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NORM-VET

Usage of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Norway

Norsk overvåkingssystem for antibiotikaresistens hos mikrober (NORM)

Veterinærinstituttet
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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 food production.

Several countries have implemented monitoring programmes for antimicrobial resistance and usage of antimicrobial agents in recent years. Some programmes focus primarily on human usage and resistance in human pathogens, whereas others also include data concerning veterinary medicine, food production and the environment. The EU supported this broad approach in 1998 through the Copenhagen recommendations and at later follow-up conferences. The World Health Organization (WHO), the Food and Agriculture Organization (FAO), and the World Animal Health Organization (OIE) have, through several expert consultations, emphasised the importance of a One Health approach to monitoring of antimicrobial drug usage and resistance. Several reports and recommendations have been published in this regard including the WHO Global Action Plan adopted at the World Health Assembly in May 2015.

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. Again, the importance of monitoring both the human and veterinary sectors, including food production, was emphasised. The action plan recognised the need for ongoing surveillance as a fundamental component of the strategy for containment of antimicrobial resistance. The NORM and NORM-VET programmes were consequently established in order to provide and present microbiologically and epidemiologically valid 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 drug 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 by 2020 compared to 2012.

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 the veterinary and food production sectors was established in 2000 and is coordinated by the Norwegian Veterinary Institute. The usage of antimicrobial agents is based on reporting of wholesalers' data for humans and animals, which was made mandatory from January 1st 2002, as well as human prescription data reported to the Norwegian Institute of Public Health. Data on the usage of feed additives, i.e. coccidiostat growth promoters, are collated at the Norwegian Food Safety Authority which is also responsible for the Veterinary Prescription Register; data from this register have been applied to present data by fish species.

This report, which is the seventeenth annual joint report from NORM and NORM-VET, presents data for 2016. In addition to resistance data, the NORM/NORM-VET reports present data on the usage of antimicrobial agents in humans and animals in Norway. The present report, together with earlier reports and reports from the years to come, form a basis for the detection, interpretation and evaluation of trends regarding usage of antimicrobial agents and occurrence of resistance in Norway. The NORM and NORM-VET programmes are valuable tools for setting policies, assessing risks and evaluating interventions.

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 2017

SAMMENDRAG

Dette er den syttende 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 2016. Data fra relevante prosjekter som ikke er med i de kontinuerlige overvåkingsprogrammene, presenteres også.
Både 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, Universitets-sykehuset Nord-Norge i Tromsø. NORM-VET koordineres av Veterinærinstituttet i Oslo. Programmene utgir en felles årsrapport.

Forbruk av antibiotika til dyr

Salget av antibiotika til matproduserende landdyr har vært stabilt siden 2000, med små fluktuasjoner; i 2016 var salget på 5452 kg. Regjeringens handlingsplan mot antibiotikaresistens (2015-2020) har satt som målsetning at forbruket av antibiotika til matproduserende landdyr skal reduseres med minst 10 % sammenlignet med 2013. I perioden 2013-2016 ble salget av veterinære antibakterielle midler til matproduserende landdyr redusert med 4 %, målt i kg. Når salgsdata ble korrigert for estimert biomasse dyr (mg/PCU) var reduksjonen 2 %. Det må trekkes frem at husdyrnæringen allerede i 1996 satte som mål å redusere antibiotikaforbruket til matproduserende dyr med 25 % over en periode på fem år med 1995 som referanseår, og fra 1996-2000 ble det observert en nedgang på 40 % (i kg). Siden da har det årlige salget vært på samme nivå med små variasjoner. Andelen av salget som utgjøres av rene penicillinpreparater har økt fra 19 % i 1993 til 60 % i 2016. For samme periode ble salget av aminoglykosider redusert fra 32 % til 9 % av totalsalget. Årsaken til dette er redusert bruk av kombinasjonspreparater med penicillin og dihydrostreptomycin. For antibiotika klassifisert av WHO som kritisk viktige for humanmedisinen og med høyest prioritet (CIA), ble salget til bruk på matproduserende landdyr redusert fra 1,8 % til 0,6 % av totalsalget fra 1993 til 2016. Det ble ikke solgt antibiotika i klassen polymyksiner (høyest prioriterte CIA) til dyr i perioden 1993-2016 i Norge. Salg av veterinære antibakterielle midler til matproduserende landdyr, som kan brukes til flokkbehandling, var lavt i perioden 1993-2016; i 2016 utgjorde salget av slike preparater 6 % (i kg) av totalsalget av antibakterielle midler til matproduserende landdyr.

Forbruket av antibiotika i norsk akvakultur er svært lavt. I 2016 var forskrivningen av antibiotika til behandling av oppdrettsfisk kun på 201 kg aktiv substans, noe som er det laveste nivået siden 1981. Forbruket av antibiotika til oppdrettsfisk ble redusert med 99 % (kg) fra 1987 til 1996 og har siden vært relativt konstant. Dette kan i hovedsak tilskrives vaksinering av all oppdrettsfisk med effektive vaksiner mot de mest aktuelle bakteriesykdommene.

Salget (i kg) av veterinære antibakterielle preparater som kun er godkjent til hund og katt økte med 16 % i perioden 1993-2016 (fra 345 kg til 400 kg), men viste noen fluktuasjoner. Økningen kan delvis forklares ved at antallet veterinærpreparater til hund og katt har økt og at det derved brukes mindre antibakterielle preparater godkjent til humanmedisin. I tillegg har særlig antallet hunder økt. Fram til 2007 ble salget dominert av kombinasjonspreparater med sulfa-trimethoprim, men siden da har salget vært dominert av penicillinpreparater. I Regjeringens handlingsplan mot antibiotikaresistens (2015-2020) ble det satt som målsetning å redusere forbruket av antibiotika til kjæledyr med minst 30 % sammenlignet med 2013. Fra 2013-2016 ble salget (i kg) av antibakterielle midler til hund og katt redusert med 24 %.

I desember 2014 vedtok fjørfenæringen å fase ut all bruk av narasin som fôrtilsetningsmiddel til slaktekylling i løpet av 2016. Utfasingen startet i februar 2015 og var fullført allerede i juni 2016. Regjeringens handlingsplan mot antibiotikaresistens (2015-2020) har satt som målsetning at «narasin og andre koksidiostatika med antibakteriell virkning er faset ut av kyllingproduksjonen forutsatt at dette ikke går utover dyrehelse og dyrevelferd eller øker bruken av antibiotika til behandling». Foreløpige analyser av data fra Veterinært legemiddelregister viser at utfasingen ikke medførte økning i terapeutisk bruk av antibiotika til fjørfe; i 2015 og 2016 var det bare noen få kyllingflokker som ble behandlet med antibakterielle midler.

Forbruk av antibiotika hos mennesker

Totalt antibiotikasalg inkluderer alt forbruk hos mennesker i Norge dvs. primærhelsetjenesten og institusjoner. I 2016 gikk det totale salget av antibakterielle midler til systemisk bruk i mennesker (J01 utenom methenamine) ned med 5 % sammenlignet med 2015; fra 15,5 til 14,6 DDD/1000 innbyggere/døgn, forbruket er redusert med 11 % siden 2012. Andelen smalspektrede penicilliner (J01CE) av totalt salg (J01 utenom metenamin) er redusert; i år 2000 var andelen 32 % av det totale salget, og i 2016 var andelen 26 %.
Rundt 85 % av totalt antall DDD av antibakterielle midler brukes i primærhelsetjenesten, dvs. utenfor helseinstitusjoner. Bruken av antibiotika i primærhelsetjenesten har gått ned siden 2012. I primærhelsetjenesten i 2016 var penicilliner mest brukt, J01C (41 % av DDD), etterfulgt av tetracykliner, J01A (19 %). De fire hyppigst brukte antibiotika i 2016 var fenoksymetylpenicillin, pivmecillinam, doksycyklin, og amoxicillin. Disse fire representerte omtrent halvparten av alle forskrevne resepter og rundt halvparten av alle solgte DDD.
Antibiotikasalg (i DDD) til sykehus utgjorde 7 % av totalt salg av antibakterielle midler til mennesker i 2016. I norske sykehus ble det gjennomsnittlig brukt 76 DDD/100 liggedøgn i 2016, dette er en økning på 24 % siden 2006. Antall DDD/sykehusinnleggelse (3,2 i 2016) økte med 5 % i samme periode. Terapimønster av antibakterielle midler i sykehus endres ikke mye fra ett år til et annet. I sykehus, ble penicilliner (J01C) mest brukt (omtrent halvparten av bruken, målt i DDD), cefalosporiner er den nest største antibiotikagruppen; 17 % av alle DDD. Det er store variasjoner mellom sykehus, både målt i volum (DDD/100 liggedøgn) av antibiotika som brukes og i terapiprofil. Variasjonene kan ikke forklares med forskjeller i aktivitet eller pasientsammensetning alene.

Resistens hos indikatorbakterier fra dyr, mat og fôr

I et internasjonalt perspektiv er forekomsten av antibiotikaresistens i bakterier fra dyr, mat og fôr i Norge fortsatt lav. Forekomst av resistens hos visse bakterier kan være indikator på selektivt antibiotikapress i ulike populasjoner. I et land som Norge hvor forbruket av veterinære antibakterielle midler er svært lavt, og den generelle forekomsten av antibiotikaresistens er lav, kan imidlertid forekomst av antibiotikaresistens være påvirket av andre faktorer. I 2016 viser funnene at internasjonal handel med mat og fôr kan introdusere bakterier med resistens mot kritisk viktige antibiotika som definert av WHO. Et annet eksempel på dette er introduksjonen av cefalosporinresistente *Escherichia coli* til norsk kylling via import av avlsdyr. I 2016 viser resultatene at forekomsten hos kylling og i kyllingkjøtt har blitt betydelig redusert.
NORM-VET følger de krav til overvåking av antibiotikaresistens som er satt i EU-regelverket. I tillegg overvåkes/ kartlegges bakterier og resistensformer fra dyr, matvarer og fôr ut i fra nasjonale hensyn. Eksempler på dette er kartlegging av methicillinresistente *Staphylococcus aureus* (MRSA), og målrettede selektive undersøkelser av spesielle resistensformer slik som f.eks. kinolon- eller kolistinresistens. Slike undersøkelser benyttes når forekomsten av resistens er så lav at det ellers er vanskelig å detektere resistente bakterier.

I 2016 ble 181 *E. coli* fra kyllingflokker og 156 *E. coli* fra kalkunflokker undersøkt, og av disse var henholdsvis 79,0 % og 73,7 % fullt følsomme for de antibiotika som var med i testpanelet.
Totalt 185 prøver fra kyllingflokker og 175 prøver av kyllingkjøtt ble undersøkt med målrettede selektive metoder for påvisning av cefalosporinresistente *E. coli*. Cefalosporinresistente *E. coli* ble påvist i henholdsvis 10,8 % og 9,7 % av prøvene, alle forårsaket av $bla_{\text{CMY-2}}$ genet. Dette er en betydelig reduksjon sammenliknet med tidligere år. Videre undersøkelser viste at i majoriteten av de positive prøvene var cefalosporinresistente bakterier tilstede kun i et svært lavt antall. Disse gunstige resultatene er sannsynligvis et resultat av tiltak iverksatt av industrien. *E. coli* som er resistente mot cefalosporiner ble også påvist i 10,2 % av 156 prøver fra kalkunflokker, og i 2,3 % av 128 prøver av kalkunkjøtt. Som for kylling, viste kvantifiseringen at bakteriene var tilstede i et svært lavt antall. Ved undersøkelse av tilfeldige *E. coli* fra de samme prøvene fra kylling- og kalkunflokker, ble det ikke påvist noen cefalosporinresistente *E. coli*.

Totalt 125 norskproduserte og 54 importerte oster, både pasteuriserte og upasteuriserte, ble undersøkt. Det ble isolert 50 *E. coli;* 10 fra pasteuriserte og 40 fra upasteuriserte oster, 29 isolater var fra norskproduserte og 21 fra importerte oster. Av disse, var 74 % fullt følsomme for alle antibiotika det ble testet for. De aller fleste produktene hvor det ble påvist resistente bakterier var upasteuriserte. Det var mer resistens i bakteriene av utenlandsk opprinnelse, og noen av disse var også multiresistente.

Det ble undersøkt 359 prøver av forskjellige typer sjømat (ikke fisk), både norskproduserte (n=180) og importerte (n=179) produkter. Det ble påvist *E. coli* fra 64 prøver, omtrent 50 % fra hver kategori, hvor 73 % av alle isolatene var fullt følsomme for alle antibiotika det ble testet for. Resistens mot kun ett antibiotika ble påvist i 15,6 % av isolatene, mens de siste 11,4 % av isolatene var resistente mot mellom to og syv forskjellige antibiotika. Selektive undersøkelser påviste kinolonresistente *E. coli* i 2,5 % av prøvene og da hovedsakelig fra importerte prøver. Ett av disse importerte isolatene viste seg også å være resistent mot kolistin forårsaket av det plasmidmedierte overførbare genet *mcr-1*. Andre selektive undersøkelser påviste cefalosporinresistent *E. coli* i én prøve, samt *Enterobacter* sp. med nedsatt følsomhet for karbapenemer fra en annen prøve, begge prøvene var importerte. Som for osteprøvene, indikerer sjømatresultatene at antibiotikaresistente bakterier er mer vanlige i importerte enn i norskproduserte produkter, og at enkelte av disse bakteriene også er multiresistente. I tillegg viser resultatene at enkelte av disse importerte sjømatproduktene er kontaminert med bakterier resistente mot antibiotika definert av WHO som kritisk viktige for behandling innen humanmedisinen.
Skjell filtrerer store mengder vann og oppkonsentrerer frie og partikkelbundne bakterier, inkludert antibiotikaresistente bakterier, som havner i sjøen gjennom kloakk og avrenning fra land. I tillegg til å representere et næringsmiddelprodukt, kan skjell slik benyttes som en indikator på antibiotikaresistens i miljøet. I 2016 ble det undersøkt 261 *E. coli* fra skjell prøvetatt ved ulike norske produksjonssteder. Hele 91,6 % av disse var fullt følsomme for de antibiotika de ble testet for. Ved selektiv metodikk ble cefalosporinresistente *E. coli* påvist i 3,3 % av prøvene, og kinolonresistente *E. coli* i 12,8 % av prøvene.

Prøver fra rødrev og ville fugler kan også brukes som indikatorer på forekomst av antibiotikaresistens i miljøet. I 2016 ble det undersøkt avføring fra 528 rødrev og 357 ville fugler. Totalt ble 434 *E. coli* fra rødrev og 303 *E. coli* fra villfugl testet, hvorav 91,7 % av isolatene fra rødrev og 91,4 % av isolatene fra villfugl var fullt følsomme for de antibiotika de ble testet for. Ved målrettet selektiv metodikk ble det påvist cefalosporinresistente *E. coli* i 3,2 % av prøvene fra rødrev og 8,7 % av prøvene fra villfugl, samt kinolonresistente *E. coli* fra 14,8 % av rødrevprøvene og 5,6 % av villfuglprøvene. Fra en prøve fra villfugl ble det også påvist *Enterobacter* sp. med nedsatt følsomhet for karbapenemer.

Fra 155 prøver av tørrfôr til storfe og svin, ble det kun påvist seks *E. coli*-isolater hvorav kun ett var resistent og da kun mot ampicillin. I tillegg ble det undersøkt 85 prøver av hundefôr. Fra disse ble det isolert 64 *E. coli*, alle fra våtfôr. 85,9 % av disse var fullt følsomme for alle de antibiotika det ble testet for. Selektive undersøkelser påviste cefalosporinresistente *E. coli* fra 17,6 % av prøvene, hovedsakelig forårsaket av $bla_{\text{CMY-2}}$ genet. Kinolonresistente *E. coli* ble påvist fra 51,8 % av prøvene. Ett av disse isolatene, fra importert fôr, var også resistent mot kolistin, og det plasmidmedierte overførbare genet *mcr-1* ble påvist.

NORM-VET 2016 rapporten presenterer også resultatene fra overvåkingsprogrammet for methicillinresistente *Staphylococcus aureus* (MRSA) hos gris i Norge. Totalt 872 besetninger ble undersøkt i 2016. MRSA CC398 t034 ble påvist i kun én besetning (0,13 %). Mink ble også undersøkt for MRSA i 2016. Prøver fra 121 gårder med mink ble undersøkt, inkludert gårder som hadde importert dyr de siste årene. MRSA ble ikke påvist.

Resistens hos zoonotiske bakterier og andre enteropatogene bakterier

Zoonosebakterier isolert fra dyr
I 2016 ble det undersøkt 19 *Salmonella* spp. isolater fra dyr og ett isolat fra hundefôr. Alle isolatene var fullt følsomme for de antibiotika de ble testet for.
Totalt 114 *Campylobacter jejuni* isolater fra kyllingflokker ble testet. Av disse var 82,5 % fullt følsomme for de antibiotika som er inkludert i testpanelet. Resistens mot ett eller to antibiotika ble påvist i 14 % av isolatene, mens 3,5 % var resistente mot tre av de testede antibiotika. Resistens mot kinoloner var mest vanlig.

Kliniske isolater av tarmpatogene bakterier fra mennesker
For kliniske *Salmonella*-isolater fra mennesker sett under ett var forekomsten av multiresistens (MDR) 8.3 %, mens forekomsten av bredspektrede beta-laktamaser (ESBL) holdt seg under 2 %. Når det gjelder blodkulturisolater (n=66), var forekomsten av MDR høyest for *Salmonella* spp. (alle serovarer unntatt *S.* Typhi, Paratyphi, Typhimurium og Enteritidis). Forekomsten av resistens var høyere for flere antibiotika i *S.* Typhimurium-gruppen (inkludert *S. enterica* serovar 4,[5],12:i:-) enn hos andre *Salmonella* serovarer. Forekomsten av resistens mot tetracyklin og ampicillin var også økende i forhold til tidligere år denne bakteriegruppen.

Når det gjelder *Campylobacter*, var det økende resistens mot tetracykliner og kinoloner hos isolater ervervet ved innenlandssmitte, men forekomsten var fortsatt betydelig lavere enn for utenlandssmittede isolater. I tillegg ble det observert en økende resistens mot erytomycin uavhengig av smittested.

De fleste tilfeller av *Shigella*-infeksjoner i Norge kan knyttes til smittekilder i utlandet. Antibiotikaresistens var følgelig utbredt hos *Shigella*-isolater. En økende forekomst av resistens mot kinoloner ble observert hos både *S. sonneii* og *S. flexneri*. Forekomsten av ESBL hos *Shigella* var 5 % i 2016.

Antibiotikaresistens hos *Yersinia enterocolitica* ligger stabilt lavt, bortsett fra artens iboende resistens mot ampicillin.

Resistens hos kliniske isolater fra mennesker

Forekomsten av antibiotikaresistente kliniske bakterie-isolater fra mennesker var fortsatt lav i 2016. Det ble påvist tolv tilfeller av methicillinresistente *Staphylococcus aureus* (MRSA) blant de 1255 blodkulturisolatene (1,0 %) som ble inkludert i NORM-protokollen. Dette samsvarer godt med tall fra laboratorienes datasystemer som rapporterte totalt 20 MRSA blant 1768 (1,1 %) *S. aureus* fra blodkultur og spinalvæske i 2016. Andelen er på samme nivå som i 2014 (0,8 %) og 2015 (0,7 %). Meldesystemet for infeksjonssykdommer (MSIS) registrerte 887 tilfeller av MRSA-infeksjon i 2016 mot 832 i 2014 og 785 i 2015. De fleste tilfellene var fra pasienter med sårinfeksjoner og abscesser. MRSA utgjør fortsatt en svært liten andel av *S. aureus*-isolater fra sårprøver (17 av 1090, 1,6 %) hvilket er en svak økning fra 2014 (1,3 %) og 2015 (1,2 %). MSIS registrerte videre 1651 tilfeller av MRSA-kolonisering mot 1035 i 2014 og 1448 i 2015. Det totale antallet MRSA-meldinger økte dermed fra 2233 i 2015 til 2538 i 2016 (+ 14 %). Overvåkingen viser at det totale antallet MRSA-registreringer øker, og dette skyldes økning av både antallet infeksjoner og koloniseringer. Det påvises fortsatt svært få alvorlige infeksjoner. Økningen i antall meldte koloniseringer kan skyldes en reell økning av MRSA-forekomsten, men kan også skyldes høyere testaktivitet.

Blodkulturisolater av *E. coli* og *Klebsiella* spp. viste stabil forekomst av resistens mot bredspektrede antibiotika i 2016. Forekomsten av resistens og nedsatt følsomhet for gentamicin hos *E. coli* var 6,7 % i 2016 sammenliknet med 8,6 % i 2014 og 6,4 % i 2015. Forekomsten av resistens og nedsatt følsomhet for ciprofloxacin i *E. coli* økte fra 11,9 % i 2015 til 17,1 % i 2016, men dette skyldes endringer i brytningspunktene for resistens. Ved korreksjon for disse endringene har forekomsten vært stabil de siste tre årene. Det er en klar samvariasjon mellom forbruket av fluoro-kinoloner og nedsatt følsomhet for denne antibiotika-gruppen. *Klebsiella* spp. hadde lavere forekomst av resistens mot aminoglykosider og fluorokinoloner enn *E. coli*.

Produksjon av bredspektrede betalaktamaser (ESBL) er blitt et utbredt problem i mange land, og forekomsten har også vært økende i Norge. Til sammen 113/1940 *E. coli* (5,8 %) og 39/855 *Klebsiella* spp. (4,6 %) fra blodkultur ble rapportert som ESBL-positive i 2016. Forekomsten er stabil for *E. coli* (5,8 % i 2014; 6,5 % i 2015) og svakt økende for *Klebsiella* spp. (3,4 % i 2014; 2,9 % i 2015). Andelen av ESBL-positive isolater var fortsatt høyere blant *E. coli* fra blodkulturer (5,8 %) enn fra urinprøver (3,0 %). Karbapenemaseproduserende *Enterobacteriaceae* (CPE), *Pseudomonas aeruginosa* og *Acinetobacter* spp. har vært meldepliktige til MSIS siden juli 2012. Antallet pasienter meldt med CPE økte fra 30 i 2015 til 33 i 2016, mens antallet karbapenemaseproduserende *P. aeruginosa* (n=5) og *Acinetobacter* spp. (n=16) var uendret fra 2015.

Blant *Haemophilus influenzae*-isolater fra systemiske infeksjoner (n=81) var 17,3 % betalaktamase positive og 11,1 % resistente mot cefuroxim som ved kromosomal betalaktamresistens. Elleve av 22 *Neisseria meningitidis*-isolater fra systemiske infeksjoner hadde nedsatt følsomhet for penicillin G, men de var fortsatt følsomme for andre relevante antibiotika. *Neisseria gonorrhoeae* (n=381) viste nedsatt følsomhet for penicillin G (97,1 %) og azitromycin (35,9 %). Hele 48,6 % var resistente mot ciprofloxacin, men dette er tross alt en nedgang fra 62,2 % i 2015. Ni isolater (2,4 %) var også resistente mot cefixim, men alle var følsomme for ceftriaxon.

Det ble påvist tre enterokokkisolater fra blodkultur med klinisk signifikant vankomycinresistens i 2016 (2 VanB *E. faecium* og 1 VanB *E. faecalis*). Forekomsten av nedsatt følsomhet for ampicillin i *E. faecium* ligger stabilt rundt 80-90 %. Høygradig gentamicinresistens ble påvist i 18,8 % av *E. faecalis* og 38,3 % av *E. faecium*, dette er på omtrent samme nivå som henholdsvis 13,1 % og 39,3 % i 2015. Alle *E. faecium*-isolater med høygradig gentamicinresistens hadde samtidig nedsatt følsomhet for ampicillin. Det ble ikke påvist linezolidresistente enterokokker i 2016.

Det ble påvist nedsatt følsomhet for penicillin G hos 5,1 % av *Streptococcus pneumoniae* fra blodkultur/spinalvæske. Dette er tilnærmet uendret fra 2014 (5,5 %) og 2015 (7,5 %). To blodkulturisolatisolater var penicillinresistente og hadde samtidig nedsatt følsomhet for cefalosporiner. Forekomsten av makrolidresistens var 5,6 % sammenliknet

med 4,8 % i 2015. Det var høyere forekomst av nedsatt følsomhet for penicillin G (8,2 %) og erytromycin (11,1 %) i pneumokokkisolater fra luftveiene sammenliknet med systemiske isolater.

Streptococcus pyogenes (betahemolytiske streptokokker gruppe A) fra blodkultur hadde uendret forekomst av erytromycinresistens (3,5 %). Forekomsten av resistens og nedsatt følsomhet for erytromycin blant *Streptococcus agalactiae* (betahemolytiske streptokokker gruppe B) var 21,7 % sammenliknet med 21,0 % i 2014 og 26,9 % i 2015. Alle isolatene var følsomme for penicillin.

I alt 298 tilfeller av tuberkulose ble meldt til MSIS i 2016. Det ble utført resistensbestemmelse av 228 *Mycobacterium tuberculosis* isolater. Elleve isolater (4,8 %) fra pasienter smittet i henholdsvis Afrika (n=4), Asia (n=3), Norge (n=2) og Europa utenom Norge (n=2) ble klassifisert som multiresistente.

Det ble utført resistensbestemmelse av 213 *Candida* blodkulturisolater av ti ulike species. De vanligste artene var *C. albicans* (n=137), *C. glabrata* (n=35), *C. parapsilosis* (n=15) og *C. tropicalis* (n=13). Det ble kun påvist enkelte non-albicans isolater med ervervet resistens mot fluconazol, men som forventet var det høy forekomst av resistens mot azoler hos *C. glabrata*. Alle *C. albicans* var fullt følsomme for de undersøkte midlene. Nøyaktig speciesbestemmelse er avgjørende for å forutsi iboende resistens og velge effektiv behandling. Resultatene er i samsvar med tidligere studier fra Norge.

Konklusjon

Antibiotikaresistens er fortsatt et begrenset problem i Norge når det gjelder bakterier fra både mennesker og dyr. Det lave forbruket av antibiotika og det fordelaktige forbruksmønsteret må opprettholdes for å bevare denne gunstige situasjonen. Resultatene som presenteres i denne rapporten, viser at norske strategier for antibiotikabruk og antibiotikaresistens hittil har vært vellykkede både i husdyrholdet og i helsevesenet. Faren for økende resistensproblemer er imidlertid til stede i form av økt antibiotikaforbruk i Norge og import av resistente bakterier fra andre land. Det er derfor nødvendig med fortsatt aktiv innsats for å sikre at vi også i fremtiden kan gi effektiv antibiotikabehandling til dem som trenger det. NORM/NORM-VET-rapporten er et viktig bidrag til arbeidet med å forebygge utvikling og spredning av antibiotikaresistens.

SUMMARY

This is the seventeenth 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 2016. 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, Oslo. A joint NORM/ NORM-VET report is issued annually.

Usage of antimicrobial agents in animals

Sales of antimicrobial veterinary medicinal products (VMPs) for terrestrial food producing animals in Norway have been stable since 2000; only minor fluctuations are observed. In 2016, 5,450 kg active substance was sold. In the National Strategy against Antibiotic Resistance (2015–2020) it is set as a target to reduce the usage of antimicrobials for terrestrial food producing animals by 10% by 2020, with 2013 as reference year. In the period 2013-2016, the usage has been reduced by 4% when measured in kg, while the reduction was 2% when measured in mg/PCU (PCU = population correction unit). Of note is that the Norwegian husbandry organization already in 1996 set a target of 25% reduction of use of antimicrobial VMPs for terrestrial food producing animals in five years with 1995 as reference year. This goal was achieved after three years and in the period 1996-2000 a 40% reduction was observed. The sales patterns of antimicrobial VMPs for terrestrial food producing animals have gradually become more favourable as the proportion of penicillin usage has increased; the proportion accounted for by pure penicillins rose gradually from 19% of total sales in 1993 to 60% in 2016. In this period the sales of aminoglycosides decreased from 32% to 9% of the total sales; this is due to a reduction in the use of combination preparations of penicillin and dihydrostreptomycin in food producing animals. The sales of the antimicrobial VMPs defined by the World Health Organization (WHO) as highest priority critically important antimicrobials (CIA) for human medicine, i.e. third and higher generations' cephalosporins, fluoroquinolones and macrolides for terrestrial food producing animals, have been very low. In 1993 the sales accounted for 1.8% of the total sales while in 2016 the corresponding figure was 0.6%. During 1993-2016 there have been no sales of polymyxin VMPs, (belong to highest priority CIA), in Norway. The sales of antimicrobial VMPs applicable for group treatment of terrestrial food producing animals have been low during the years 1993-2016; in 2016 the sales of such formulations accounted for only 6% (kg) of the total sales.

In 2016, the total sales of antimicrobial agents for therapeutic use in farmed fish were 201 kg of active substance. The sales of antimicrobial VMPs in Norwegian aquaculture declined by approximately 99% from 1987 to 1996 and have thereafter remained relatively constant. This reduction was mainly attributed to the introduction of effective vaccines and full scale vaccination of salmonids.

For veterinary antibacterial products approved for companion animals (dogs and/or cats) only, the sales increased by 16% (from 345 to 400 kg) during the period 1993-2016, but fluctuations were observed. The increase can partly be explained by an increase of antimicrobial VMPs marketed for companion animals, which was followed by reduced use of antimicrobials marketed for human use. In addition the number of companion animals, in particular dogs, has increased. In the National Strategy against Antibiotic Resistance (2015–2020) it is set as a target to reduce the usage of antimicrobials for companion animals by 30% by 2020 with 2013 as reference year. From 2013 to 2016 the sales (kg) of antimicrobial VMPs marketed for companion animals have been reduced by 24%.

In February 2016, the Norwegian poultry industry launched a project with the aim of phasing out the use of narasin as coccidiostat feed additive in the broiler industry by the end of 2016. Subsequently, this aim was included in the National Strategy against Antibiotic Resistance, but indicating that phasing out of narasin should not be followed by an increase in therapeutic use of antibiotics in broilers. The goal was reached already in June 2016 and since then no narasin coccidiostat feed additive has been sold. Preliminary analysis of data for 2015 and 2016 obtained from the Norwegian Veterinary Prescription Register shows that only a couple of broiler flocks were subjected to antibiotic treatment.

Usage of antimicrobial agents in humans

Overall antibiotic sales include all consumption in humans in Norway i.e. primary care and institutions. In 2016, the overall sales of antibacterials for systemic use in humans (J01 excluding methenamine) decreased by 5% compared to 2015; from 15.5 to 14.6 DDD/1,000 inhabitants/day. Antibiotics are prescription-only drugs in Norway. The overall consumption has decreased by 11% since 2012. The proportion of narrow-spectrum penicillins (J01CE) of total sales (J01, excluding methenamine) was 32% of overall sales in year 2000 and has decreased to 26% in 2016.

Around 85% of the total human sales of antibacterials are used in primary care, i.e. outside health institutions. Sales of antibiotics to outpatients have decreased since 2012. For ambulatory care, the most important antibiotic group in 2016 were penicillins, J01C (41% of DDDs) and tetracyclins, J01A (19%). The four most commonly prescribed antibiotics for outpatients in 2016 were phenoxymethylpenicillin, pivmecillinam, doxycycline, and amoxicillin. These four represented approximately half of all prescriptions and DDDs.

In 2016, the antibacterial sales (in DDDs) to hospitals represented 7% of total sales of antibacterials for human use in the country. In 2016, a mean use of 76 DDD/100 bed days was observed, an increase by 24% in the last 10 years. The DDD/admission (2016; 3.2) increased by 5% in the same period. Therapy pattern of antibacterials in hospitals does not change much from one year to another. In hospitals, 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 indicator bacteria from animals, food and feed

From an international perspective, the prevalence of antimicrobial resistance in bacteria from animals, food and feed in Norway is still low. Prevalence of antimicrobial resistance among bacteria of the normal enteric microflora can serve as an indicator for the selective antimicrobial pressure in various populations. However, in a country with low usage of antimicrobial veterinary medicinal products and generally low levels of antimicrobial resistance, the detected prevalence may be influenced by other factors as well. In 2016, findings demonstrated that international trade with food and feed can represent a route for introduction of important resistant bacteria. Another exampel of this is cephalosporin resistant *Escherichia coli* in broilers, introduced to Norway through import of breeding animals. In NORM-VET 2016, the results show a substantial reduction of *E. coli* resistant to third generation cephalosporins in broilers and meat thereof.

In 2016, *E. coli* resistant to third generation cephalosporins were detected by selective methods in 10.8% of 185 caecal samples and 9.7% of 175 meat samples. Quantifications showed that in the majority of these samples, the cephalosporin resistant *E. coli* were present at very low levels. Genotyping showed that all isolates contained the $bla_{\text{CMY-2}}$ gene. This is a substantial reduction of *E. coli* resistant to third generation cephalosporins in broiler flocks and broiler meat compared to previous years ($p<0.001$). The reduction is a likely result of the measures implemented by the industry to reduce the occurrence in broilers. For turkey, *E. coli* resistant to third generation cephalosporins were detected by selective methods in 10.2% of 156 caecal samples and in 2.3% of 128 meat samples. Genotyping showed that about half of the isolates contained the $bla_{\text{CMY-2}}$ gene and the remaining half had mutations in the chromosomally located *ampC* gene. Similar to the broiler samples, quantifications showed that in the majority of samples, cephalosporin resistant isolates were present at very low levels. Among the indicator *E. coli*, none showed decreased sensitivity towards the cephalosporins tested for. In total, 181 and 156 indicator *E. coli* isolates from broiler and turkey flocks, respectively, were included for testing. Of these, 79.0% and 73.7% of the isolates, respectively, were susceptible to all the antimicrobial agents in the test panel. From both animal species, most of the resistant *E. coli* were resistant to one antimicrobial agent (predominantly sulfamethoxazole or ampicillin).

Samples of 125 domestically produced and 54 imported cheeses were investigated in 2016. About half of the cheeses in each of these categories were made from pasteurised milk, the other half were made from un-pasteurised milk. *E. coli* isolates were obtained from 50 of the samples; differing significantly between the pasteurised and un-pasteurised milk with a total of ten and 40 isolates, respectively. Among the isolates obtained, 29 isolates were from domestic and 21 were from imported cheese. In total, 74% of the isolates obtained from cheese were susceptible to all antimicrobial agents in the test panel. Altogether, 8.0% of the isolates were resistant to one antimicrobial agent, 14.0% to two, three or four antimicrobial agents, while two isolates were resistant to six and seven antimicrobial agents, respectively. Selective screening detected a few quinolone resistant isolates. The results indicate that antimicrobial resistance is more common in imported than in domestic products, and that some of these are multidrug resistant. Moreover, the majority of the antimicrobial resistant *E. coli* were of un-pasteurised origin.

A total of 359 seafood samples comprising of shellfish such as blue mussels, scallops, oysters, scampi etc. of both domestic (n=180) and imported (n=179) origin were tested in 2016. *E. coli* isolates were obtained from 17.8% of these samples, approximately 50% from each category. Altogether, 15.6% of the isolates were resistant to one antimicrobial agent, while 11.4% of the isolates were resistant to from two up to seven antimicrobial agents. By selective screening, quinolone resistant *E. coli* was detected from 2.5% of these samples. One of these quinolone resistant isolates, from a sample of imported origin, had additional resistance to colistin, and the plasmid-mediated gene, *mcr-1,* was detected. Moreover, from one sample *E. coli* resistant to third generation cephalosporins was detected, and carbapenemase-producing Entero-bacteriaceae, i.e an *Enterobacter asburiae*, was detected from another of these samples, both of imported origin. Similar to the cheese samples, the results indicate that antimicrobial resistance is more common in imported than in domestic products, and that some of these are multidrug resistant. Moreover, some of these imported seafood products are contaminated with bacteria resistant to antimicrobials defined by the WHO as critically important for treatment of human infections.

A total of 261 *E. coli* from 391 batches of bivalve molluscs collected at Norwegian rearing localities were tested for antimicrobial resistance in 2016. In total, 4.2% of the isolates were resistant to one antimicrobial agent, 2.7% to two, 0.4% to three and 1.1% to four or more antimicrobial agents. By selective screening, *E. coli* resistant to third generation cephalosporins were detected in thirteen of the bivalve mollusc batch samples (3.3%). Genotyping showed that the majority of these carried $bla_{\text{CTX-M}}$ genes, of which two additionally had $bla_{\text{CMY-2}}$ genes and four additionally had $bla_{\text{TEM-1}}$ genes. Quinolone resistant *E. coli* was detected in 50 (12.8%) of the bivalve molluscs batch samples by selective screening. Five isolates showed additional resistance to cephalosporins. Genotyping showed that four of these contained $bla_{\text{CTX-M}}$ genes, of which two also carried $bla_{\text{CMY-2}}$. No carbapenemase-producing *Enterobacteri-aceae* were detected by the selective screening methods. Bivalve molluscs filtrate large volumes of water and actively retain free and particle-bound bacteria, including antimicrobial resistant bacteria that reaches the sea through sewage and runoff from land. In addition to representing a food product, bivalves may therefore be used as an indicator for antimicrobial resistance in the environment.

