemia episodes mixed infections with other yeasts were observed (*Rhodotorula mucilaginosa* and *Saccharomyces cervisiae*).

We received nine different *Candida* species from patients with bloodstream infections. Over time we observe a slow shift in species distribution, but acquired resistance in *Candida* spp is rare and in Norway species identification still predicts the susceptibility pattern of *Candida* spp. in patients without long-term antifungal treatment.

Candida albicans is the most common species (n=116, 58.3%) declining from 65.7% in 2018. The number of *Candida glabrata* isolates is still low (n=29, 14.6%) compared to 18.5% last year, whereas the number of *C. parapsilosis* (n=19) and its sibling species *C. metapsilosis* (n=1) and *C. orthopsilosis* (n=2), has risen from seven to 22 (11.1%) and *C. tropicalis* nearly doubled from 4.5% to 8.5% (n=17) in 2019. The number of other species is low, but *Candida dubliniensis* increased from 3.9 to 5.5% (n=11). There were also three *C. krusei* candidemias and one *C. lusitaniae* candidemia.

All isolates were susceptibility tested for amphotericin B, fluconazole, voriconazole, caspofungin, anidulafungin and micafungin by E-test according to the manufacturer's instructions (AB bioMérieux). Unexpected susceptibility patterns were confirmed by EUCAST standardised broth microdilution method at Statens Serum Institut in Copenhagen. The results are presented in Tables 89-96.

All *C. albicans* isolates, except one *C. albicans* with micafungin MIC 0.032 mg/L, were susceptible to all drugs tested, and all but one *C. parapsilosis* (n=19) belonged to the wild-type, in 2019 still categorised as "intermediately susceptible" to echinocandins.

Fluconazole resistance was observed in one *C. orthopsilosis* (MIC 256 mg/L), one *C. tropicalis,* (MIC 256 mg/L), and one *C. dubliniensis* (MIC 32 mg/L). Otherwise no acquired fluconazole resistant stains were found. Breakpoints for fluconazole (S $\leq$ 0.002 mg/L, R> 32 mg/L) in *C. glabrata* categorise the wild-type as intermediately susceptible and in 2019 31% of *C. glabrata* isolates were categorised as resistant. All *C. krusei* are inherently resistant to fluconazole (n=3).

C. dubliniensis is closely related to *C. albicans*. Breakpoints were established for itraconazole, posaconazole and voriconazole in 2018. From 2020 breakpoints of amphotericin B and fluconazole against *C. albicans* will be adopted for *C. dubliniensis*. One *C. dubliniensis* isolate with high fluconazole MIC also expressed high MICs against other azoles. No echinocandin breakpoints are set, but one *C. dubliniensis* isolate with anidulafungin and micafungin MICs of > 4 mg/L was regarded resistant.

The wild-type populations of *C. albicans, C. dubliniensis, C. parapsilosis* and *C. tropicalis* are considered susceptible to voriconazole, and all isolates with defined breakpoints, with exception of the three fluconazole resistant isolates, were found susceptible to voriconazole in 2019. The intermediate category was introduced for *C. albicans, C. dubliniensis, C. parapsilosis* and *C. tropicalis* in 2018 to acknowledge that increased exposure can be obtained by intravenous dosing. There is not enough information available for the response to voriconazole of infections caused by *Candida* spp. with higher MICs, and there is insufficient evidence that *C. glabrata* and *C. krusei* are good targets for therapy with voriconazole. No breakpoints have been set. There is still insufficient evidence that *Candida* spp. is a good target for therapy with isavuconazole and breakpoints have not been set.

All tested isolates were susceptible to amphotericin B. Amphotericin B is not recommended treatment of *C. lusitaniae* (n=1) infections as *C. lusitaniae* has high MICs or develop resistance during treatment.

To implement the new definition of the "I" category from "Intermediate" to "Susceptible, Increased exposure" and an "Area of Technical Uncertainty" (ATU) for the antifungal agents, the EUCAST-AFST (Subcommittee on Antifungal Susceptibility Testing) has reviewed all, and revised some, clinical antifungal breakpoints. The changes were released in a revised breakpoint table v. 10.0 in February 2020, but are *not* adopted retrospectively in this report.

From 2020 *C. albicans* with micafungin MIC 0.03 mg/L and anidulafungin MIC 0.016 mg/L will be regarded sensitive and EUCAST-AFST recommend reporting such isolates as "sensitive" with the following comment: "Isolates susceptible to anidulafungin with micafungin MIC of 0.03 mg/L do not harbour an *fks* mutation conferring resistance to the echinocandins". Anidulafungin resistant *C. albicans* with micafungin MIC 0.03 mg/L will be regarded as micafungin resistant.

The new definition of the "I" category from "Intermediate" to "Susceptible, Increased exposure" is not applicable for the echinocandins and *C. parapsilosis* as no dose escalation option exists for the echinocandins. Given that the clinical response of echinocandins is not statistically different from that of other agents despite the intrinsic target gene alteration, EUCAST-ASFT from 2020 therefore regards the *C. parapsilosis* wild-type susceptible to echinocandins. Fluconazole breakpoints in *C. glabrata* are also redefined ("I " $\leq$ 16 mg/L) to acknowledge the use of fluconazole in a high dose in some clinical situations.

From 2020 breakpoints of amphotericin B and fluconazole against *C. albicans* will be adopted for *C. dubliniensis* given that these species are similar in terms of antifungal susceptibility to these agents and in terms of virulence.

Appendix 1:
Collection of data on usage of antimicrobial agents in animals

Data sources

Sales data at wholesalers level

In Norway, all medicinal products for animals are prescription-only medicines – this includes both veterinary medicinal products (VMPs) and human medicinal products (HMPs). The latter can be prescribed according to the so-called cascade (Directive 2001/82/EC, Article 10) – i.e. if there is no VMP authorised for the condition HMP is allowed to be used. For food producing species it requires that a maximum residue level (MRL) has been assigned for the active substance in question or that it is shown that MRL is not nessecary.

Both VMPs and HMP have to be dispensed through pharmacies that are supplied by wholesalers. Medicated feed (manufactured from premix VMPs) is supplied to the end user by feed mills and is currently only used for farmed fish; this is due to the small size of livestock herds in Norway and the low use of group/flock treatments. Group treatment of livestock (terrestrial animals) with antibacterial agents is administered through drinking water or as top-dressing on the feed.

Wholesalers and feed mills in Norway are mandated to provide sales statistics for veterinary medicinal products, including when supplied as medicated feed, to the Norwegian Institute of Public Health (NIPH). Data on sales of each product presentation (name, form, strength and pack size) of the included VMPs were obtained from the NIPH. One exception; antibacterials for farmed fish for the years 2013-2019 were obtained from the Veterinary Prescription Register (VetReg). Veterinarians in Norway are not allowed to dispense VMPs, except for treatments until a pharmacy can provide the VMPs. In such cases the medicinal products have to be sold at cost price.

Prescription data

The Norwegian Food Safety Authority established the Veterinary Prescription Register (VetReg) for farmed fish 1st January 2011 and for terrestrial animals 1st January 2012. The veterinarians are mandated to report any administration and deliveries of VMPs and HMPs to VetReg for all terrestrial food-producing animals and horses while it is voluntary for all other animal species such as companion animals. Pharmacies and feed mills have to report all deliveries, i.e. for all terrestrial animals and farmed fish, to veterinarians or animal owners, including medicines prescribed for companion animals and HMPs.

For farmed fish the reporting of prescription of antibacterials has been shown to be complete for the years 2013-2018 (1), and this was the case also for 2019 data; VetReg data are used for farmed fish for these years. For 2012-2014 the quality of the prescription data from VetReg on antibacterials for terrestrial food producing animals was unsatisfactory (unpublished data). For oral paste and intramammaries data quality was unsatisfactory for the entire period 2012-2019, resulting in that amounts used could not be calculated. The number of prescriptions was used to obtain a picture of the prescribing per species for these formulations. In this analysis only 2015-2019 data for injectables, oral powders and oral solution from VetReg have been used (2); these were calculated to express kg antibacterials prescribed/used and the outputs were compared to sales data for the corresponding forms obtained from NIPH for the years 2015-2019. The results show that the VetReg data covers around two third of the sales data for VMP injectables**,** oral powders and oral solution. It could not be identified whether the data are represenative for the prescribing of VMPs by animal species, but the VetReg data is nevertheless believed to give a rough picture of the prescription of antibacterial classes by formulation and animal species. VetReg data have therefore been used as an additional souce in order to assess changes according to targets set in the National Strategy against Antibiotic Resistance (2015-2020) (3).