Samples from red foxes and wild birds can also be used as indicators on antimicrobial resistance in the environment. In 2016, faecal swab samples from a total of 528 red foxes and 357 wild birds were examined. A total of 434 *E. coli* from red fox and 303 *E. coli* from wild birds were tested. In total, 4.1% of the isolates were resistant to one antimicrobial agent, 2.1% to two, 1.1% to three and 0.9%

to four antimicrobial agents. Of the wild bird isolates, 4.6% were resistant to one antimicrobial agent, 1.0% to two, 1.7% to three and 1.3% to four or more antimicrobial agents. By use of selective method, *E. coli* resistant to third generation cephalosporins were found in 3.2% of the red fox samples, and in 8.7% of the wild bird samples. Genotyping showed that three isolates carried the $bla_{\text{CMY-2}}$ gene, seven had mutations in the chromosomally located *ampC* gene, while the last seven isolates carried $bla_{\text{CTX-M}}$ genes and some had additional $bla_{\text{TEM-1}}$ genes. From wild birds, the genotyping showed that one isolate carried the $bla_{\text{CMY-2}}$ gene, one the bla_{DHA} gene, and six had mutations in the chromosomally located *ampC* gene, while 23 isolates had a cephalosporin resistance profile corresponding to an ESBL phenotype (16 with $bla_{\text{CTX-M}}$ gr 1 genes, five $bla_{\text{CTX-M}}$ gr 9 genes, two $bla_{\text{SHV-12}}$ genes, and eight with additional $bla_{\text{TEM-1}}$ genes). Quinolone resistant *E. coli* was found in 14.8% of the 528 red fox and in 5.6% of the 358 wild bird samples by selective screening. No carbapenemase-producing Enterobacteriacae was detected by selective screening of the red fox samples, while one wild bird sample was positive and an *Enterobacter asburiae* was detected. The wild bird samples were also screened by selective methods for the presence of colistin resistant *E. coli,* with no such findings.

In 2016, a total of 155 samples of dry feed for cattle and swine was investigated and *E. coli* was detected from six of the samples. Only one isolate was resistant to only to one antimicrobial (ampicillin). A total of 85 dog feed samples were also investigated, and *E. coli* was obtained from 64, all from wet dog feed. Of these, three isolates was resistant to one antimicrobial, two to two antimicrobials, two to three antimicrobials and two to four antimicrobials. By selective screening, *E. coli* resistant to third generation cephalosporins were detected from 17.6% of the dog feed samples. Genotyping showed that 14 isolates contained the $bla_{\text{CMY-2}}$ gene and that one had mutations in the chromosomally located *ampC* gene. Quinolone resistant *E. coli* was detected from 51.8% of the samples. One of these, from an imported product, showed additional resistance toward colistin, and the plasmid-mediated colistin resistance gene *mcr-1* was identified.

The NORM-VET 2016 report includes a summary of the results of the surveillance programme of methicillin resistant *Staphylococcus aureus* (MRSA) in pigs in Norway in 2016. In total, 872 herds were investigated. MRSA CC398 t034 was found in one herd only (0.13%). Minks waere also subjected to investigation of the occurrence of MRSA in 2016. Samples from 121 mink farms were included in the survey, including farms with previous import of live animals. MRSA was not detected in any of the samples.

Resistance in zoonotic bacteria and non-zoonotic enteropathogenic bacteria

Animal isolates

In 2016, a total of 19 *Salmonella* spp. isolates from animals and one isolate from dog feed were susceptibility tested. All isolates were fully susceptible to all antimicrobial agents in the test panel.

A total of 114 *Campylobacter jejuni* isolates from broiler flocks were susceptibility tested. In total, 82.5% of the included isolates were susceptible to all antimicrobial agents included in the test panel. Resistance to one or two antimicrobial agent was detected in 7% and 7% of the isolates, respectively, while 3.5% of the isolates were resistant to three antimicrobial agents. Resistance to the quinolones ciprofloxacin and nalidixic acid were the most frequently identified resistance determinants.

Human clinical enteropathogenic isolates

The frequency of multidrug resistance (MDR) in human clinical isolates of all *Salmonella* serovars was 8.3%, and the frequency of ESBLs less than 2%. Among the 66 *Salmonella* blood culture isolates, the highest frequency of MDR was found in *Salmonella* serovars other than *S.* Typhi, *S.* Paratyphi and the *S.* Typhimurium-group. Antimicrobial resistance in general was more prevalent in the *S.* Typhimurium-group (including *S. enterica* serovar 4,[5],12:i-) than in other serovars, and resistance to ampicillin and tetracycline was still increasing in this group.

For *Campylobacter*, domestically acquired isolates were increasingly resistant to quinolones and tetracycline. However, resistance had not yet reached the same level as seen in isolates acquired abroad. In addition, isolates were seen increasingly resistant to erythromycin, irrespective of place of acquisition.

Most cases of shigellosis were acquired abroad and widespread resistance was observed. An increasing trend of quinolone resistance was observed in both *S. sonnei* and *S. flexneri* isolates. The ESBL prevalence in *Shigella* was 5% in 2016 compared to 20% in 2015. Antimicrobial resistance in *Yersinia enterocolitica* remains low, except for its intrinsic resistance to ampicillin.

Resistance in human clinical isolates

The prevalence of resistance in human clinical isolates was still low in Norway in 2016. Only twelve methicillin resistant *Staphylococcus aureus* (MRSA) blood culture isolates were detected among the 1,255 strains included in the NORM protocol (1.0%). During 2016, the total number of systemic *S. aureus* isolates from blood cultures and cerebrospinal fluids was 1,768, including 20 MRSA strains (1.1%). This prevalence is at the same level as in 2014 (0.8%) and 2015 (0.7%). The Norwegian Surveillance System for Communicable Diseases (MSIS) registered 887 cases of MRSA infections in 2016 compared to 832 in 2014 and 785 in 2015. The majority of MRSA cases were reported to be wound infections and/or abscesses. The prevalence of MRSA among non-invasive *S. aureus* isolates is still very low at 1.6% (17/1,090), but this is a slight increase from 1.3% in 2014 and 1.2 % in 2015. Furthermore, MSIS registered 1,651 cases of MRSA colonisation in 2016 compared to 1,035 in 2014 and 1,448 in 2015. The total number of MRSA notifications thus increased from 2,233 in 2015 to 2,538 in 2015 (+ 14%). The results indicate an increasing number of MRSA notifications caused by increases of both infections and colonisations. The prevalence of invasive disease has remained stable at a low level. The increased number of reported colonisations probably reflects a higher prevalence in the population, but may also be a consequence of increased test activity.

Antimicrobial resistance to broad-spectrum antimicrobials in *E. coli* and *Klebsiella* spp. blood culture isolates remained stable in 2016. The prevalence of gentamicin non-

susceptibility in *E. coli* was 6.7% in 2016 compared to 8.6% in 2014 and 6.4 % in 2015. The prevalence of *E. coli* non-susceptibility to fluoroquinolones increased from 11.9% in 2015 to 17.1% in 2016, but this was entirely due to changes in the breakpoints for resistance. After correction for these changes, the prevalence of non-susceptibility has been stable over the last three years. There is a clear correlation between the total usage of fluoroquinolones and non-susceptibility to these agents. The prevalence of resistance to aminoglycosides and fluoroquinolones is lower in *Klebsiella* spp. isolates than in *E. coli*.

Extended spectrum beta-lactamases (ESBL) have emerged as a significant clinical problem in many countries, including Norway. A total of 113/1,940 (5.8%) *E. coli* and 39/855 (4.6%) *Klebsiella* spp. blood culture isolates were reported with this phenotype in 2016. The prevalence is stable for *E. coli* (5.8% in 2014; 6.5% in 2015) and slightly increasing for *Klebsiella* spp. (3.4% in 2014; 2.9% in 2015). The proportion of ESBL positive isolates is higher among *E. coli* from blood cultures (5.8%) than among urinary tract isolates (3.0%). Carbapenemase-producing Entero-bacteriaceae (CPE), *Pseudomonas aeruginosa* and *Acinetobacter* spp. have been notifiable to MSIS since July 2012. The number of patients reported with CPE increased from 30 in 2015 to 33 in 2016, whereas the numbers of carbapenemase-producing *P. aeruginosa* (n=5) and *Acinetobacter* spp. (n=16) were unchanged from 2016.
Among *Haemophilus influenzae* isolates from systemic infections (n=81), 17.3% displayed beta-lactamase production and 11.1% were resistant to cefuroxime, thus indicating chromosomal resistance to beta-lactam antibiotics. Eleven of 22 *Neisseria meningitidis* isolates from systemic infections displayed reduced susceptibility to penicillin G, but all remained susceptible to other relevant antibiotics. *Neisseria gonorrhoeae* isolates (n=381) demonstrated non-susceptibility to penicillin G (97.1%) and azithromycin (35.9%). Resistance to ciprofloxacin was widespread (48.6%), but still lower than in 2015 (62.2%). Nine isolates (2.4%) were resistant to cefixime but remained susceptible to ceftriaxone.

Three enterococcal blood culture isolates with clinically significant vancomycin resistance were detected in 2016 (two VanB *E. faecium* and one VanB *E. faecalis*). The prevalence of non-susceptibility to ampicillin in *E. faecium* has stabilised around 80-90%. High-level gentamicin resistance (HLGR) was detected in 18.8% of *E. faecalis* and 38.3% of *E. faecium* isolates, which is at the same level as 13.1% and 39.3% in 2015, respectively. All HLGR *E. faecium* isolates were also non-susceptible to ampicillin. Enterococcal resistance to linezolid was not detected in 2016.

Non-susceptibility to penicillin G was detected in 5.1% of *Streptococcus pneumoniae* isolates from blood cultures and cerebrospinal fluids. This is unchanged from 5.5% in 2014 and 7.5% in 2015. Two blood culture isolates were resistant to penicillin G and at the same time showed reduced susceptibility to cephalosporins. The prevalence of macrolide resistance was 5.6% compared to 4.8% in 2015. The prevalence of non-suscpetibility to penicillin G (8.2%) and erythromycin (11.1%) was higher in strains from the respiratory tract than in systemic isolates.

Streptococcus pyogenes (group A streptococcus) isolates from blood cultures had a stable prevalence of erythromycin resistance (3.5%). The prevalence of macrolide non-susceptibility in *Streptococcus agalactiae* (group B streptococcus) was 21.7% compared to 21.0% in 2014 to 26.9% in 2015. All isolates were susceptible to penicillin G.

A total of 298 cases of tuberculosis were reported to MSIS in 2016. Susceptibility testing was performed on 228 *Mycobacterium tuberculosis* isolates. Eleven isolates (4.8%) originating from Africa (n=4), Asia (n=3), Norway (n=2) and Europe excluding Norway (n=2) were classified as multidrug resistant (MDR).

Susceptibility testing was performed on 213 *Candida* spp. blood culture isolates of ten different species. The most common species were *C. albicans* (n=137), *C. glabrata* (n=35), *C. parapsilosis* (n=15) and *C. tropicals* (n=13). All *C. albicans* isolates were fully susceptible to the substances examined. 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 clinically important microbes in Norway. The relatively low usage of antimicrobial agents as well as appropriate patterns of use must be maintained to preserve this rather favourable situation. The data presented in this report show that Norwegian antimicrobial policies in food production and healthcare have been successful. However, the situation may rapidly change if the use of antimicrobial agents in Norway increases or resistant clones are imported from abroad. A continued effort is needed to prevent the development and spread of antimicrobial resistance and thereby ensure the effectiveness of antimicrobials when such treatment is needed. The NORM/NORM-VET report is a vital component in the work aimed at preventing the development and spread of antimicrobial resistance in Norway.

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 January 1st, 2017.
Data provided by Statistics Norway.

Age group	All	Males	Females
0 to 4 years	303,493	155,857	147,636
5 to 14 years	634,217	324,906	309,311
15 to 24 years	668,322	345,519	322,803
25 to 44 years	1,430,973	734,707	696,266
45 to 64 years	1,346,490	686,761	659,729
65 years and older	874,822	401,380	473,442
All age groups	5,258,317	2,649,130	2,609,187

TABLE 2. Livestock population in Norway in 2016.
Data provided by the Register of Production Subsidies as of 31.07.2017.

	Number* of	
Animal category	Herds	Animals
Cattle	14,000	870,000
Dairy cows only**	7,700	197,000
Suckling cow only**	4,300	75,700
Combined production (cow)**	680	31,600
Goat	1,300	68,500
Dairy goat**	300	33,900
Sheep	14,600	2,469,000
Breeding sheep > 1 year**	14,500	951,000
Swine	2,200	822,000
Breeding animal > 6 months**	1,100	49,800
Fattening pigs for slaughter**	2,000	448,000
Laying hen flocks > 250 birds	580	4,278,000
Broilers	670[1]	75,850,900[2]
Turkey, ducks, geese for slaughter (flock > 250 birds)	160	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]Register of Production Subsidies.

TABLE 3. Production volume of the most important species in Norwegian aquaculture during the time period 1992-2016. *Data provided by the Norwegian Directorate of Fisheries updated by 18.06.2017.*

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[3]	1,235,263	87,446	0	308	1,461	2,131	12	11

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

Import of live animals

Import of live animals (excluding fish and companion animals) to Norway in 2016 was 27 cattle (including 9 yaks), 5 camelids and 39,645 day old chicks from broiler, turkey, duck and guinea fowl.

USAGE OF ANTIMICROBIAL AGENTS

USAGE IN ANIMALS

Kari Grave and Kari Olli Helgesen

Therapeutic usage of veterinary antimicrobial agents

Overall, the sales in Norway of antibacterial veterinary medicinal products (VMPs) for therapeutic use in terrestrial food producing animals and companion animals in 2016 were 5,852 kg. Annual sales for use for these animal categories, in the period 1993-2016, are shown in Figure 1. The data are based on sales from drug wholesalers (see Appendix 1) of veterinary antibacterial agents for therapeutic use to Norwegian pharmacies. This includes pharmaceutical formulations approved for food producing animals, including horses, and companion animals. Thus, the figures represent national sales data for veterinary antibacterial agents (see Appendix 1 for inclusion criteria).

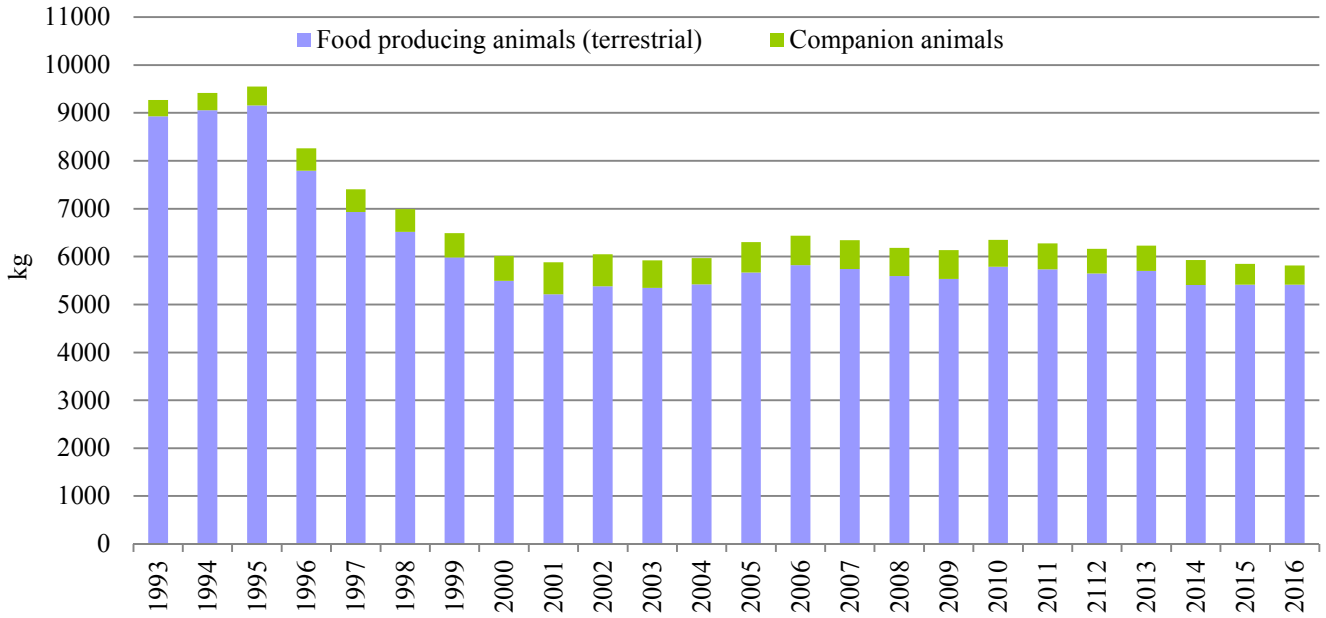

FIGURE 1. Total sales, in kilograms active substance, for food producing animals (terrestrial, including horses) and companion animals, of antibacterial veterinary medicinal products for therapeutic use in Norway in 1993-2016.

Food producing animals (terrestrial)

In 1996, the husbandry organizations (for food producing animals) in Norway set a target for reduction of the usage of antibacterial VMPs, in weight of active substance, by 25% in five years, with 1995 indicated as the reference year. That target was reached already after three years; in 1998 the sales of antibacterial VMPs for use in terrestrial food producing animals had been reduced by 29%. From 1993 to 2016 the sales, in kg active substance, of antibacterial VMPs for use in terrestrial food producing animals have decreased by 39%. In the same period, the proportion of sales of VMPs containing only penicillins increased from 19% to 60% (Figure 2), while the sales of VMPs containing aminoglycoside for use in food producing terrestrial animals decreased from 32% to 9% of the total sales. This change was mainly due to a reduction in the use of combination preparations of penicillin and dihydrostreptomycin (Figure 2). The proportion of pure sulphonamides preparations accounted for 78% of all sales of sulphonamides. This proportion decreased gradually and since 2001, almost all sales of sulfonamides have been accounted for by combination preparation of sulfa+ trimethoprim.

In the National Strategy against Antibiotic Resistance it is set as a target to reduce usage of antibacterial agents in terrestrial food producing species by 10% by 2020 with 2013 as the reference year. Figure 3 shows the sales, in kg active substance, of antibacterial VMPs for therapeutic use in terrestrial food producing animals (including horses) normalised by the biomass “at risk” (population correction unit = PCU). The observed decrease in the sales, in mg/PCU, from 2013-2016 was 2% (see information about calculation of PCU in Appendix 1); when measured in kg active substance the corresponding figure was 4%. Since the prescribing patterns have been stable across 2013 to 2016, the figures are not assumed to be biased by changes towards products with higher or lower dosing per treatment.

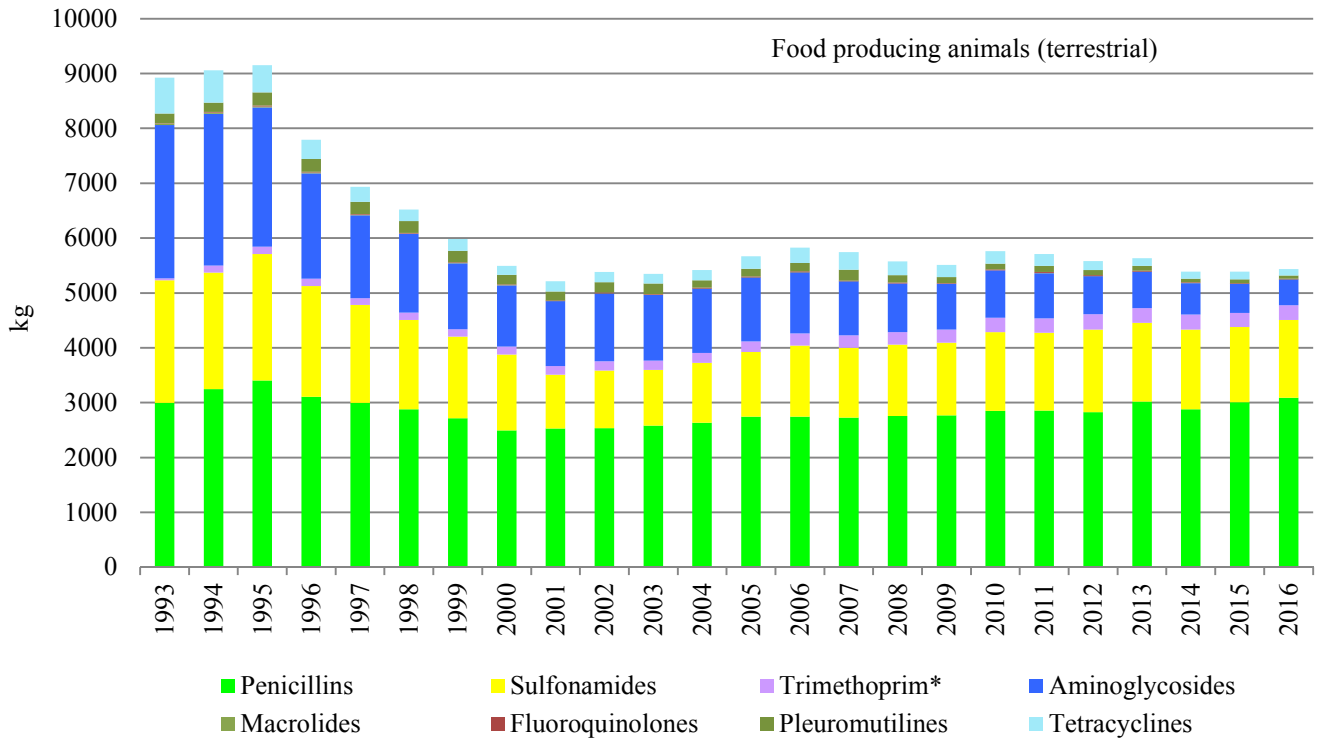

FIGURE 2. Sales, in kilograms active substance, of antibacterial veterinary medicinal products (VMPs) for therapeutic use in terrestrial food producing animals (including horses) in Norway for the years 1993-2016. In addition, minor amounts of amphenicols (range 17-27 kg) were sold in 2008-2016. There were minor sales (< 0.05 kg) of a third generation cephalosporin VMP for the years 2012-2016. *Includes minor amounts of baquiloprim 1994-2000.

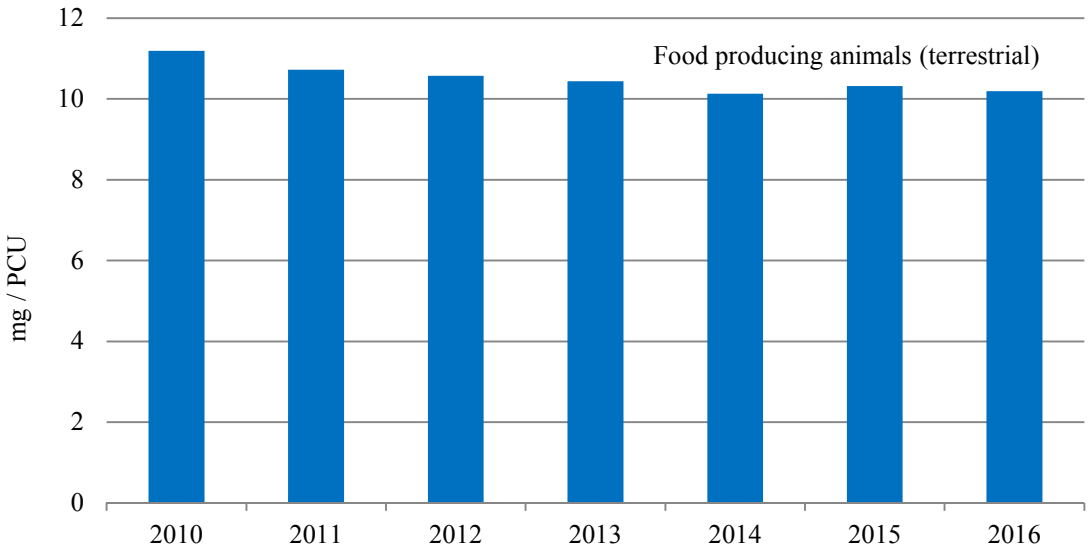

FIGURE 3. Sales (mg/PCU) in Norway, in mg active substance, of antibacterial veterinary medicinal products for therapeutic used in terrestrial food producing animals (including horses) normalised by the population correction unit (PCU) for the years 2010-2016. PCU=Population Correction Unit (see Appendix 1).

The sales of the antibacterial VMPs defined by the World Health Organization (WHO) as the Critical Important Antimicrobials (CIA) with highest priority for human medicine i.e. third and higher generation cephalosporins, quinolones (fluoroquinolones and other quinolones), macrolides, glycopeptides and from 2017 also polymyxins (colistin) for use in food producing terrestrial animals in Norway, has decreased substantially from 1993 to 2016 (Figure 4). Since the late 1990s the sales of the highest priority CIA have been dominated by fluoroquinolones, which in turn is mainly accounted for by VMPs for treatment of individual animals (injectables). The sales of the priority CIA were reduced from 1.8% to 0.6% of the total annual sales of antibacterial VMPs for food producing animals during the years 1993-2016.

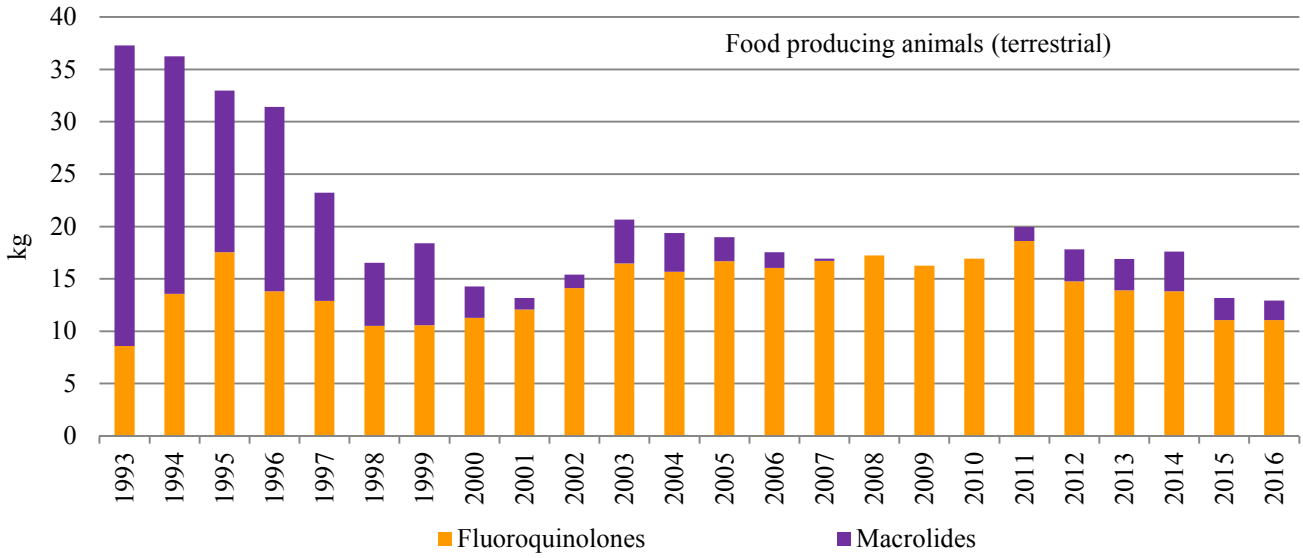

FIGURE 4. Sales, in kilograms of active substance, of antibacterial veterinary medicinal products (VMPs) containing the priority CIAs, fluoroquinolones and macrolides, for therapeutic use in terrestrial food producing animals (including horses) in Norway during 1993-2016. Minor sales (< 0.05 kg) of a third generation cephalosporin VMP were observed for the years 2012-2016; the sales of polymyxins (colistin) were zero for the period 1993-2016. Glycopeptides are not allowed to be used in food producing animals.

In Norway, antibacterial VMPs for treatment of food producing terrestrial animals (including horses), except from broilers, is dominated by pharmaceutical forms for treatment of individual animals (Figure 5), and primarily injectables. This reflects that the production is characterised by small herds, but it can partly also be explained by therapeutic traditions. In 2016, only 6% of the sales of antibiotic VMPs for food producing terrestrial animals were for VMPs for group treatment (oral treatment).

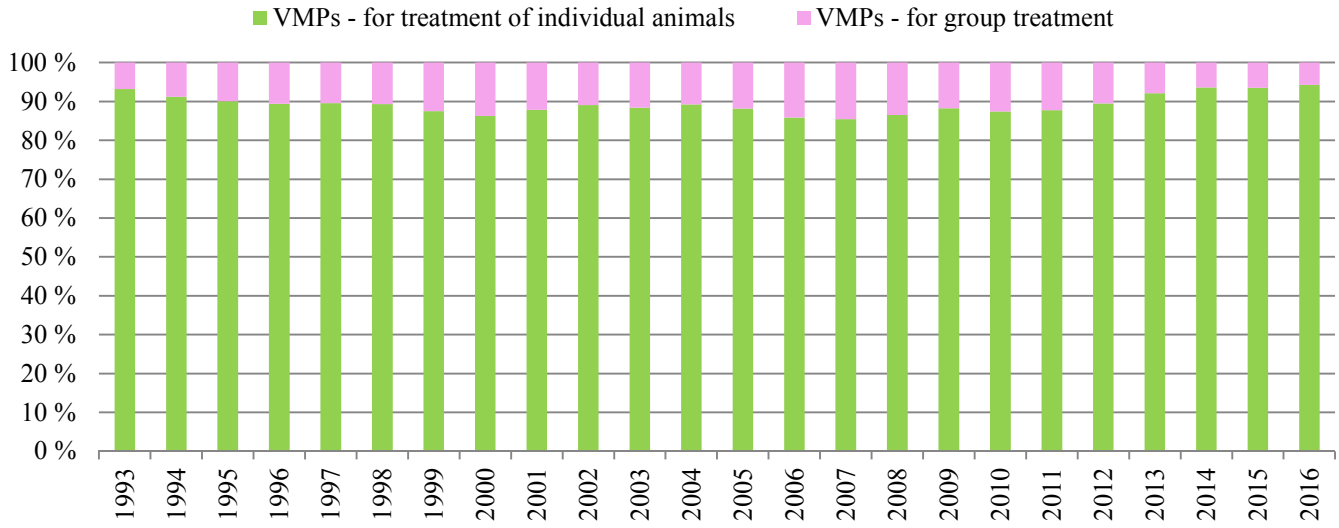

FIGURE 5. Percentage distribution of sales in Norway of antibacterial veterinary medicinal products (VMPs) marketed for treatment of individual food producing terrestrial animals (bolus, injectables, intramammary preparations, intrauterine preparations, oral paste and some tablet VMP presentations – 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). Numbers are based on sales of VMPs in kilograms active substance.

One of the targets stated in the National Strategy against Antibiotic Resistance is to phase out the use of narasin as coccidiostat feed additive in the Norwegian broiler industry, without increasing of the usage of therapeutic antibiotics and without compromising the animal welfare (see chapter on ionophore coccidiostat feed additives). Preliminary analysis of data obtained from the Veterinary Prescription Register (VetReg) for 2015 and 2016 indicate that only a couple of broiler flocks were subjected to treatment with antibiotics during this period.

Farmed fish

The annual sales of antibacterial VMPs for use in farmed fish peaked in 1987 when the sales amounted to 48 tonnes (Figure 6). Since then the sales have declined by approximately 99%. Note that for the last couple of years, the sales of antibacterial VMPs for use in farmed fish have shifted from other quinolones to amphenicols (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 and to some extent also to improved health management. The sales of antibacterials for use in aquaculture normalised by PCU for fish is shown in Figure 7.

TABLE 4. Sales (in kilograms of active substance) of antibacterial agents, for therapeutic use in farmed fish, in Norway in the period 2005-2016. For 2005-2014 the data represent sales data from feed mills collected by the Norwegian Institute of Public Health; for 2015 and 2016, data represent prescription data obtained from the Veterinary Prescription Register (See Appendix 1). Note that data include sales for use in cleaner fish (not food producing) except from 2015 and 2016.

Classes/active substance	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
Tetracyclines												
Oxytetracycline	8	0	19	23	40	10	1	1	0	0	0	0
Amphenicols												
Florfenicol	202	302	139	166	303	275	336	191	300	403	188	136
Quinolones												
Flumequine	28	7	18	1	1	0	0	0	0	0	0	0
Oxolinic acid	977	1,119	406	681	926	308	212	1,399	672	108	85	66
Combinations												
Spectinomycin + lincomycin (2+1)	0	50	66	70	43	57	0	0	0	0	0	0
Total	**1,215**	**1,478**	**648**	**941**	**1,313**	**649**	**549**	**1,591**	**972**	**511**	**273**	**201**

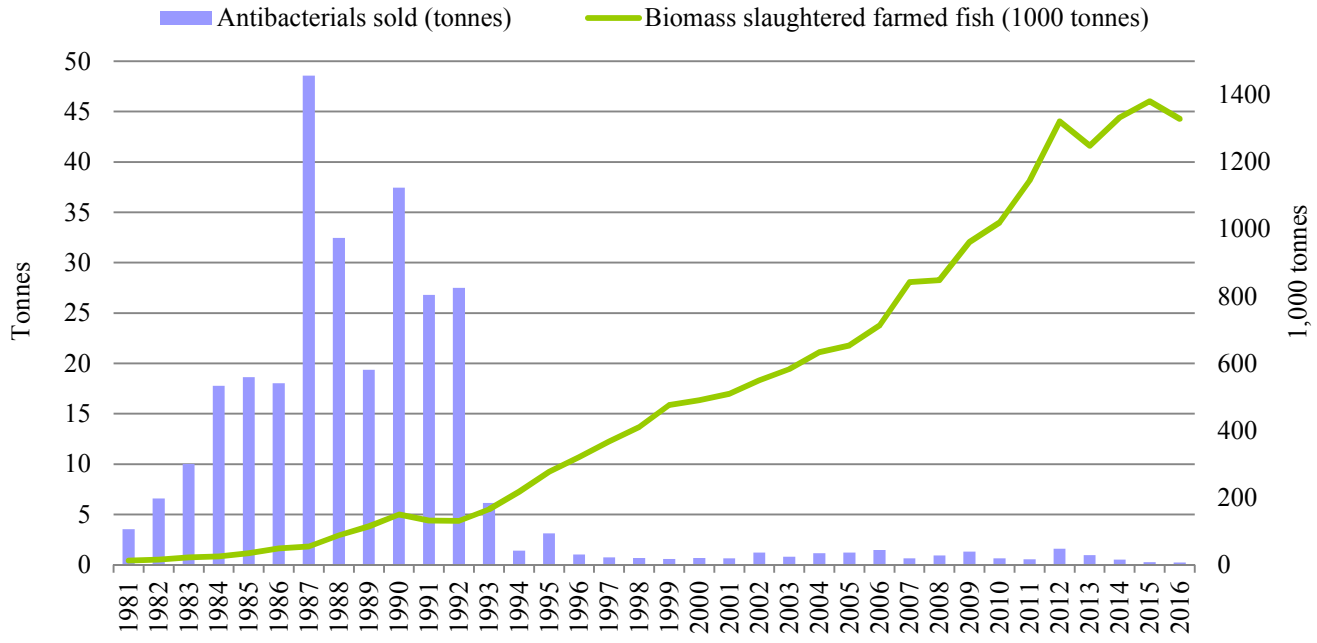

FIGURE 6. Total sales, in tonnes of active substance, of antibacterial veterinary medicinal products (VMPs) for therapeutic use in farmed fish in Norway in the period 1981-2016 versus produced biomass (slaughtered) farmed fish in 1,000 tonnes. For 1981-2014 the data represent sales data provided by the Norwegian Institute of Public Health; for 2015 and 2016 data represent prescription data obtained form the Veterinary Prescription Register. Data on slaughtered biomass farmed fish were obtained from Statistics Norway.

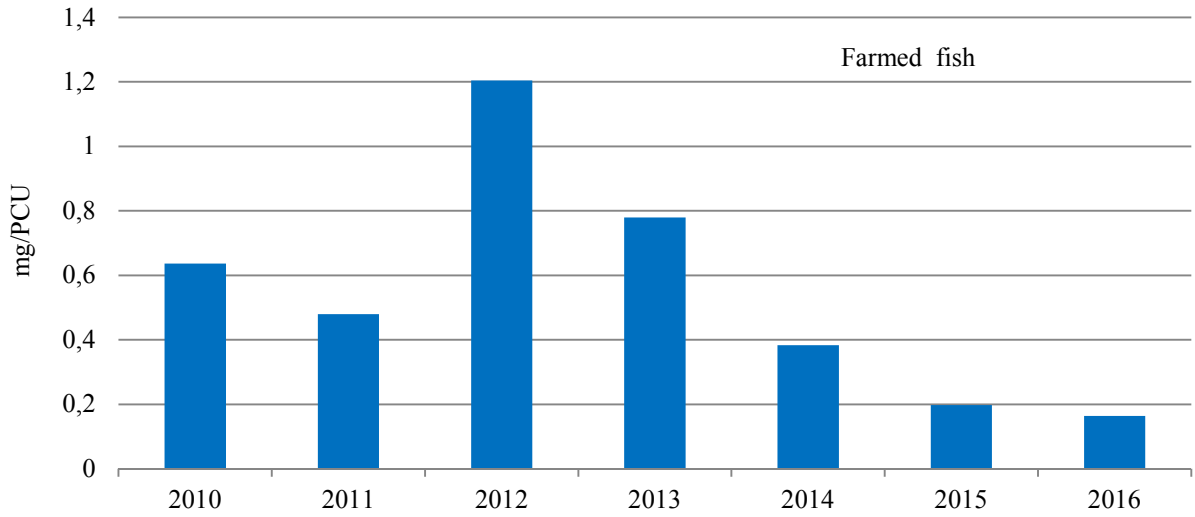

FIGURE 7. Sales (mg/PCU) in Norway, in mg active substance, of antibacterial veterinary medicinal products (VMPs), for therapeutic use in farmed fish, normalised by the population correction unit (PCU) for the years 2010-2016. For 2010-2014 the data represent sales data provided by the Norwegian Institute of Public Health; for 2015 and 2016 data represent prescription data obtained from the Veterinary Prescription Register. Data on slaughtered biomass farmed fish were obtained from Statistics Norway.