Ionophore coccidiostat feed additives

Data on sales of coccidiostat feed additives have been collected from the Norwegian Food Safety Authority.

Antibacterial included in the data set

The Anatomical Therapeutic Chemical vet (ATCvet) classification system was used to identify the VMPs to be included in the data. Sales of VMPs belonging to the ATCvet codes shown in the table below were collected from the NIPH for terrestrial animals, for farmed fish data for QJ01 were collected from VetReg. This is identical to the inclusion criteria by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (4). For the estimation of prescription of HMP antibacterials belonging to the ATC, codes J01 and J04AB are included (extracted from VetReg data).

Antibacterial veterinary medicinal products included in the data

Categories	ATCvet codes
Intestinal use	QA07AA;QA07AB
Intrauterine use	QG01AA; QG01AE, G01BA; QG01BE; QG51AA; QG51AG
Systemic use	QJ01
Intramammary use	QJ51
Antiparasitic agents[1]	QP51AG

[1] Only sulfonamides

Antibacterial veterinary medicinal products sold on special exemption from market authorisation are included in the sales data and prescription data. Dermatological preparations (QD) and preparations for sensory organs (QS) are not included in the data which is in accordance with the ESVAC protocol (4).

Data source animal population data - Denominator
A population correction unit (PCU) has been established as a denominator for the reporting of ESVAC sales data. In this report, PCU has been used as denominator for sales of antibacterial VMPs. It is emphasised that the PCU is purely a surrogate for the animal population at risk.

The animal categories included in the PCU as well as the calculation methodology are identical to ESVAC and is detailed in the ESVAC 2016 report (3). The PCU for each terrestrial animal category is calculated by multiplying numbers of livestock animals (dairy cows, sheep, sows and horses) and slaughtered animals (cattle, goat, pigs, sheep, poultry, rabbits and turkeys) by the theoretical weight at the most likely time for treatment.

The PCU is calculated for each species, weight class and/or production type, as follows:

- Number of animals slaughtered × estimated weight at treatment
- Number of livestock × estimated weight at treatment

The total PCU is calculated according to the above data.

1 PCU = 1 kg of animal biomass.

For farmed fish, fish biomass live-weight slaughtered is used as PCU in ESVAC reports. Data on animal population, including farmed fish, used to calculate PCU were obtained from Statistics Norway (https://www.ssb.no).

Indicators
The National Strategy against Antibiotic Resistance (2015-2020) (3) does not specify which indicators to be used in order to measure progress in terms of reduction of usage of antibacterials in animals. In 2017, ECDC, EFSA and EMA jointly established a list of harmonised outcome indicators to measure progress in reducing the usage of antimicrobials and antimicrobial resistance both in humans and food-producing animals. In order to measure the overall effect of policy interventions/management measure to reduce the consumption for food producing animals the proposed indicator is overall sales in mg/PCU (mg active substance/population correction unit) (5). Therefore, the indicators used to report the usage of antibacterials in the current report are kg active substance and for food producing animals also mg/PCU.

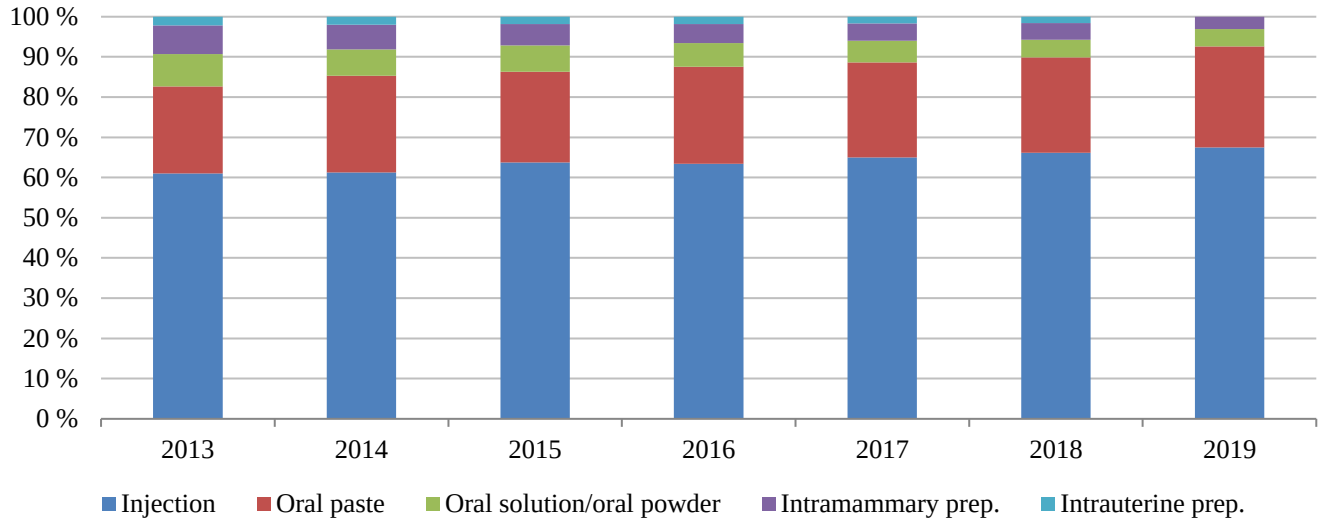
Analysis of the overall sales data
The sales data for each VMP presentation were calculated to express weight of active substance. In order to comply with the ESVAC standards, sales of prodrugs - e.g. procaine benzylpenicillin and penethamate hydriodide - have been converted to the corresponding values for the active ingredient, here benzylpenicillin (4).

The sales data of antibacterial VMPs for terrestrial animals have been split into sales for food producing animals (including horses) and companion animals. Sales of antibacterial VMPs for companion animals refer to sales of tablets, oral solution and oral paste that are approved solely for companion animals; in addition dihydro-streptomycin tablets of pack size 10 pieces have been included in the data on sales for companion animals (no sales after 2004). The other antibacterial VMPs are assumed sold for use only in food producing animals (including horses). There is some use of injectable VMPs in companion animals thus the usage for this animal category is slightly underestimated and thus slightly over-estimated for food producing animals. Sales of VMPs for food producing animals have been further stratified into VMPs for treatment of individual food producing animals - bolus, oral paste injectables, intramammary preparations, intrauterine preparations and some tablets (dihydro-streptomycin pack size 20 and 100) and for group treatment (oral solution and oral powder).

<u>Estimation of sales for cattle, pigs, sheep, goat and poultry</u>
The national strategy does not specify for which food producing terrestrial animals the reduction should cover. Because cattle, pigs, sheep, and poultry accounted for approximately 99% of the Norwegian meat production in 2019 (https://www.ssb.no/slakt) these species as well as goats were selected to evaluate the goals set down in the national strategy (3).

The sales data for 2013-2019 have been further refined in order to obtain estimates on the usage in cattle, pigs, sheep, goat and poultry that are more accurate in terms of identifying changes across time. Sales data from wholesalers show that oral paste approved for horses accounted for 21% to 25% of the total annual sales of antibacterial VMPs for terrestrial food producing animals during 2013-2019 (Figure). Data on prescribtions per animal species obtained from the Veterinary Prescription Register (VetReg) has been used as supportive information to the sales data for this refinement.

VetReg data show that for the years 2015-2019, on average 96% (range 95% to 97%) of the number of prescriptions of antibacterial oral paste VMPs were for horses showing that off-lable use for other animal species of oral paste was negligible. Oral paste (numerator) and PCU for horses (denominator) have been excluded from the analysis of data for the estimation of usage of antibacterial VMPs for cattle, pigs, sheep, goat and poultry. Intramammaries have been excluded from the analysis of the VetReg data regarding prescribed amounts (kg) due to data quality issues (2).



Proportion of sales (wholesalers) in Norway of antibacterial veterinary medicinal products (VMPs) approved for one or more of the food producing animal species, including horses, by pharmaceutical forms in the period 2013-2019. Of note, there were no sales of antibacterial VMP intrauterine devices in 2019.

The usage of HMPs for cattle, pigs, sheep, goat and poultry was estimated by use of the following data from VetReg:

- Delivery to animal owners from pharmacies of antibacterial HMPs for use in these species, plus
- Veterinarians' use/delivery of antibacterial HMP for these species. Note that due to underreporting by veterinarians the data represents an underestimate.

Estimation of sales of HMPs for dogs and cats

Veterinarians reported almost no use of HMPs for companion animals to VetReg; this is due to the fact that veterinarians are not mandated to report use of medicines for companion animals to VetReg. It should be noted that the sales from pharmacies to veterinarians of antibacterial HMPs applicable for use in dogs and cats were negligible. The amounts, in kg active substance, of usage of antibacterial HMPs for companion animals were estimated by use of the following data from VetReg:

- Delivery from pharmacies to animal owners of antibacterial HMPs for use in dogs and cats
- Delivery from pharmacies to veterinarians of antibacterial HMP tablets and of oral solution and oral powder for solution suitable for companion animals.

References:

1. Kari Grave and Kari Olli Helgesen. Antibacterials for farmed fish – prescribing, usage and diagnoses 2013 - 2017 (In Norwegian: Antibakterielle midler til oppdrettsfisk – rekvirering, forbruk og diagnose 2013 - 2017). Rapport 5: Veterinærinstituttet, 2018.
2. Kari Grave and Petter Hopp. Veterinary Prescription Register – data quality for antibacterials (In Norwegian: Veterinært legemiddelregister (VetReg) – datakvalitet for antibakterielle midler). Rapport 29: Veterinærinstituttet, 2017
3. National Strategy against Antibiotic Resistance (2015 - 2020) (in Norwegian). Nasjonal strategi mot Antibiotikaresistens 2015 – 2020. (https://www.regjeringen.no/contentassets/5eaf66ac392143b3b2054aed90b85210/strategi_antibiotikaresistens_230615.pdf)
4. EMA, 2016. European Surveillance of Veterinary Antimicrobial Consumption (ESVAC). Web Based Sales Data and Animal Population Data Collection Protocol (version 2). (http://www.ema.europa.eu/docs/en_GB/document_library/Other/2015/06/WC500188365.pdf).
5. EMA, 2017. Joint ECDC, EFSA and EMA scientific opinion on a list of outcome indicators as regards surveillance of antimicrobial resistance and antimicrobial consumption in humans and food-producing animals (http://www.ema.europa.eu/docs/en_GB/document_library/Report/2017/10/WC500237745.pdf).

Appendix 2:
Collection of data on usage of antimicrobial agents in humans

Data sources
In Norway, antimicrobials are prescription only medicines, and only allowed sold through pharmacies. These data are collected from three databases: the Norwegian drug wholesales statistics database, the hospital pharmacies drug statistics database, and the Norwegian Prescription Database (NorPD).

The Norwegian Institute of Public Health collects data on drug use from wholesalers. The wholesales database covers total sales of antimicrobials in Norway and is based on sales of medicaments from drug wholesalers to pharmacies and health institutions in Norway. The figures presented should be regarded as maximum figures based on the assumption that all medicaments sold are actually consumed. The actual drug consumption will probably be somewhat lower. Data are available since the beginning of the seventies.

Data on antibacterial use in hospitals are retrieved from *Sykehusapotekenes Legemiddelstatistikk* (the hospital pharmacies drug statistics database) which is a cooperation of LIS, Legemiddel Innkjøp Samarbeid (Drug Purchasing Cooperation) and the four regional pharmaceutical health trusts operating the hospital pharmacies in Norway. *Sykehusapotekenes Legemiddelstatistikk* collects sales data from each pharmacy delivering drugs to hospitals. Data are collected as sales from the pharmacy to hospital wards. Data have been available since 2006. The National Centre for the use of antibiotics in hospitals (*Nasjonal kompetanse-tjeneste for antibiotikabruk i spesialisthelsetjenesten*) has analysed the data according to activity (admission and bed days).

Population statistics per 1st January are collected from Statistics Norway. Information on bed days and admissions are collected from the Norwegian Patient Register at the Norwegian Directorate of Health. The definition of bed days is: "*the number of whole days an admitted patient disposes a bed*". An admission is defined as: "*admission of patient where the medical interventions usually are complex and requires hospitalisation for one or more days*" (2).

Data on the use in ambulatory care are retrieved from NorPD, a nation-wide prescription database situated at the Norwegian Institute of Public Health. This database includes all prescriptions being prescribed to out-patients in Norway. For analyses on prescriptions and DDDs, all prescriptions and DDDs to outpatients are included. For the results on annual prevalence (number of individuals per population group being prescribed antibiotics within a year) only prescriptions to individuals with national ID numbers are included. The data give us the exact population prevalence of antibacterial use in the total population in ambulatory care. More information is available at www.fhi.no. Data are available from 2004.

Drug Classification
The data are categorised according to the ATC classification system (1). Defined Daily Doses (DDDs) are employed as units of measurement. The ATC/DDD index of 2020 is used for all years.

Unit of measurement
The ATC/DDD system is recommended by the WHO to serve as a tool for drug utilisation research in order to improve quality of drug use. One component of this is the presentation and comparison of drug consumption statistics at international and other levels.

The use of defined daily dose (DDD) as a unit of measurement, simplifies and improves the evaluation of drug consumption over time, nationally and internationally. The DDD is a theoretical unit of measurement, and does not necessarily reflect the recommended or Prescribed Daily Dose.

The basic definition of the unit is:
The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults.

The DDDs for antibacterials are as a main rule based on the use in infections of moderate severity. Some antibacterials are only used in severe infections and their DDDs are assigned accordingly. The DDDs assigned are based on daily treatment.

Inclusion criteria
The antibacterials for human use included in this report belong to ATC group J01 *antibacterials for systemic use*. Oral vancomycin (A07AA09), fidaxomycin (A07AA12) and oral and rectal metronidazole (P01AB01) are also included in some figures. Antibacterials used in dermatological preparations (ATC group D) and preparations intended for sensory organs (ATC group S) are not included in the material, except for mupirocin, which is included in one table.

References
1. WHO Collaborating Centre for Drug Statistics Methodology. ATC index with DDDs 2020. WHO Collaborating Centre, Oslo
2. Definitions Norwegian Directorate of Health https://volven.helsedirektoratet.no/begrep.asp?id=452&catID=12

Appendix 3: Sampling, microbiological methods and data processing in NORM-VET

Sampling strategy

The clinical isolates included in NORM-VET 2019 were *Escherichia coli*, *Staphylococcus pseudintermedius*, and *Streptococcus canis* originating from clinical infections in dogs. The isolates were retrieved through clinical submissions to the Norwegian Veterinary Institute. The *E. coli* were included were from urinary tract infections (n=89) and from various other infections such as respiratory or skin (n=43) between the years 2016 to 2018. The *S. pseudintermedius* (n=157) and the *S. canis* (n=123) were sampled in 2017 and 2018 from various infections and organs. In total seven of the 157 *S. pseudintermedius* isolates were classified as methicillin resistant *S. pseudintermedius* (MRSP) in the diagnostic laboratory and the *mecA* gene was detected by PCR. One isolate per submission was susceptibility tested.

Caecal samples from cattle under one year of age and fattening pig were collected at slaughter throughout the year by the Norwegian Food Safety Authority (NFSA), following the specifications set by the European Food Safety Authority (EFSA; EFSA Journal 2014;12(5):3686). One individual caecal sample was included per herd, in total 319 and 298 samples from cattle and pigs, respectively, except from one cattle and one pig herd where samples were collected twice. The included indicator bacteria *E. coli*, *Enterococcus faecalis* and *E. faecium* were retrieved from these samples. The caecal samples were also used for selective isolation of *E. coli* resistant to extended-spectrum cephalosporins (ESC), and carbapenemase-producing *Enterobacteriaceae* (CPE). In addition, the caecal samples from pigs were used for selective isolation of *Campylobacter coli*. Faecal samples (one animal/herd), nasal swabs (ten animals/herd) and environmental cloths (two/herd) from 63 goat herds were collected at the farm by the NFSA in connection with the surveillance programmes for *Brucella* and paratuberculosis. The faecal samples were used for retrieving indicator *E. coli*, and for selective isolation of *E. coli* resistant to ESC, CPE, and quinolone resistant *E. coli* (QREC). The nasal swabs and environmental cloths were pooled and used for selective isolation of methicillin resistant *Staphylococcus aureus* (MRSA).