For the years 2011 to 2016, the major proportion of prescriptions was for fish in the pre-ongrower phase (Figure 8). The number of prescriptions of antibacterial VMPs for Atlantic salmon ongrowers was negligible during the period 2011-2016, despite that Atlantic salmon represents approximately 95% of the biomass farmed fish produced in Norway. This gives a strong indication for the vaccines used being efficient and that the coverage of vaccination of fingerlings (included in data on pre-ongrower phase) is complete.

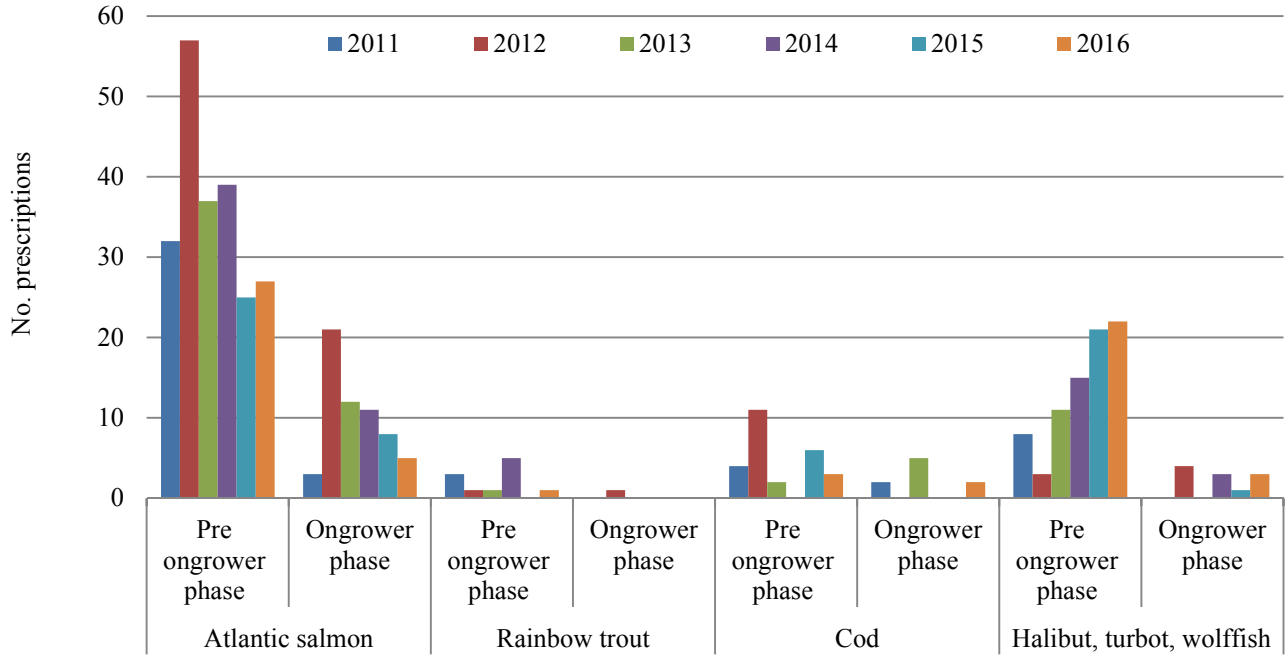

FIGURE 8. Number of prescriptions of antibacterial agents by fish species, split into pre-ongrowing phase and ongrower phase, for 2011-2016. Data were obtained from the Veterinary Prescription Register.

Companion animals (dogs and cats)

For veterinary antibacterial products approved solely for companion animals (includes tablets and some oral solution, injectable and oral paste), an increase of 16% in the sales was observed from 1993-2016 (Figures 1 and 9). The prescribing patterns have changed significantly during the period 1993-2016 and vary between the various classes. Furthermore, the number of antibacterial VMPs marketed for dogs and cats increased substantially during the period – in 1993 only 12 antibacterial VMP presentations (name, form, strength and pack size) were authorised for dogs and cats, while in 2016 the corresponding number was 50. When the availability of therapeutic alternatives was low, human antibacterial products were more frequently prescribed for dogs and cats. Since use of human antibacterials are not included in the statistics and because of differences in dosing between the antibacterials, the observed increase across the years 1993-2016 should be interpreted with caution. The observed peak in the sales of sulfonamides+trimethoprim in companion animals in 2001-2002 was probably due to use in sheep of a sulfonamide-trimethoprim VMP marketed for companion animals because of a withdrawal in 2001 of a product used for mastitis in sheep.

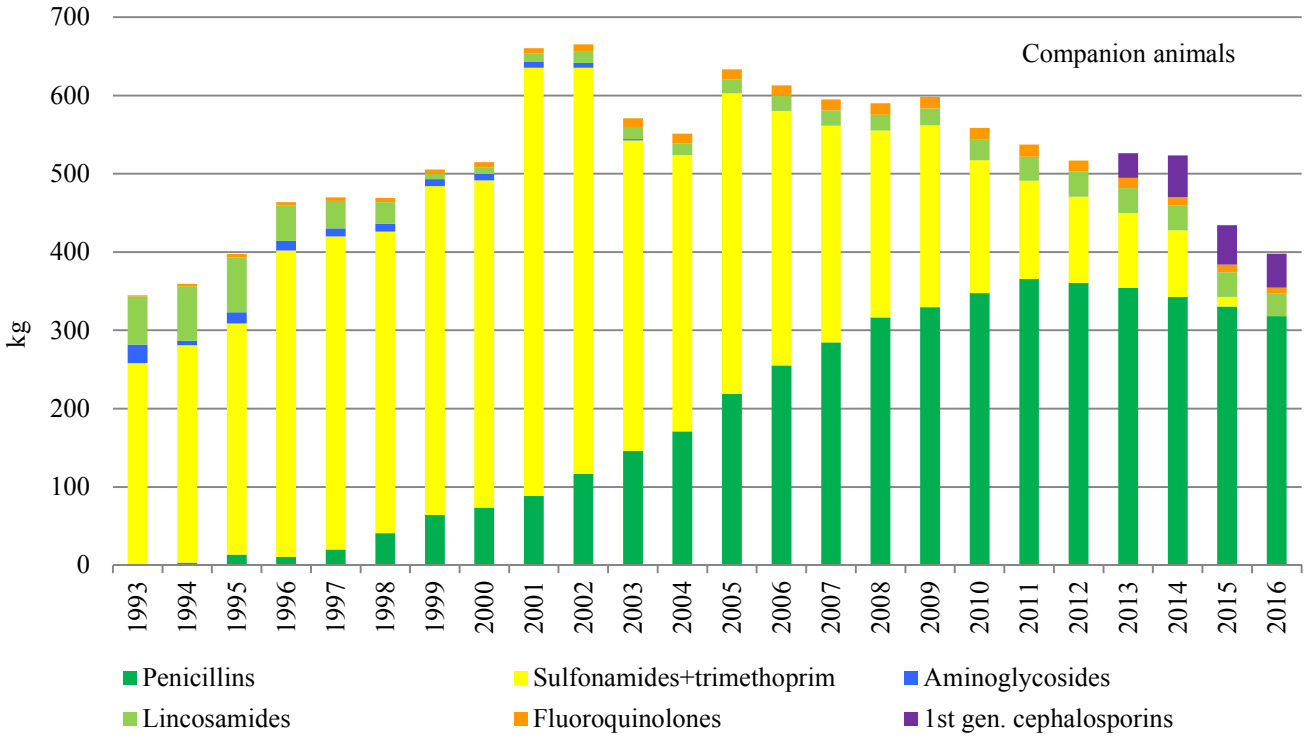

FIGURE 9. Sales in Norway, in kilograms active substance, of antibacterial veterinary medicinal products (VMPs) marketed for therapeutic use in companion animals only (injectables, oral paste, oral solution and tablets) for the years 1993-2016. In addition, minor amounts of a third generation cephalosporin injectable VMP (0.6-1.1 kg) were sold annually during 2008 -2016 and minor amounts of macrolide VMPs (0.4-5 kg) during 1996-2003.

Since the first penicillin VMP tablets were marketed for companion animals in 1994 the proportion of penicillin sales of total antibacterial VMPs sales for companion animals has increased from 1% to 80%. In 2016, approximately 84% of the sales of penicillin products marketed solely for companion animals were for the combination amoxicillin and clavulanic acid (Figure 10).
The sales of the highest priority CIA for humans, for use in companion animals, varied during 1993-2016, peaking in 2008-2011. In that period the sales of these CIAs accounted for around 0.8% of the total annual sales (kg) of antibacterial VMPs for companion animals (Figure 11).
The National Strategy against Antibiotic Resistance has set a target for reduction of usage of antibacterial agents in companion animals by at least 30% by 2020 with 2013 as the reference year. During the period 2013-2016 the sales, in kg active substance, of antibacterial VMPs marketed solely for dogs and cats, decreased by 24%.

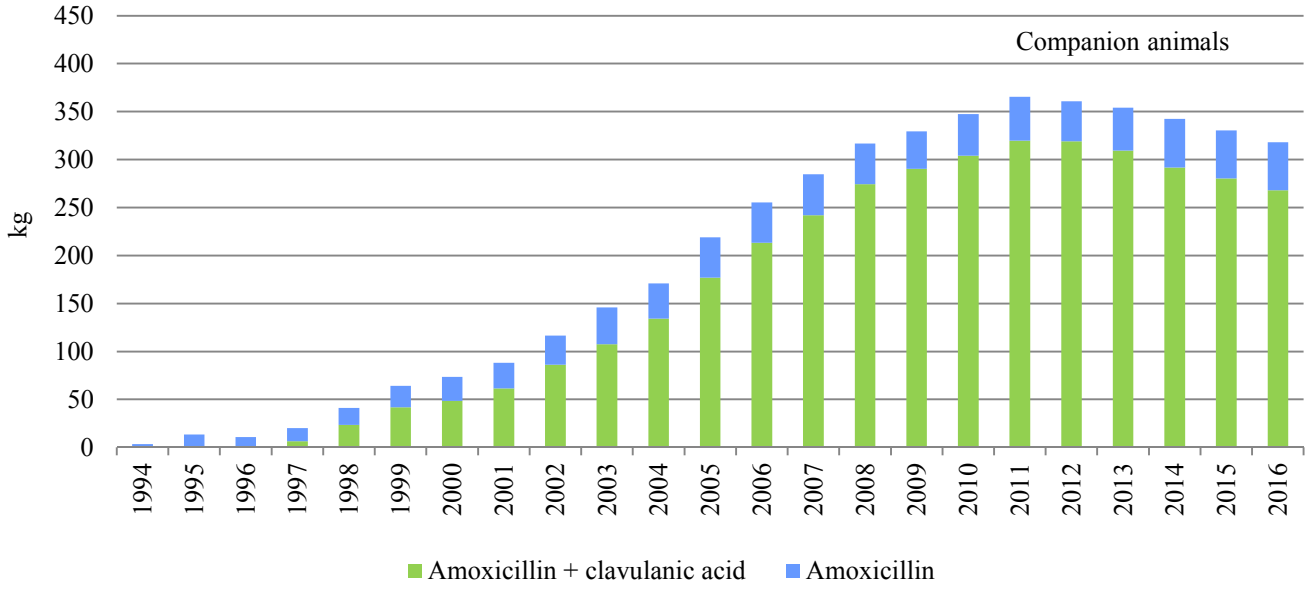

FIGURE 10. Sales, in kg active substance, of penicillin veterinary medicinal products (VMPs) for companion animals (dogs and cats), during 1995-2016.

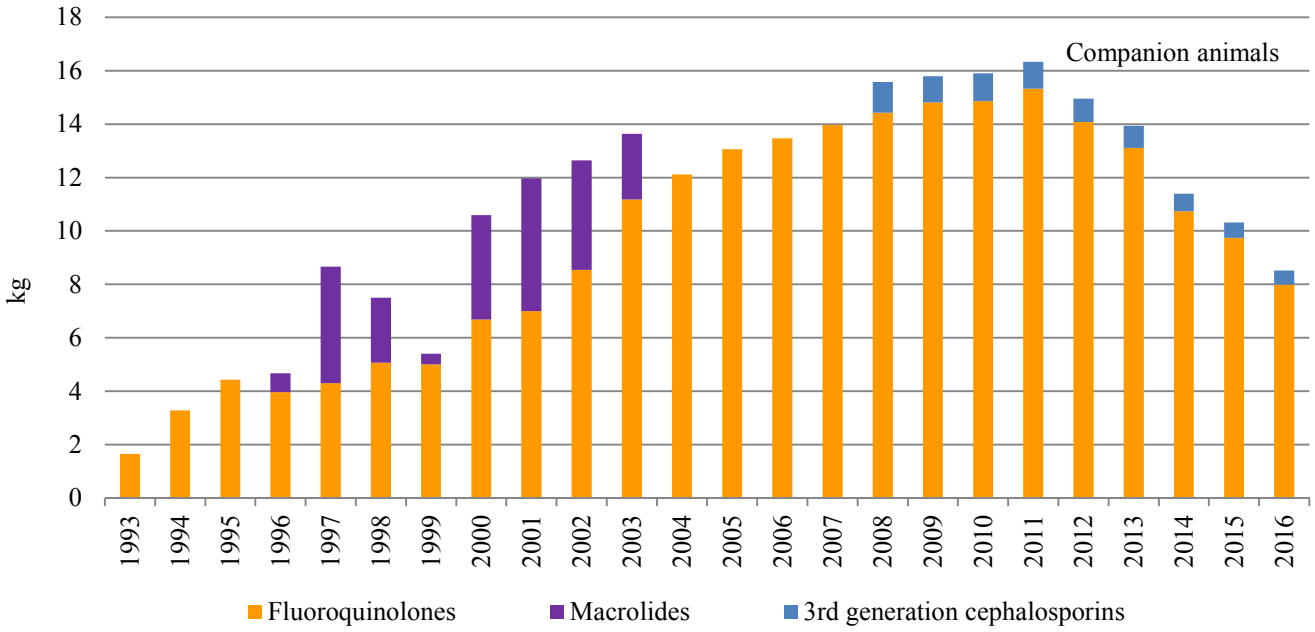

FIGURE 11. Sales in Norway, in kilograms active substance, of antibacterial veterinary medicinal products containing the highest priority critical important antimicrobials for humans – fluoroquinolones, macrolides and third generation cephalosporins - for therapeutic use in companion animals (dogs and cats) in 1993-2016.

Antimicrobial and coccidiostat feed additives

Due to a reported association between use of avoparcin as antimicrobial growth promoter and the occurrence of vancomycin resistant enterococci in 1995, the Norwegian food animal production industry immediately decided phasing out all use of antimicrobial growth promoters (AGPs) with instant stop of using of avoparcin (Table 5). In 1996 and 1997, the sales of zinc bacitracin were only 64 kg and 27 kg, respectively; since 1997 no AGPs have been used for animals in Norway. Data in Table 5 on sales of AGPs in 1995 are given for historical reference.

TABLE 5. Total sales, in kilograms of active substance, of ionophore coccidiostat feed additives in Norway 2007-2016; data for 1995 also include antimicrobial growth promoters and are given for historical reference. Data were obtained through annual reports from the Norwegian Food Safety Authority.

Active substance	1995	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
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	17	16	63	0	0	0	0	0	164	0
Monensin	3,422	919	896	885	805	1,060	1,080	1,174	1,313	1,081	874
Salinomycin	214	0	0	0	0	0	0	0	0	0	0
Narasin	24	7,065	9,212	8,621	9,080	9,394	10,378	12,345	12,409	9,126	562
Total ionophore coccidiostats	**4,656**	**8,001**	**10,124**	**9,569**	**9,885**	**10,454**	**11,458**	**13,519**	**13,722**	**10,371**	**1,436**

*Sold only part of the year

Following the phasing out of avoparcin in 1995, narasin was introduced as coccidiostat feed additive in the Norwegian broiler production due to its effect on *Clostridium perfringens*. From 1996, the sales of coccidiostat feed additives for use in Norwegian broiler production have almost exclusively been for narasin. In February 2015, the Norwegian poultry industry launched a project with the aim to phase out narasin use in the broiler production by the end of 2016. This goal was reached already by June 2016 (Figure 12).

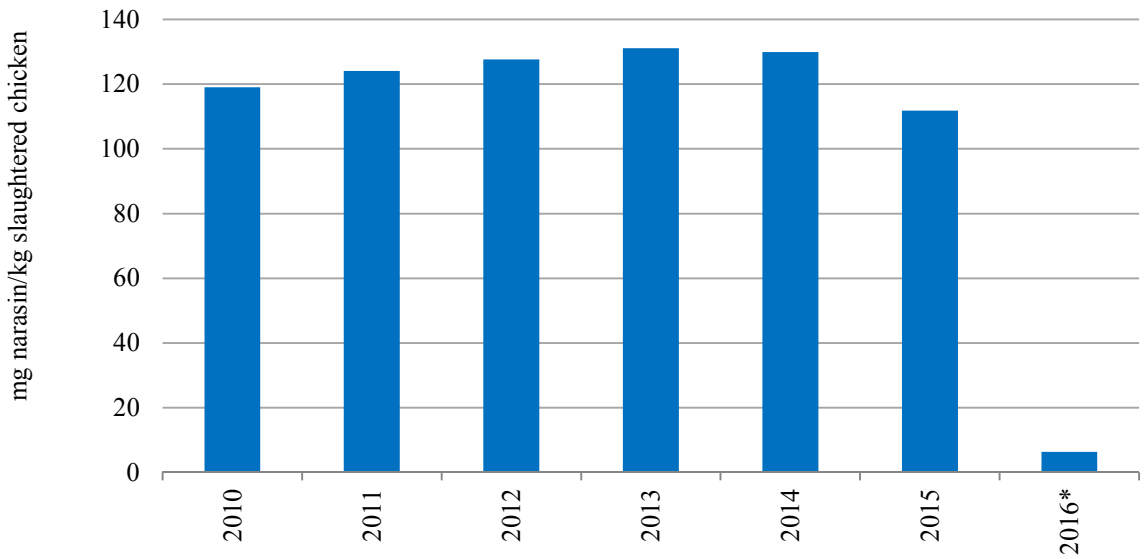

FIGURE 12. Sales, in mg active substance, of narasin coccidiostat feed additive per kg slaughtered chicken produced in Norway during 2010-2016. *No sales from June 2016.

USAGE IN HUMANS

Per Espen Akselsen, Hege Salvesen Blix, Morten Lindbæk, Marion Neteland

Overall antibiotic sales

In 2016, the total sales of antibacterials for systemic use in humans (J01 excluding methenamine) decreased by 5% from 15.5 in 2015 to 14.6 DDD/1,000 inhabitants/day. Antibiotics are prescription-only drugs in Norway. The overall antibiotic sales include all consumption in humans in Norway i.e. primary care and institutions. The overall consumption has decreased by 11% since 2012, when a *Mycoplasma pneumoniae* epidemic caused higher prescription rates of macrolides and tetracyclines. Increased sales of ATC group J01 *antibacterials* in the first decades of this century are mainly caused by increased use of penicillins and the urinary antiseptic methenamine (Table 6, Figure 13).

The proportion of narrow-spectrum penicillins (J01CE) of total sales (J01, excluding methenamine) has decreased over the years; from 32% in 2000 to 26% in 2016.

TABLE 6. Human usage of antibacterial agents in Norway 2009-2016 by ATC groups. The usage is presented as DDD (Defined Daily Doses)/1,000 inhabitants/day and in % change 2015-2016. Methods for collection of data on human usage of antimicrobial agents is presented in Appendix 2.

ATC	Groups of substances	2009	2010	2011	2012	2013	2014	2015	2016	Change (%) 2015-2016
J01A	Tetracyclines	3.09	3.12	3.47	3.87	3.54	3.46	3.38	3.16	- 7
J01B	Amphenicols	0.002	0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-
J01CA	Penicillins with extended spectrum	3.15	3.19	3.21	3.34	3.35	3.46	3.27	3.14	- 4
J01CE	Beta-lactamase sensitive penicillins	4.47	4.44	4.47	4.30	4.09	3.88	3.88	3.73	- 4
J01CF	Beta-lactamase resistant penicillins	0.80	0.82	0.88	0.90	0.79	0.91	0.89	0.90	- 3
J01CR	Combination of penicillins	0.02	0.03	0.03	0.04	0.05	0.08	0.09	0.10	+ 15
J01D	Cephalosporins, monobactams, carbapenems	0.58	0.55	0.56	0.55	0.52	0.48	0.45	0.43	- 3
J01E	Sulfonamides and trimethoprim	0.94	0.87	0.87	0.87	0.86	0.88	0.88	0.85	- 4
J01F	Macrolides, lincosamides and streptogramins	1.89	2.01	2.31	2.26	1.94	1.67	1.51	1.33	- 12
J01G	Aminoglycosides	0.07	0.07	0.07	0.08	0.07	0.08	0.08	0.08	-
J01M	Quinolones	0.71	0.73	0.75	0.75	0.72	0.68	0.61	0.54	- 8
J01X*	Other antibacterials	0.46	0.47	0.49	0.47	0.45	0.43	0.41	0.38	-
J01	**Total exclusive of methenamine**	**16.2**	**16.3**	**17.1**	**17.4**	**16.4**	**16.0**	**15.5**	**14.6**	**- 5**
J01XX05	Methenamine	3.19	3.37	3.44	3.57	3.70	3.86	4.00	4.09	+ 2
J01	**Total all antimicrobial agents**	**19.4**	**19.7**	**20.6**	**21.0**	**20.1**	**19.9**	**19.4**	**18.7**	**- 4**

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

The beta-lactamase sensitive penicillin-group (J01CE), penicillins with extended spectrum (J01CA) and the tetracyclines (J01A) were the three most used antibacterial groups in Norway in 2016. All, but two antibiotic groups, have decreased. The two groups increasing were; penicillins with betalactamase inhibitors (increased by 15% since 2015), and the urinary prophylactic agent methenamine. Methenamine has been continously increasing over the years and has in 2016 the largest amounts of DDDs in ATC group J01. Methenamine accounts for 91% of subgroup J01X and 22% of total antibacterial use (Figures 13-14). Of the tetracyclines (J01A), doxycycline is the most frequently used, followed by lymecycline, mainly indicated for acne (Table 7). In 2016, the penicillins (ATC group J01C) accounted for 43% of the total antibacterial use in Norway (Figure 14). Over the years there has been a shift towards use of more broad-spectrum penicillins. Penicillins with extended spectrum (J01CA) now represent 40% of the penicillin group compared to 25% in 1996 (Figures 14 and 17). This is mainly due to increasing use of amoxicillin and pivmecillinam. Pivmecillinam is used for urinary tract infections, at the expense of the subgroup of sulfonamides and trimethoprim, which has decreased over the years (Figure 13). The use of the group J01F macrolides, lincosamides and streptogramins has followed a wavy pattern over the years; the internal pattern within the group has remained relatively unchanged (Figure 18). 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. Since 2012, the use has decreased and is now at the same level as in 1990. In the latest years, sales of ATC group J01D (cephalosporins, monobactams and carbapenems) have decreased, mainly due to decreased use of first and second generation cephalosporins (Table 6 and 7, Figure 19). The quinolones represent only a small fraction (3%) of total antibacterial sales (Figure 14) and the use has steadily decreased since 2012 (Table 6). Ciprofloxacin is the main substance accounting for 96% of the quinolone group in 2016.

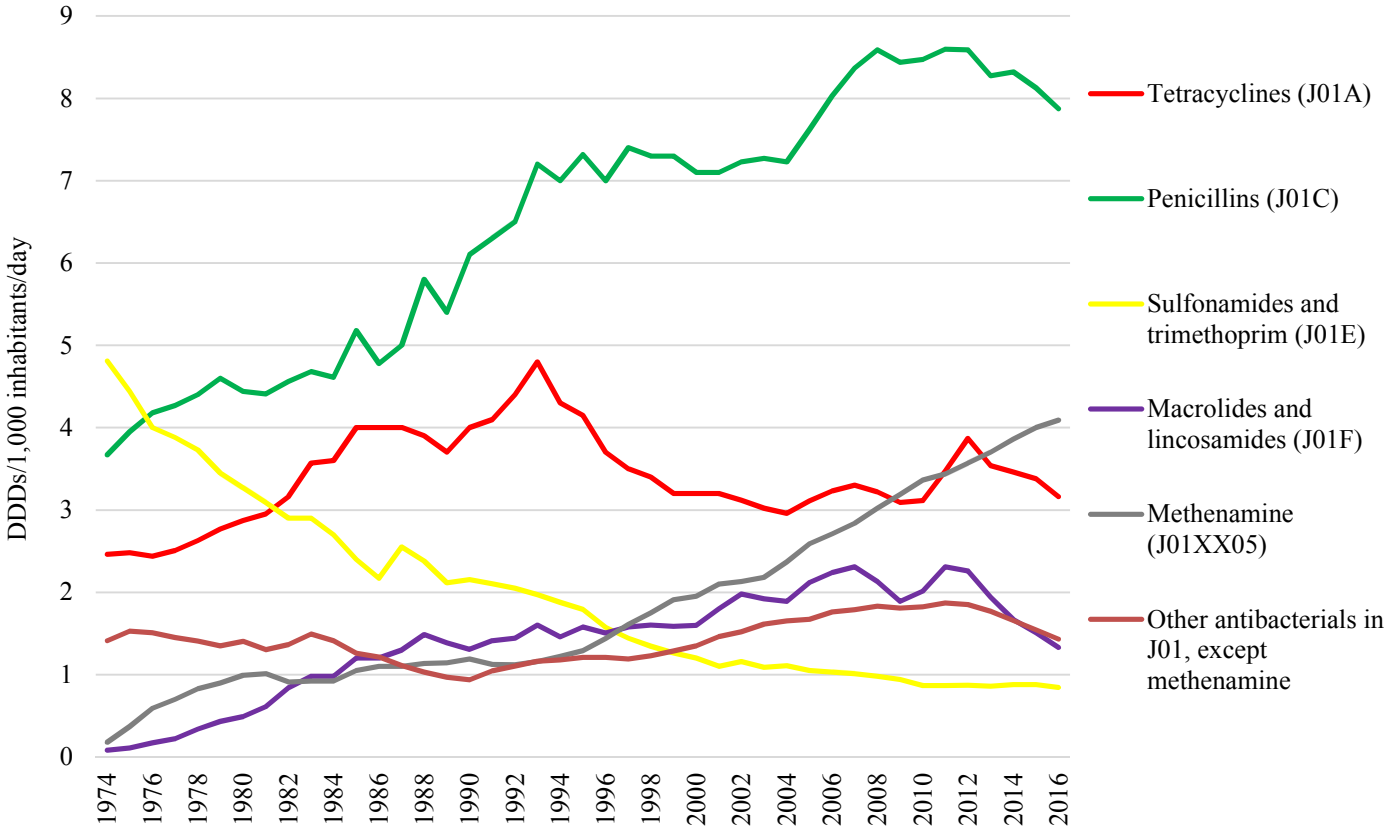

FIGURE 13. Sales of penicillins (J01C), tetracyclines (J01A), macrolides, lincosamides and streptogramines (J01F), sulfonamides and trimethoprim (J01E), methenamine (J01XX05), and other antibacterials in Norway 1974-2016. Other types of antibacterials include all other antibacterials in ATC group J01.

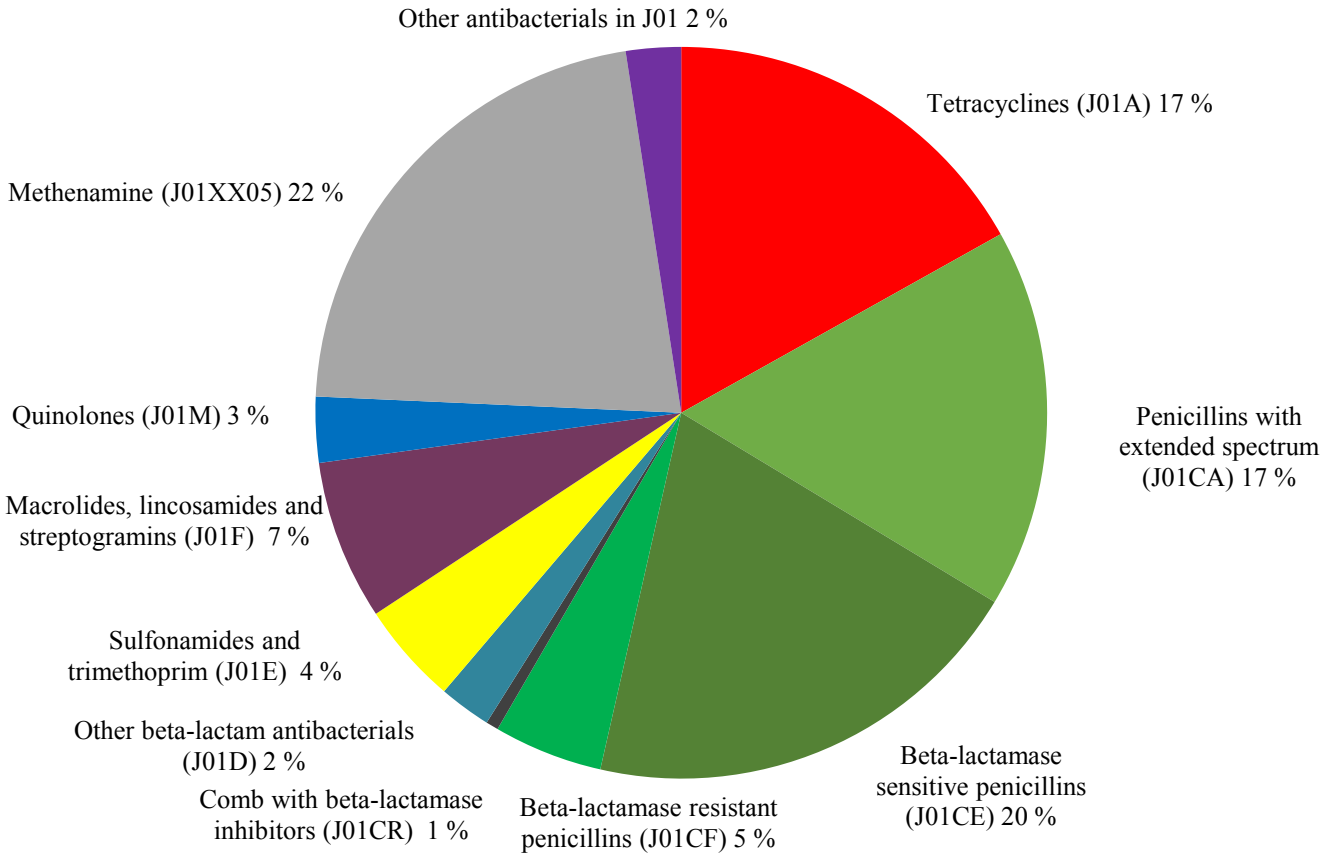

FIGURE 14. Relative amount of antibacterial agents for systemic use in 2016 in Defined Daily Doses (DDDs), total sales in the country.

Total usage in humans, animals and fish, measured in weight of active substance

In 2016, the overall sales in Norway of antibacterials for use in humans, terrestrial animals and farmed fish measured in weight of active substance was 56.6 tonnes (Figure 15). Of the total sales of antibacterials in Norway, human medicinal products accounted for 89.2%. Sales of veterinary medicinal products (VMPs) for use in terrestrial animals accounted for 10.4% and the use in fish only for 0.4% of total sales. Since 2005, increased sales of antibacterials in humans have been observed, however in 2016, the level of sales in tonnes, when excluding methenamine, is now the same as in 2005. During these years the sales for use in terrestrial animals have been relatively stable, while for farmed fish the sales varied during the period 2005-2016 but is substantially lower than in 2005.

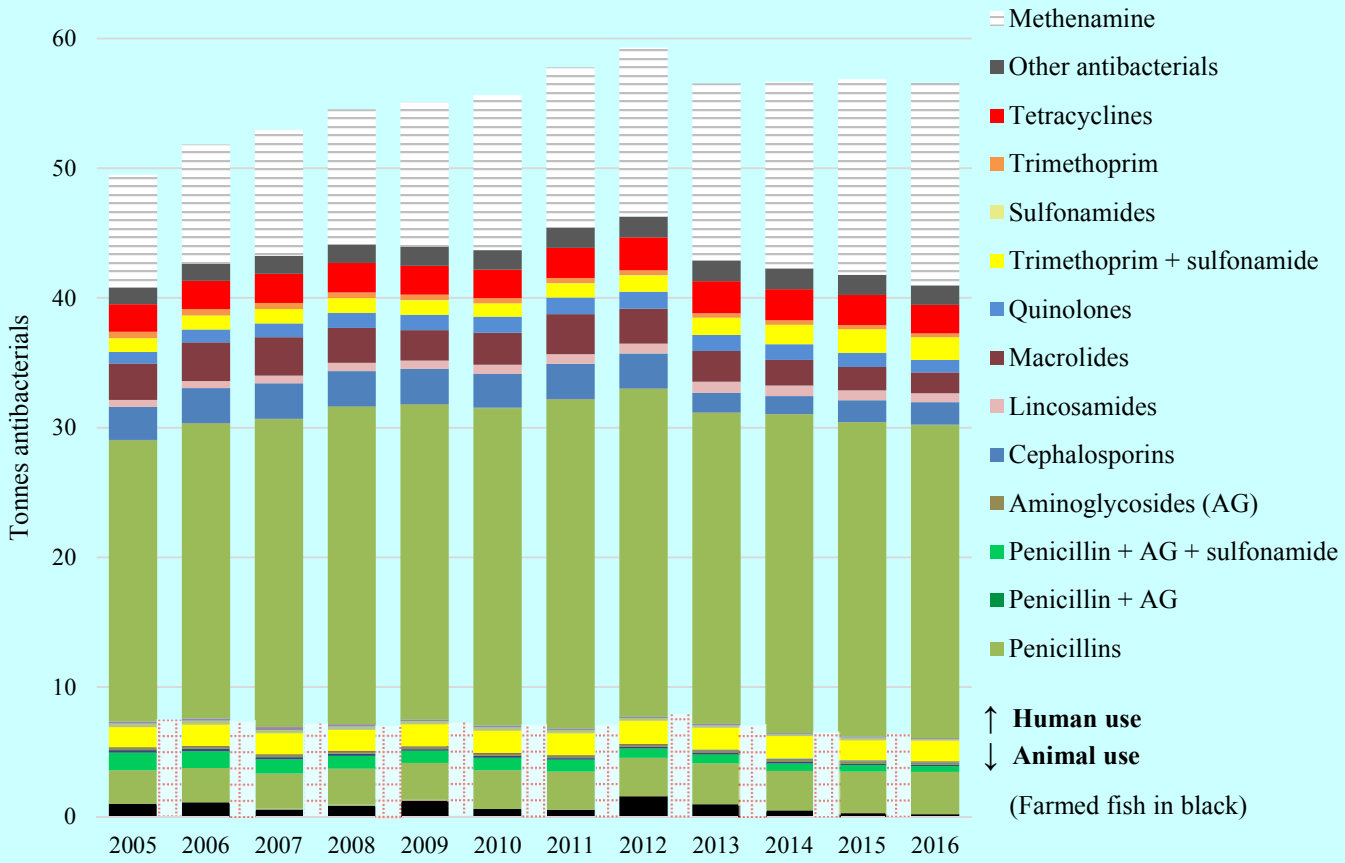

FIGURE 15. Sales, in tonnes of active substance, of antibacterials for humans, animals and fish, for the years 2005-2016. The use in farmed fish is shown at the bottom (blue colour).

Oral formulations are dominating in human medicine while for veterinary medicine the dominating formulations are the parenteral ones. In human medicine the oral formulations represent 82% of the total weight followed by parenteral formulations (18%). For food producing terrestrial animals the sales of parenteral formulations antimicrobial VMPs accounted for 62% of the total sales. In companion animals, the sales of oral VMPs were totally dominating (99.9%). Sales of other formulations e.g. for eye, ear and skin are low; in humans, sales of eye and ear preparations were 34 kg and dermatological preparations 105 kg and the corresponding figures for veterinary preparations were 49 kg and 12 kg, respectively.

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Usage in animals, aquaculture and humans of "Highest Priority Critically Important Antimicrobials" for human medicine

The World Health Organization has classified certain antimicrobial classes as "Highest Priority Critically Important Antimicrobials" for human medicine in the so-called CIA list[1]. The CIA list is intended for public health and animal health authorities, practicing physicians and veterinarians, and other interested stakeholders involved in managing antimicrobial resistance to ensure that critically important antimicrobials are used prudently both in human and veterinary medicine. It is intended as a reference to help formulate and prioritize risk assessment and risk management strategies for containing antimicrobial resistance due to human and non-human antimicrobial use. As a consequence of the detection of the plasmid-mediated *mcr-1* gene in 2015 that was followed by numerous reports on its occurrence in food producing animals, the WHO list of priority CIAs was updated by including polymyxins in April 2017.

The justification for the classification is summarized as follows[1]:

Quinolones
Quinolones are known to select for quinolone resistant *Salmonella* and *E. coli* in animals. At the same time, quinolones are one of few available therapies for serious *Salmonella* and *E. coli* infections. Given the high incidence of human disease due to *Salmonella* and *E. coli*, the absolute number of serious cases is substantial.

Cephalosporins (third and higher generation)
Cephalosporins (third and higher generation) are known to select for cephalosporin resistant *Salmonella* and *E. coli* in animals. At the same time, third and higher generation cephalosporins are one of few available therapies for serious *Salmonella* and *E. coli* infections in humans, particularly in children. Given the high incidence of human disease due to *Salmonella* and *E. coli*, the absolute number of serious cases is substantial.

Macrolides and ketolides
Macrolides and ketolides are known to select for macrolide resistant *Campylobacter* spp. in animals, especially *Campylobacter jejuni* in poultry. At the same time, macrolides are one of few available therapies for serious *Campylobacter* infections, particularly in children, for whom quinolones are not recommended for treatment. Given the high incidence of human disease due to *Campylobacter* spp., especially *Campylobacter jejuni*, the absolute number of serious cases is substantial.

Glycopeptides
Glycopeptides are known to select for glycopeptide resistant *Enterococcus* spp. in food animals (e.g. when avoparcin was used as a growth promoter, vancomycin resistant enterococci (VRE) developed in food animals and were transmitted to people). At the same time, glycopeptides are one of the few available therapies for serious enterococcal infections. Given the high number of cases, the previously documented occurrence of transmission of VRE to people from food animals, and the very serious consequences of treatment failures in such cases, glycopeptides are classified as being of the highest priority.

Polymyxins
Polymyxins (e.g. colistin) are known to select for plasmid mediated polymyxin resistant *E. coli* in food animals. At the same time, intravenous polymyxins are one of few available therapies for serious *Enterobactericeae* and *Pseudomonas aeruginosa* multidrug resistant infections in people in healthcare settings in many countries, especially in seriously ill patients in critical care. Given the high incidence of human disease due to *Enterobactericiae*, the absolute number of serious cases where colistin is needed can be considered substantial.

In Norway, only a small proportion of all antibiotics classified as Highest Priority Critically Important Antimicrobials for human medicine are used in animals and in farmed fish (Figure 16). In 2016, 0.6% of total sales of priority CIAs in Norway were used in terrestrial food producing animals. The proportion of priority CIAs sold for use in farmed fish has varied over the years, but in 2016, 2.1% of all sales of CIA were for farmed fish. Of note is that there have been no sales of neither glycopeptides nor polymyxins in veterinary medicine in Norway during 1993-2016. In total, 97.2% of the sales of priority CIAs in Norway in 2016 were for use in human medicine.

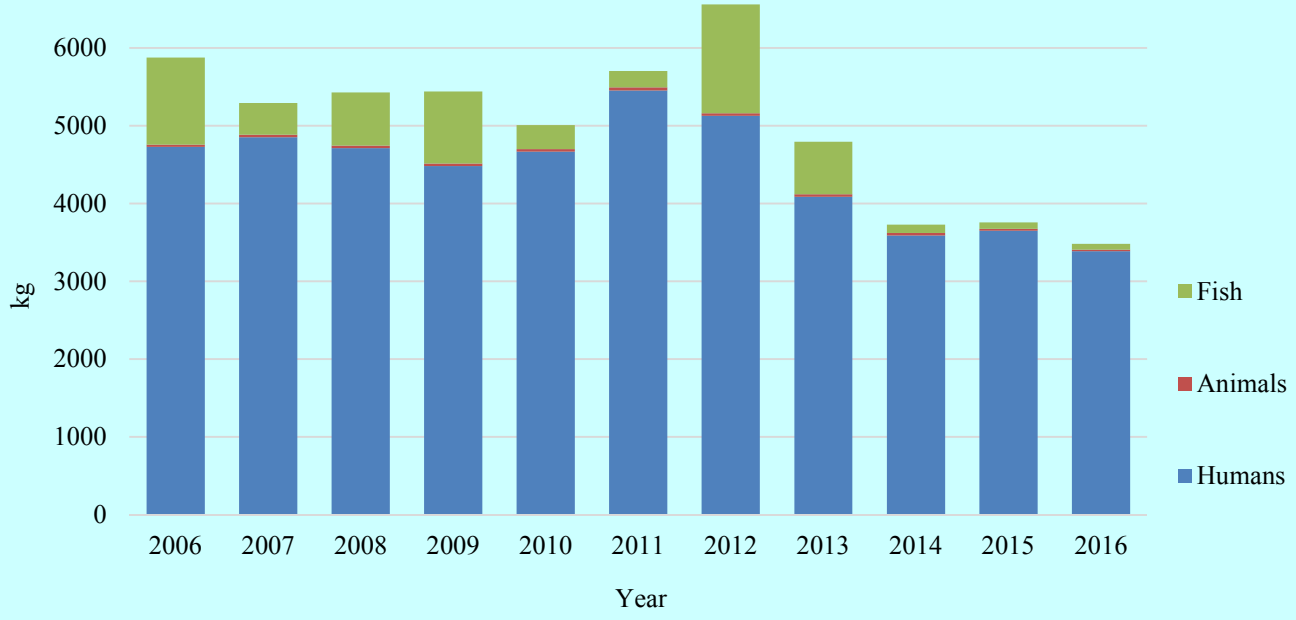

FIGURE 16. Trends in the sales of the WHO Highest Priority Critically Important Antimicrobials (CIAs) for human medicine since the first CIA list was published in 2005[2]. Data for 2006-2016 are separated into sales for use in humans, terrestrial animals and farmed fish in kilograms active substance.

References:

1. World Health Organization 2017. http://who.int/foodsafety/cia/en/2
2. World Health Organization 2005. http://apps.who.int/iris/bitstream/10665/43330/1/9241593601_eng.pdf?ua=1&ua=1

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TABLE 7. Human usage of single antibacterial agents for systemic use in Norway 2011-2016. Sales 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	2011	2012	2013	2014	2015	2016
J01A - Tetracyclines	J01A A02	Doxycycline	2.09	2.36	2.02	1.97	1.94	1.94
	J01A A04	Lymecycline	0.76	0.90	1.00	0.96	0.96	0.96
	J01A A06*	Oxytetracycline	0.03	-	<0.001	<0.001	<0.001	<0.001
	J01A A07	Tetracycline	0.58	0.62	0.54	0.50	0.45	0.40
	J01A A08*	Minocycline	0.002	0.006	0.009	0.003	0.002	0.002
	J01A A12	Tigecycline	<0.001	<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	<0.001
J01CA - Penicillins with extended spectrum	J01C A01	Ampicillin	0.09	0.09	0.10	0.12	0.11	0.13
	J01C A04	Amoxicillin	1.39	1.45	1.41	1.46	1.39	1.31
	J01C A08	Pivmecillinam	1.73	1.78	1.84	1.87	1.76	1.69
	J01C A11	Mecillinam	0.008	0.008	0.008	0.008	0.006	0.005
J01CE - Beta-lactamase sensitive penicillins	J01C E01	Benzylpenicillin	0.24	0.24	0.22	0.24	0.22	0.23
	J01C E02	Phenoxymethyl-penicillin	4.23	4.07	3.86	3.64	3.66	3.50
	J01C E08*	Benzathine benzylpenicillin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
J01CF - Beta-lactamase resistant penicillins	J01C F01	Dicloxacillin	0.74	0.76	0.58	0.72	0.73	0.74
	J01C F02	Cloxacillin	0.14	0.14	0.21	0.19	0.16	0.17
	J01C F05*	Flucloxacillin	<0.001	<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	0.004	0.007	0.012	0.013	0.016
	J01C R05	Piperacillin and enzyme inhibitor	0.03	0.03	0.04	0.07	0.08	0.09
J01DB - First gen. cephalosporins	J01D B01	Cefalexin	0.19	0.18	0.17	0.14	0.12	0.10
	J01D B03	Cefalotin	0.08	0.08	0.08	0.09	0.09	0.09
J01DC - Second gen. cephalosporins	J01D C02	Cefuroxime	0.09	0.08	0.07	0.06	0.04	0.04
J01DD - Third gen. cephalosporins	J01D D01	Cefotaxime	0.12	0.12	0.12	0.12	0.12	0.12
	J01D D02	Ceftazidime	0.01	0.01	0.01	0.01	0.01	0.01
	J01D D04	Ceftriaxone	0.03	0.03	0.03	0.02	0.02	0.02
J01DF - Monobactams	J01D F01	Aztreonam	<0.001	<0.001	0.001	0.001	0.001	0.001
J01DH - Carbapenems	J01D H02	Meropenem	0.04	0.05	0.05	0.05	0.04	0.04
	J01D H03	Ertapenem	0.002	0.002	0.002	0.002	0.003	0.002
	J01D H51	Imipenem and enzyme inhibitor	0.002	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
	J01DI54	Ceftolozane and enzyme inhibitor						<0.001
J01E - Sulfonamides and trimethoprim	J01E A01	Trimethoprim	0.55	0.51	0.49	0.46	0.42	0.38
	J01E C02	Sulfadiazine						0.001
	J01E E01	Sulfamethoxazole and trimethoprim	0.32	0.36	0.37	0.40	0.44	0.44
J01F - Macrolides, lincosamides and streptogramins	J01F A01	Erythromycin	1.18	1.06	0.85	0.75	0.68	0.60
	J01F A02	Spiramycin	0.01	0.01	0.006	0.005	0.004	0.003
	J01F A06	Roxithromycin			<0.001	<0.001	<0.001	<0.001
	J01F A09	Clarithromycin	0.37	0.39	0.30	0.23	0.18	0.14
	J01F A10	Azithromycin	0.44	0.48	0.41	0.35	0.35	0.35

ATC group	ATC code	Substance	2011	2012	2013	2014	2015	2016
	J01FS15	Telithromycin		<0.001	<0.001	<0.001	<0.001	<0.001
	J01F F01	Clindamycin	0.32	0.33	0.37	0.34	0.31	0.28
J01G - Aminoglycosides	J01GA01*	Streptomycin	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	J01G B01	Tobramycin	0.03	0.03	0.03	0.02	0.02	0.02
	J01G B03	Gentamicin	0.05	0.05	0.05	0.05	0.06	0.06
	J01G B06*	Amikacin	0.001	0.001	0.001	0.001	0.001	0.001
J01M - Quinolones	J01M A01	Ofloxacin	0.03	0.02	0.02	0.01	0.01	0.01
	J01M A02	Ciprofloxacin	0.71	0.72	0.70	0.65	0.59	0.52
	J01MA12*	Levofloxacin	0.002	0.002	0.001	0.002	0.002	0.003
	J01MA14*	Moxifloxacin	0.006	0.004	0.005	0.007	0.008	0.009
J01X - Other antibacterials	J01X A01	Vancomycin	0.01	0.01	0.01	0.02	0.02	0.02
	J01X A02	Teicoplanin	0.001	0.001	0.001	<0.001	<0.001	<0.001
	J01X B01	Colistin	0.004	0.004	0.005	0.006	0.005	0.007
	J01X C01	Fusidic acid	0.005	0.005	0.004	0.004	0.004	0.003
	J01X D01	Metronidazole	0.07	0.07	0.06	0.05	0.04	0.03
	J01X E01	Nitrofurantoin	0.39	0.37	0.36	0.35	0.34	0.31
	J01XX01	Fosfomycin		<0.001	<0.001	<0.001	<0.001	<0.001
	J01X X05	Methenamine	3.44	3.57	3.70	3.86	3.99	4.09
	J01XX08	Linezolid	0.01	0.01	0.007	0.007	0.009	0.01
	J01XX09	Daptomycin	<0.001	0.001	0.001	<0.001	0.001	0.001
Antibiotics in other ATC groups	J04A	Rifampicin**	0.12	0.13	0.13	0.13	0.12	0.12
	A07AA09	Vancomycin	0.001	0.002	0.002	0.002	0.002	0.002
	A07AA11	Rifaximin	0.002	0.004	0.007	0.012	0.028	0.043
	A07AA12	Fidaxomicin		<0.001	<0.001	<0.001	<0.001	<0.001
	P01AB01	Metronidazole	0.24	0.23	0.24	0.24	0.24	0.24
	D06AX09/ R01AX06*	Mupirocin (grams)	93	145	174	174	225	185

*Drugs not licensed in the Norwegian market in 2016. ** Given as the amount DDD/1,000 inhabitants/day of rifampicin (i.e. total amount in plain and combination products).

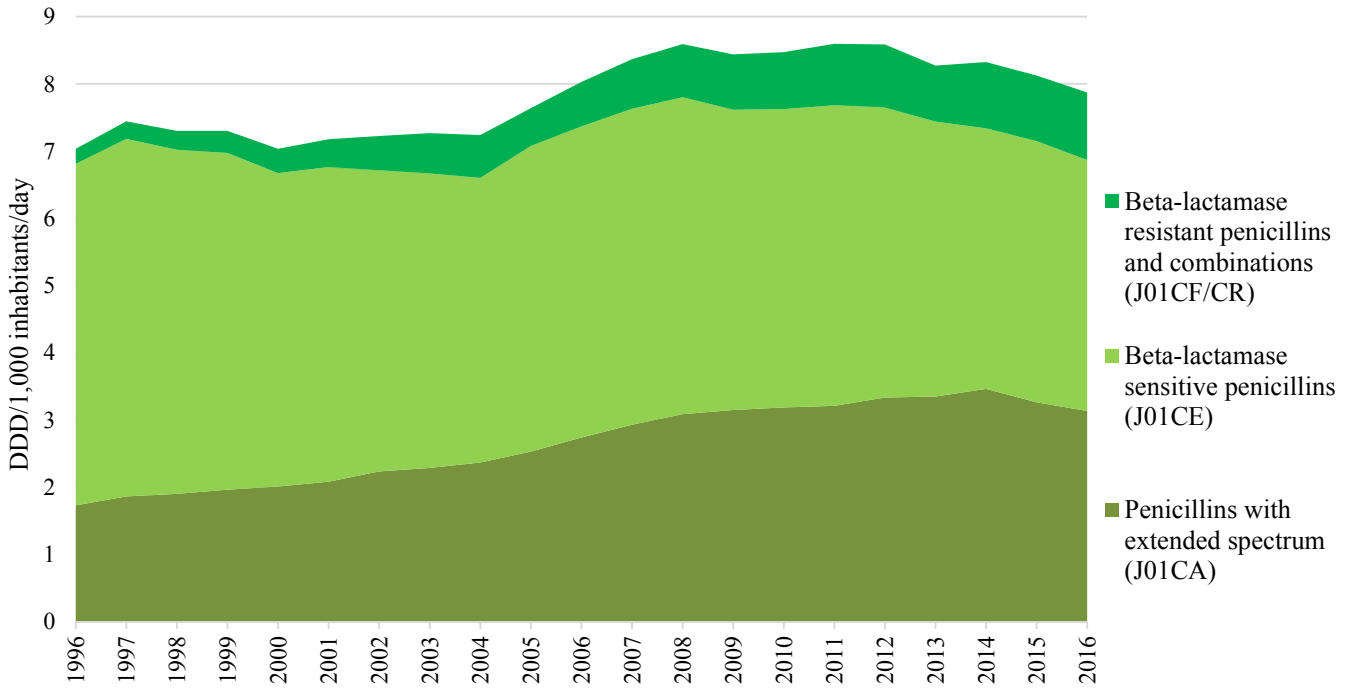

FIGURE 17. Sales of penicillins (J01C) in Norway 1996-2016 and changes within groups of penicillins.

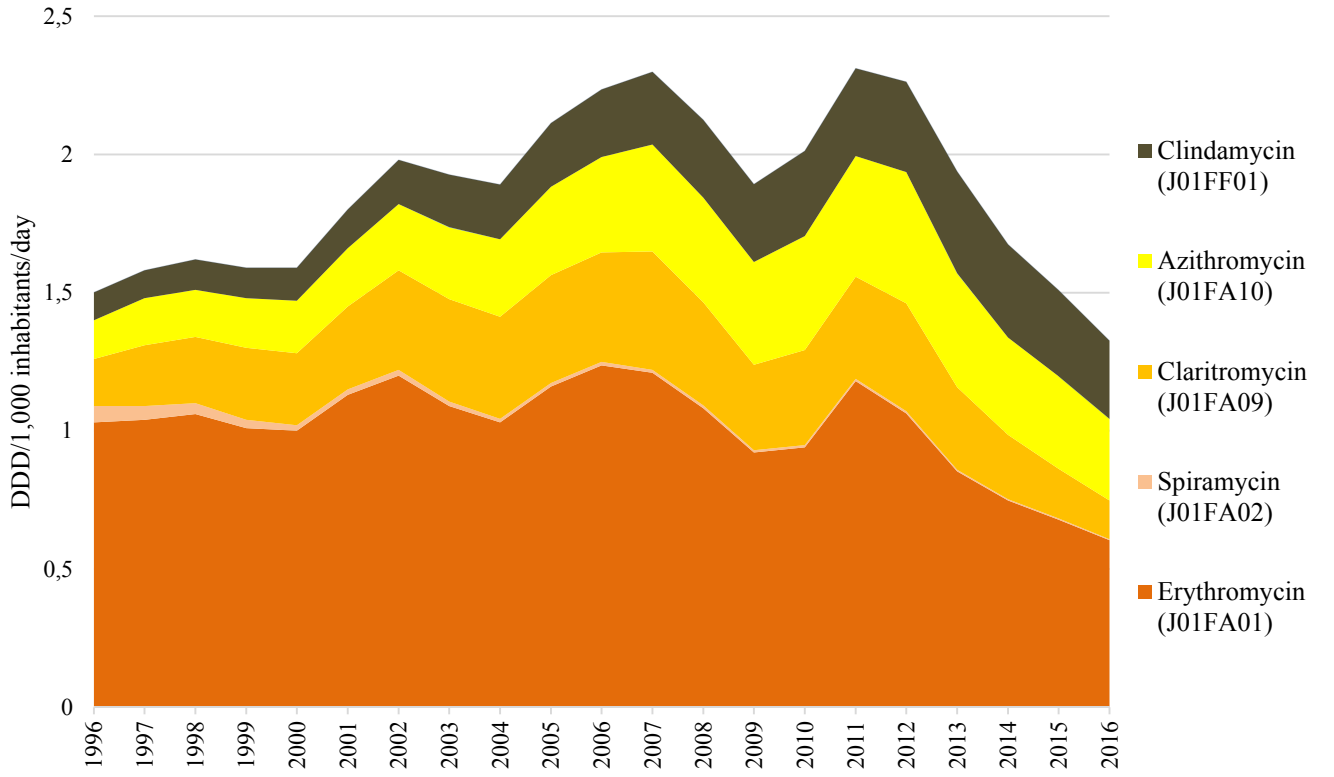

FIGURE 18. Sales of macrolides, lincosamides and streptogramins (J01F) in Norway 1996-2016.

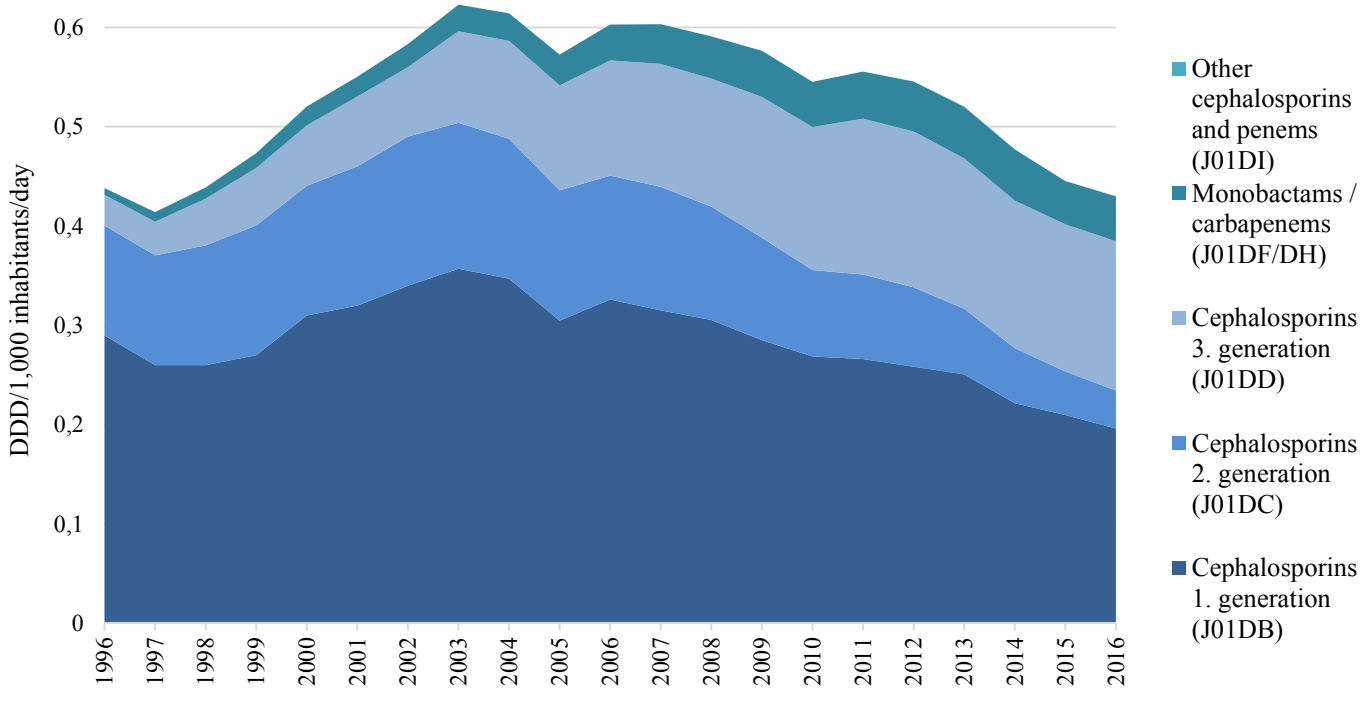

FIGURE 19. Sales of cephalosporins, monobactams and carbapenems (J01D) in Norway 1996-2016 and changes within generations of cephalosporins and monobactams/carbapenems.

Antibiotic usage in primary care

Around 85% of the total human sales of antibacterials are used in primary care, i.e. outside healthcare institutions. Antibacterials are prescription-only drugs in Norway and all prescriptions (included those prescribed from hospitals to out-patients) to persons living in Norway are captured in these figures.

Sales of antibiotics to outpatients have decreased since 2012. For ambulatory care, the most important antibiotic groups in 2016 were penicillins, J01C (41% of DDDs), tetracyclins, J01A (19%) and macrolides and lincosamides, J01F (8%). The four most commonly prescribed antibiotics for outpatients in 2016 were phenoxymethylpenicillin, pivmecillinam, doxycycline, and amoxicillin. These four represented 52% of all prescriptions and 47% of all DDDs. The urinary antiseptic methenamine represented only 9% of prescriptions, but 23% of the DDDs in primary care of ATC group J01 antibacterials for systemic use.

Geographical variation

The usage of antibacterials varies among the 19 Norwegian counties. The county using the least is using around 72% (in DDDs) of the county using the most (Figure 20). Over the years, and measured in DDDs/1,000 inhabitants/day, the same counties seem to be high-use counties and low-use counties, respectively. A North-South gradient has existed over years – the counties in northern Norway have low consumption while counties in the south have higher consumption. The pattern is almost the same when looking at the number of prescriptions/1,000 inhabitants. None of the counties have reached the national target of 250 prescriptions per 1,000 inhabitants per year (Figure 21).

Females use more antibiotics than males; 25% of the females purchased at least one antibiotic course (methenamine is excluded) in 2016 compared to 17% of the males. The gender pattern is similar in all regions in the country (Figure 22). The highest use is found among young children, young women and the elderly (Figure 23). Among those who use antibacterials, the elderly population use more; more than one in three elderly persons are prescribed antibiotics each year. This is also apparent with regard to the number of prescriptions and volum (amount measured in DDDs); for those above 75 years 2.1-2.3 prescriptions are dispensed every year compared to 1.5 for younger persons (Figure 24).

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. In 2016, dentists most often prescribed phenoxymethylpenicillin (72% of all antibiotic DDDs prescribed by dentists) followed by amoxicillin (12%) and clindamycin (6%).

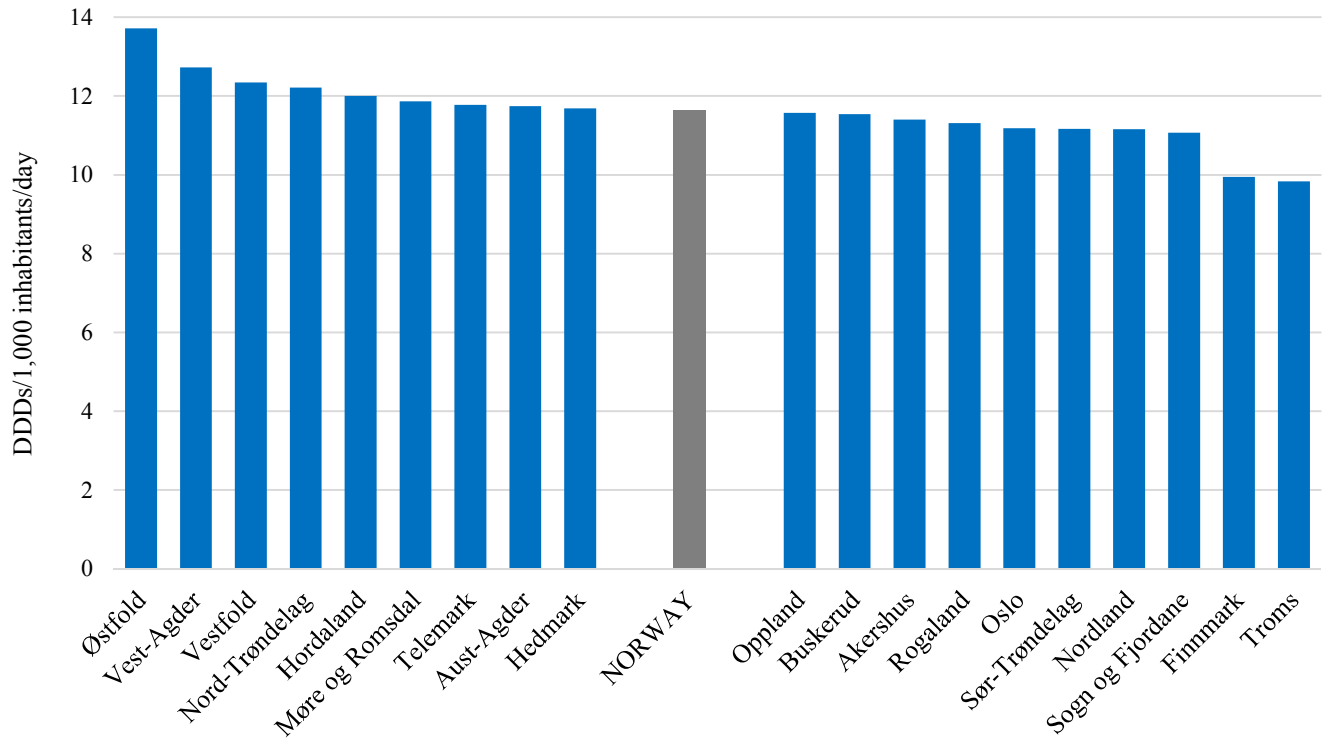

FIGURE 20. Sales of antibacterial agents for systemic use (ATC group J01, excluding methenamine) in outpatients in the different counties of Norway in 2016. Measured as number of DDD/1,000 inhabitants/day. Data from NorPD (i.e. health institutions not included).

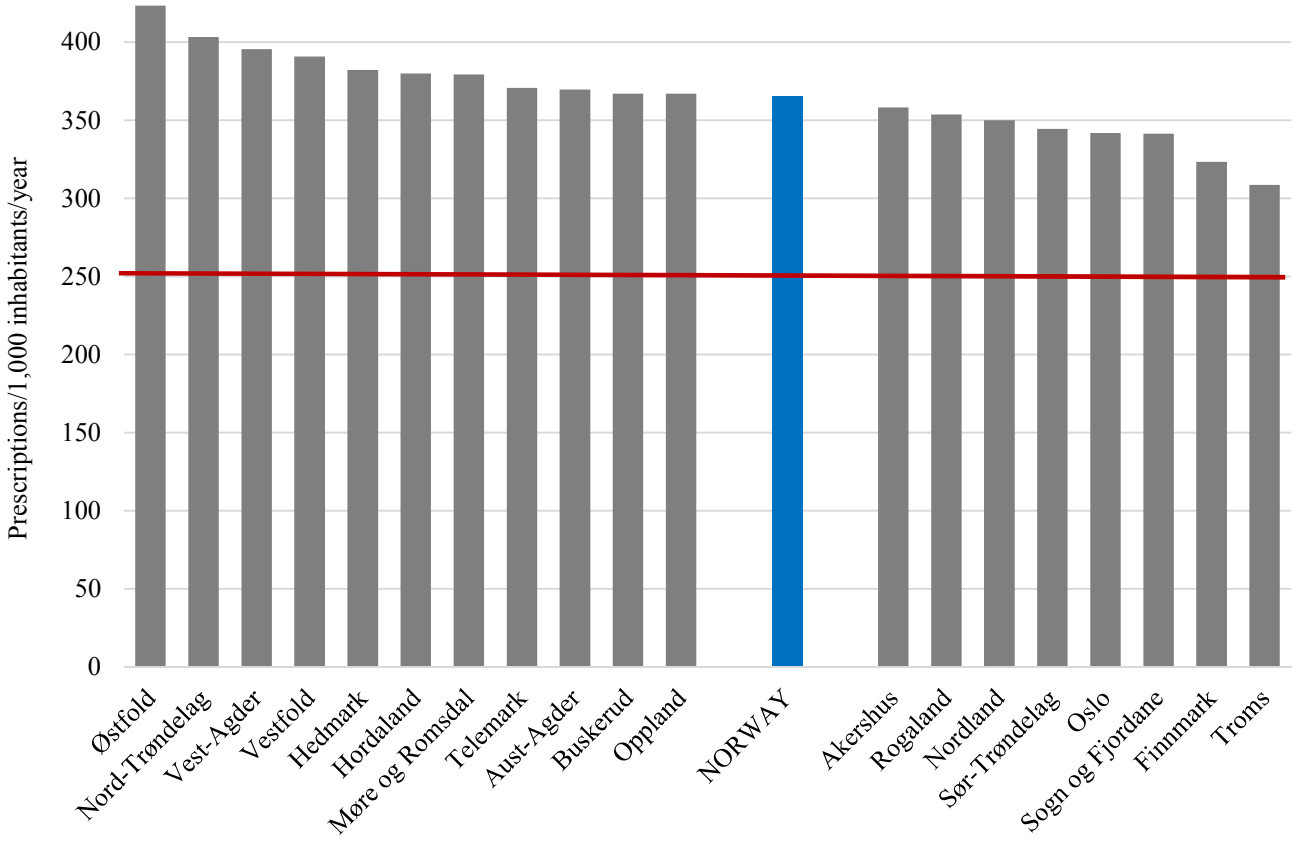

FIGURE 21. Sales of antibacterial agents for systemic use (ATC group J01, excl. methenamine) in outpatients in the different counties of Norway in 2016. Measured as number of prescriptions/1,000 inhabitants. Data from NorPD (excl. health institutions). Red line; target set by the National Strategy against Antibiotic Resistance 2015-2020.

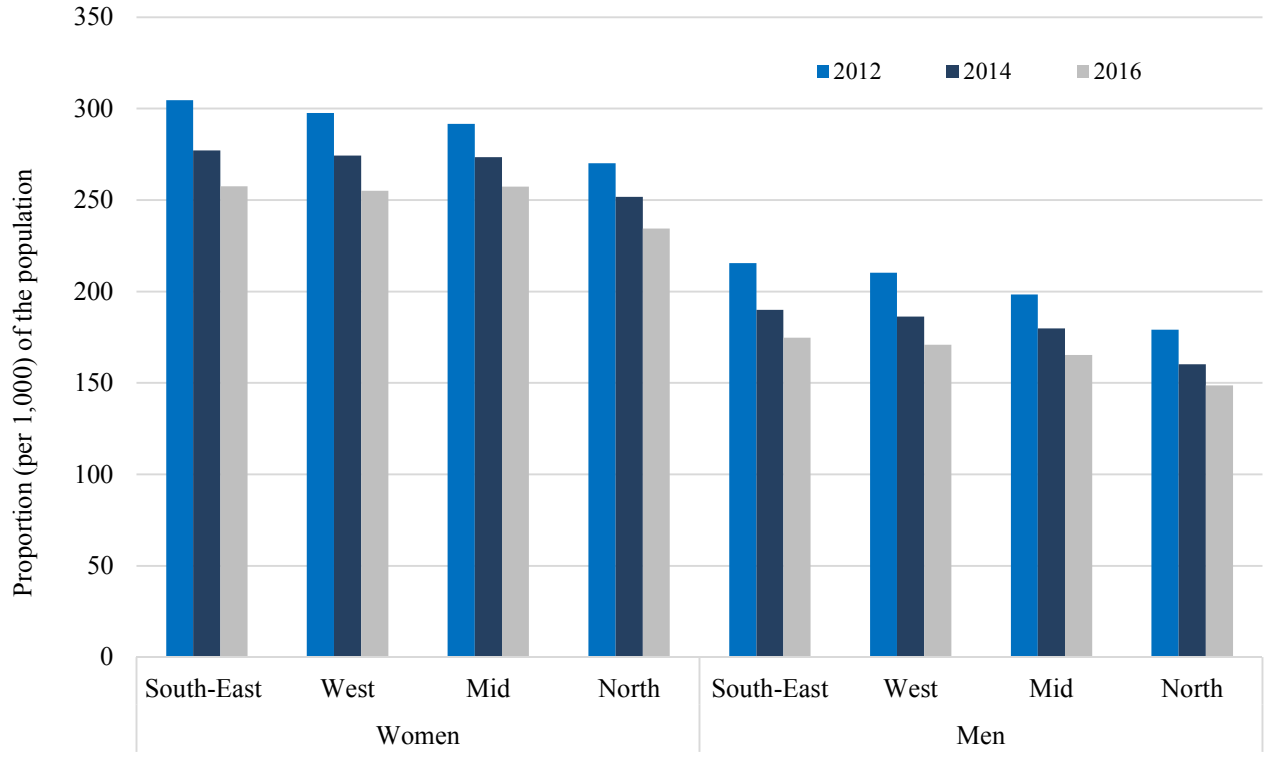

FIGURE 22. One year prevalence of systemic antibacterial use in ambulatory care by gender and health region in Norway for the years 2012, 2014 and 2016. Antibacterials included are antibacterials for systemic use (ATC group J01, excluding methenamine), vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and metronidazole (P01AB01).

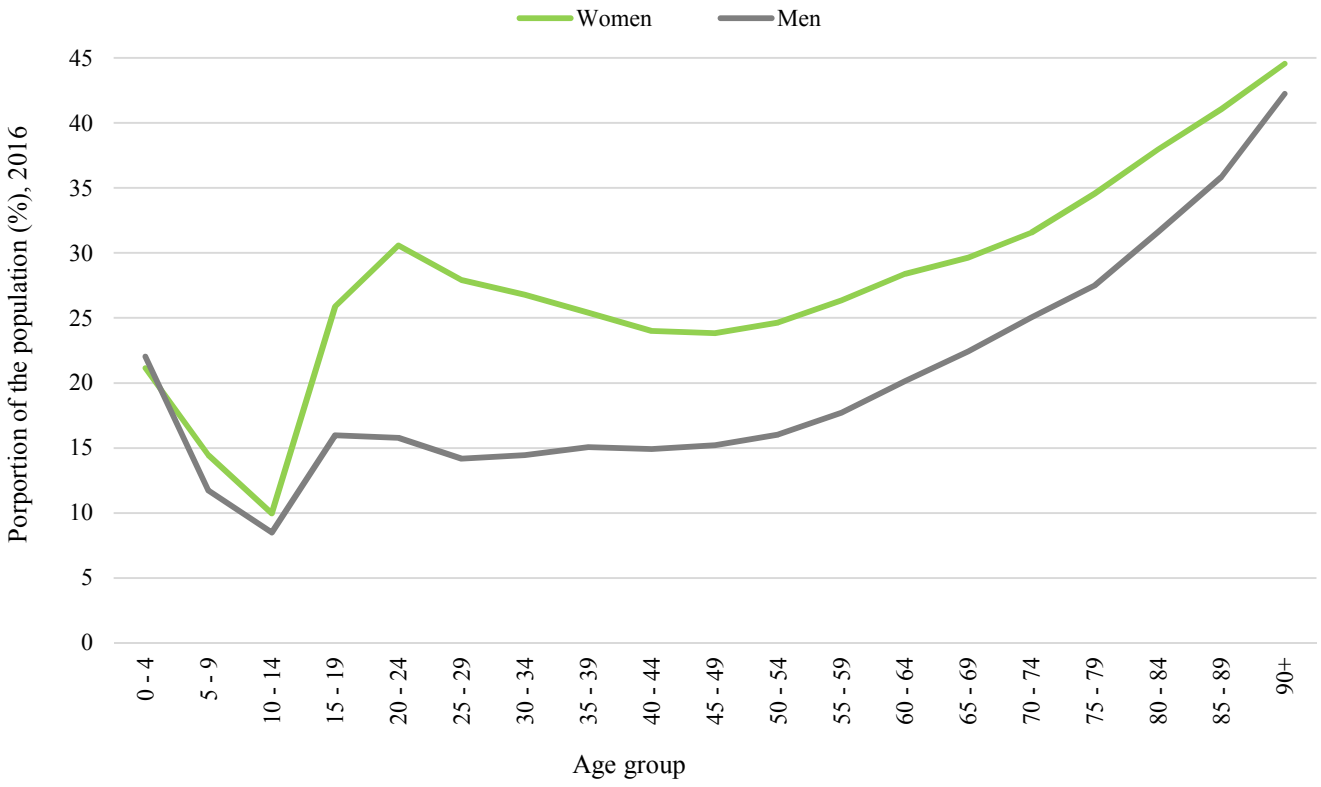

FIGURE 23. Proportion (%) of the population having dispensed at least one prescription of antibacterials (one year prevalence) in ambulatory care by gender and age in Norway, 2016. Antibacterials included are antibacterials for systemic use (ATC group J01, excluding methenamine), vancomycin (A07AA09), rifaximin (A07AA11), fidaxomicin (A07AA12) and metronidazole (P01AB01). Prevalence in age groups above 65+ is adjusted according to number of individuals living outside institutions.