Faecal and oral/nasal/perineum swab samples from a total of 245 dogs were collected by practicing veterinarians throughout the year. Faecal samples from 40 dogs and 15 oral/nasal/perineum swab samples were not examined, leaving 205 and 230 samples for further analyses, respectively. The sampled dogs were between 0 and 14 years of age and from all over the country. A total of 30 dogs had been out of the country last six months and 38 had been treated with antibiotics last six months. The included indicator *E. coli* were retrieved from the faecal swab samples. The same samples were also used for selective isolation of *E. coli* resistant to ESC, CPE, QREC, and colistin resistant *E. coli* (COL-R). The nasal/perineum swabs were used for retrieving *S. pseudintermedius* and for selective isolation of MRSP and MRSA.

All food samples were collected by the NFSA. Beef and pork samples, 349 and 352, respectively, were collected at retail in all regions of Norway following the specifications set by EFSA (EFSA Journal 2014;12(5):3686). Samples were collected without taking place of origin into account. A total of 198 samples of leafy greens and leafy herbs were collected. The 147 leafy greens samples comprised both imported and domestically produced, washed and unwashed leafy salads and included a variety of salad types. The 51 leafy herbs were all imported and comprised a variety of washed and unwashed leafy herbs. Only one sample from each production batch was included. All the food samples were analysed using selective isolation for *E. coli* resistant to ESC and CPE. The leafy greens and leafy herbs samples were also subjected to isolation of indicator *E. coli*, and selective isolation of QREC and COL-R.

A total of 73 samples of raw dog feed were included. The samples were collected by the NFSA in connection with a surveillance programme in feed. Only one sample per production batch was included. The raw dog feed samples were used for retrieving *E. coli*, *E. faecalis* and *E. faecium* indicator bacteria, in addition to selective isolation for *E. coli* resistant to ESC, CPE, QREC, COL-R and vancomycin resistant *Enterococcus* spp. (VRE).

Indicator isolates of *E. coli*

Sample material, i.e. caecal content from one cattle and one fattening pig per herd and faecal material from one dog and one goat, were plated directly onto MacConkey agar (Difco) and incubated at 44±0.5°C for 20±2h. From samples of vegetables and raw dog feed, 25±0.5 g sample material was homogenised in 225 mL buffered peptone water (BPW-ISO) and incubated at 37±1°C for 20±2h according to the protocol from the European Union Reference Laboratory for Antimicrobial Resistance (EURL-AR; https://www.eurl-ar.eu/protocols). From the overnight enrichment broth 10-20 µL were plated onto MacConkey agar and incubated at 44±0.5°C for 20±2h. From all sample types, typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood and incubated at 37±1°C for 20±2h. Colonies were identified as *E. coli* by typical colony appearance and a positive indole reaction.

Indicator isolates of *E. faecalis* and *E. faecium*

Sample material, i.e. caecal content from one cattle and one fattening pig per herd and faecal material from one dog were plated directly onto Slanetz and Bartley agar (Oxoid, Oslo, Norway) and incubated at 44±0.5°C for 24-48h. From raw dog feed samples, 10-20 µL of the overnight BPW-ISO broth were plated onto Slanetz and Bartley agar before further incubation. From all sample types, typical colonies were subcultured on blood agar incubated at 37±1°C for 20±2h. Colonies were identified as *E. faecalis* and/or *E. faecium* using Matrix Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics GmbH, Bremen, Germany).

Enrichment of samples before selective isolation

All samples were enriched prior to plating onto selective media. A total of 1±0.1 g caecal sample material, and faecal material from goat was homogenised with 9 mL of BPW-ISO. Faecal swab samples from dogs were inoculated in 5

mL of BPW-ISO. A total of 25±0.5 g sample material of beef, pork, leafy greens and leafy herbs, and raw dog feed were homogenised with 225 mL of BPW-ISO. Samples were incubated at 37±1°C for 20±2h according to the protocol from the EURL-AR (http://www.eurl-ar.eu/233-protocols.htm). After incubation, 10-20 µL of the enrichment broth was plated onto selective media as described in the sections below.

***E. coli* resistant to extended-spectrum cephalosporins**
Aliquots from the overnight BPW-ISO broth from all caecal, faecal, beef, pork, leafy green and leafy herbs, and raw dog feed samples were plated onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar (Difco) containing 2 mg/L ceftazidime. The agar plates were incubated at 44±0.5°C for 20±2h. Presumptive *E. coli* resistant to ESC were subcultured onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and blood agar, and confirmed as *E. coli* using MALDI-TOF MS before further testing for cephalosporinase production.

Quinolone resistant *E. coli*
Aliquots from the overnight BPW-ISO broth from faecal, leafy greens and leafy herbs, and raw dog feed samples were plated onto MacConkey agar (Difco) containing 0.06 mg/L ciprofloxacin. Plates were incubated at 44±0.5°C for 20±2h. Presumptive QREC were subcultured onto MacConkey agar (Difco) containing 0.06 mg/L ciprofloxacin and blood agar and confirmed as *E. coli* using MALDI-TOF MS before further phenotypical testing.

Carbapenemase-producing *Enterobacteriaceae*
Aliquots from the overnight BPW-ISO broth from all caecal, faecal, beef, pork, leafy greens and leafy herbs, and raw dog feed samples were plated onto CHROMID® CARBA and CHROMID® OXA-48 agar (bioMérieux, Marcy l'Etoile, France). The plates were incubated at 35±2°C for 24-48 h. Presumptive CPE were subcultured on respective selective CHROMID® agar and blood agar, and species confirmed using MALDI-TOF MS before further phenotypical testing.

Colistin resistant *E. coli*
Aliquots from the overnight BPW-ISO broth from faecal, leafy greens and leafy herbs, and raw dog feed samples were plated onto SuperPolymyxin agar (Oxoid) and incubated at 44±0.5°C for 20±2h (Nordmann *et al.* 2016). Presumptive COL-R colonies were selected, subcultured on blood agar and Super-Polymyxin agar, and confirmed as *E. coli* using MALDI-TOF MS before further phenotypical testing.

Vancomycin resistance *Enterococcus* spp.
Aliquots from the overnight BPW-ISO broth from the raw dog feed samples were plated onto Slanetz and Bartley agar containing 4 mg/L vancomycin before incubation at 44±0.5°C for 24-48h. Presumptive positive colonies were selected, subcultured on blood agar and confirmed as *E. faecalis* or *E. faecium* using MALDI-TOF MS before further phenotypical testing.

Staphylococcus pseudintermedius
Nasal/perineum swabs from dogs were analysed for *S. pseudintermedius* by plating sample material directly onto blood agar containing washed bovine blood cells before incubation at 37±1°C for 20±2h. Presumptive *S. pseudintermedius* were selected, subcultured on blood agar and species confirmed using MALDI-TOF MS before further phenotypical testing.

MRSA and MRSP
Pooled nasal swabs and environmental cloths from goat herds were analysed for MRSA and oral/nasal/perineum swabs from dogs were analysed for both MRSA and MRSP. Sample material were incubated in Mueller-Hinton broth containing 6.5% NaCl at 37±1°C for 18-24 hours. A loopful of the overnight broth (10 µL) was plated onto Brilliance™ MRSA2 agar plate (Oxoid) (EFSA journal 2012:10 (10):2897). Suspected colonies were subjected to species identification using MALDI-TOF MS before further phenotypical testing.

Streptococcus canis
All isolates retrieved from clinical samples were species identified using MALDI-TOF MS. For isolates difficult to distinguish from *Streptococcus dysgalactiae* using MALDI-TOF MS, the Lancefield grouping was performed using Oxoid™ Streptococcal grouping kit (Oxoid) and also the ability for the bacteria to ferment trehalose (*S. canis* are negative, while *S. dysgalactiae* are positive).

Genotyping
For genotyping of presumptive resistant isolates, the procedure was either performed by conventional PCR or whole genome sequencing (WGS). For presumptive *E. coli* resistant to ESC, PCR was performed for the identification of the genotypes $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, multiplex PCR for plasmid-mediated AmpC genes, or PCR for the $bla_{\text{CMY-2}}$ gene (Pérez-Pérez *et al.* 2002, Hasman *et al.* 2005, Briñas *et al.* 2002, Sundsfjord *et al.* 2004). For *E. coli* isolates with an AmpC beta-lactamase resistance profile where no plasmid-mediated AmpC genes were detected, amplification of the promoter and attenuator regions of the chromosomal *ampC* gene was performed to detect any mutation causing an upregulation of the chromosomally located *ampC* gene in *E. coli* (Agersø *et al.* 2012, Peter-Getzlaff *et al.* 2011, Tracz *et al.* 2007). For presumptive MRSA or MRSP isolates, real-time PCR for the detection of *mecA* and *nuc* genes together with a conventional PCR for the *mecC* gene was performed (Tunsjø *et al.* 2013, Stegger *et al.* 2012). WGS was performed at the NVI on an Illumina® MiSeq (Illumina, San Diego, California, USA). Paired end reads were subjected for analysis using ResFinder V.3.2 for both aquired genes and chromosomal point mutations (PointFinder) using the online tool at the Centre for Genomic Epidemiology web site (https://cge.cbs.dtu. dk/services/ResFinder/).