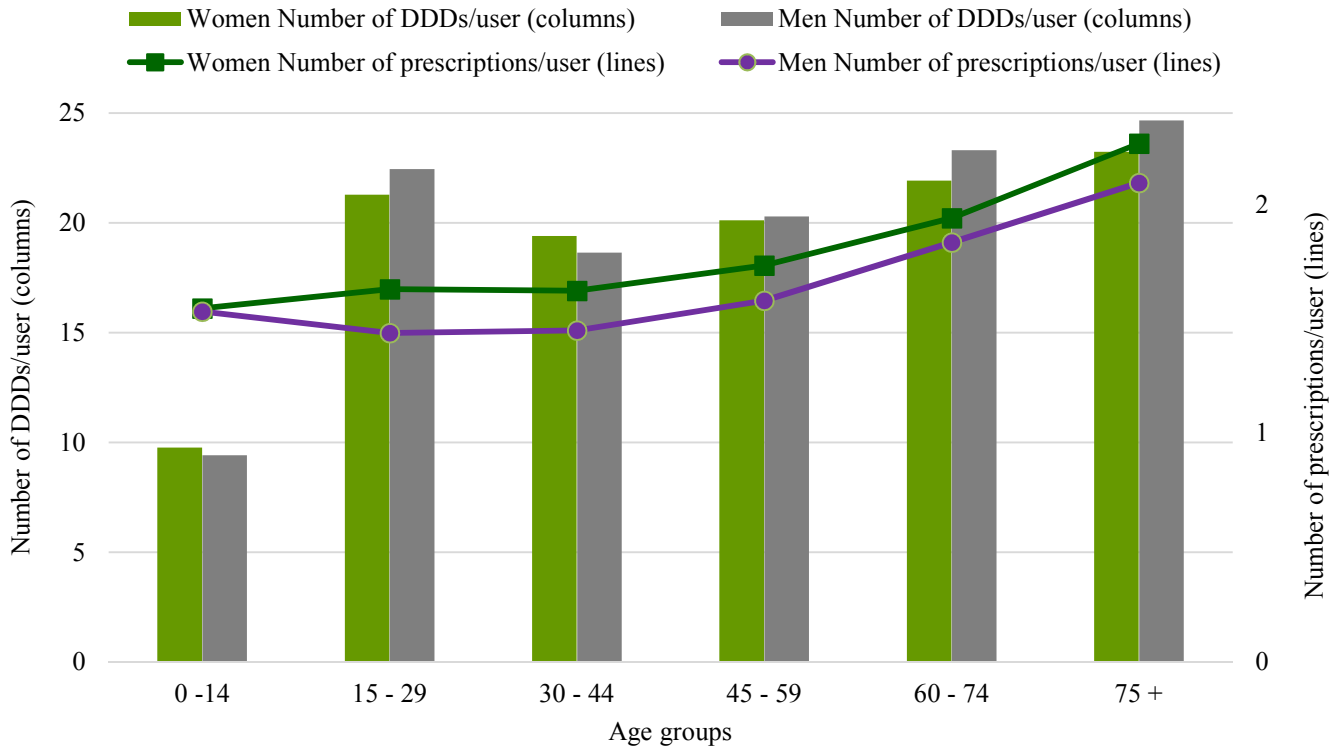

FIGURE 24. Mean number of prescriptions per person and mean number of DDDs per person among users of antibacterials in ambulatory care by gender and age in Norway, 2016. Antibacterials included are antibacterials for systemic use (ATC group J01, excluding. methenamine).

Antibiotic prescribing in dentistry

The sales of antibiotics to dentists and/or to dental clinics measured in DDDs decreased by 7% in 2016 compared with 2015. Moreover, there was a 8% reduction in prescriptions from 33.4 to 30.7 prescriptions per 1,000 inhabitants/year for J01 and oral metronidazole (P01AB01). Phenoxymethylpenicillin is most commonly prescribed followed by amoxicillin, clindamycin and oral metronidazole. In 2016, these antibiotic substances represented 72%, 12%, 6% and 4% of all DDDs of antibiotics prescribed by dentists, respectively. There has been a shift towards more broad-spectrum antibiotics over the years; in 2007, the phenoxymethylpenicillin proportion (i.e. of DDDs) was higher (76%), while the amoxicillin proportion was lower (7%). In dentistry, women are more often prescribed antibiotics than men. The age group 60-69 years stands for the highest consumption of antibiotics (J01 and P01AB01) prescribed by dentists, around 4% of the population in this age group population were prescribed antibiotics in 2016. In the age group younger and older, the proportions are around 3%.

Dentists account for approximately 5% of all antibiotics prescribed in outpatient care in 2016. Corresponding to outpatient medical care, there are great differences between the counties. The prevalence of use varies between 1.5% of the population in Finnmark to 3.1% in Vest-Agder. The total sales of antibiotics (J01 and metronidazole), measured as DDD/1,000 inhabitants/ year, decreased in 18 out of 19 counties in 2016 compared with 2015, range +2% to -11%. The reduction of the overall prescription in dentistry for 2016 to a level close to 2008 level may be accounted for by increased focus on antimicrobial resistance (AMR) in general (National Strategy against Antibiotic Resistance 2015-2020), as well as increased focus towards dental professionals via different channels (Norwegian Dental Association and the teaching institutions in Oslo, Bergen and Tromsø).

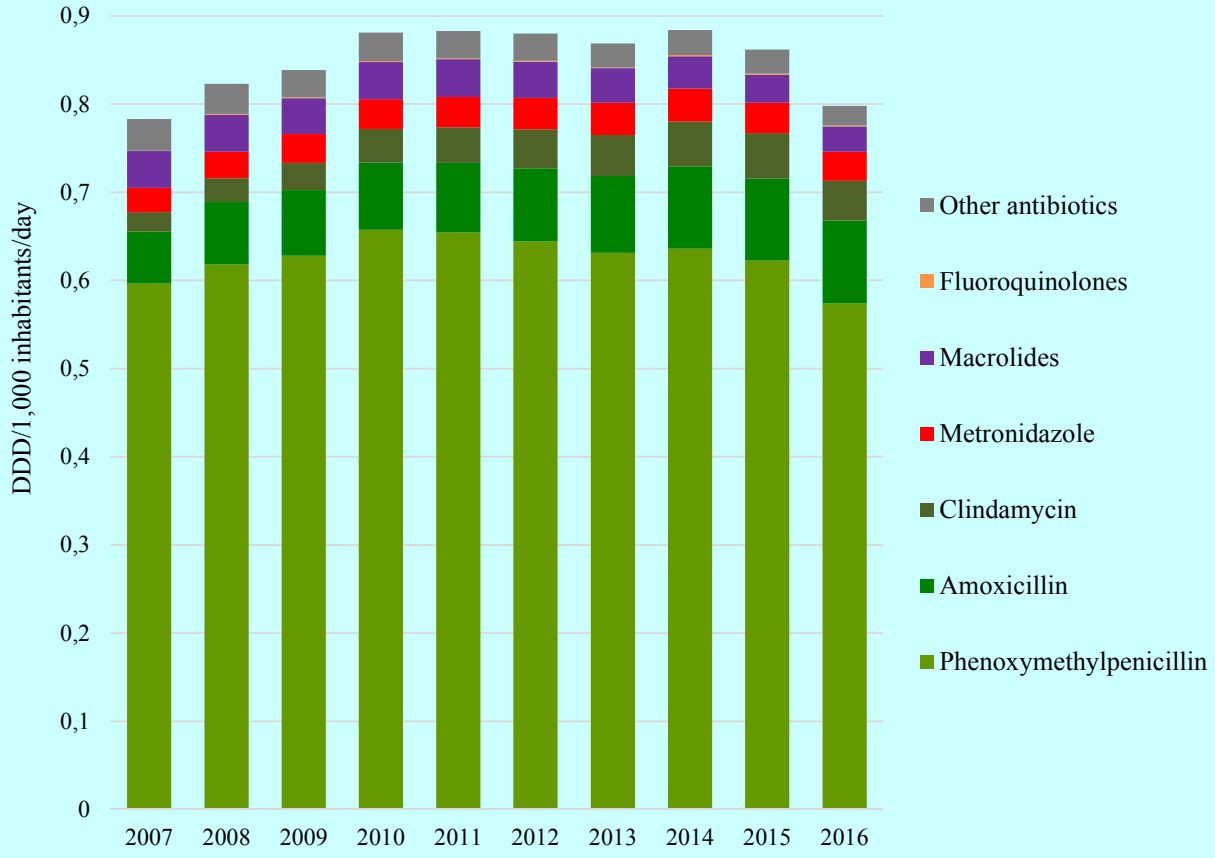

FIGURE 25. Antibiotics (J01 and oral metronidazole (P01AB01), prescribed in Norway for the years 2007-2016, measured in DDDs per 1,000 inhabitants per day.

Hege Salvesen Blix, Department of Pharmacoepidemiology, Norwegian Institute of Public Health; Sissel Torheim, Department of Cohort studies, Norwegian Institute of Public Health; and Morten Enersen, Department of Oral Biology, Faculty of Dentistry, University of Oslo, Norway.

Antibiotics used in the treatment of urinary tract infections in primary care

Increased and inappropriate use of antibiotics is a global concern due to its contribution to the increase of antimicrobial resistance. Urinary tract infections (UTI) is a leading cause of antibiotic use. This quantitative study aims to describe the treatment of UTI in Norway, and by that trying to understand where it is necessary to intervene to promote appropriate use of antibiotics.

Materials and methods

Data on antibiotic use were collected from the Norwegian Prescription Database (NorPD) for all men and women in the age span 1-79 years in Norway for the period 2006-2016. Indications for antibiotic use is not included in NorPD, but we defined urinary tract antibiotics (UTI-AB) to be antibiotics recommended for treatment of urinary tract infections according to Norwegian national clinical guidelines; pivmecillinam, trimethoprim, sulfamethoxazole-trimethoprim, ciprofloxacin and nitrofurantoin. In addition, ofloxacin was included. The measurements presented are annual prevalence and prescriptions.

Results

An observed decrease of UTI-AB for treatments of urinary tract infection was found for 2015 and 2016 compared to previous years. However, the pattern of use of UTI-AB has not changed. Women are the main consumers of UTI-AB, and the use increases from the age of 15. Furthermore, the consumption increases in both genders when they surpass the age of 50 (Figure 26).

Women are mainly treated with pivmecillinam (Figure 27), while the main substances used in men are sulfamethoxazole-trimethoprim and ciprofloxacin (Figure 27). Children are mainly prescribed trimethoprim and sulfamethoxazole-trimethoprim, possibly due to their availability as mixtures (data not shown).

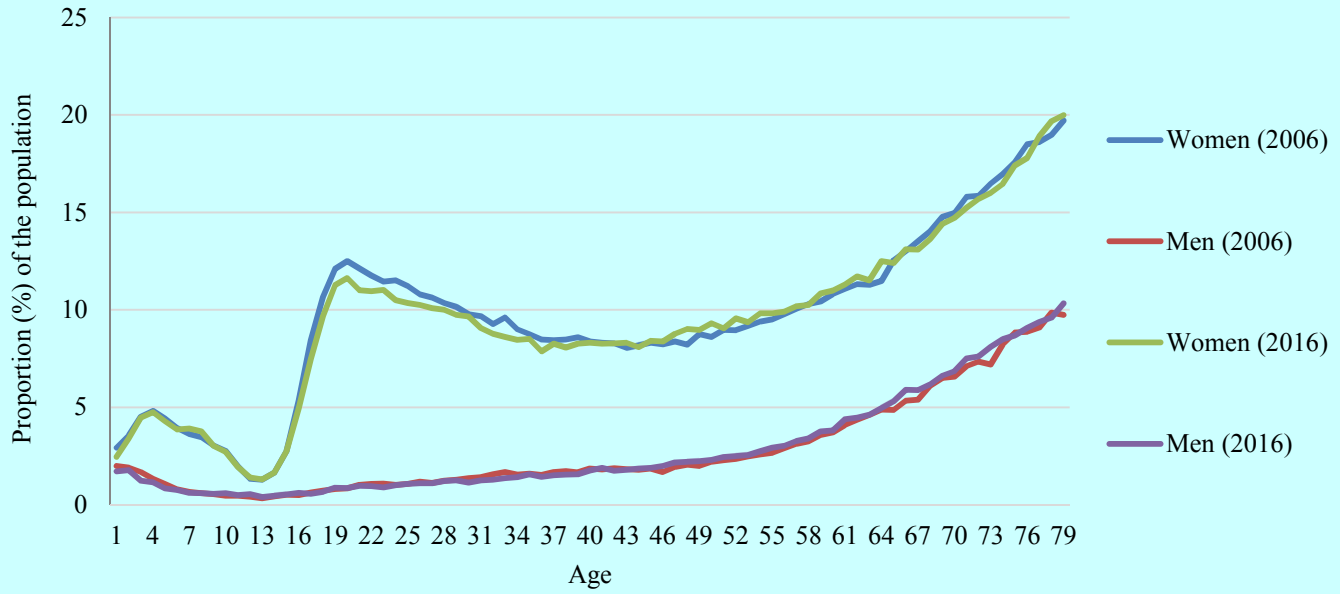

FIGURE 26. The figure shows prevalence (%) of urinary tract antibiotics (UTI-AB) in females and males in 2006 and 2016.

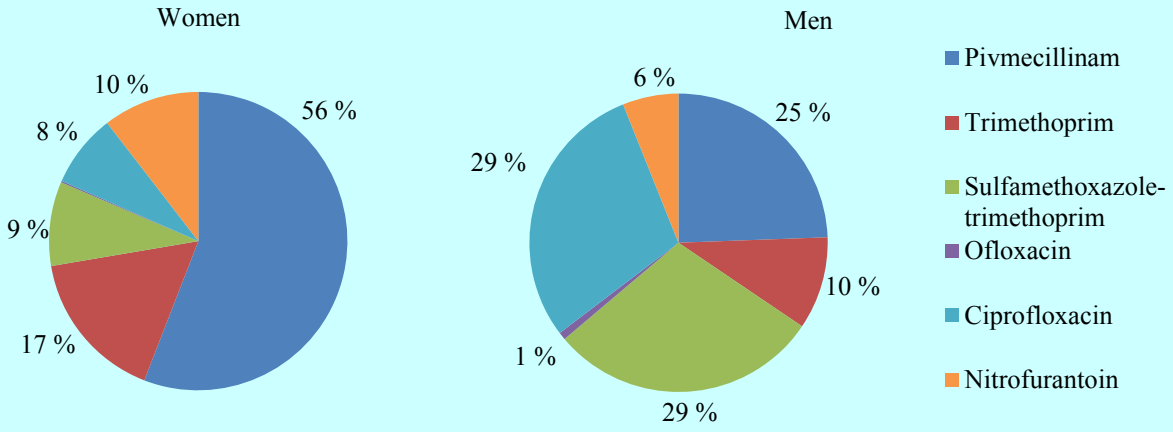

FIGURE 27. Distribution of urinary tract antibiotics (UTI-AB) prescriptions for women and men (20-79 years) in 2016.

Lina Janabi, School of Pharmacy, University of Oslo; and Hege Salvesen Blix, Department of Pharmacoepidemiology, Norwegian Institute of Public Health, Oslo, Norway.

Antibiotic usage in hospital care

In 2016, the antibacterial sales (in DDDs) to hospitals represented around 7% of total sales of antibacterials for human use in the country. The therapy pattern of antibacterials in hospitals does not change much from one year to another (Figure 28).

Penicillins (J01C) represent 50% of the use measured in DDDs in hospitals (J01CE 17%, J01CA 15% and J01CF 12%, J01CR 6%). The second largest group is the cephalosporins; 17% of all DDDs, the dominant subgroup being third generation cephalosporins (J01DD). In 2016, two single substances accounted for 24% of all antibacterial DDDs in hospitals; benzylpenicillin (14%) and cloxacillin (10%). Seven substances accounted for 54% of DDDs used in hospitals. These were benzylpenicillin, cloxacillin, ampicillin, cefotaxime, cefalotin, piperacillin/tazobactam and doxycycline. In hospital care, cefalotin and doxycycline are almost exclusively used for surgical prophylaxis.

Figure 29 shows annual trends in national antibiotic use in hospitals by hospital activity data (bed days and admissions) instead of population statistics. The two measurements together show the interplay between shorter hospital stays and intensity of antibiotic treatment.

Seven selected groups that are mainly used in hospitals are shown in Figure 30. Since 2006, there has been an increase in the use of the combination of piperacillin/tazobactam and the aminoglycosides. The use of third generation cephalosporins decreased in 2014 to a lower level which seems to be stabilized in 2015 and 2016. The use of second generation cephalosporins has been decreasing over many years. The use of carbapenems decreased for the first time in 2015, at least partly due to the worldwide shortage of meropenem, and in 2016 the use did not increase much. It should be noted that only parenteral formulations of second and third generation cephalosporins as well as carbapenems are lisenced in Norway.

There are large variations in volume of antibiotics used, measured in DDD/100 bed days, and in therapy profile between the hospitals. Figure 31 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 composistion alone.

A National Guideline for Antibiotic Use in Hospitals was published in 2013. The recommendations emphasises the use of narrow-spectrum antibiotics where possible. Figure 32 shows the proportions of preferred antibiotics (J01CA, J01CE, J01CF, J01EE01, J01GB) and antibiotics that are considered resistance drivers (J01CR, J01DC, J01DD, J01DH, J01FF01, J01MA, J01XA, J01XX08) for all Norwegian hospitals/health trusts. The differences are relatively small and the trends are much the same as in Figure 31 with the exception that the university hospitals use a lower proportion of preferred antibiotics. The proportion of preferred antibiotics is >60% in all hospitals/ health trusts except the trust of Oslo University Hospital. One factor that may partially explain higher relative use of resistance driving antibiotics in this hospital trust is that it has several national assignments and is the only hospital doing solid organ transplants.

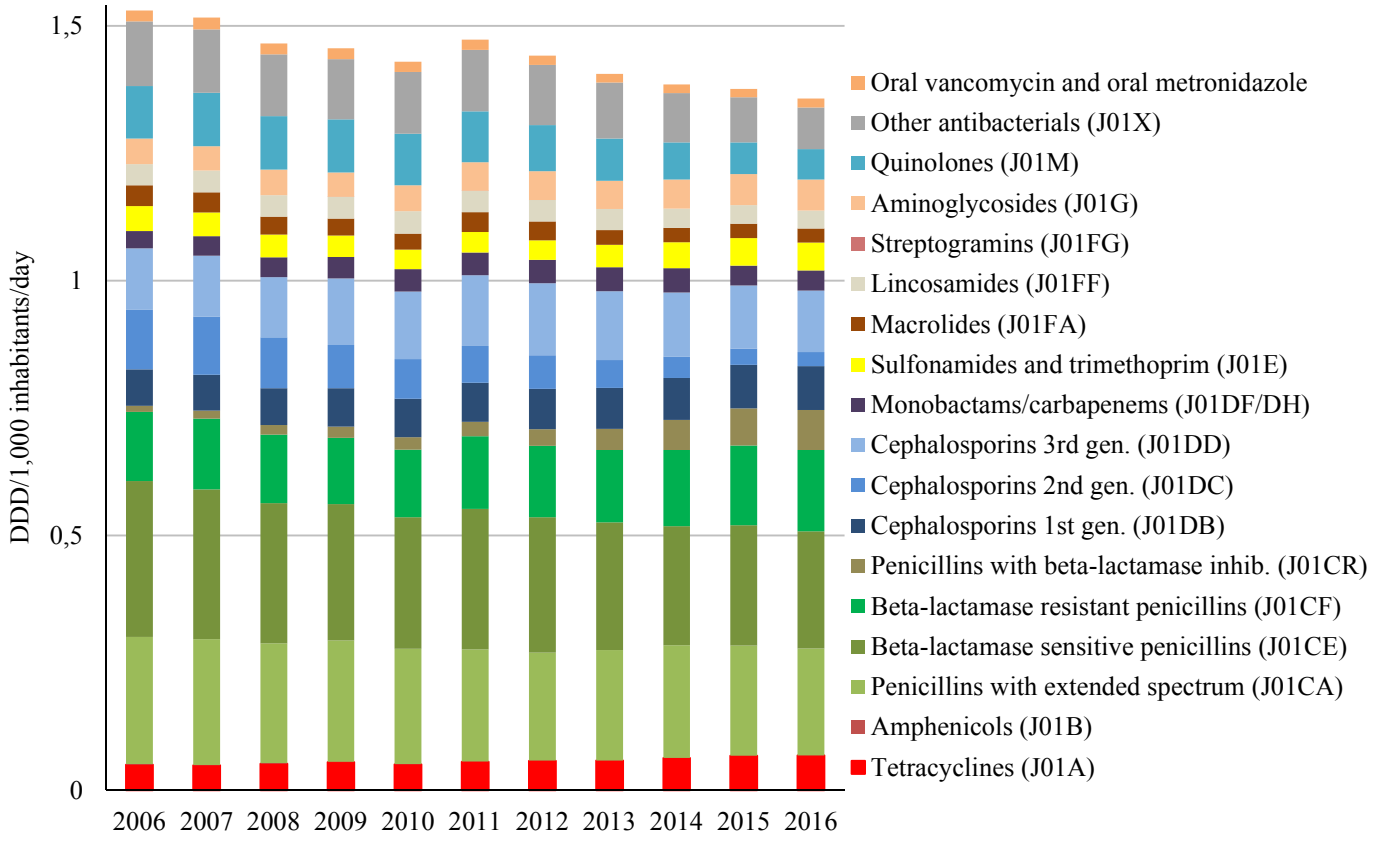

FIGURE 28. Use of antibacterial agents for systemic use in Norwegian hospitals 2006-2016, measured in DDD/1,000 inhabitants/day.

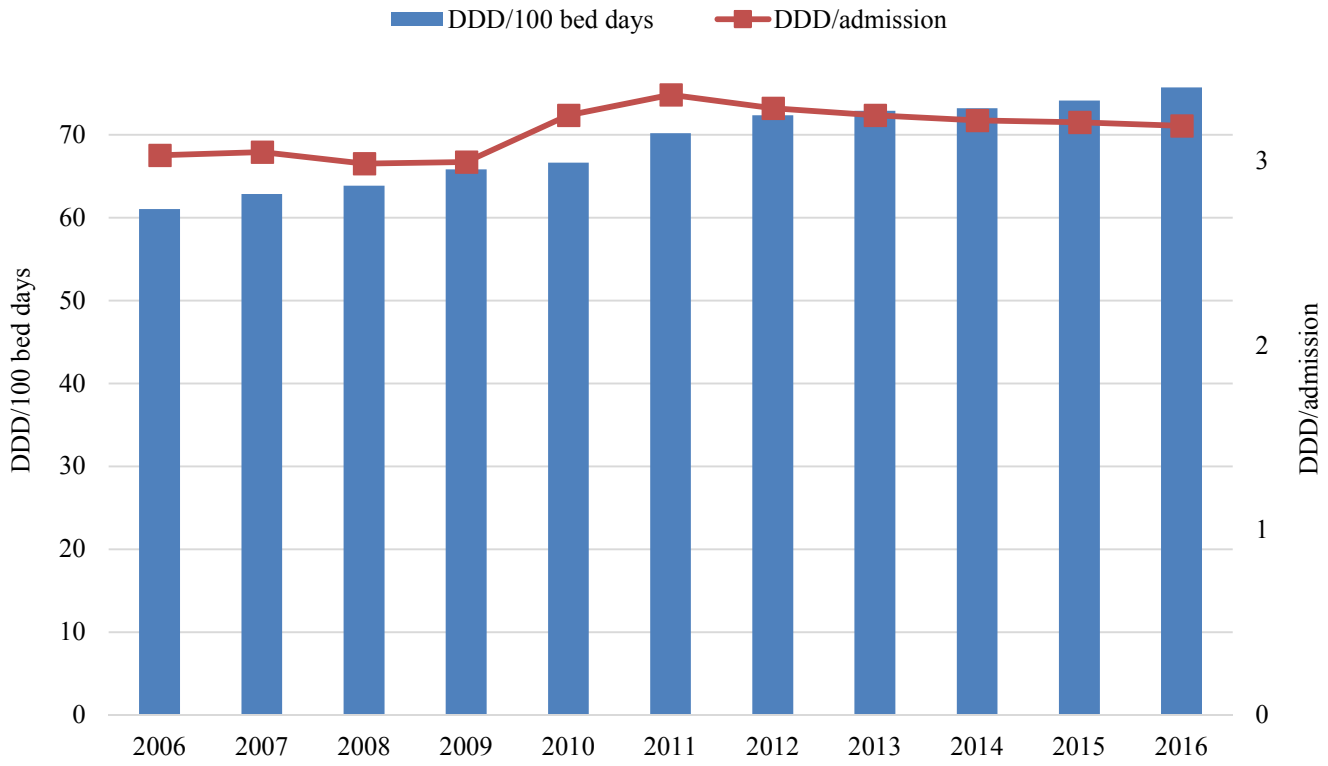

FIGURE 29. Total use of antibiotics in Norwegian hospital (somatic) 2006-2016, measured in DDD/100 bed days (blue bars) and DDD/admission (red line). Antibiotics are defined as J01 antibacterials for systemic use, A07AA09 vancomycin (oral), A07AA12 fidaxomycin and P01AB01 metronidazole (oral and rectal).

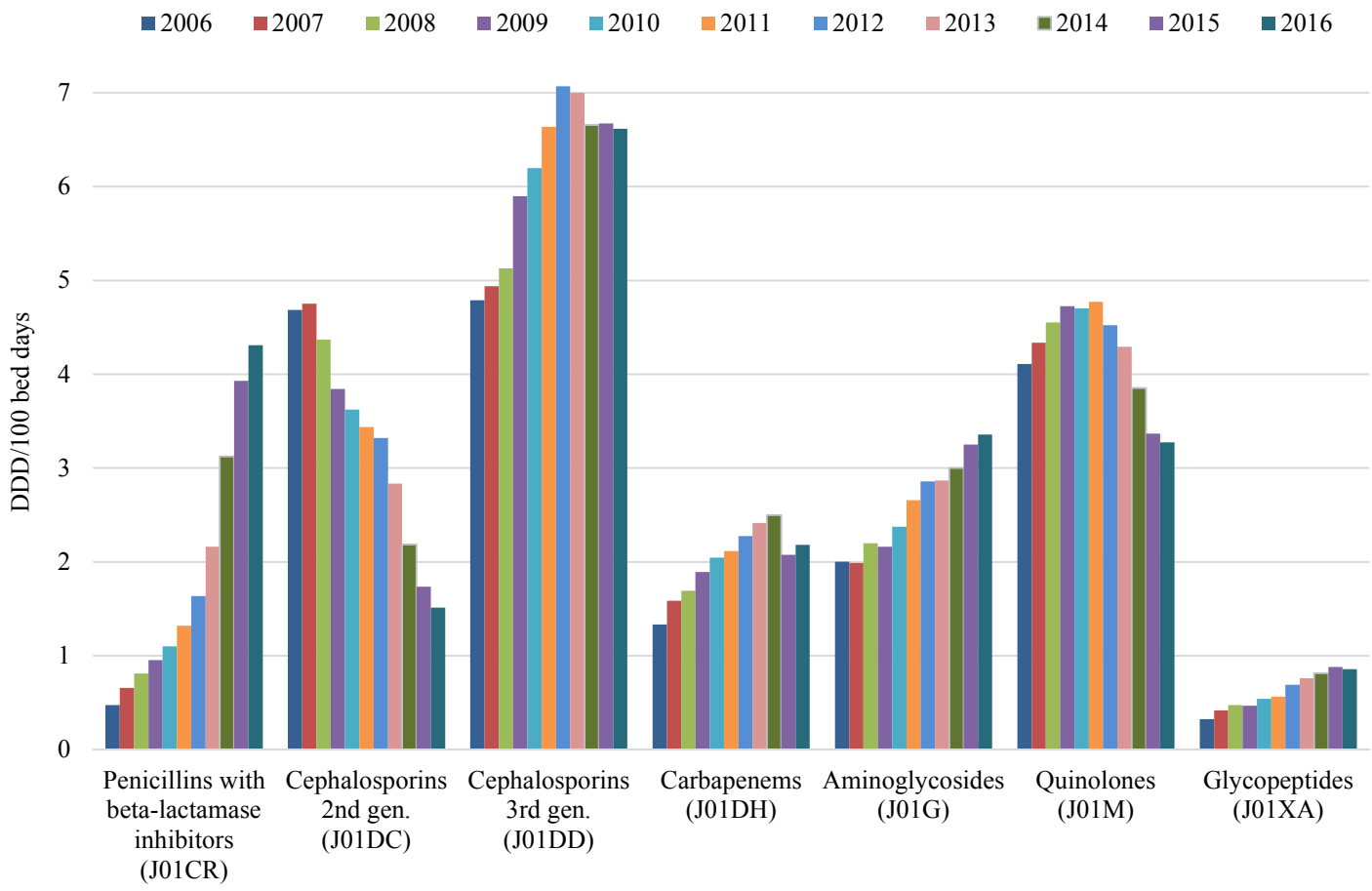

FIGURE 30. Use of selected antibacterial agents for systemic use (ATC group J01CR, J01DC, J01DD, J01DH, J01G, J01M and J01XA) in Norwegian hospitals in 2006-2016, measured in DDD/100 bed days.

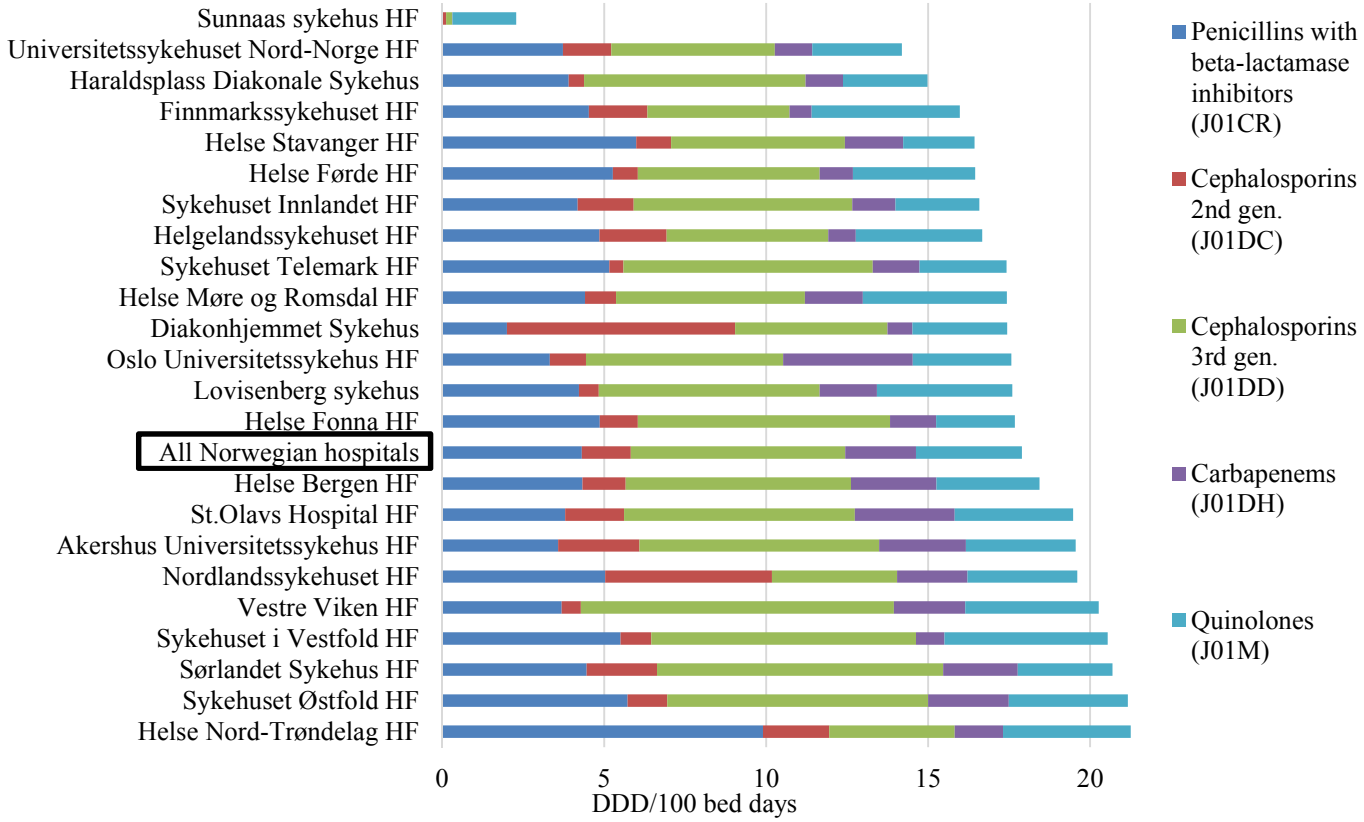

FIGURE 31. Use of selected antibacterial agents for systemic use (ATC group J01CR, J01DC, J01DD, J01DH and J01M) in Norway, presented per hospital/health trust, in 2016, measured in DDD/100 bed days.

FIGURE 32. Proportions (% DDDs) of preferred antibiotics and antibiotics that are considered resistance drivers (J01CR, J01DC, J01DD, J01DH, J01M, J01XA and J01XX08) in Norwegian hospitals, presented per hospital/health trust, in 2016.

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 antibiotics 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 are presented; reduction of average number of prescriptions (target; 250 precriptions per 1,000 inhabitants per year) and the reduction (in DDDs) of antibiotics for respiratory tract infections by 20%. Figure 33 shows total human use (J01) and use of antibiotics for respiratory tract infections in Norway since 2012 according to national targets. Figure 34 shows proportional change (2012-2016) in usage in ambulatory care in Norwegian municipalities with more than 5,000 inhabitants. In many municipalities the use of antibacterials has decreased since 2012, but in 6% of the municipalities, usage has increased. Furthermore, since 2012, there has been a reduced number of users (annual prevalence) in all age groups. The largest reduction is seen in small children, the lowest reduction for young adults and the elderly above 75 years. Moreover, number of prescriptions to men is reduced more than in women; 20% reduction in prescriptions pr 1,000 in men vs. 18% in women. The highest reductions in prescriptions per 1,000 are observed in children 0-14 year olds and presumingly their parents (i.e. age group 30-44 years) (approx. 23% less prescriptions pr 1,000 in 2016 compared to 2012).
For hospitals, the main target is 30% reduction in combined use of five selected groups of antibiotics. Figure 35 shows the annual variation of total hospital use of these groups in the years 2006-2016 according to the national target. Figure 36 shows how the use of these five groups have changed in the different Norwegian hospitals/health trusts in relation to the national target; a reduction by 30 % (marked by a grey line in the figure). There are substantial variations between the hospitals, which partly may be explained by differences in use in the base year 2012. Another point is that some, but not all hospitals, had established antibiotic stewardship programs in 2016. The National Action Plan requires all Norwegian hospitals/health trusts to implement antibiotic stewardship programs (ASPs) at the latest by 2017.

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 updated National Antibiotic Treatment Guidelines for ambulatory care, nursing homes, dentists and hospitals.