Susceptibility testing
Isolates were tested for antimicrobial susceptibility using a broth microdilution method at the NVI, Oslo. Minimum inhibitory concentration (MIC) values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the tested bacteria. Epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 25.03.2020) with some exceptions as explained further in Appendix 7. See Appendix 6 for definitions of cut-off values. The table below gives an overview of which panel was used for which clinical isolate.

Overview of which Sensititre® TREK panel was used for which clinical isolate:

Clinical isolate tested	**Sensititre® TREK panel**
Streptococcus canis	STP6F
Escherichia coli	EUVSEC
Staphylococcus pseudintermedius	EUST

Quality assurance systems

The following susceptible bacteria were included as quality control on a regular basis: *E. coli* ATCC 25922, *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213 and *Streptococcus pneumoniae* CCUG 33638. In addition to the regular susceptible bacteria, the following bacteria received from EURL-AR were included: *Acinetobacter baumanii* 2012-70-100-69 (EUVSEC and EUVSEC2 panel), and *E. faecium* 2012-70-76-8 and *E. faecalis* 2012-70-103-3 (EUVENC panel). The following resistant bacteria were tested on a regular basis: *E. coli* CCUG 37382, *E. coli* K8-1 (ESBL), *E. coli* K5-20 (AmpC), *E. coli* 2012-60-1176-27 (*mcr-1*) and *E. coli* KP37 (*mcr-2*). The results were approved according to reference values given by EUCAST when available. Additional control strains were included when necessary. The laboratories at the NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit. Loughborough. UK) and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

Data processing

Susceptibility data were recorded and stored in the sample registration system at the NVI as discrete values (MIC). Data management was performed both in SAS-PC System® v 9.4 for Windows (SAS Institute Inc. Cary. NC. USA) and in R version 3.6.2 Copyright (C) 2019 (The R Foundation for Statistical Computing Platform), while the statistical analysis was performed in R. All changes and differences yielding a p-value < 0.05 were considered as statistically significant. The 95% confidence intervals were calculated using the exact binomial test.

Appendix 4:
Sampling, microbiological methods and data processing of zoonotic and non-zoonotic enteropathogenic bacteria in NORM and NORM-VET

NORM-VET enteropathogenic bacteria
Sampling strategy – animals and food

Salmonella
Isolates of *Salmonella* spp. were retrieved from the Norwegian *Salmonella* control programme for live animals. Additional isolates were obtained from clinical submissions or necropsies at the Norwegian Veterinary Institute (NVI). One isolate of each serovar per incident was included for susceptibility testing.

Campylobacter coli
Sample material, i.e. caecal content from one fattening pig per herd were plated directly onto mCCDA agar and incubated under microaerophilic conditions at 41.5±0.5°C for 48h. Typical colonies were subcultured on blood agar and confirmed as *Campylobacter coli* using Matrix Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry (MALDI-TOF MS, Bruker Daltonics GmbH).

Pathogenic *Yersinia enterocolitica*
Isolates of pathogenic *Yersinia enterocolitica* were retrieved from a surveillance programme for pathogenic *Yersinia entericolitica* in minced pork meat in 2019. Samples were collected by The Norwegian Food Safety Authority (NFSA). Pathogenic *Y. enterocolitica* were isolated on CIN agar, confirmed by using MALDI-TOF MS, and tested for presence of the *ail*-gene by real-time PCR.

Susceptibility testing animal isolates
Isolates were tested for antimicrobial susceptibility at the NVI, Oslo. MIC values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the bacteria to be tested as recommended by Decision 2013/652/EU, see table below. For animal isolates, epidemiological cut-off (ECOFF) values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 25.03.2020) were used, with some exceptions as explained further in Appendix 7.

Overview of which Sensititre® TREK panel was used for which clinical isolate:

Bacteria tested	Sensititre® TREK panel
Salmonella spp.	EUVSEC
Camplylobacter coli	EUCAMP2
Yersinia enterocolitica	EUVSEC

Quality assurance systems NORM-VET
The following susceptible bacteria were included as quality controls on a regular basis: *E. coli* ATCC 25922 and *C. jejuni* ATCC 33560. In addition to the regular susceptible bacteria, the following bacterium received from EURL-AR were included: *C. coli* 2012-70-443-2 (EUCAMP2 panel). The NVI and the Reference Laboratory for Enteropathogenic Bacteria/NIPH have a quality assurance system according to the requirements of NS-EN ISO/IEC 17025. The participating laboratories at the NVI are accredited according to the requirements of NS-EN ISO/IEC 17025 and participate in external quality assurance programmes for veterinary pathogens (Veterinary Laboratories Agency Quality Assurance Unit Loughborough. UK) and for resistance monitoring (EURL for Antimicrobial Resistance in Denmark).

Data processing animal isolates
Susceptibility data were recorded and stored in the sample registration system at NVI as discrete values (MIC). Data management was performed both in SAS-PC System® v 9.4 for Windows (SAS Institute Inc. Cary. NC. USA) and in R version 3.6.2 Copyright (C) 2019 (The R Foundation for Statistical Computing Platform), while the statistical analysis was performed in R. Significance tests for differences between proportions of resistant isolates were calculated using Pearson's Chi-squared Test or Fisher's Exact Test for Count Data as appropriate. All changes and differences yielding a p-value < 0.05 were considered as statistically significant. The 95% confidence intervals were calculated using the exact binomial test.

NORM – enteropathogenic bacteria
Sampling strategy - humans
All human isolates of *Salmonella*, *Yersinia enterocolitica* and *Shigella* were obtained from clinical cases. One isolate per patient or one isolate per recognised outbreak was included for susceptibility testing. *Campylobacter* isolates from a selection of a little less than 10% of campylobacteriosis cases registered were submitted in the following way: Five regional laboratories submitted the first five independent isolates each month to the NRL for Enteropathogenic Bacteria at the Norwegian Institute of Public Health.

Identification of bacteria – human isolates
The reference analyses on human clinical isolates of enteropathogenic bacteria were performed according to conventional methods described in standard reference literature (e.g. Murray PR & al.: Manual of Clinical Microbiology, 8th edition ASM-Press, Washington 2003 and Ewing WH: Edwards and Ewing's Identification of *Enterobacteriaceae*, 4. edition, Elsevier, New York 1986).

Susceptibility testing human isolates
Salmonella spp., *Yersinia* spp. and *Shigella* spp. isolates from humans were susceptibility tested at the NRL for Enteropathogenic Bacteria at the NIPH by agar disk diffusion tests according to the EUCAST standardised method for AMR testing of non-fastidious bacteria. *Campylobacter* isolates from humans were tested for antimicrobial susceptibility using MIC Test Strips (Liofilchem).
For human isolates EUCAST clinical or epidemiological breakpoints for *Enterobacteriaceae*, version 10.0 2020 were used if defined. In absence of clinical breakpoints, ECOFFs based on national zone distributions were used

(e.g. tetracycline). Pefloxacin was used to infer ciprofloxacin resistance in *Salmonella.*
Isolates with reduced susceptibility to cefotaxime or ceftazidime were tested for the presence of $ESBL_A$ by a double disk approximation test (BD Sensidisc), and for the presence of $ESBL_M$ by an AmpC detection test (Liofilchem MIC test strips). Isolates with reduced susceptibility to meropenem were forwarded to the Norwegian National Advisory Unit on Detection of Antimicobial Resistance (K-res) for further analyses.

Genotyping
For presumptive $ESBL_A$ and $ESBL_M$ isolates, Spades assembled whole genome sequences (Illumina generated) were screened for acquired antimicrobial resistance genes using the ResFinder 3.2 software and database online with default threshold and length settings. (https://cge.cbs.dtu.dk/services/ResFinder/).

Quality assurance systems human isolates
The NRL for Enteropathogenic Bacteria at the NIPH is accredited according to the requirements of NS-EN ISO/IEC 17025. *E. coli* ATCC 25922 was used as quality control strain for AMR testing of non-fastidious *Enterobacteriaceae.* The NRL participated in the external quality assessment programme of ECDC for *Salmonella* spp. and *Campylobacter* for antimicrobial susceptibility testing.

Data processing human isolates
The NRL at the NIPH stored susceptibility data of human isolates as either millimeter zone diameters or MIC values. The results were further analysed by WHONET 5.6 with the aid of the BacLink programme, both developed by Dr. John Stelling.

Appendix 5: Sampling, microbiological methods and data processing in NORM

General considerations

NORM is based on a combination of periodic sampling and testing in primary diagnostic laboratories and annual results from national reference laboratories for specific micro-oganisms. Isolates are included from defined clinical conditions such as respiratory tract infections, wound infections, urinary tract infections, and septicaemiae. For enteric infections see Appendix 4. 2019 was the twentieth year of surveillance, and all 22 diagnostic laboratories in Norway participated in the surveillance system in addition to eleven reference laboratories. All diagnostic laboratories followed the same sampling strategy and used identical criteria for the inclusion of microbial strains. Only one isolate per patient and infectious episode was included unless otherwise stated. All microbes were identified using conventional methods as described in the ASM Manual of Clinical Microbiology. The surveillance period started in the beginning of January, and consecutive isolates were included for defined time periods for each surveillance category. The surveillance categories and sampling periods in 2019 were as follows: *E. coli* in blood cultures (6 months); *Klebsiella* spp., *Staphylococcus aureus*, *Enterococcus* spp. and *Pseudomonas aeruginosa* in blood cultures (9 months); *Streptococcus agalactiae* and *Candida* spp. from blood cultures (12 months); *Neisseria meningitidis* from blood cultures and cerebrospinal fluids (12 months); *S. aureus* (1 week) and *S. pyogenes* (3 weeks) from wound specimens; *S. pyogenes* from respiratory tract samples (3 weeks); *E. coli* from urinary tract infections (3 days); *Klebsiella* spp. and *P. aeruginosa* from urinary tract infections (3 weeks); *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae* from all samples (12 months). *N. meningitidis* from blood cultures and cerebrospinal fluids was analysed at the the Norwegian Institute of Public Health (NIPH) in Oslo. *N. gonorrhoeae* was analysed at NIPH and Oslo University Hospital (OUS)/Ullevål. *Candida* isolates were analysed at OUS/Rikshospitalet. MRSA and *S. agalactiae* isolates were analysed at St. Olav University Hospital in Trondheim. *M. tuberculosis* isolates were analysed at NIPH, OUS/Ullevål and OUS/Riks-hospitalet.

Susceptibility testing

E. coli, *Klebsiella* spp., *Enterococcus* spp., *S. aureus* and *P. aeruginosa* isolates were examined according to the EUCAST disk diffusion method using antibiotic disks and Mueller Hinton II agar from either Oxoid or Beckton Dickinson. Suitable antibiotics were selected for each bacterial species, and the results were interpreted according to the most recent breakpoints from NordicAST, which are harmonised with EUCAST. Beta-lactamase production in *S. aureus* and *N. gonorrhoese* was examined by nitrocefin disks, acidometric agar plates (3.6 mg/L penicillin G and phenol red) or clover leaf test. *Enterococcus* strains were screened for glycopeptide resistance using vancomycin 6 mg/L BHI agar. *S. pyogenes*, *S. agalactiae*, *N. meningitidis* and *N. gonorrhoeae* were susceptibility tested using MIC gradient tests (bioMerieux or Liofilchem) on MH II agar supplemented with 5% lysed horse blood or GC agar with 1% haemoglobin and Isovitalex (*N. gonorrhoeae*). Susceptibility testing of *Candida* spp. isolates was performed by MIC gradient tests using RPMI agar containing 2% glucose and MOPS. Resistance values were recorded as mm inhibition zone sizes or MIC values in order to monitor trends in the occurrence of resistance.
M. tuberculosis isolates were tested using BACTEC MGIT 960 systems. All three test laboratories participate in the WHO external DST quality control programme. They were also tested for mutations in the *rpoB* gene to detect rifampicin resistance.

Confirmation of resistance phenotypes

E. coli and *Klebsiella* spp. with reduced susceptibility to third generation cephalosporins were examined for ESBL production using ESBL combination MIC gradient tests (Liofilchem), disks (BD) or tablets (Rosco) according to the instructions of the manufacturer. *S. aureus* isolates with reduced susceptibility to cefoxitin were examined by *mecA* PCR for confirmation of methicillin resistance (MRSA). *Enterococcus faealis* and *E. faecium* isolates displaying growth on the vancomycin screening agar were examined by *van* PCRs. The MLS phenotype of erythromycin resistant *S. aureus* and *S. pyogenes* isolates was analysed using the double disk diffusion (DDD) synergy assay with erythromycin and clindamycin disks.

Quality control

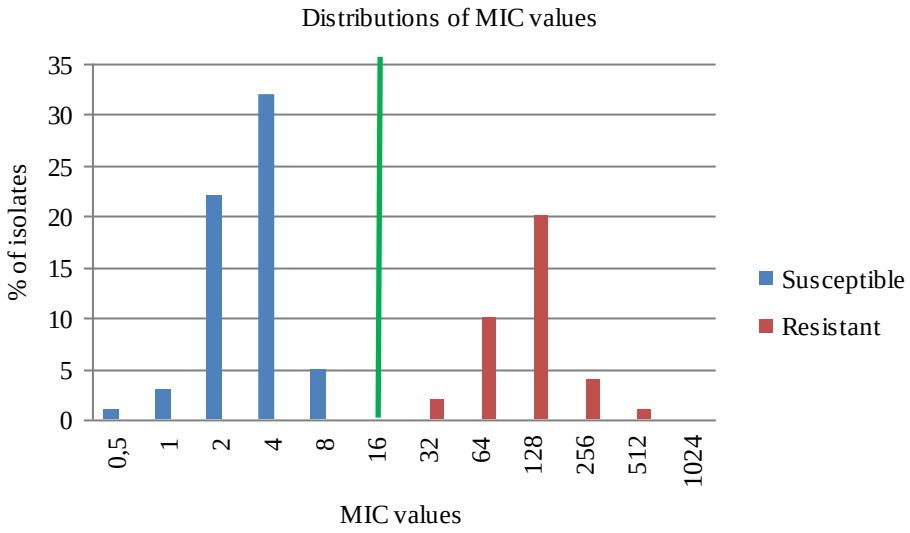
The following strains were used for quality control: *E. coli* ATCC 25922, *K. pneumoniae* ATCC 700603 (ESBL positive), *E. faecalis* ATCC 29212, *E. faecalis* ATCC 51299, *S. pneumoniae* ATCC 49619, *S. pneumoniae* TIGR4, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300 (heterogeneous MRSA), *S. aureus* CCUG 35600 (homogeneous MRSA), *N. gonorrhoeae* CCUG 26213/ATCC 49266, *N. gonorrhoeae* WHO L, *C. albicans* ATCC 90028, *C. krusei* ATCC 6258 and *C. parapsillosis* ATCC 22019.

Data processing

The specially designed web-based eNORM computer programme was used for registration and storage of patient data, sample data and resistance data. The results were further analysed by WHONET 5.6 with the aid of the BacLink programme, both developed by Dr. John Stelling. The distribution of microbial species in blood culture was based on extraction of routine data from the laboratory information systems of the participants. All isolates of the same species recovered within one month after the initial finding were considered duplicates and omitted from the survey. No attempts were made to evaluate the clinical significance of each finding.

Appendix 6:
Definitions and classification of resistances used in this report

General guidelines for the reader of this report
The results presented in the NORM and NORM-VET programmes are not directly comparable. This is because the sampling and the classification of resistance differs between the programmes. Clinical breakpoints are used for the classification within NORM, while epidemiological cut-off values (ECOFF) are used for the classification of resistance within NORM-VET. EUCAST definitions of clinical breakpoints and ECOFF values are presented at http://www.eucast.org. The terms and usage of these two ways of classification of resistance are further explained below. The ECOFF would normally be lower for minimum inhibitory concentration (MIC) values and higher for disk diffusion diameters compared to the clinical breakpoints. However, this is not always the case.