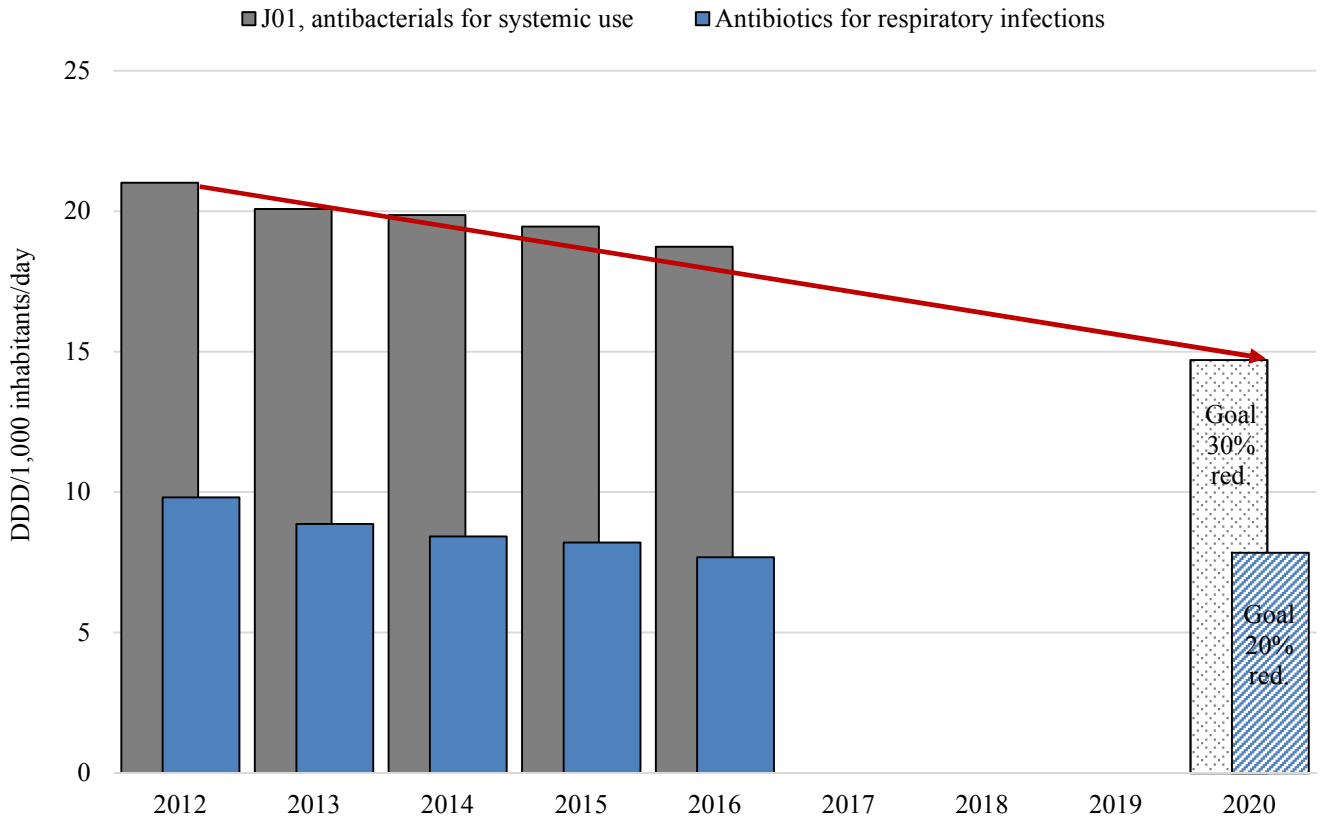

FIGURE 33. Total human use of antibacterial agents for systemic use (ATC group J01, including methenamine) and sales of antibiotics for respiratory tract infections (amoxicillin, phenoxymethylpenicillin, macrolides and doxycycline) in Norway in 2012-2016 measured in DDD/1,000 inhabitants/day. According to the National Action Plan, the target for 2020 is 30% reduction, measured in DDDs. Bars shows measured use 2012-2016 (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.

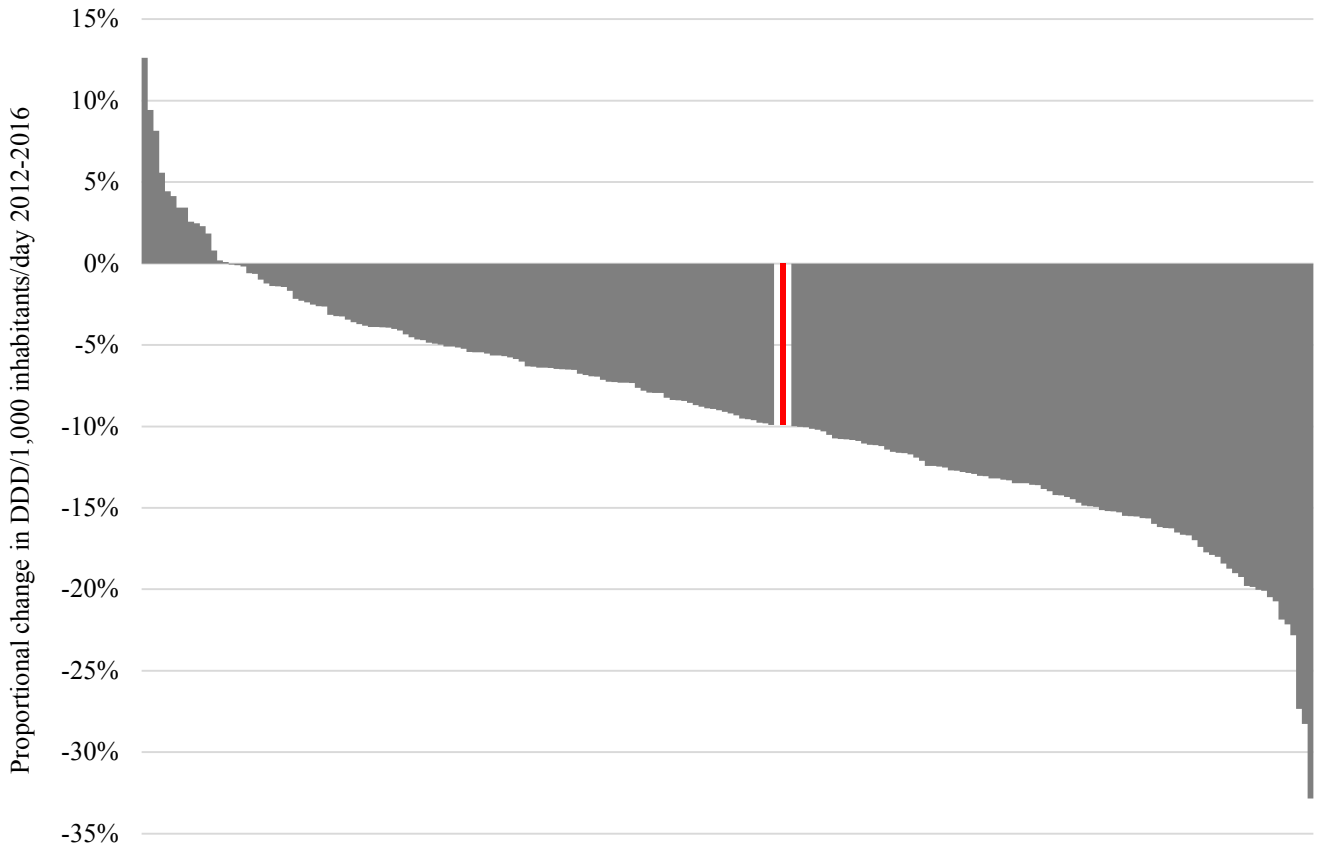

FIGURE 34. Proportional change (%), measured in DDD/1,000 inhabitants/day, of use of antibacterial agents for systemic use (ATC group J01) in outpatients in the 198 largest municipalities (more than 5,000 inhabitants) in Norway in 2016. Data from NorPD (i.e. excluding health institutions). Red bar; National average; in 2016, 10 % reduction since 2012.

FIGURE 35. Use of selected broad-spectrum antibacterial agents for systemic use (ATC J01CR05, ATC group J01DC, J01DD, J01DH and J01M) in Norwegian hospitals in 2006-2016, measured in DDD/100 bed days. According to the National Action Plan, the target for hospitals is 30% reduction (measured in DDDs) by 2020 compared to 2012.

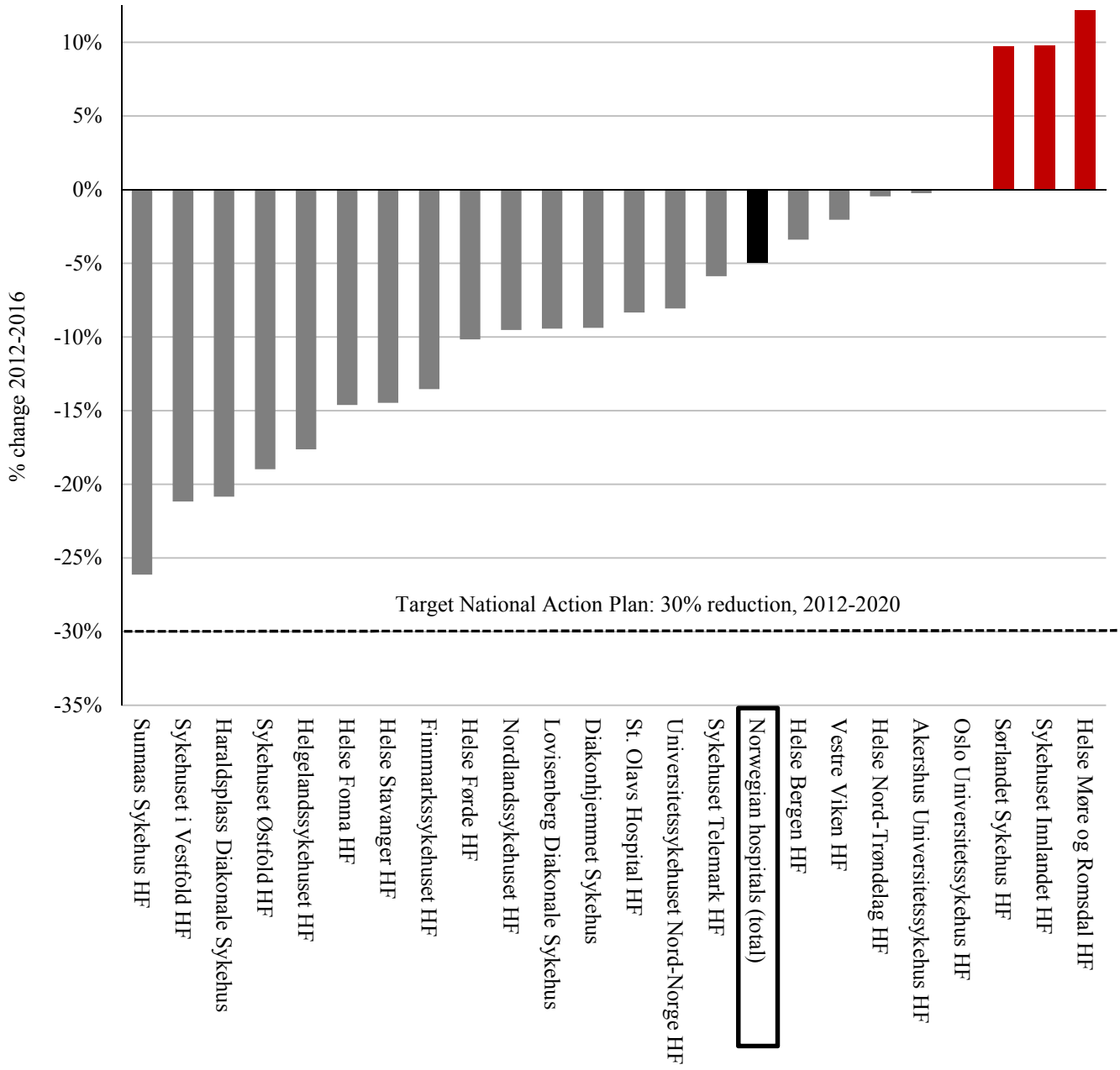

FIGURE 36. Proportional change (%) in DDD/100 bed days in the use of selected broad-spectrum antibacterial agents for systemic use (ATC group J01CR, J01DC, J01DD, J01DH and J01M) in Norwegian hospital trusts 2006-2016, measured in DDD/100 bed days. According to the National Action plan, the target for hospitals is 30% reduction (measured in DDDs) by 2020 compared to 2012. Black bar: National average.

Antimycotic usage in Norway

The use of antimycotics for systemic use has been increasing in Norway, more so in ambulatory care than in hospitals (Figure 37). In 2016, hospital use of antimycotics represented 23% of total use measured in DDDs. Fluconazole is the most commonly used agent in both settings. In July 2013, a warning regarding the use of oral ketoconazole was issued due to increased risk of liver damage. This resulted in decreased use of ketoconazole in ambulatory care (red part of the bars). In ambulatory care, mainly oral formulations are used. Of total DDDs, 2% of the DDDs was for parenteral use and in hospitals, 62 % was parenteral use.

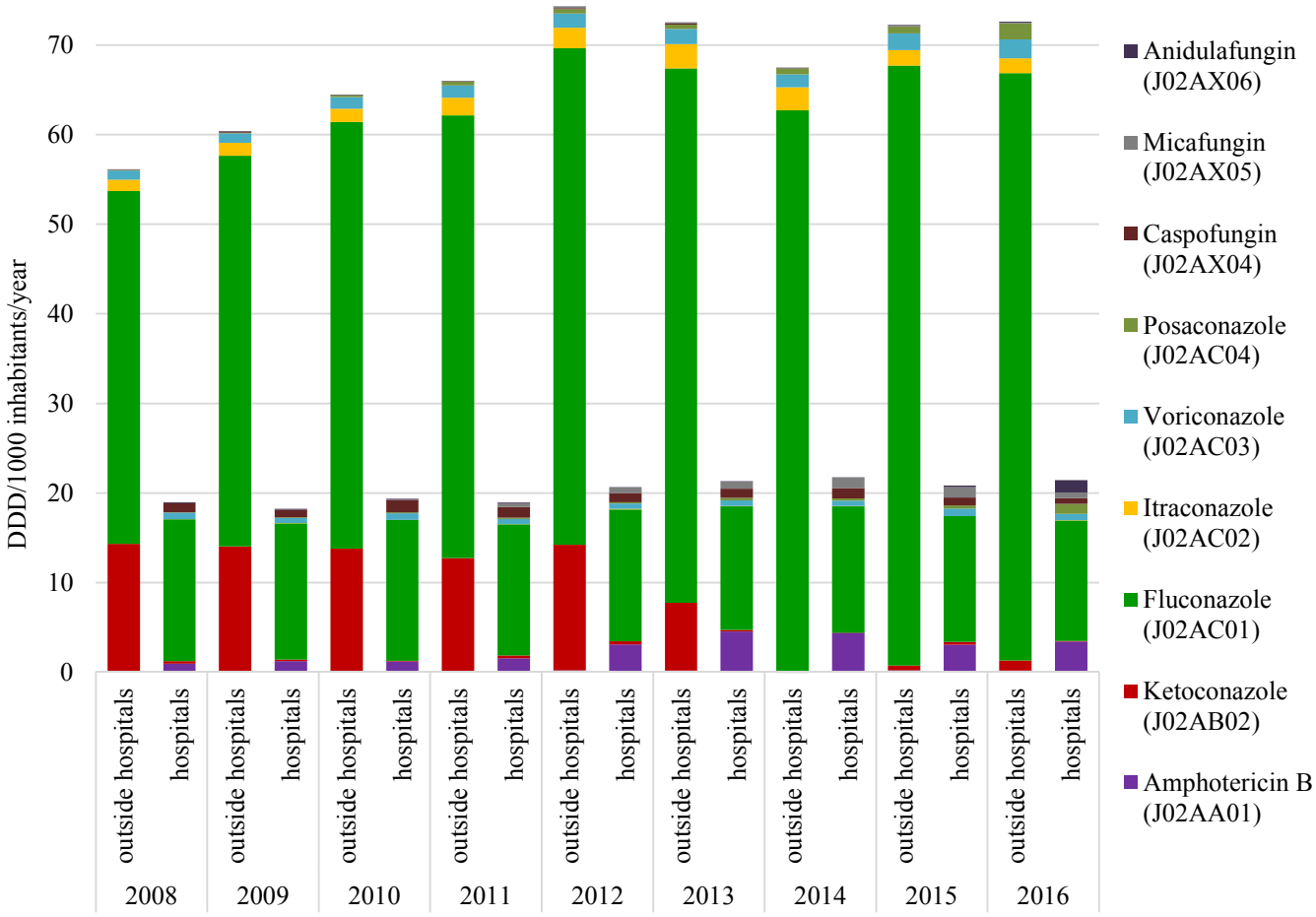

FIGURE 37. Proportions of antimycotics for systemic use in Norway for ambulatory care and hospitals 2008-2016, measured in DDD/1,000 inhabitants/year.

OCCURRENCE OF ANTIMICROBIAL RESISTANCE

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 normal enteric microflora from healthy animals, as well as from feed and food, is important to get an overview of the prevalence of antimicrobial resistance, detect trends and evaluate effects of interventions.

Bacterial resistance to critically important antimicrobials, such as third generation cephalosporins, quinolones and carbapenems, has received special attention over the last years. These are defined by the WHO as critically important for treatment of human infections, and monitoring the resistance to these substances in the bacterial population is therefore of special interest. A reservoir of such resistant bacteria in food production animals and the food chain is of concern as they may have an impact on resistance development in human bacterial populations.

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). In addition, antimicrobial testing of bacteria from other sources than those included in this decision, or investigation of presence of specific antimicrobial resistant bacteria by selective methods, are included. The use of selective methods are especially relevant for low prevalent sources, as it enables early detection of important resistance mechanisms; thereby enabling these to be monitored and characterised.

In NORM-VET, *Escherichia coli* and *Enterococcus* spp. are used as indicator bacteria. Selective methods for detection of *E. coli* resistant to third generation cephalosporins were included in NORM-VET from 2011, and for quinolone resistant *E. coli* from 2013. From 2015 a selective method for detection of carbapenemase-producing *Enterobacteriaceae*, and from 2016 a selective method for colistin resistant *E. coli* were implemented as well.

In 2016, animal samples included caecal samples of broiler and turkey flocks, as well as red foxes and wild birds. Food samples included broiler and turkey meat, cheese, seafood and bivalve molluscs. Feed samples included dry feed for cattle and swine, and both dry and wet feed for dogs. These samples were all analysed for the presence of *E. coli*. In addition, the results from screening of methicillin resistant *Staphylococcus aureus* in mink and swine are described (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 2016. Sampling, laboratory methods and data processing are described in Appendix 3.

PRODUCTION ANIMALS

Escherichia coli from broilers and turkey

Caecal samples from a total of 185 broiler flocks and 156 turkey flocks were examined and *E. coli* isolates were obtained from 181 (97.8%) and 156 (100.0%) samples, respectively. One isolate per positive sample was susceptibility tested. The results are presented in the text, in Table 8 and Figures 38-41.

TABLE 8. Antimicrobial resistance in isolates of *Escherichia coli* from caecal samples from broiler and turkey flocks (n=181 and n=156, respectively) in 2016.

		Resistance (%)		Distribution (%) of MIC values (mg/L)*															
Substance	Sample	[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	Broiler	3.3	[1.2-7.1]								95.0	1.7		0.6	0.6	1.1	1.1		
	Turkey	8.3	[4.5-13.8]								91.0	0.6			1.3	2.6	4.5		
Tigecycline	Broiler	1.1	[1.3-3.9]					95.6	3.3	0.6	0.6								
	Turkey	1.3	[0.2-4.6]					98.1	0.6	0.6	0.6								
Chloramphenicol	Broiler	0	[0.0-2.0]										100						
	Turkey	0.6	[0.0-3.5]										98.7	0.6				0.6	
Ampicillin	Broiler	3.8	[1.6-7.8]							4.4	44.8	43.6	3.3			0.6	3.3		
	Turkey	12.8	[8.0-19.1]							2.6	34.6	48.7	1.3				12.8		
Cefotaxime	Broiler	0	[0.0-2.0]					100											
	Turkey	0	[0.0-2.3]					100											
Ceftazidime	Broiler	0	[0.0-2.0]						100										
	Turkey	0	[0.0-2.3]						100										
Meropenem	Broiler	0	[0.0-2.0]		100														
	Turkey	0	[0.0-2.3]		100														
Sulfamethoxazole	Broiler	7.2	[3.9-12.0]										91.7	0.6	0.6				7.2
	Turkey	10.9	[6.5-16.9]										84.6	3.2	1.3				10.9
Trimethoprim	Broiler	1.7	[0.3-4.8]					95.6	1.7	1.1						1.7			
	Turkey	3.2	[1.0-7.3]					92.9	3.2	0.6						3.2			
Azithromycin	Broiler	ND									51.4	41.4	6.6	0.6					
	Turkey	ND									46.2	47.4	6.4						
Gentamicin	Broiler	0.6	[0.0-3.0]						70.7	24.3	4.4	0.6							
	Turkey	1.3	[0.2-4.6]						78.8	17.9	1.9		0.6	0.6					
Ciprofloxacin	Broiler	6.1	[3.1-10.5]	91.7	2.2			1.1		5.0									
	Turkey	1.3	[0.2-4.6]	93.6	5.1			0.6		0.6									
Nalidixic acid	Broiler	6.1	[3.1-10.5]									92.3	1.5			0.6	0.6	5.0	
	Turkey	0.6	[0.0-3.5]									99.4						0.6	
Colistin	Broiler	0	[0.0-2.0]							100									
	Turkey	0.6	[0.0-3.5]							99.4		0.6							

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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.

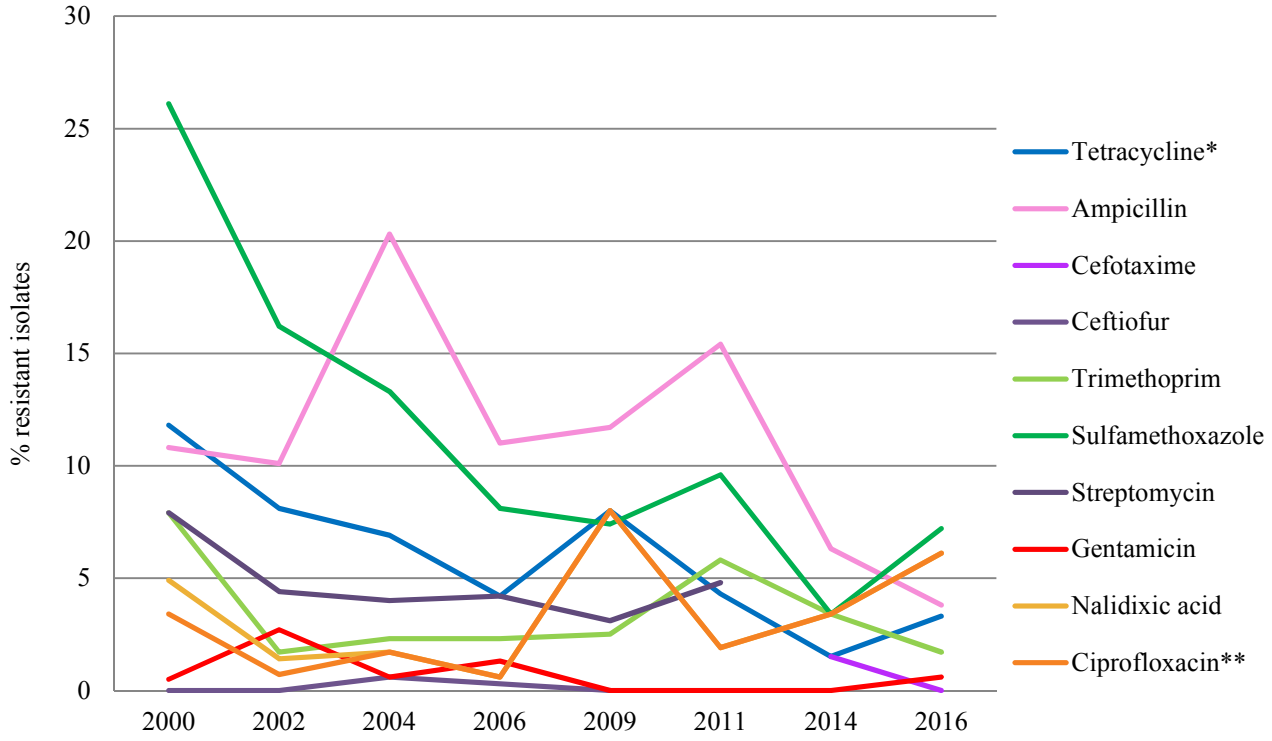

FIGURE 38. Prevalence of resistance to various antimicrobials in *Escherichia coli* from broiler isolates in 2000-2016. The cut-off values used in NORM-VET 2016 were applied. *Oxytetracycline in 2002 and 2004. **Enrofloxacin before 2006.

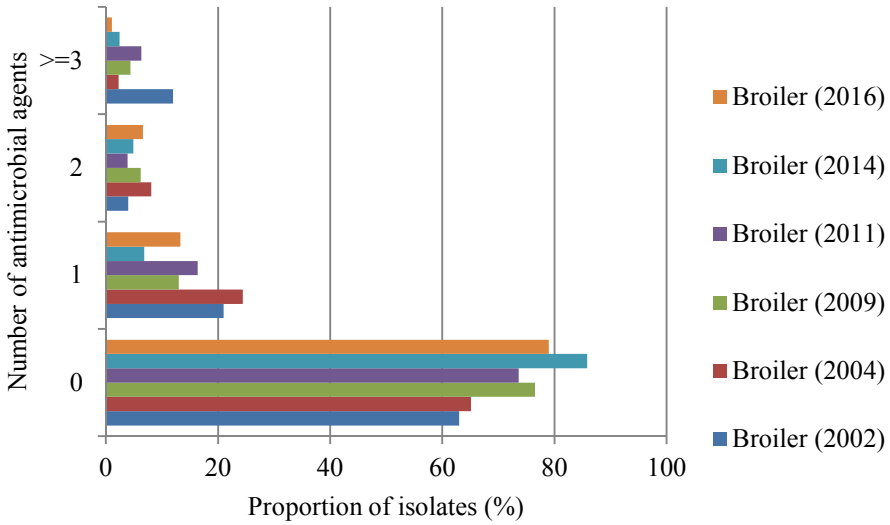

FIGURE 39. Antimicrobial resistance profile for *Escherichia coli* faecal isolates from broiler in 2002-2016. Proportions of isolates susceptible to all or resistant to one, two, and three or more antimicrobial agents are illustrated. The antimicrobial agents tested for varies between the years and this probably has an effect on the results.

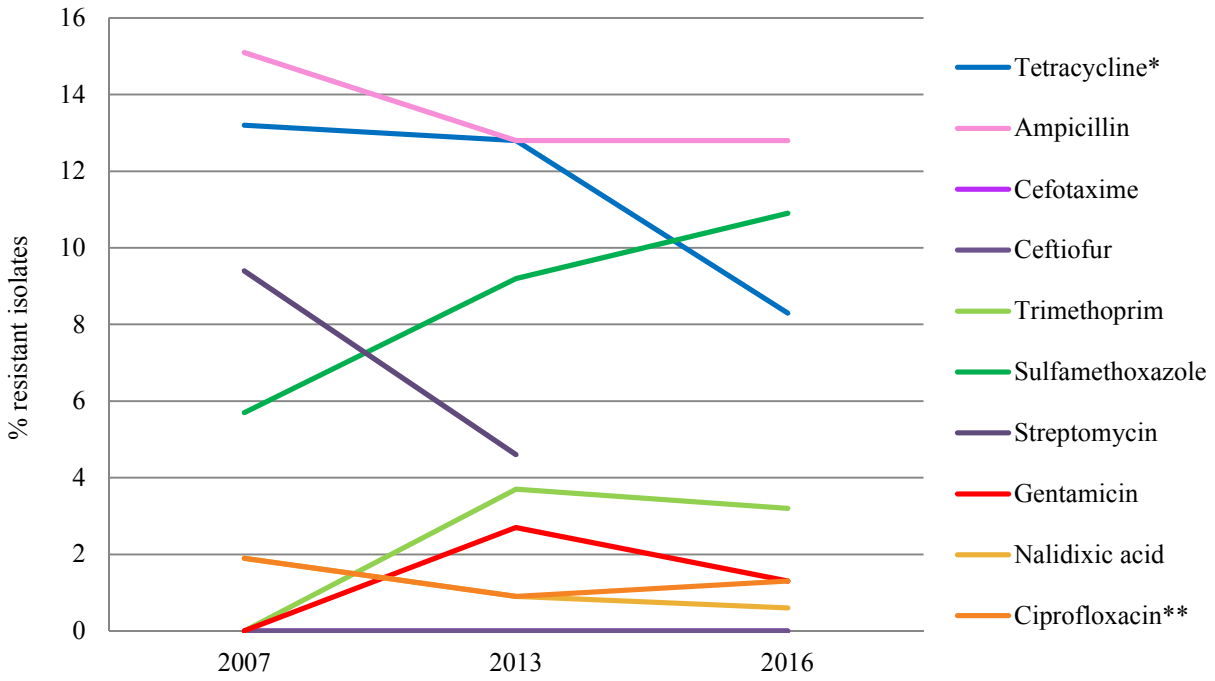

FIGURE 40. Prevalence of resistance to various antimicrobials in *Escherichia coli* from turkey isolates in 2007-2016. The cut-off values used in NORM-VET 2016 were applied. *Oxytetracycline in 2002 and 2004. **Enrofloxacin before 2006.

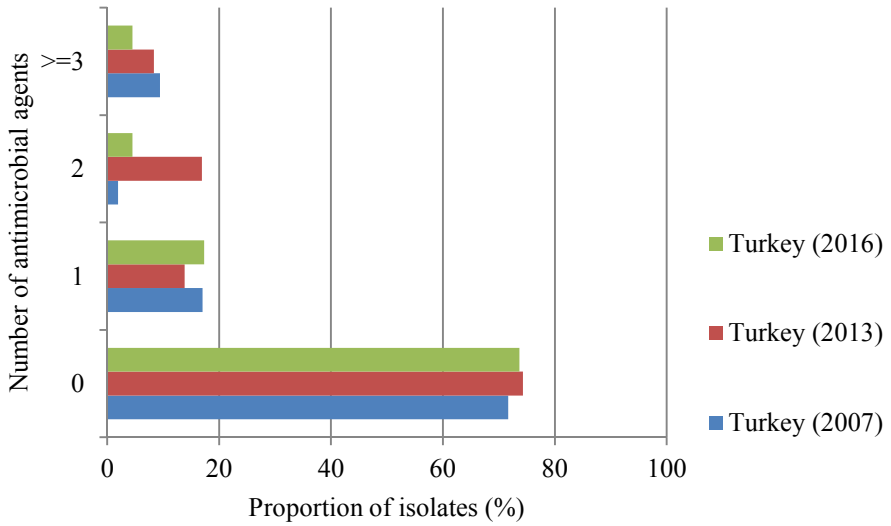

FIGURE 41. Antimicrobial resistance profile for *Escherichia coli* faecal isolates from turkey in 2007-2016. Proportions of isolates susceptible to all or resistant to one, two, and three or more antimicrobial agents are illustrated. The antimicrobial agents tested for varies between the years and this probably has an effect on the results.

RESULTS AND COMMENTS

BROILER

The 2016 data showed that 79.0% of the *E. coli* isolates from broiler caecal samples were susceptible to all antimicrobial agents included. Altogether, 13.3% of the isolates were resistant to one antimicrobial agent (predominantly sulfamethoxazole or ampicillin), 6.6% to two (nalidixic acid and ciprofloxacin), and 1.1% to three antimicrobial agents (Figure 38). In total, 21% of the isolates were resistant, indicating a high occurrence of resistance in broilers according to the EFSA classification described in Appendix 6. Resistance to sulfamethoxazole was the most frequently identified resistance determinant, followed by resistance to ciprofloxacin, nalidixic acid and ampicillin.

Compared to the 2014 data, the results indicate that the proportion of isolates being resistant to one or two antimicrobial agents has increased and the proportion being fully susceptible has decreased. When comparing to data pre 2014, the proportion of isolates being fully susceptible seem to have increased. There has, however, been a change in the panel of antimicrobial agents tested for compared to pre 2014, and this may have an effect on the comparison result. Nevertheless, since the start of NORM-VET in 2000, the prevalence of resistance to some antimicrobial agents in *E. coli* from broilers has indeed decreased as illustrated in Figure 40, especially resistance to sulfamethoxazole, tetracycline and ampicillin ($p<0.001$).

None of the *E. coli* isolates from broilers displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime, indicating a prevalence below 2.0%. This is similar to the occurrence detected in previous years. In addition, a selective method was used to investigate the occurrence of *E. coli* resistant to third generation cephalosporins in the same broiler caecal sample material (page 50). Quantitative methods were also applied (see separate presentation on page 52).

Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in 6.1% [95% CI: 3.1-10.6%] of the isolates. This is an increase from 2014 and 2011 where 3.4% and 2.0% of the indicator *E. coli* displayed resistance to quinolones, respectively, though lower than the peak of 8.0% detected in 2009. However, the observed changes are not significant, and further monitoring is needed to assess whether this is a true increasing trend.

In an international perspective, the occurrence of resistance among *E. coli* from Norwegian broiler is low, though the occurrence varies markedly between countries reporting to EFSA with the Nordic countries having the lowest resistance levels (EFSA and ECDC Summary report 2014). This favourable situation is probably due to the very limited use of antibiotics in the Norwegian broiler production.

TURKEY

The 2016 data showed that 73.7% of the *E. coli* isolates were susceptible to all antimicrobial agents included. Altogether, 17.3% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin or sulfamethoxazole), 4.5% to two and 3.2% to three and 1.3% to four antimicrobial agents (Figure 40). In total, 26.3% of the isolates were resistant, indicating a high occurrence of resistance in turkey according to the EFSA classification described in Appendix 6. Resistance to ampicillin and sulfamethoxazole were the most frequently identified resistance determinant, followed by resistance to tetracycline and trimethoprim.

Comparison to previous results as shown in Figure 41 is difficult as there has been a change in the panel of antimicrobial agents tested for, and this may have had an effect on the results. Nevertheless, the resistance to tetracycline has decreased from 13.2% in 2007 and 12.8% in 2013, to 8.3% in 2016, while the resistance to sulfamethoxazole seems to have increased from 5.7% in 2007 and 9.2% in 2013, to 10.9% in 2016. These changes are, however, not statistically significant. Moreover, the sampling procedure varies between these years, and this may have had an impact on the result. Further monitoring is therefore needed to follow the situation.

None of the isolates displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime, indicating a prevalence below 2.3%. This is in concordance with the results from previous years. In addition, a selective method was used to investigate the occurrence of *E. coli* resistant to third generation cephalosporins in the same caecal sample material (page 51). Quantitative methods were also applied (see separate presentation on page 52).

Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in 1.3% [95% CI: 0.2-4.6%] of the isolates. This is in concordance with the results from previous years.

One isolate showed decreased sensitivity to colistin. None of the plasmid-mediated resistance genes *mcr-1* or *mcr-2* were identified, indicating that the resistance phenotype might be due to chromosomal mutations.

In an international perspective, the occurrence of resistance among *E. coli* from Norwegian turkey is low, though the occurrence varies markedly between countries reporting to EFSA with the Nordic countries having the lowest resistance levels (EFSA and ECDC Summary report 2014). This favourable situation is probably due to the limited use of antibiotics in the Norwegian turkey production.

Cephalosporin resistant *Escherichia coli* from broiler and turkey

In 2016, selective screening for *E. coli* resistant to third generation cephalosporins was performed on samples from broiler and turkey flocks. A total of 185 broiler and 156 turkey caecal samples were screened. The results are presented in the text and in Table 9.

TABLE 9. Antimicrobial resistance in cephalosporin resistant *Escherichia coli* isolates from caecal samples from broiler and turkey flocks (n=20 and n=16, respectively) in 2016.

			Distribution (n) of MIC values (mg/L)*															
Substance	Sample	n resistant	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Broiler	9								11				2	6	1		
	Turkey	5								11					4	1		
Tigecycline	Broiler	0					20											
	Turkey	0					16											
Chloramphenicol	Broiler	0										20						
	Turkey	0										16						
Ampicillin	Broiler	20												1	2	17		
	Turkey	16													1	15		
Cefotaxime	Broiler	19					1			1	3	15						
	Turkey	16								3	4	1	8					
Ceftazidime	Broiler	19						1		1	2	13	3					
	Turkey	16								1	2	2	11					
Meropenem	Broiler	0		20														
	Turkey	0		16														
Sulfamethoxazole	Broiler	11										9						11
	Turkey	2										11	2		1			2
Trimethoprim	Broiler	0					17	3										
	Turkey	0					14	2										
Azithromycin	Broiler	ND								10	7	3						
	Turkey	ND								3	11	2						
Gentamicin	Broiler	9						8	3			1	6	2				
	Turkey	0						14	1	1								
Ciprofloxacin	Broiler	0	17	3														
	Turkey	0	14	2														
Nalidixic acid	Broiler	0									20							
	Turkey	0									16							
Colistin	Broiler	0							20									
	Turkey	0							16									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

BROILER

By use of the selective method, *E. coli* resistant to third generation cephalosporins were found in 10.8% [95% CI: 6.7-16.2%] of the 185 broiler caecal samples. As described above, no cephalosporin resistant isolates were found by using the standard non-selective procedure, indicating that the within-flock prevalence is low. These aspects are further investigated by quantitative methods (see separate presentation on page 52).

Nine of the isolates were only resistant to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. Ten of the isolates were additionally resistant to sulfamethoxazole, nine to gentamicin and tetracycline (Table 9). One isolate showed decreased susceptibility to the carbapenem ertapenem (data not shown in table), with MIC values above the EUCAST cut-off value at 0.06 mg/L, i.e. MIC 0.12-0.25 mg/L. However, none of the isolates were resistant to imipenem or meropenem (data not shown), strongly indicating that there were no carbapenemase-producing *Enterobacteriaceae* present as ertapenem is known to have a lower specificity to detect carbapenemase-producing *Enterobacteriaceae* than imipenem and meropenem (Cohen *et al.* 2010).

All isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype, and real-time PCR showed that all isolates contained the bla_{CMY-2} gene. This is in concordance with the 2014 results, indicating that the bla_{CMY-2} gene is the dominating cause of cephalosporin resistance in broilers.