Epidemiological cut-off values
The ECOFF may indicate emerging resistance in the bacterial populations. Based on the distribution of the MIC values or the inhibition zone diameter distribution, each bacterial population could (in an ideal case) be divided into two populations by a biphasic curve as shown in the example above. The curve to the left (blue) shows the susceptible or wild-type distribution whereas the curve to the right (red) shows the resistant or non-wild-type distribution. The green line indicates a possible ECOFF value applicable to the distributions in the example.
However, for several bacterial populations and corresponding tested antimicrobial substances these distributions may be overlapping. A part of the population within the overlapping area may carry resistance mechanisms and others not. In the area with the non-wild-type distribution, new resistance mechanisms are responsible for the resistance either alone or in addition to the resistance mechanisms present at lower MIC values. In order to establish MIC values for each specific bacterial population and antimicrobial agent, large amounts of data are collected and assessed. In the NORM-VET part of this report, we have mainly used the ECOFF values recommended by EUCAST. However, for some combinations of bacteria and antimicrobial agents these were not applicable to our data. In these cases, ECOFF values defined based on the actual MIC distributions obtained in the NORM-VET programme were used. We applied the normalised resistance interpretation (NRI) method with permission from the patent holder, Bioscand AB, TÄBY, Sweden (European patent No 1383913, US Patent No. 7,465,559). The automatic and manual excel programmes were made available through courtesy of P. Smith, W. Finnegan, and G. Kronvall and were applied on the clinical isolates of *S. canis* to define ECOFFS.

Clinical breakpoints
Clinical breakpoints are defined in order to indicate if treatment of a specific pathogen is likely to succeed or not. Other factors like dosage and formulations also affect the clinical result. The MIC values are ideally obtained for the pathogen *in vitro*, and this is compared with the predetermined clinical breakpoint to determine whether the organism is likely to respond *in vivo*.

Term used to describe antimicrobial resistance levels
In this report the level of resistance (i.e. the percentage of resistant isolates among the tested isolates) in the NORM-VET programme have been classified according to the levels presented in The European Union Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018 by EFSA Journal 2020;18(3):6007 as follows:

Rare:	< 0.1%
Very Low:	0.1% to 1%
Low:	> 1% to 10%
Moderate:	> 10% to 20%
High:	> 20% to 50%
Very high:	> 50% to 70%
Extremely high:	> 70%

Appendix 7: Cut-off values NORM-VET

Epidemiological cut-off (ECOFF) values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 23.03.2020) were used. ECOFFs for *Staphylococcus aureus* were used for *S. pseudintermedius*. For additional antimicrobial agents not defined in the EUCAST recommendations, EFSA recommended cut-off was used or cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme as described in Appendix 6. This was applied on the clinical isolates of *Streptococcus canis*, and on trimethoprim for the *S. pseudintermedius*.

Overview of antimicrobial classes and agents tested for with their corresponding epidemiological cut-off values, that are used in NORM-VET 2019:

Antimicrobial class	Antimicrobial agents	*Escherichia coli*	*Salmonella* spp.	*Yersinia enterocolitica*	*Campylobacter coli*	*Enterococcus faecalis / E. faecium*	*Staphylococcus aureus / S. pseudintermedius*	*Streptococcus canis*
Tetracyclines	Tetracycline	>8	>8	>4	>2	>4	>1	ND
	Tigecycline	>0.5	>1[#]	ND		>0.5/>0.25*		>0.25
Amphenicols	Chloramphenicol	>16	>16	ND		>32	>16	>8
Penicillins with extended spectrum	Ampicillin	>8	>8	ND		>4		
	Temocillin	(>16)						
Beta-lactamase sensitive penicillins	Benzylpenicillin						>0.125	NA
Combinations of penicillins	Amoxicillin and enzym inhibitor (clavulanic acid)							NA
2nd generation cephalosporins	Cefoxitin	(>8)					>4	
	Cefuroxime							NA
3rd generation cephalosporins	Cefotaxime	>0.25	>0.5	>1				NA
	Ceftazidime	>0.5	>2	ND				
	Ceftriaxone							NA
Combinations of 3rd generation cephalosporins and clavulanic acid	Cefotaxime/clavulanate	(>0.25)						
	Ceftazidime/clavulanate	(>0.5)						
4th generation cephalosporins	Cefepime	(>0.125)						NA
Carbapenems	Meropenem	>0.125	>0.125	ND				NA
	Ertapenem	(ND)						>1
	Imipenem and enzyme inhibitor	(>0.5)						
Trimethoprim and derivatives	Trimethoprim	>2	>2	>4			>8	
Sulfonamides	Sulfamethoxazole	>64	>256[#]	ND			>128	
Combinations of sulfonamides and trimethoprim, incl. derivates	Sulfamethoxazole and trimethoprim							>1
Macrolides	Erythromycin				>8	>4	>1	>0.5
	Azithromycin	ND	ND	ND				>0.5
Lincosamides	Clindamycin						>0.25	>0.25
Streptogramins	Quinupristin and dalfopristin					ND	>1	

Antimicrobial class	Antimicrobial agents	*Escherichia coli*	*Salmonella* spp.	*Yersinia enterocolitica*	*Campylobacter coli*	*Enterococcus faecalis / E. faecium*	*Staphylococcus aureus / S. pseudintermedius*	*Streptococcus canis*
Streptomycins	Streptomycin				>4		>16	
Other aminoglycosides	Gentamicin	>2	>2	ND	>2	>32	>2	
	Kanamycin						>8	
Fluoroquinolones	Ciprofloxacin	>0.064	>0.064	>0.25	>0.5	>4/>8*	>1	
	Levofloxacin							>2
	Moxifloxacin							>2
Other quinolones	Nalidixic acid	>8	>8	ND	>16			
Glycopeptid antibacterials	Vancomycin					>4	>2	>1
	Teicoplanin					>2		
Polymyxins	Colistin	>2	>2[#]	ND				
Steroid antibacterials	Fusidic acid						>0.5	
Pleuromutilins	Tiamulin						>2	
Other antibacterials	Linezolid					>4	>4	>4
	Daptomycin					>4/>8**		>0.25
Other antibiotics for topical use	Mupirocin						>1	
	Rifampicin						>0.016	

ND=not defined, NA=not applicable, (X)=only ESBL/AmpC suspected isolates tested as described in Commission implementing decision of 12. Nov 2013 on the monitoring and reporting of antimicrobial resistance in zoonotic and commensal bacteria (2013/652/EU), data not shown in the report tables. [#] Cut-off defined by EFSA. *> 0.5 for *E. faecalis*, > 0.25 for *E. faecium*, **> 4 for *E. faecalis*, > 8 for *E. faecium*

Appendix 8: Breakpoints NORM

NORM data are categorised according to the breakpoints of the Nordic Committee on Antimicrobial Susceptibility Testing (NordicAST) which are harmonised with EUCAST breakpoints. NordicAST breakpoints are available at www.nordicast.org.

Antimicrobials	MIC (mg/L)		*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Salmonella* spp.	*Shigella* spp.	*Yersinia enterocolitica*	*Campylobacter jejuni*	*Campylobacter coli*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
	S	R																		
Amikacin	≤ 16	> 16			■															
Amphotericin B	≤ 1	> 1															■	■	■	■
Ampicillin	≤ 4	> 8												■						
	≤ 8	> 8	■					■	■	■										
Amoxi-Clav*	≤ 8	> 8	■	■																
	≤ 32	> 32	■	■																
Anidulafungin	≤ 0.002	> 4																		■
	≤ 0.03	> 0.03															■			
	≤ 0.06	> 0.06																■	■	
Aztreonam	≤ 0.001	> 16			■															
Cefepime	≤ 1	> 4	■	■																
Cefixime	≤ 0.125	> 0.125					■													
Cefoxitin	≥ 22 mm	< 22 mm											■[1]							
Cefotaxime	≤ 1	> 2	■	■				■	■	■										
Ceftazidime	≤ 0.001	> 8			■															
	≤ 1	> 4	■	■				■	■	■										
Ceftriaxone	≤ 0.125	> 0.125				■	■													
	≤ 0.5	> 2																		
Cefuroxime	≤ 0.001	> 8	■	■																
Chloramphenicol	≤ 2	> 2				■														
	≤ 8	> 8						■	■	■										
Ciprofloxacin	≤ 0.001	> 0.5			■															
	≤ 0.001	> 1											■							
	≤ 0.03	> 0.03				■														
	≤ 0.03	> 0.06					■													
	≤ 0.25	> 0.5	■	■					■	■										
	≤ 0.5	> 0.5									■	■								
Clindamycin	≤ 0.25	> 0.5											■							
	≤ 0.5	> 0.5													■	■				
Erythromycin	≤ 0.25	> 0.5													■	■				
	≤ 1	> 2											■							
	≤ 4	> 4									■									
	≤ 8	> 8										■								

Antimicrobials	MIC (mg/L) S	MIC (mg/L) R	*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Salmonella* spp.	*Shigella* spp.	*Yersinia enterocolitica*	*Campylobacter jejuni*	*Campylobacter coli*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Fluconazole	≤ 0.002	> 32																■		
	≤ 2	> 4															■		■	■
Fosfomycin	≤ 32	> 32	■																	
Fusidic acid	≤ 1	> 1											■							
Gentamicin	≤ 1	> 1											■							
	≤ 2	> 2	■	■							■[2]	■[2]								
	≤ 128	> 128												■						
Imipenem	≤ 0.001	> 4			■									■						
Linezolid	≤ 4	> 4											■	■						
Mecillinam	≤ 8	> 8	■	■																
Meropenem	≤ 2	> 8	■	■	■			■	■	■										
Micafungin	≤ 0.002	> 2																		■
	≤ 0.016	> 0.016															■			
	≤ 0.03	> 0.03																■		
Mupirocin	≤ 1	> 256											■							
Nitrofurantoin	≤ 64	> 64	■																	
Penicillin G	≤ 0.06	> 0.25				■														
	≤ 0.06	> 1					■													
	≤ 0.25	> 0.25													■	■				
Pefloxacin	≥ 24 mm	< 24 mm						■[3]												
Pip-Tazo**	≤ 0.001	> 16			■															
	≤ 8	> 16	■	■																
Rifampicin	≤ 0.06	> 0.5											■							
	≤ 0.25	> 0.25				■														
Spectinomycin	≤ 64	> 64					■													
Tetracycline	≤ 0.5	> 1					■													
	≤ 1	> 2											■		■	■				
	≤ 2	> 2				■					■	■								
	≥ 17 mm	< 17 mm						■[2]	■[2]	■[2]										
Tigecycline	≤ 0.25	> 0.25												■						
	≤ 0.5	> 0.5	■										■							
Tobramycin	≤ 2	> 2			■															
Trimethoprim	≤ 4	> 4	■	■																

Antimicrobials	MIC (mg/L)		*Escherichia coli*	*Klebsiella* spp.	*Pseudomonas aeruginosa*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Salmonella* spp.	*Shigella* spp.	*Yersinia enterocolitica*	*Campylobacter jejuni*	*Campylobacter coli*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
	S	R																			
TMS***	≤ 1	> 2													■						
	≤ 2	> 4	■	■									■								
Vancomycin	≤ 2	> 2											■			■					
	≤ 4	> 4												■							
Voriconazole	≤ 0.06	> 0.25															■				
	≤ 0.125	> 0.25																		■	■

*Amoxi-Clav= Amoxicillin-Clavulanic acid. **Pip-Tazo=Piperacillin-Tazobactam. ***TMS Trimethoprim-sulfamethoxazole. Breakpoints for the combination are given for the trimethoprim component only. [1]Epidemiological cut-off value based on the wild-type distribution by EUCAST. [2] Breakpoints according to national zone distributions. [3] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion. Susceptibility to ciprofloxacin is inferred from pefloxacin disk diffusion according to EUCAST clinical breakpoints (v. 10.0).

Appendix 9: References used in this report

- Agersø Y, Aarestrup FM, Pedersen K, Seyfarth AM, Struve T, Hasman H. Prevalence of extended-spectrum cephalosporinase (ESC)-producing *Escherichia coli* in Danish slaughter pigs and retail meat identified by selective enrichment and association with cephalosporin usage. *J Antimicrob Chemother*. 2012 Mar;67(3):582-8.
- Agersø Y, Torpdahl M, Zachariasen C, Seyfarth AM, Hammerum AM, & Nielsen EM (2012). Tentative Colistin Epidemiological Cut-Off Value for Salmonella spp. *Foodborne Pathogens and Disease*, *9*(4), 367-369.
- Briñas L, Zarazaga M, Sáenz Y, Ruiz-Larrea F, Torres C. Beta-lactamases in ampicillin-resistant *Escherichia coli* isolates from foods, humans, and healthy animals. *Antimicrob Agents Chemother*. 2002 Oct;46(10):3156-63.
- Day MJ, Hopkins KL, Wareham DW, et al. Extended-spectrum beta-lactamase-producing Escherichia coli in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. *Lancet Infect Dis.* Dec 2019;19(12):1325-1335.
- Dorado-Garcia A, Smid JH, van Pelt W, et al. Molecular relatedness of ESBL/AmpC-producing Escherichia coli from humans, animals, food and the environment: a pooled analysis. *J Antimicrob Chemother.* Feb 1 2018;73(2):339-347.
- EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). EU Summary Report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2017. EFSA Journal 2019;17(2):5598
- EFSA (European Food Safety Authority) and ECDC (European Centre for Disease Prevention and Control). The European Union Summary Report on Antimicrobial Resistance in zoonotic and indicator bacteria from humans, animals and food in 2017/2018. EFSA Journal 2020;18 (3):6007,166pp.https://doi.org/10.2903/j.efsa.2020.6007
- EURL-AR Laboratory protocol. Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* from caecal samples. February 2018. Version 6. https://www.eurl-ar.eu/protocols.aspx
- EURL-AR Laboratory protocol. Isolation of ESBL-, AmpC- and carbapenemase-producing *E. coli* in fresh meat. February 2018. Version 6. https://www.eurl-ar.eu/protocols.aspx
- Hasman H, Mevius D, Veldman K, Olesen I, Aarestrup FM. beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. *J Antimicrob Chemother.* 2005 Jul;56(1):115-21.
- Manageiro V, Jones-Dias D, Ferreira E, Caniça M. Plasmid-Mediated Colistin Resistance (*mcr-1*) in *Escherichia coli* from Non-Imported Fresh Vegetables for Human Consumption in Portugal. *Microorganisms*. 2020;8(3):429.
- Nordmann P, Jayol A, Poirel L. A Universal Culture Medium for Screening Polymyxin-Resistant Gram-Negative Isolates. *J Clin Microbiol.* 2016 May;54(5):1395-9.
- NORM/NORM-VET 2004. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2005. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2008. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2009. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2011. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2012. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2013. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2014. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2015. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2016. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2016. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2017. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2017. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2018. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- NORM/NORM-VET 2018. Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway. Tromsø / Oslo 2019. ISSN: 1502-2307 (print) / 1890-9965 (electronic).
- Pérez-Pérez FJ, Hanson ND. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol.* 2002 Jun; 40(6): 2153-62.
- Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, Böttger EC, Zbinden R, Bloemberg GV. Detection of AmpC beta-lactamase in *Escherichia coli*: comparison of three phenotypic confirmation assays and genetic analysis. *J Clin Microbiol.* 2011 Aug;49(8):2924-32.
- Stegger M, Andersen PS, Kearns A, Pichon B, Holmes MA, Edwards G, Laurent F, Teale C, Skov R, Larsen AR. Rapid detection, differentiation and typing of methicillin-resistant *Staphylococcus aureus* harbouring either *mecA* or the new mecA homologue *mecALGA251*. *CMI.* 2011 18, 395-400.
- Sundsfjord A, Simonsen GS, Haldorsen BC, Haaheim H, Hjelmevoll SO, Littauer P, Dahl KH. Genetic methods for detection of antimicrobial resistance. *APMIS* 2004;112:815–37.
- Tracz DM, Boyd DA, Hizon R, Bryce E, McGeer A. Ofner-Agostini M. Simor AE. Paton S. Mulvey MR. Canadian Nosocomial Infection Surveillance Program. ampC gene expression in promoter mutants of cefoxitin-resistant *Escherichia coli* clinical isolates. *FEMS Microbiol Lett.* 2007 May;270(2):265-71.
- Tunsjø HS, Follin-Arbelet B, Clausen NM, Ness Y, Leegaard TM, Bemanian V. A rapid, high-throughput screening method for carriage of methicillin-resistant *Staphylococcus aureus*. *APMIS.* 2013 Sep;121(9):865-70.
- WHO. Critically important antimicrobials for human medicine, 6th revision. Geneva: World Health Organization; 2019.
- Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother*. 2012 Jul 10. PMID: 22782487 doi: 10.1093/jac/dks261
- Zurfluh K, Poirel L, Nordmann P et al. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-β-lactamase-producing *Enterobacteriaceae* in river water and imported vegetable samples in Switzerland. *Antimicrob Agents Chemother.* 2016;60:2594-5.