The current findings by the selective method show that there has been a substantial reduction of *E. coli* resistant to third generation cephalosporins in broiler flocks compared to previous years ($p<0.001$). In 2014, a total of 35.7% of the

broiler caecal samples were found positive for *E. coli* resistant to third generation cephalosporins. This reduction was expected, and is likely a result of the measures implemented by the industry to reduce the occurrence in broilers. There is no selection pressure from cephalosporin usage in Norway, but the poultry production is dependent on import of breeding animals. These animals have been shown to be the source of introduction, and the industry has therefore taken measures to limit the number of imported breeding animals carrying *E. coli* resistant to third generation cephalosporins.

In an international perspective, the prevalence of *E. coli* resistant to third generation cephalosporins in Norwegian broilers is low though the occurrence varies markedly between countries reporting to EFSA (EFSA and ECDC Summary report 2014). As the monitoring using selective methods was not mandatory in EU in 2014, and with very few countries reporting data on positive/negative samples to EFSA, prevalence of *E. coli* resistant to third generation cephalosporins in samples was not assessed and thereby directly comparable prevalence data are not available.

The situation regarding *E. coli* resistant to third generation cephalosporins in broilers in Norway is similar to the situation in Sweden. A Swedish report from 2014 (Egervärn *et al.* 2014), concluded that food on the Swedish market, including food with *E. coli* resistant to third generation cephalosporins from broilers, was a limited contributor to the prevalence of *E. coli* resistant to third generation cephalosporins within the human healthcare sector. Results from a Norwegian study show that Norwegian patients have *E. coli* resistant to third generation cephalosporins and resistance plasmids highly similar to isolates from retail chicken meat. However, these cephalosporin resistant *E. coli* is a very limited proportion of the total cases of human *E. coli* resistant to third generation cephalosporins (Berg *et al.* 2017).

TURKEY

By use of the selective method, *E. coli* resistant to third generation cephalosporins were found in 10.2% [95% CI: 5.9-16.1%] of the 156 turkey caecal samples. As described above, no cephalosporin resistant isolates were found by using the non-selective procedure, indicating that the within-flock prevalence is low. These aspects are further investigated by quantitative methods (see separate presentation on page 52).

Nine of the isolates were only resistant to beta-lactams, i.e. ampicillin and the third generation cephalosporins cefotaxime and ceftazidime. In addition, there was some resistance observed for tetracycline and sulfamethoxazole (Table 9). None of the isolates were resistant to ertapenemen, imipenem or meropenem (data not shown), antimicrobial agents used to identify carbapenemase-producing *Enterobacteriaceae*.

All isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype, and genotyping showed that eight isolates contained the $bla_{\text{CMY-2}}$ gene and the remaining eight isolates had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression. Compared to previous results, it seems to have been an increase in cephalosporin resistant *E. coli* due to presence of the plasmid mediated $bla_{\text{CMY-2}}$ gene from 1.5% [95% CI: 0.2-5.4%] in 2013 to 5.1% [95% CI: 2.2-9.9%] in 2016. In contrast, it seems to have been a decrease in cephalosporin resistance due to chromosomal mutations from 12.9% [95% CI: 7.7-20.0%] in 2013 to 5.1% [95% CI: 2.2-9.9%] in 2016. However, these observed differences are non-siginificant and there has been a change in sampling procedure from boot swabs to caecal material that may have had an effect on the observed results. Boot swab sampling mirrors the prevalence in the turkey house, while the caecal samples mirror prevalence in the sampled animals.

In an international perspective, the occurrence of cephalosporin resistant *E. coli* in Norwegian turkey is low, though the occurrence varies markedly between countries reporting to EFSA (EFSA and ECDC Summary report 2014). As the monitoring using selective methods was not mandatory in EU in 2014, and with very few countries reporting data on positive/negative samples to EFSA, prevalence of *E. coli* resistant to third generation cephalosporins in samples were not assessed and thereby direct comparable prevalence data are not available.

Carbapenemase-producing *Enterobacteriaceae* from broilers and turkey

Selective screening for carbapenemase producing *Enterobacteriaceae* was performed on a total of 185 broiler and 156 turkey caecal samples. No carbapenemase-producing *Enterobacteriaceae* were detected. 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.

Quantification of cephalosporin resistant *Escherichia coli* in caecal and meat samples from broilers and turkey

A selective method for detection of cephalosporin resistant *E. coli* has been implemented in NORM-VET since 2011 (1), showing that cephalosporin resistant *E. coli* in broilers are frequently present. However, the analyses performed by non-selective methods (indicator *E. coli*) indicate that cephalosporin resistant *E. coli* is present at low levels in the intestinal microflora. In a risk assessment perspective it is therefore of importance to assess the proportion of cephalosporin resistant *E. coli* in relation to the overall amount of *E. coli*. Such quantification was included in NORM-VET for the first time in 2014 (2).

Material and methods

In NORM-VET 2016, selective screening for cephalosporin resistant *E. coli* was performed on caecal samples from 185 broiler and 156 turkey flocks, and on 175 poultry and 128 turkey meat samples. Of these, quantification was performed on caecal samples from 20 broiler flocks and seven turkey flocks, and meat samples from eleven broilers and one turkey, all identified as positive for bla_{CMY-2} producing *E. coli*. Caecal samples were mixed 1:10 in peptone glycerol and diluted tenfold. From each dilution, 10 µL were plated by running drop method (3) onto a square MacConkey (MC) agar plate (Difco); one with 1 mg/L cefotaxime and one without. Meat samples were mixed 1:9 in MC broth containing 1 mg/L cefotaxime. Subsequently, 45 mL and 10 mL of the broth were transferred to new containers and diluted tenfold. The broth was incubated over night before plating 10 µL of each dilution on square MC agar with 1 mg/L cefotaxime. The plate count was determined as the highest dilution with visible growth. The colonies were verified as *E. coli* and the bla_{CMY-2} gene was confirmed (4). For the caecal samples, the proportion of cephalosporin resistant *E. coli* due to presence of the bla_{CMY-2} gene in relation to the total number of the *E. coli* was calculated.

Results and discussion

The occurrence of cephalosporin resistant *E. coli* among the total caecal *E. coli* was less than 0.1 % in 95.0% of the broiler caecal samples, and less than 0.01 % in all the turkey caecal samples (Table 10). All samples from broiler and turkey meat had very low levels of cephalosporin resistant *E. coli* present (≤ 0.2 cfu/g). The results indicate that the majority of broiler flocks positive for cephalosporin resistant *E. coli* have very low levels of these bacteria present among the caecal *E. coli*. Furthermore, the levels of contamination on broiler meat samples are generally very low. Compared to the 2014 quantification results on broilers, there has been a reduction in cephalosporin resistant *E. coli* present both among the caecal *E. coli* and per gram meat. It has also been a reduction in the occurrence of cephalosporin resistant *E. coli* in the broiler production in general (see chapters on cephalosporin resistant *E. coli* in poultry and poultry meat). These results show that the measures implemented by the industry to reduce the occurrence in broilers are now giving positive results with only low levels of cephalosporin resistant *E. coli* present in Norwegian broiler and turkey food production chain.

TABLE 10. Number of isolates identified as cephalosporin resistant *Escherichia coli* due to presence of the bla_{CMY-2} gene in relation to the total number of the *E. coli* of the gut flora (caecum) of broilers and turkeys sampled in 2016.

Cephalosporin resistant *E. coli* (%)	Species	No of samples	Proportion (%) of samples
0.00001	Broiler	1	5.0
	Turkey	-	-
0.0001	Broiler	8	40.0
	Turkey	2	28.6
0.001	Broiler	7	35.0
	Turkey	2	28.6
0.01	Broiler	5	10.0
	Turkey	3	42.9
0.1	Broiler	1	5.0
	Turkey	-	-
1	Broiler	1	5.0
	Turkey	-	-

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Main findings from the first finalized research project on cephalosporin resistant *Escherichia coli* in Norwegian poultry

Escherichia coli displaying resistance towards extended-spectrum cephalosporins was detected in samples from the broiler production pyramid using a selective method for detection in the NORM-VET programme in 2011 [1]. As the use of antimicrobials in Norwegian broiler production is almost non-existent [2], it was assumed that other factors than selection pressure from antimicrobial use was associated with the occurrence of cephalosporin resistant *E. coli*. As a response to these findings, the research project "Increasing occurrence of antimicrobial resistance in the food chain – epidemiology and preventive measures against ESBL-producing *E. coli*" was started in 2013. The aim of the project was to give scientifically based advice to authorities and the broiler industry regarding preventive measures related to introduction, persistence and spread of cephalosporin resistant *E. coli*.

Data on cephalosporin resistant *E. coli* collected in the NORM-VET programme (2011-2014), two independent projects on breeding flocks and an epidemiological study performed from late 2013 to early 2015 formed the basis for the project. All cephalosporin resistant *E. coli* displayed an AmpC phenotype, and the vast majority carried the $bla_{\text{CMY-2}}$ gene. AmpC-producing *E. coli* were detected at all levels of the Norwegian broiler production pyramid throughout Norway [3]. The risk for occurrence of cephalosporin resistant *E. coli* was associated with the status of the previous broiler flock in the broiler house, number of parent flocks supplying day-old chickens to the broiler flock, routines for disinfection between production cycles, and transport personnel entering the room where the broilers are raised. Overall, the results indicated that a high level of biosecurity, including implementation of thorough disinfection routines, will contribute to minimizing the risk of cephalosporin resistant *E. coli* occurring in Norwegian broiler flocks [4]. These preventive measures have already been implemented by the Norwegian poultry industry.

Thorough characterization of isolates revealed that cephalosporin resistance was generally mediated by $bla_{\text{CMY-2}}$ on conjugative IncK or IncI1 plasmids. Furthermore, a large group of closely related isolates belonging to multilocus sequence type (ST) 38, and a group belonging to ST1158 was identified. These isolates harboured $bla_{\text{CMY-2}}$ on IncK plasmids. Interestingly, *E. coli* ST38 and IncK and IncI1 plasmids highly similar to those found in Norway have also been reported from other European countries, indicating that some successful clones and plasmids are circulating in the European broiler production. In addition, plasmid stability systems were identified on both IncK and IncI1 plasmids. The presence of such systems may contribute to the persistence of cephalosporin resistant *E. coli* in the Norwegian broiler production despite the lack of antimicrobial selection pressure [5].

Transfer experiments revealed the ability of IncK and IncI1 plasmids to transfer into other bacterial hosts in biofilm. Furthermore, transfer of an IncK plasmid into *Serratia* spp. was observed. Bacteria in biofilm have an increased ability to survive cleaning and disinfection. Environmental bacteria can also have intrinsic properties making them more tolerant to some disinfectants. Therefore, the results indicate that biofilms and environmental bacteria may act as reservoirs for cephalosporin resistant *E. coli* and plasmids encoding cephalosporin resistance in the broiler production [6].
Further details can be found in the PhD thesis "Cephalosporin resistant *Escherichia coli* in the Norwegian broiler production pyramid – genetic characterization and determination of risk factors" by Solveig Sølverød Mo (NFR grant no. 225165/E40). The thesis is available upon request (solveig.mo@vetinst.no).

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Surveillance of methicillin resistant *Staphylococcus aureus* (MRSA) in pigs in Norway in 2016

Some methicillin resistant *Staphylococcus aureus* (MRSA) are adapted to animal hosts, including pigs, and are therefore called livestock associated MRSA (LA-MRSA). From Europe, these have 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 (1). The rationale behind this strategy was to avoid the pig population becoming a 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, t11 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.

In 2016, a total of 872 herds were included in the survey, 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 specialized finisher herd (0.13%; 95% CI: 0.0 - 0.72). Follow up testing of contact herds, revealed two other herds positive for the same CC and *spa*-type, and eradication was initiated.

Further details of the surveillance can be found in the report "The surveillance programme for methicillinresistant *Staphylococcus aureus* in pigs in Norway 2016" (3).

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Methicillin resistant *Staphylococcus aureus* (MRSA) in mink in Norway 2016

Norway has an eradication strategy for methicillin resistant *Staphylococcus aureus* (MRSA) in pig as described in text box on MRSA in pigs, with a yearly surveillance programme implemented from 2014 (1-3). The rationale behind this strategy is to avoid the pig population becoming a reservoir of MRSA with the potential of zoonotic transmission. A successful implementation of this strategy depends, among other factors, on the knowledge of occurrence in other animal species, so that possible preventive measures can be taken. MRSA has over the last years also been reported commonly found in mink in Denmark (4). In the same time period, Norwegian mink farmers have imported live mink from Denmark. Therefore, mink was subjected for investigation of the occurrence of MRSA in 2016.
Samples from 121 mink farms were included in the survey (5). All farms with previous import of live animals were included in the survey. MRSA was not detected in any of the samples. The result indicate that Norwegian mink has very low level to absence of MRSA, and that import of mink from Denmark has not led to a widespread distribution of MRSA in Norwegian mink farms.
Further details of the survey in mink can be found in the report "A survey on methicillin resistant *Staphylococcus aureus* in mink in Norway 2016" (5).

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Methicillin resistant *Staphylococcus aureus* CC398 in humans and pigs in Norway: A One Health perspective on introduction and transmission

Staphylococcus aureus is a cause of infections in humans and animals, and methicillin resistant *Staphylococcus aureus* (MRSA) are associated with increased morbidity, mortality and costs [1, 2]. In the last decade, there has been a rapid increase in livestock-associated MRSA (LA-MRSA) among production animals, and especially pigs, in different countries [3]. These MRSA variants are adapted to animals, especially pigs and are usually, but not exclusively, belonging to the clonal complex (CC) 398. Documentation has accumulated globally on LA-MRSA as an important zoonotic agent and recently LA-MRSA has been recognized as an important cause of human infections in countries with a low overall level of MRSA in humans, such as Denmark [4].

Norway has an essentially closed pig population with an annual production of approximately 1.6 million slaughtered pigs. From the initial traceable findings of MRSA CC398 in Norwegian pig herds in 2013, Norwegian authorities have adopted a unique strategy for control of LA-MRSA in the pig population. This strategy includes a surveillance programme covering the entire swine population, a "search and destroy" policy at pig farm level for elimination of LA-MRSA from affected farms and recommendations on targeted screening of personnel before working in pig herds. The rationale behind this strategy is to avoid the swine population from becoming a permanent MRSA reservoir with zoonotic transmission to the human population.

In a recent publication, we described experiences from the first two years with the control strategy. LA-MRSA findings were investigated by contact tracing, epidemiological data and whole-genome sequencing of LA-MRSA isolates from all positive pig farms and humans associated with positive animals. Isolates from all human MRSA CC398 cases detected through notification to the Norwegian Surveillance System for Communicable Diseases (MSIS) during the period 2008-2014 were also included. Our findings highlighted points of epidemiological importance concerning prevention and control of LA-MRSA in pig herds. Humans were identified as the most probable source of introductions of LA-MRSA to the closed Norwegian pig population and further domestic spread occurred mainly by trade of animals, and to some extent via MRSA positive persons. Interestingly, no transmission of LA-MRSA from Norwegian pig herds to the general public was observed in the study period from 2008-2014 [5]. Eradication of LA-MRSA from positive pig holdings through depopulation, stringent washing and disinfection and repopulation from MRSA negative holdings seems to be possible and successful in most cases. The control strategy has involved a close collaboration between several institutions; The Norwegian Food Control Authority, the Norwegian Veterinary Institute, the Norwegian Institute of Public Health, the Norwegian Reference Laboratory for MRSA and Statens Serum Institut, Copenhagen, Denmark.

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The text above is as summary of main findings in the article "*Methicillin-Resistant Staphylococcus aureus CC398 in Humans and Pigs in Norway: A "One Health" Perspective on Introduction and Transmission"* published in Clinical Infectious Diseases, 2016. 63(11):1431-8.

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WILD ANIMALS

Escherichia coli from red fox and wild birds

Faecal swab samples from a total of 528 red foxes and 357 wild birds were examined and *E. coli* isolates were obtained from 434 (82.2%) foxes and 303 (84.9%) wild birds. One isolate per positive sample was susceptibility tested. The results are presented in the text, in Table 11 and Figure 42.

Part of the data on red fox was also included in a survey conducted for the Ministry of Climate and Environment to map the occurrence of antimicrobial resistance in the environment (separate presentation page 61).

TABLE 11. Antimicrobial resistance in isolates of *Escherichia coli* from faecal swab samples from red fox and wild birds (n=434 and n=303, respectively) in 2016.

		Resistance (%)		Distribution (%) of MIC values (mg/L)*															
Substance	Sample	[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	Fox	2.8	[1.4-4.8]								96.1	1.2				1.6	1.2		
	Bird	3.6	[1.8-6.4]								92.7	3.6			0.3	1.7	1.7		
Tigecycline	Fox	0.2	[0.0-1.3]					97.5	2.3				0.2						
	Bird	0.3	[0.0-1.8]					92.4	7.3	0.3									
Chloramphenicol	Fox	0.5	[0.1-1.7]										98.8	0.7				0.5	
	Bird	0.7	[0.1-2.4]										99.3				0.7		
Ampicillin	Fox	3.7	[2.1-5.9]							0.9	32.9	56.5	6.0	0.2	0.2	3.2			
	Bird	3.6	[1.8-6.4]							3.0	34.3	55.8	3.3	0.7		0.3	2.6		
Cefotaxime	Fox	0.0	[0.0-0.8]					100											
	Bird	0.0	[0.0-1.2]					100											
Ceftazidime	Fox	0.2	[0.0-1.3]					0.2	99.5	0.2									
	Bird	0.0	[0.0-1.2						100										
Meropenem	Fox	0.0	[0.0-0.8]		100														
	Bird	0.0	[0.0-1.2]		100														
Sulfamethoxazole	Fox	3.7	[2.1-5.9]										94.7	1.4		0.2			3.7
	Bird	2.6	[1.1-5.1]										94.6	2.6	0.3			0.3	2.3
Trimethoprim	Fox	1.4	[0.5-3.0]					92.2	6.0	0.5						1.4			
	Bird	2.3	[0.9-4.7]					84.5	12.9	0.3						2.3			
Azithromycin	Fox	ND	ND									57.1	35.0	7.6	0.2				
	Bird	ND	ND								33.7	54.5	10.6	0.7	0.3		0.3		
Gentamicin	Fox	0.5	[0.1-1.7]						68.0	27.6	3.9	0.5							
	Bird	0.0	[0.0-1.2]						88.8	9.6	1.7								
Ciprofloxacin	Fox	1.2	[0.4-2.7]	90.6	7.8	0.5		0.9					0.2						
	Bird	2.3	[0.9-4.7]	88.4	9.2			1.0	0.3				0.3	0.7					
Nalidixic acid	Fox	1.4	[0.5-3.0]									97.5	1.2		0.2		0.5	0.7	
	Bird	2.0	[0.7-4.2]									97.4	0.7				0.3	1.7	
Colistin	Fox	0.5	[0.1-1.7]						0.5	98.6	0.5	0.2	0.2						
	Bird	0.3	[0.0-1.8]							99.3	0.3	0.3							

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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.

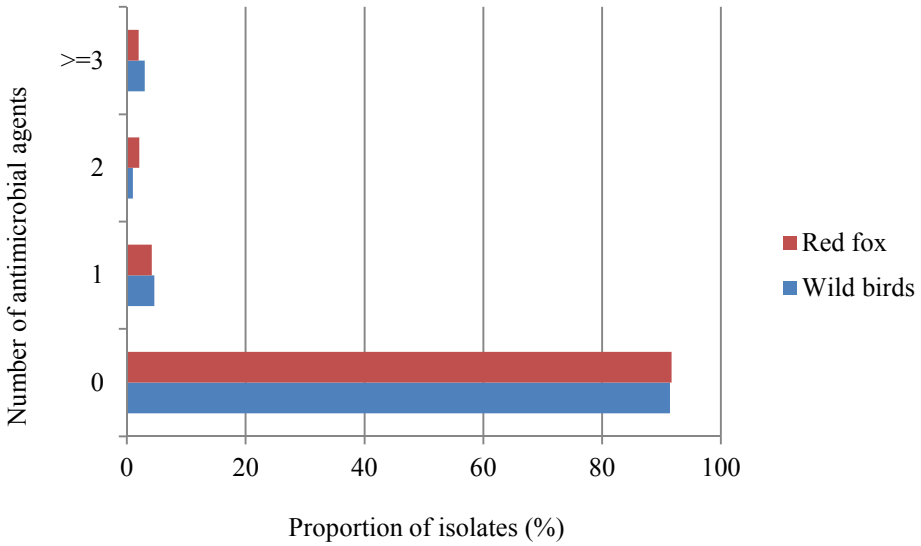

FIGURE 42. Antimicrobial resistance profile for *Escherichia coli* faecal isolates from red fox and wild birds in 2016. Proportions of isolates susceptible to all or resistant to one, two, and three or more antimicrobial agents are illustrated.

RESULTS AND COMMENTS

RED FOX

The 2016 data indicate a low occurrence of resistance among *E. coli* from faecal samples of red fox. In total, 91.7% of the isolates were susceptible to all antimicrobial agents included. Altogether, 4.1% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin), 2.1% to two, 1.1% to three and 0.9% to four antimicrobial agents (Figure 42). Resistance to ampicillin, sulfamethoxazole and tetracycline were the most frequently identified resistance determinants.

Two isolates showed decreased sensitivity to colistin. None of the plasmid mediated genes *mcr-1* and *mcr-2* were identified, indicating that the resistance phenotype is due to chromosomal mutations. Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in 1.4% [95% CI:0.5-3.0%] of the isolates. None of the isolates displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime, indicating a prevalence below 0.8%. Selective methods were used on the same sample material to investigate the occurrence of critically important antimicrobials more thoroughly.

The 2016 results are similar to previous results from 2010. There has, however, been a change in the panel of antimicrobial agents tested for, and comparisons should therefore be done with caution.

WILD BIRDS

The 2016 data indicate a low occurrence of resistance among *E. coli* from faecal samples of wild birds. In total, 91.4% of the isolates were susceptible to all antimicrobial agents included. Altogether, 4.6% of the isolates were resistant to one antimicrobial agent (predominantly ampicillin or tetracycline), 1.0% to two, 1.7% to three and 1.3% to four or more antimicrobial agents (Figure 42). Resistance to ampicillin and tetracycline were the most frequently identified resistance determinants, followed by resistance to sulfamethoxaxole, ciprofloxacin and nalidixic acid.

One isolate showed decreased sensitivity to colistin. None of the plasmid mediated genes *mcr-1* and *mcr-2* were identified, indicating that the resistance phenotype is due to chromosomal mutations. None of the isolates displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime, indicating a prevalence below 1.2%. Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in 2.3% [95% CI:0.9-4.7 %] of the isolates. Selective methods were used on the same sample material to investigate the occurrence of these critically important antimicrobials more thoroughly.

This was the first time wild birds were included in NORM-VET, and comparison to previous surveillance data on wild birds are therefore not possible. Interestingly, the results on wild birds and red foxes were very similar (Figure 42), indicating that these species may be exposed to similar levels of antimicrobial resistance drivers such as contact with areas with high human influence. However, in contrast to red fox, wild birds may be migrating and thereby enabling dissemination of resistance from other more distant sources. Moreover, finding of *E. coli* resistant to third generation cephalosporins is no surprice as several international studies have reported such findings over the years (reviewed in Bonnedahl and Järhult 2014).

Cephalosporin resistant *Escherichia coli* from red fox and wild birds

Selective screening for *E. coli* resistant to third generation cephalosporins was performed on the samples from both red fox and wild birds. A total of 528 red fox and 358 wild bird samples were screened. Results are presented in the text and in Tables 12 and 16.

TABLE 12. Antimicrobial resistance in isolates of *Escherichia coli* resistant to third generation cephalosporins from red fox and wild birds (n=17 and n=31, respectively) in 2016.

			Distribution (n) of MIC values (mg/L)*															
Substance	Sample	n resistant	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Fox	3								14					1	2		
	Bird	16								14	1			2	7	7		
Tigecycline	Fox	1					15	1	1									
	Bird	0					27	4										
Chloramphenicol	Fox	0										17						
	Bird	2									1	27	1		1	1		
Ampicillin	Fox	17														17		
	Bird	31														31		
Cefotaxime	Fox	17							2	5	2	8						
	Bird	31							3	3	2	23						
Ceftazidime	Fox	17							2	2	4	8	1					
	Bird	31						3	3	1	10	5	9					
Meropenem	Fox	0		17														
	Bird	0		31														
Sulfamethoxazole	Fox	4										12	1					4
	Bird	11										13	6	1				11
Trimethoprim	Fox	3					10	4							3			
	Bird	11					18	2							11			
Azithromycin	Fox	ND								5	7	3		2				
	Bird	ND								3	21	2	1	1		3		
Gentamicin	Fox	1						10	5	1					1			
	Bird	7						22	2					3	4			
Ciprofloxacin	Fox	3	11	3			1		1				1					
	Bird	18	12	1			3	11				1	3					
Nalidixic acid	Fox	2									15						2	
	Bird	6									12	6	7		1	1	4	
Colistin	Fox	0							17									
	Bird	0							31									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

RED FOX

By use of the selective method, *E. coli* resistant to third generation cephalosporins were found in 17 (3.2% [95% CI: 1.9–5.1%]) of the 528 red fox samples. In total, ten of the cephalosporinase producing *E. coli* from red fox faecal samples were resistant only to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. In addition, there was some resistance observed for sulfamethoxazole, tetracycline, trimethoprim, gentamicin and the quinolones ciprofloxacin and nalidixic acid (Table 12). Three of the isolates were resistant to five, seven or nine of the antimicrobials, respectively.

Ten isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype, and real-time PCR showed that three isolates contained the $bla_{\text{CMY-2}}$ gene, while the remaining seven isolates had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression. Seven isolates had a cephalosporin resistance profile corresponding to an ESBL phenotype. Genotyping showed that six carried the $bla_{\text{CTX-M}}$ group 1 genes, and one carried $bla_{\text{CTX-M}}$ group 9 genes. Additional $bla_{\text{TEM-1}}$ genes were detected in two isolates. An overview of the cephalosporin resistant genotypes and the isolates´ antimicrobial resistance patterns are shown in Tables 15 and 16.

One isolate showed decreased susceptibility to the carbapenem ertapenem (Table 15), with MIC values above the EUCAST cut-off value at 0.06 mg/L, i.e. MIC 0.12-0.25 mg/L. However, none of the isolates were resistant to imipenem or meropenem (data not shown), strongly indicating that there was no carbapenemase-producing *Enterobacteriaceae* present as ertapenem is known to have a lower specificity to detect carbapenemase producing *Enterobacteriaceae* than imipenem and meropenem (Cohen *et al.* 2010).

Selective methods for isolation of *E. coli* resistant to third generation cephalosporins have not been performed on red fox samples previously, and comparison to previous years is therefore not possible. See also separate presentation (page 61) on the survey conducted for the Ministry of Climate and Environment.

WILD BIRDS

By use of the selective method, *E. coli* resistant to third generation cephalosporins were detected from 31 (8.7% [95% CI: 6.0-12.1]) of the 358 wild bird samples. In total, nine of the isolates were resistant only to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. Three of the isolates showed MIC values for ceftazidime just below the EUCAST breakpoint at 0.5 mg/L. However, this is considered to be within the method's normal variation. In addition, there was some resistance observed for sulfamethoxazole, tetracycline, trimethoprim, gentamicin and the quinolones ciprofloxacin and nalidixic acid (Table 12). Two of the isolates were resistant to chloramphenicol. A total of 20 of the isolates (64.5% [95% CI:45.4-80.8%]) were resistant to five, or more (up to eight) of the antimicrobials tested.

Eight isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype. Real-time PCR showed that one isolate carried the $bla_{\text{CMY-2}}$ gene. Further genotyping showed that one isolate carried the bla_{DHA} gene, while the remaining six had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression. Altogether, 23 isolates had a cephalosporin resistance profile corresponding to an ESBL phenotype. Genotyping showed that 16 isolates contained genes from $bla_{\text{CTX-M}}$ group 1, five from $bla_{\text{CTX-M}}$ group 9, and two the $bla_{\text{SHV-12}}$ genes. Additional $bla_{\text{TEM-1}}$ genes were detected in eight isolates. An overview of the cephalosporin resistant genotypes and the isolates antimicrobial resistance patterns are shown in Tables 15 and 16.

In addition to the 31 detected *E. coli* isolates resistant to third generation cephalosporins, one isolate of *Klebsiella pneumoniae* was detected. The *K. pneumoniae* isolate carried the bla_{DHA} gene responsible for the third generation cephalosporin resistance (Table 15).

This was the first investigation of *E. coli* from wild birds in NORM-VET, and comparison to previous surveillance data are therefore not possible. However, finding of *E. coli* resistant to third generation cephalosporins is no surprise as several studies have reported such findings over the years (reviewed in Bonnedahl and Järhult 2014).

Quinolone resistant *Escherichia coli* from red fox and wild bird

In 2016, selective screening for *E. coli* resistant to quinolones was performed on samples from red fox and wild birds. A total of 528 red fox and 358 wild bird samples were screened. Results are presented in Table 13 and in the text.

TABLE 13. Antimicrobial resistance in quinolone resistant isolates of *Escherichia coli* from red fox (n=81) and wild birds (n=74) in 2016.

Substance	Sample	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	Fox	27.1	[17.9-38.2]								72.8					6.2	21.0		
	Bird	52.7	[40.7-64.4]								45.9	1.4			1.4	27.0	24.3		
Tigecycline	Fox	0	[0.0-4.4]					97.5	2.5										
	Bird	0	[0.0-4.9]					90.5	9.5										
Chloramphenicol	Fox	7.4	[2.8-15.8]										91.4	1.2			2.5	4.9	
	Bird	13.5	[6.7-23.5]										83.8	2.7	2.7	2.7	5.4	2.7	
Ampicillin	Fox	34.6	[24.3-46.0]							2.5	21.0	42.0	1.2	3.7	29.6				
	Bird	48.7	[36.9-60.64]							1.4	18.9	28.4	2.7	1.4		2.7	44.6		
Cefotaxime	Fox	3.7	[0.8-10.4]					96.3		1.2		1.2	2.5						
	Bird	9.5	[3.9-18.5]					90.5	1.4	1.4			6.8						
Ceftazidime	Fox	3.7	[0.8-10.4]						96.3		1.2		1.2	1.2					
	Bird	9.5	[3.9-18.5]						90.5	1.4		1.4	1.4	5.4					
Meropenem	Fox	0	[0.0-4.4]		100														
	Bird	0	[0.0-4.9]		100														
Sulfamethoxazole	Fox	23.5	[14.8-34.2]											69.1	3.7	3.7			23.5
	Bird	32.4	[22.0-44.3]										52.7	14.9					32.4
Trimethoprim	Fox	25.9	[16.8-36.9]					61.7	11.1	1.2						25.9			
	Bird	32.4	[22.0-44.3]					56.8	9.5	1.4					4.1	28.4			
Azithromycin	Fox	ND	ND								42.0	34.6	17.3	2.5	2.5	1.2			
	Bird	ND	ND								12.2	60.8	16.2	4.1	2.7		4.1		
Gentamicin	Fox	2.5	[0.3-8.6]						66.7	24.7	6.2					2.5			
	Bird	9.5	[3.9-18.5]						73.0	14.9	2.7	1.4			4.1	4.1			
Ciprofloxacin	Fox	93.8	[86.2-98.0]	1.2		4.9	13.6	54.3	7.4	9.9			4.9	3.7					
	Bird	98.6	[92.7-100.0]			1.4	10.8	43.2	21.6	2.7		2.7	6.8	10.8					
Nalidixic acid	Fox	81.5	[71.3-89.2]									4.9	6.2	7.4	2.5	11.1	23.5	44.4	
	Bird	71.6	[59.9-81.5]									2.7	10.8	14.9	4.1	5.4	18.9	43.2	
Colistin	Fox	0	[0.0-4.4]							100									
	Bird	0	[0.0-4.9]							97.3	2.7								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

RED FOX

A total of 81 *E. coli* were isolated. Of these 78 isolates were resistant to quinolones when MICs were determined (Table 13). Quinolone resistant *E. coli* was found in 78 (14.8% [95% CI: 11.9-18.1%]) of the 528 red fox samples. In total, 55.1% of the isolates showed decreased sensitivity only to quinolones (ciprofloxacin and/or nalidixic acid). Additional resistance to one, two or three antimicrobial agents was found in 33.3% of the isolates (tetracycline, ampicillin, sulfamethoxazole or trimethoprim), while 11.5% were resistant to additonal four or more antimicrobial agents.

None of the isolates showed decreased sensitivity to colistin. Three (3.8%) of the 78 isolates showed additional resistance to the third generation cephalosporins cefotaxime or ceftazidime. Two isolates had a cephalosporin resistance profile corresponding to an ESBL phenotype. Genotyping showed that these two contained genes from $bla_{\text{CTX-M}}$ group 1. The remaining isolate had a cephalosporin resistance profile corresponding to an AmpC phenotype and genotyping showed that it had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression. Selective methods for isolation of quinolone resistant *E. coli* have not been performed on red fox samples previously, and comparison to previous years are therefore not possible. See also separate presentation below on survey conducted for the Ministry of Climate and Environment.

WILD BIRDS

By use of the selective method, quinolone resistant *E. coli* were found in 74 (5.6% [95% CI: 3.4-8.5%]) of the 358 wild bird samples. In total, 27.0% of the isolates showed decreased sensitivity only to quinolones (ciprofloxacin and/or nalidixic acid). Additional resistance to one, two or three antimicrobial agents was found in 45.9% of the isolates (tetracycline, ampicillin, sulfamethoxazole or trimethoprim), while 27.0% were resistant to additonal four or more antimicrobial agents.

None of the isolates showed decreased sensitivity to colistin. Seven (9.5%) of the isolates showed additional resistance to the third generation cephalosporins cefotaxime and/or ceftazidime. Six isolates had a cephalosporin resistance profile corresponding to an ESBL phenotype. Genotyping showed that five contained genes from $bla_{\text{CTX-M}}$ group 1 and one the $bla_{\text{SHV-12}}$ gene. Additional $bla_{\text{TEM-1}}$ genes were detected in two isolates. The remaining isolate was not confirmed as resistant to cefotaxime or ceftazidime on the Sensititre® TREK EUVSEC2 plate. The finding of *E. coli* resistant to third generation cephalosporins in wild birds is no surprise as several studies have reported such findings over the years (reviewed in Bonnedahl and Järhult 2014). This was the first investigation of wild birds in NORM-VET, and comparison to previous surveillance data is therefore not possible.

Carbapenemase-producing *Enterobacteriacae* spp. from red fox and wild birds

A total of 514 red fox and 357 wild bird samples were screened for the presence of carbapenemase-producing Enterobacteriacae. One isolate, an *Enterobacter asburiae*, from wild birds was resistant to the carbapenems meropenem, imipenem and ertapenem. This isolate will be subjected to whole genome sequencing for further characterization of the responsible gene mechanisms.

Colistin resistant *Escherichia coli* from wild birds

Selective screening for detection of colistin resistant *E. coli* was performed on samples from wild birds. A total of 358 wild bird samples were screened and no colistin resistant *E. coli* were detected.

A survey conducted for the Ministry of Climate and Environment

A subset of the samples from foxes presented in NORM/NORM-VET 2016 was included in a survey conducted for the Ministry of Climate and Environment to map the occurrence of antimicrobial resistance (AMR) in the environment. The aim of the survey was to estimate the burden of antimicrobial resistance in three different environments in Norway; from areas with almost no human influence to areas with high human influence. The red fox is a top predator species, and can acquire AMR bacteria from consumption of prey, as well through interaction with human waste and infrastructure (e.g. garbage and sewage). Further, the red fox is distributed throughout Norway, and is hence a good indicator species for monitoring AMR in its environmental habitat.

Material and methods

In total 387 foxes selected from the Norwegian monitoring programme for *Echinococcus multilocularis* were included. One sample per fox was analysed. The samples received were divided into three different groups based on human population density to reflect possible exposure to drivers for antimicrobial resistance related to human activity as follows:

- low exposure ≤ five inhabitants per km^2 (n=98).
- medium exposure = between five and 200 inhabitants per km^2 (n=200).
- high exposure ≥ 200 inhabitants per km^2 (n=89).

Two strategies for detection of resistant bacteria were used;

1. Non-selective culturing and inclusion of one randomly chosen *E. coli* (indicator *E. coli*) from each sample for testing of resistance against 13 different antimicrobials.
2. Selective screening for *E. coli* resistant to third generation cephalosporins, carbapenems, quinolones or colistin, and for enterococci resistant to vancomycin.

Results

The occurrence of resistance in indicator *E. coli* was low, as 92.3% were susceptible to all antimicrobials included in the test panel. However, the occurrence of AMR differed significantly between the medium and high population density areas with 4.7% and 15.2% resistant to at least one antimicrobial substance, respectively. Multidrug resistance (i.e. isolates resistant to ≥ 3 antimicrobial substances) was detected in only 2.4% of the isolates (Figure 43).

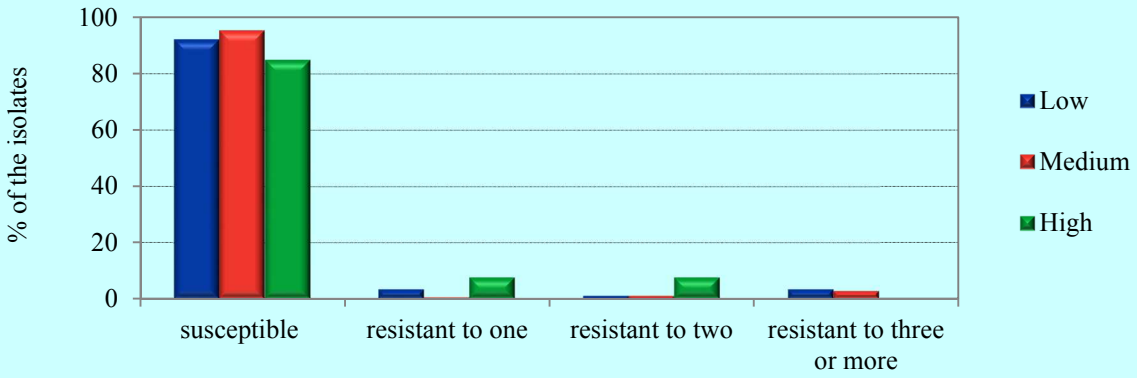

FIGURE 43. The antimicrobial resistance profiles among *Escherichia coli* isolates (N=326) from wild red foxes in Norway, according to human population density (low, medium, high).

Selective screening for *E. coli* resistant to third generation cephalosporins, carbapenems, quinolones or colistin, and for enterococci resistant to vancomycin was performed on a total of 387 faecal samples. *E. coli* displaying resistance towards carbapenems or colistin or enterococci displaying resistance towards vancomycin were not detected.

The overall occurrence of *E. coli* resistant to third generation cephalosporins was 3.4%, differing between the exposure areas as follows; 3.4%, 3.5% and 6.0% in the low, medium and high exposure areas, respectively. In the low exposure areas, resistance to third generation cephalosporins was found to be due to chromosomal mutations resulting in an up-regulation of the chromosomal *ampC* gene. In the medium and high exposure areas, however, $bla_{\text{CTX-M}}$ genes were detected in four out of ten isolates.

The overall occurrence of quinolone resistant *E. coli* was 12.9%, with the lowest occurrence in the low exposure areas with 9.2% compared to medium and high exposed areas with 14.5% and 16.9%, respectively. In total, 32 out of the 50 detected quinolone resistant isolates (64%) originated from foxes hunted in the south eastern part of Norway. However, there was a high number of samples (51.6%) obtained from this part of Norway.

The results from the present study indicate that human population density, which reflects human activities, is a driver for the occurrence of AMR in Norwegian wildlife. Further details of the survey can be found in the report "Antimicrobial resistance in the Norwegian environment - red fox as an indicator" Rapport 11 – 2017. http://www.vetinst.no/rapporter-og-publikasjoner/rapporter/2017/antimicrobial-resistance-in-the-norwegian-environment-red-fox-as-an-indicator.

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INDICATOR BACTERIA FROM FOOD

Madelaine Norström, Jannice Schau Slettemeås, Gro Johannessen, Cecilie Svanevik, Bjørn Tore Lunestad and Anne Margrete Urdahl

Cephalosporin resistant *Escherichia coli* from broiler and turkey meat

In 2016, selective screening for *E. coli* resistant to third generation cephalosporins was performed on samples from broiler and turkey meat. A total of 175 broiler and 128 turkey meat samples were screened. Results are presented in the Tables 14-15 and in the text

TABLE 14. Antimicrobial resistance in cephalosporin resistant *Escherichia coli* isolates from meat samples from broiler and turkey flocks (n= 17 and n=3, respectively) in 2016.

			Distribution (n) of MIC values (mg/L)*															
Substance	Sample	n resistant	0.015	0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	Broiler	3								14					1	2		
	Turkey	0								3								
Tigecycline	Broiler	0					17											
	Turkey	0					3											
Chloramphenicol	Broiler	0										17						
	Turkey	0										3						
Ampicillin	Broiler	17													2	15		
	Turkey	3														3		
Cefotaxime	Broiler	17								1	2	14						
	Turkey	3									2	1						
Ceftazidime	Broiler	17								1	1	10	5					
	Turkey	3										2	1					
Meropenem	Broiler	0		17														
	Turkey	0		3														
Sulfamethoxazole	Broiler	2										14		1			2	
	Turkey	0										3						
Trimethoprim	Broiler	1					16								1			
	Turkey	0					3											
Azithromycin	Broiler	ND								2	11	4						
	Turkey	ND								1	2							
Gentamicin	Broiler	3						12	2				3					
	Turkey	0						1	2									
Ciprofloxacin	Broiler	0	14	3														
	Turkey	0	3															
Nalidixic acid	Broiler	0									17							
	Turkey	0									3							
Colistin	Broiler	0							17									
	Turkey	0							3									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

BROILER MEAT

E. coli resistant to third generation cephalosporins, i.e. cefotaxime and/or ceftazidime, were found in 17 (9.7%, [95% CI: 5.8-15.1]) out of 175 meat samples. The majority (n=13) of the isolates was only resistant to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. In addition, there was some resistance observed for tetracycline, sulfamethoxazole, trimethoprim and gentamicin (Table 14).

All isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype, and real-time PCR showed that all isolates contained the bla_{CMY-2} gene. The responsible resistance gene mechanisms are shown in Table 15, together with an overview of what other antimicrobial agents the isolates showed decreased susceptibility to. None of the isolates showed decreased susceptibility to the carbapenems ertapenem, imipenem or meropenem.

The selective method for the detection of *E. coli* resistant to third generation cephalosporins has been included in

NORM-VET since 2011. This selective method showed that 43% [95% CI: 36.7-49.2] of the broiler flocks and 32.2% [95% CI: 25.9-39.1%] of the broiler meat samples were positive for *E. coli* resistant to third generation cephalosporins encoded by the plasmid mediated bla_{CMY-2} gene (NORM-VET 2011, NORM-VET 2012). The current findings by the selective method show that there has been a reduction of *E. coli* resistant to third generation cephalosporins in broiler meat samples compared to previous years ($p<0.001$). This reduction was probably achieved as a result of the measures implemented by the industry to reduce the occurrence in broilers and thereby contamination to broiler meat. There is no selection pressure from cephalosporin usage in Norway, but the poultry production is dependent on import of breeding animals. These imported animals have been shown to be the source of introduction, and the industry has therefore taken measures to limit the occurrence of *E. coli* resistant to third generation cephalosporins in imported breeding animals.

In an international perspective, the prevalence of *E. coli* resistant to third generation cephalosporins in Norwegian broilers and meat thereof is quite low, though the occurrence varies markedly between countries reporting to EFSA (EFSA and ECDC Summary report 2014). The monitoring using selective methods was not mandatory in 2014, and with very few countries reporting data on positive/negative samples, prevalence of *E. coli* resistant to third generation cephalosporins in meat samples was not assessed and directly comparable prevalence data are therefore not available.

The situation regarding *E. coli* resistant to third generation cephalosporins in broiler meat in Norway has been considered similar to the situation in Sweden. A Swedish report from 2014 (Egervärn *et al.* 2014), concluded that food on the Swedish market, including the *E. coli* resistant to third generation cephalosporins from broilers, was a limited contributor to the prevalence of *E. coli* resistant to third generation cephalosporins within the human healthcare sector. Results from a Norwegian study show that Norwegian patients and retail chicken meat have highly similar *E. coli* resistant to third generation cephalosporins and resistance plasmids, though this is a very limited proportion of the total cases of human *E. coli* resistant to third generation cephalosporins (Berg *et al.* 2017).

TURKEY MEAT

E. coli resistant to third generation cephalosporins, i.e. cefotaxime and/or ceftazidime, were found in three turkey samples (2.3%, [95% CI: 0.5-6.7]) out of 128 meat samples. All three isolates were only resistant to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. All three had a cephalosporin resistance profile corresponding to an AmpC phenotype. Genotyping showed that one isolate contained the bla_{CMY-2} gene and one had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression. The last isolate was not further characterised. The resistance gene mechanisms responsible are shown in Table 15. None of the isolates showed decreased susceptibility to the carbapenems ertapenem, imipenem or meropenem.

The present results on cephalosporin resistant *E. coli* due to presence of the bla_{CMY-2} gene are in concordance with previous results from selective screening for cephalosporin resistant *E. coli* in turkey meat samples. In 2013, four samples (2.6% [95% CI: 0.7-6.4%]) were positive for bla_{CMY-2} encoded cephalosporin resistant *E. coli*, while an additional 55 (35.3% [95% CI: 27.8-43.3%]) were positive for chromosomal up-regulated AmpC cephalosporin resistant *E. coli*. The latter was not detected in 2016. The reason for this decrease is unknown.

In an international perspective, the occurrence of cephalosporin resistant *E. coli* in Norwegian turkey meat is low, though the occurrence varies markedly between countries reporting to EFSA (EFSA and ECDC Summary report 2014). The monitoring using selective methods on turkey meat is not mandatory, and with very few countries reporting data on positive/negative samples, the prevalence of *E. coli* resistant to third generation cephalosporins in meat samples was not assessed and directly comparable prevalence data are therefore not available.

Carbapenemase-producing *Enterobacteriaceae* from broiler and turkey meat

A total of 174 broiler and 128 turkey meat samples were screened for the presence of carbapenemase-producing *Enterobacteriaceae.* No carbapenemase-producing *Enterobacteriaceae* were detected. 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.

TABLE 15. Overview of *Escherichia coli* resistant to third generation cephalosporins corresponding to an AmpC phenotype identified in NORM-VET 2016, their source of origin and antimicrobial resistant patterns.

		Resistance to number of antimicrobials																												
		4	4	5	5	5	5	5	5	5	5	5	6	6	6	6	6	7	7	7	7	7	7	8	8	8	9	9	11	N
Resistance pattern	Ampicillin	12	8	2	4	2	20	1	4	1	2	5	1	1	1	2	4	1	3	1	1	1	2	8	1	2	1	1	2	94
	Cefotaxime	12	8	2	4	2	20	1	4	1	2	5	1	1	1	2	4	1	3	1	1	1	2	8	1	2	1	1	2	94
	Ceftazidime	12	8	2	4	2	20	1	4	1	2	5	1	1	1	2	4	1	3	1	1	1	2	8	1	2	1	1	2	94
	Cefoxitin	12	8	2	4	2	20	1	4	1	2	5	1	1	1	2	4	1	3	1	1	1	2	8	1	2	1	1	2	94
	Cefepime					2	20	1			2	5	1		1	2	4	1		1		1		8		2		1	2	54
	Chloramphenicol																										1			1
	Ciprofloxacin																				1				1		1		2	5
	Ertapenem												1				4	1	3									1		10
	Gentamicin																					1	2	8		2		1	2	16
	Imipenem																													0
	Nalidixic acid																										1		2	3
	Sulfamethoxazole			2	4				4	1				1		2		1	3	1			2	8	1	2	1	1	2	36
	Tetracycline																		3		1	1	2	8	1	2	1	1	2	22
	Trimethoprim													1	1					1	1				1				2	7
No. of isolates	Broiler caecal		3		2							5	1						3					6					20
	Turkey caecal	4			2		5		4	1																				16
	Red fox	4	2	1							2							1												10
	Wild bird	3				1								1						1	1				1		1			9
	Broiler meat		2				11								1							1				2				17
	Turkey meat	1					1	1																						3
	Seafood		1																											1
	Dogfood			1			3									2	4						2	2				1		15
	Bivalve molluscs					1																							2	3
Genotype	chrAmpC[1]	12		2		2			4		2			1						1										24
	bla_{CMY-2}		8		4		20			1		5	1		1	2	4	1	3			1	2	8		2	1	1	2	67
	bla_{DHA}																				1				1[2]					2
	unknown							1																						1

[1]Overexpression of chromosomally located *ampC* due to mutations in the promoter and attenuator regions.
[2]*K. pneumoniae*

TABLE 16. Overview of *Escherichia coli* resistant to third generation cephalosporins corresponding to an ESBL phenotype identified in NORM-VET 2016, their source of origin and antimicrobial resistant patterns.

		Resistance to number of antimicrobials																											
		3	4	4	5	5	5	5	6	6	6	6	6	6	7	7	7	7	8	8	8	8	8	9	9	9	10	10	11	N
Resistance pattern	Ampicillin	2	5	1	2	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	2	1	3	1	1	1	1	1	2	42
	Cefotaxime	2	5	1	2	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	2	1	3	1	1	1	1	1	2	42
	Ceftazidime		5	1	2	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	2		3	1	1	1	1	1	2	39
	Cefoxitin								1																			1	2	4
	Cefepime	2	5	1	2	1	1	1	1	1	1	1	1	1	5	1	1	1	1	1	2	1	3	1	1	1	1	1	2	42
	Chloramphenicol														5			1				1								7
	Ciprofloxacin				2	1				1			1	1		1	1		1	1	2	1	3	1	1	1	1	1	2	23
	Ertapenem								1																			1		2
	Gentamicin							1		1					5				1								1		2	11
	Imipenem																													0
	Nalidixic acid												1			1	1		1	1		1	3	1	1	1	1		2	15
	Sulfamethoxazole						1					1						1			2	1	3	1	1	1	1	1	2	16
	Tetracycline										1		1		5		1		1	1	2	1		1	1	1	1	1	2	20
	Trimethoprim										1	1				1		1		1	2		3	1	1	1	1	1	2	17
No. of isolates	Red fox		2		1		1				1								1								1			7
	Wild bird	2	2			1		1	1	1		1			5	1	1			1	2	1		1	1			1		23
	Bivalve molluscs		1	1	1								1	1				1					3			1			2	12
Genotype	$bla_{\text{CTX-M}}$ gr 1	2	5		2					1			1		5	1			1	1			3	1					2	25
	$bla_{\text{CTX-M}}$ gr 1 + $bla_{\text{TEM-1}}$			1			1							1				1			2					1	1	1		9
	$bla_{\text{CTX-M}}$ gr 9								1		1											1								3
	$bla_{\text{CTX-M}}$ gr 9 + $bla_{\text{TEM-1}}$							1				1														1				3
	$bla_{\text{SHV-12}}$					1																								1
	$bla_{\text{SHV-12}}$+ $bla_{\text{TEM-1}}$																1													1

Escherichia coli from cheese and seafood

A total of 179 cheese samples, of which 94 and 85 were of pasteurised and un-pasteurised milk, respectively, were examined. From seafood, a total of 359 samples were investigated. In total, 30 *E. coli* isolates from imported seafood and 34 *E. coli* isolates from domestic seafood was obtained. The seafood samples consisted of samples from various sources as described in Appendix 3.

One isolate per positive sample was susceptibility tested. The results are presented in the text, and in Table 17 and Figure 44.

TABLE 17. Antimicrobial resistance in isolates of *Escherichia coli* from samples from cheese n=50 (pasteurised and un-pasteurised; n=10 and n=40, respectively) and from seafood (blue mussels, scallops, oisters, scampi etc.) (n=64) collected at retail in 2016.

		Resistance (%)		Distribution (%) of MIC values (mg/L)*														
Substance	Sample	[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	Cheese	22	[11.5-36.0]								78			4	10	6	2		
	Seafood	14.1	[6.6-25.0]								84.4	1.6				3.1	10.9		
Tigecycline	Cheese	0.0	[0.0-7.1]					100											
	Seafood	0.0	[0.0-5.6]					100											
Chloramphenicol	Cheese	4	[0.5-13.7]										96				4		
	Seafood	3.1	[0.4-10.8]										95.3	1.6			3.1		
Ampicillin	Cheese	18	[8.6-31.4							4	36	40	2				18		
	Seafood	12.5	[5.6-23.2]							4.7	25.0	53.1	4.7			1.6	10.9		
Cefotaxime	Cheese	0.0	[0.0-7.1]					100											
	Seafood	0.0	[0.0-5.6]					100											
Ceftazidime	Cheese	0.0	[0.0-7.1]						100										
	Seafood	0.0	[0.0-5.6]						100										
Meropenem	Cheese	0.0	[0.0-7.1]		100														
	Seafood	0.0	[0.0-5.6]		100														
Sulfamethoxazole	Cheese	16	[7.2-29.1]										78	6					16
	Seafood	10.9	[4.5-21.2]										79.7	3.1	4.7	1.6			10.9
Trimethoprim	Cheese	12	[4.5-24.3]					82	6							12			
	Seafood	3.1	[0.4-10.8]					90.6	6.3							3.1			
Azithromycin	Cheese	ND	ND								48	38	14						
	Seafood	ND	ND								42.9	46.0	9.5	1.6					
Gentamicin	Cheese	0.0	[0.0-7.1]						80	16	4								
	Seafood	3.1	[0.4-10.8]						79.4	14.3	3.2	1.6			1.6				
Ciprofloxacin	Cheese	2.0	[0.1-10.6]	94	2	2							2						
	Seafood	7.8	[2.6-17.3]	92.2				3.1	1.6	1.6				1.6					
Nalidixic acid	Cheese	4	[0.5-13.7]									96				2		2	
	Seafood	4.7	[1.0-13.1]									93.8		1.6			1.6	3.1	
Colistin	Cheese	0.0	[0.0-7.1]							98	2								
	Seafood	1.6	[0.0-8.4]							96.9	1.6	1.6							

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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.

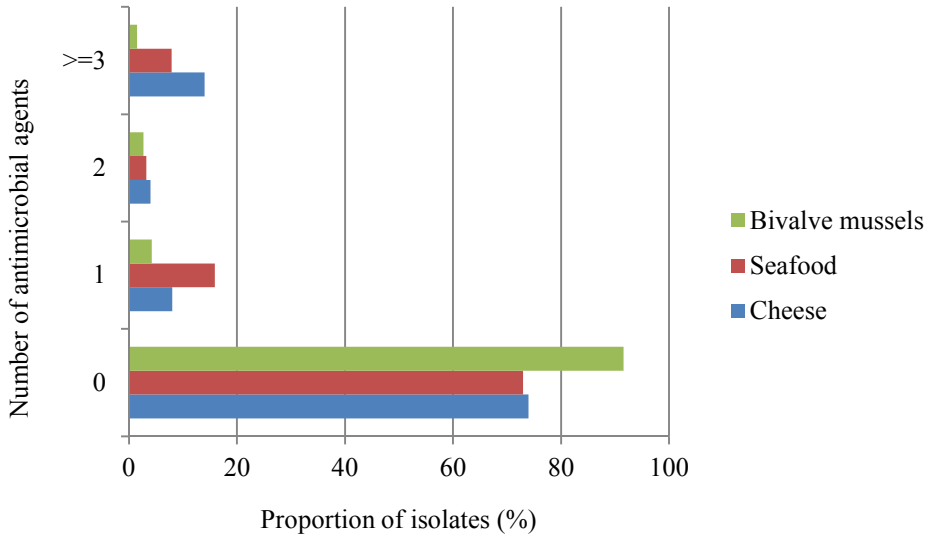

FIGURE 44. Antimicrobial resistance profile for *Escherichia coli* faecal isolates from cheese (n=50), seafood (n=64) and bivalve mussels (n=261) (Norwegian) in 2016. Proportions of isolates susceptible to all or resistant to one, two, and three or more antimicrobial agents are illustrated

TABLE 18. An overview of the antimicrobial resistance results in samples from imported and domestic cheese and seafood, respectively.

Product	No. Samples		No. Samples		No. samples	No. *E. coli*	No. res. *E. coli*	No. cephalosporin res. *E. coli*	No. quinolone-res. *E. coli*	No. carbapenemase-producing *Enterobacteriacae*
Cheese	179	Imported	54	Pasteurised	28	6	0	0	0	0
				Un-pasteurised	26	15	10	0	4	0
		Domestic	125	Pasteurised	66	4	1	0	0	0
				Un-pasteurised	59	25	2	0	2	0
Seafood	359	Imported			179	30	9	0	8*	1
		Domestic			180	34	8	1	1	0

*One colistin resistant due to presence of the plasmid mediated *mcr-1* gene.

RESULTS AND COMMENTS

CHEESE

E. coli isolates were obtained from 50 of the samples (27.9%); differing significantly between the pasteurised and un-pasteurised milk with a total of ten (5.6%) and 40 (22.3%) isolates from each group. 54 samples were imported, mainly from France, while 125 were of domestic origin. Among the isolates obtained, 29 isolates were of Norwegian origin and 21 were from imported cheese. In total, 74% [95% CI: 59.7-85.4] of the isolates obtained from cheese were susceptible to all antimicrobial agents in the test panel. Altogether, 8.0% of the isolates were resistant to one antimicrobial agent (predominantly tetracycline), 14.0% to two, three or four antimicrobial agents, while two isolates were resistant to six and seven antimicrobial agents, respectively (Figure 44). The data indicate a high occurrence of resistance among *E. coli* from these products according to the EFSA classification (Appendix 6). Resistance to ampicillin, trimethoprim, sulfamethoxazole and tetracycline were the most frequently identified resistance determinants (Table 17). None of the isolates displayed any resistance to colistin, nor the third generation cephalosporins cefotaxime or ceftazidime. Selective methods were used on the same sample material to investigate the occurrence of critically important antimicrobials more thoroughly (see result page 70).

An overview of the antimicrobial resistance results per category imported/domestic and pasteurised/un-pasteurised are shown in Table 18. The results indicate that antimicrobial resistance is more common in imported than in domestic products. Moreover, the majority of isolated *E. coli*, including the resistant *E. coli*, were of un-pasteurised origin ($p<0.05$).

This was the first investigation of cheese in NORM-VET. Further monitoring is recommended to aquire more

knowledge of antimicrobial resistance in such products, especially since these are typical products consumed without any heat treatment.

SEAFOOD

E. coli isolates were obtained from a total of 64 samples (17.8%) out of a total of 359. The origin of the samples were both from domestic and imported seafood, approximately 50% of each category.

The data indicate a high occurrence of resistance among *E. coli* from these products according to the EFSA classification (Appendix 6). In total, 73% [95% CI: 60.3-83.4] of the isolates obtained from seafood were susceptible to all antimicrobial agents in the test panel. Altogether, 15.6% of the isolates were resistant to one antimicrobial agent (tetracycline, ampicillin or sulfamethoxazole), while the remaining 11.4% of the isolates were distributed with resistance between two and up to seven antimicrobial agents. Resistance to tetracycline, ampicillin or sulfamethoxazole were the most frequently identified resistance determinants (Table 17).

None of the isolates displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime. Selective methods were used on the same sample material to investigate the occurrence of critically important antimicrobials more thoroughly (see result page 70).

One isolate showed decreased sensitivity to colistin. This isolate came from the same sample as the colistin resistant isolate described under selective screening for quinolone resistance, see below.

This was the first investigation of seafood in NORM-VET, and comparison to previous surveillance data is therefore not possible. However, the results indicate that it is more common with antimicrobial resistance (and more multidrug resistance as shown also from selective screening for quinolone reistant *E. coli*) among the isolates from seafood of imported origin ($p<0.05$). Further monitoring is recommended to aquire more knowledge of antimicrobial resistance in such products.

Cephalosporin resistant *Escherichia coli* from cheese and seafood

Selective screening for *E. coli* resistant to third generation cephalosporins was performed on samples from a total of 179 cheese and 358 seafood samples. No *E. coli* resistant to third generation cephalosporins was detected from the cheese samples, while one of the imported seafood samples was positive. The resistance mechanism encoding the cephalosporin resistance was bla_{CMY-2} (Table15).

Quinolone resistant *Escherichia coli* from cheese and seafood

CHEESE

Selective screening for quinolone resistant *E. coli* was performed on samples from a total of 179 cheese samples. Quinolone resistant *E. coli* was detected from six (3.4% [95% CI: 0.9-6.4%]) of these, of which four originated from imported products. Four of the six cheese isolates showed resistance to six or more of the antimicrobial agents included in the test panel. None of the isolates showed decreased sensitivity to colistin, nor to the third generation cephalosporins cefotaxime and ceftazidime.

SEAFOOD

Selective screening for quinolone resistant *E. coli* was performed on samples from a total 358 seafood samples. Quinolone resistant *E. coli* was detected from nine (2.5% [95% CI: 1.2-4.7%]) of these samples. Eight of the nine isolates originated from imported products.

Among the seafood isolates, one showed resistance to six or more of the antimicrobial agents included in the test panel. The others were resistant to between two to five antimicrobial agents. None of the isolates showed decreased sensitivity to the third generation cephalosporins cefotaxime and ceftazidime. However, one isolate showed resistance to colistin and the presence of the plasmid-mediated gene, *mcr-1,* was detected (see separate presentation on page 70).

Carbapenemase-producing *Enterobacteriaceae* from cheese and seafood

A total of 179 cheese and 358 seafood samples were screened for the presence of carbapenemase-producing *Enterobacteriaceae.* One isolate, an *Enterobacter asburiae*, from imported seafood was resistant to the carbapenems meropenem, imipenem and ertapenem. This isolate will be subjected to whole genome sequencing for further characterization of the responsible gene mechanisms

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Detection of *Escherichia coli* containing the plasmid-mediated colistin resistance gene *mcr-1* in samples of imported seafood and dog food in NORM-VET 2016

In November 2015, the first plasmid-mediated colistin resistance gene, *mcr-1,* was detected in an *Escherichia coli* strain from a pig in China (1). Since then, the *mcr-1* gene has been reported in *Enterobacteriaceae* from various sources in countries all over the world. Internationally, colistin is widely used in veterinary medicine, particularly for pigs and poultry, and the selection pressure in livestock production is a likely driver for persistence and dissemination of *mcr-1* (1). The sales of the polymyxin colistin have been documented through the European Surveillance of Veterinary Antimicrobial Consumption reports (http://www.ema.europa.eu/docs/en_GB/document_library/Report/2016/10/WC500214217.pdf). Historical data show that there has been no sale of polymyxin veterinary medicinal products for animals in Norway in the years 1993 to 2016 (http://www.vetinst.no/overvaking/antibiotikaresistens-norm-vet). Retrospective molecular screening of relevant strain collections has demonstrated absence of *mcr-1* isolates in samples from animals, food and feed originating from Norway for the years 2010-2015 (2).

In 2016, NORM-VET included samples of imported seafood and imported raw dog food. From two of the samples, one of each category, *E. coli* containing *mcr-1* were detected (3). The seafood sample was scampi imported from Bangladesh, while the dog food sample originated from the United Kingdom and contained turkey meat, fruit and vegetables. These samples were screened for quinolone resistant *E. coli* (QREC) using selective methods. Presumptive QREC were susceptibility tested with broth microdilution. The MICs to colistin was 4 mg/L and the isolates were subjected to further investigations.

Data from whole genome sequencing (WGS) confirmed the presence of *mcr-1* in both isolates. These findings demonstrate that international trade with food and feed can represent a route of dissemination of important resistant bacteria. Furthermore, such import may also lead to introduction of specific resistance forms into geographic areas where they are rare or absent. Only one finding of plasmid-mediated colistin resistance has been reported in humans in Norway so far (4). This was an *E. coli* with *mcr-1* isolated from a traveller returning from India with enteritis.

Until now, most *mcr-1* findings reported from countries outside Norway have been found following screening of historical isolates or WGS databases, whereas fewer findings have dealt with detection in “real time”. Our findings show the importance of risk based screening of relevant samples in order to uncover possible sources for bacteria resistant to last resort antimicrobials. Products imported from areas with a high environmental load of resistant bacteria and with higher usage of antimicrobials should receive special attention. Further monitoring is highly recommended to acquire knowledge on the frequency of the *mcr-1* gene in the years to come.

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Escherichia coli from Norwegian bivalve molluscs

A total of 391 batch samples of bivalve molluscs collected at rearing localities, were examined and *E. coli* isolates were obtained from a total of 261 samples (66.8%).

One isolate per positive sample was susceptibility tested. The results are presented in Table 19, and in the text.

TABLE 19. Antimicrobial resistance in isolates of *Escherichia coli* from batch samples of bivalve molluscs collected at rearing localities (n=261) in 2016.

	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	5.7	[3.3-9.3]								93.9	0.4			0.4	1.5	3.8		
Tigecycline	0.0	[0.0-1.4]					100											
Chloramphenicol	1.1	[0.2-3.3]										98.1	0.8			0.8	0.4	
Ampicillin	4.6	[2.4-7.9]							5.4	34.1	52.9	3.1		0.8	0.4	3.4		
Cefotaxime	0.0	[0.0-1.4]					100											
Ceftazidime	0.0	[0.0-1.4]						100										
Meropenem	0.0	[0.0-1.4]		100														
Sulfamethoxazole	3.1	[1.3-5.9]										96.2	0.8					3.1
Trimethoprim	1.1	[0.2-3.3]					97.3	1.1	0.4						1.1			
Azithromycin	ND	ND								13.4	68.2	16.5	1.5	0.4				
Gentamicin	0.4	[0.0-2.1]						80.1	18.0	1.5			0.4					
Ciprofloxacin	0.8	[0.0-2.7]	74.3	24.5	0.4		0.4					0.4						
Nalidixic acid	0.4	[0.0-2.1]									99.2	0.4					0.4	
Colistin	0.0	[0.0-1.4]							98.5	1.5								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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 2016 data indicate a low occurrence of resistance among *E. coli* from bivalve molluscs batches collected at rearing localities. In total, 91.6% [95% CI: 87.5-94.6] of the isolates were susceptible to all antimicrobial agents included. Altogether, 4.2% of the isolates were resistant to one antimicrobial agent (predominantly tetracycline), 2.7% to two, 0.4% to three and 1.1% to four or more antimicrobial agents (Figure 44). Resistance to tetracycline was the most frequently identified resistance determinant, followed by resistance to ampicillin and sulfamethoxazole. None of the isolates displayed any resistance to the third generation cephalosporins cefotaxime or ceftazidime [95% CI: 0.0–1.4%]. Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in 0.8% [95% CI: 0.1–2.7%] of the isolates. Selective methods were used on the same sample material to investigate the occurrence of these critically important antimicrobials more thoroughly (see below).

Bivalves have not previously been investigated in NORM-VET. However, a research study performed on Enterobacteriaceae (n=199) isolated from bivalves from the same rearing localities in 2014-15, identified resistance to at least one antimicrobial agent among 38% of the isolates, mainly *E. coli* (Grevskott *et al.* 2017). Resistance towards ampicillin and tetracyclines was found in 18% and 3% of the isolates, respectively. Due to methodological differences such as number of antimicrobial agents tested for, use of disk diffusion instead of broth microdilution and EUCAST clinical breakpoints instead of epidemiological breakpoints, direct comparison between the results is, however, difficult.

Bivalve molluscs filtrate large volumes of water and actively retain particles, including free and particle-bound bacteria that find their way to the marine environment. Antimicrobial agents and antimicrobial resistant bacteria reach the sea through sewage and runoff from land. In addition to representing a food product, bivalves may therefore be used as an indicator for antimicrobial resistance in the environment.

Cephalosporin resistant *Escherichia coli* from Norwegian bivalve molluscs

Selective screening for *E. coli* resistant to third generation cephalosporins was performed on a total of 391 batch samples of bivalve molluscs collected at rearing localities. Results are presented in the text and in Tables 15, 16 and 20.

TABLE 20. Antimicrobial resistance in cephalosporin resistant *Escherichia coli* (n=13) from batches of bivalve molluscs collected at rearing localities in 2016.

		Distribution (n) of MIC values (mg/L)*															
Substance	n resistant	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	≥ 512
Tetracycline	4								9					1	3		
Tigecycline	0					13											
Chloramphenicol	1										12					1	
Ampicillin	13														13		
Cefotaxime	13								1		12						
Ceftazidime	13							1	3	2	3	4					
Meropenem	0		13														
Sulfamethoxazole	7										6						7
Trimethoprim	7						5	1						7			
Azithromycin	ND								1	6			4	1	1		
Gentamicin	0						10	3									
Ciprofloxacin	9	1		3		2	4					3					
Nalidixic acid	7									5	1			1	2	4	
Colistin	0							13									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

E. coli resistant to third generation cephalosporins were detected in thirteen of the 391 bivalve molluscs batch samples (3.3% [95% CI: 1.8-5.6%]). The resistance genes responsible are shown in Tables 15-16, together with an overview of what other antimicrobial agents the isolates showed decreased susceptibility to. One isolate showed decreased susceptibility due to mutations in the promoter and attenuator regions for the chromosomally located *ampC* gene. Ten of the isolates showed decreased susceptibility due to the plasmid-encoded resistance gene $bla_{\text{CTX-M-15}}$, whereof two additionally carried $bla_{\text{CMY-2}}$ and four additionally carried $bla_{\text{TEM-1}}$. None of the isolates showed decreased susceptibility to meropenem, the preferred carbapenem used for detection of carbapenemase production.

Quinolone resistant *Escherichia coli* from Norwegian bivalve molluscs

Selective screening for quinolone resistant *E. coli* was performed on samples from a total of 391 batch samples of bivalve molluscs collected at rearing localities. Results are presented in the text and in Table 21.

TABLE 21. Antimicrobial resistance in quinolone resistant *Escherichia coli* (n=52) from bivalve molluscs batch samples collected at rearing localities in 2016.

	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	30.8	[18.7-45.1]								63.5	5.8			1.9	5.8	23.1		
Tigecycline	0	[0.0-6.9]					96.2	3.8										
Chloramphenicol	17.3	[8.2-30.3]										80.8	1.9	5.8	3.8	3.8	3.8	
Ampicillin	55.8	[39.4-53.8]								9.6	32.7	1.9	1.9	3.8	1.9	48.1		
Cefotaxime	9.6	[3.2-21.0]					90.4	1.9				7.7						
Ceftazidime	7.7	[2.1-18.5]						92.3					7.7					
Meropenem	0	[0.0-6.9]		100														
Sulfamethoxazole	34.6	[21.9-49.1]										59.6	5.8					34.6
Trimethoprim	34.6	[21.9-49.1]					59.6	3.8	1.9		1.9				32.7			
Azithromycin	ND	ND								5.8	40.4	30.8	5.8	11.5	5.8			
Gentamicin	1.9	[0.0-10.3]						67.3	23.1	7.7				1.9				
Ciprofloxacin	96.2	[86.8-99.5]	3.8			13.5	19.2	15.4	1.9			19.2	26.9					
Nalidixic acid	78.8	[62.3-88.9]									5.8	3.8	11.5	5.8	5.8	11.5	55.8	
Colistin	0	[0.0-6.9]							100									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

E. coli was isolated from a total of 52 (13.3%) of 391 bivalve molluscs batch samples in this selective screening. Of these, two isolates showed no decreased susceptibility to quinolones when MICs were determined (Table 21). Quinolone resistant *E. coli* were therefore detected in 50 (12.8% [95% CI: 9.6-16.5%]) of the bivalve molluscs batch samples. In addition to quinolone resistance, 16 isolates were resistant to one or two more antimicrobial agents (mainly ampicillin), while 18 were resistant to additional three or more antimicrobial agents (mainly to ampicilin, sulfamethoxazole, trimetho-prim and tetracycline). Five isolates showed additional resistance to cephalosporins. All five had a cephalosporin resistance profile corresponding to an ESBL phenotype of which two were resistant to cefoxitin resembling also an AmpC phenotype. Genotyping showed that four of these contained $bla_{\text{CTX-M}}$ gr 1 genes, of which two also carried $bla_{\text{CMY-2}}$ (Tables 15 and 16). The resistance genes responsible for the last cephalosporin resistant isolate could not be characterised with the methods used. None of the isolates showed decreased susceptibility to the carbapenem meropenem.

Carbapenemase-producing *Enterobacteriaceae* from Norwegian bivalve molluscs

A total of 391 bivalve molluscs batch samples collected at rearing localities were screened for the presence of carbapenemase-producing *Enterobacteriaceae.* No carbapenemase-producing *Enterobacteriaceae* were detected indicating a prevalence less than 0.94%.

INDICATOR BACTERIA FROM FEED

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Escherichia coli from feed for cattle and swine

A total of 155 samples of dry feed were sampled and examined in 2015 and 2016. *E. coli* isolates were obtained from six (3.9%) of the samples. Only one isolate was resistant and only to one antimicrobial agent (ampicillin). Selective screening for *E. coli* resistant to third generation cephalosporins and quinolones, as well as carbapenemase-producing *Enterobacteriaceae*, was performed on a total of 155 of the samples. No cephalosporin resistant or quinolone resistant *E. coli* were detected, nor any carbapenemase-producing *Enterobacteriaceae*.

Escherichia coli from dog feed

In 2016, a total of 85 samples of dog feed were included. Different feed samples have been examined; supplement feed (1), dry feed (5), dried products (3), and 76 samples of wet feed. Only from the latter category, isolates of *E. coli* were obtained, i.e. 64 (75.3 %) *E. coli* isolates. One isolate per positive sample was susceptibility tested. The results are presented in Table 22 and in the text.

TABLE 22. Antimicrobial resistance in isolates of *Escherichia coli* from samples of dog feed (wet) (n=64) in 2016.

	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	7.8	[1.7-15.2]								92.2					3.1	4.7		
Tigecycline	0	[0.0-5.6]					96.9	3.1										
Chloramphenicol	0	[0.0-5.6]										100						
Ampicillin	4.7	[1.0-13.1]							9.4	37.5	46.9	1.6				4.7		
Cefotaxime	0	[0.0-5.6]					100											
Ceftazidime	0	[0.0-5.6]						100										
Meropenem	0	[0.0-5.6]		100														
Sulfamethoxazole	7.8	[1.7-15.2]										89.1	3.1					7.8
Trimethoprim	3.1	[0.4-10.8]					89.1	7.8							3.1			
Azithromycin	ND	ND								32.8	51.6	15.6						
Gentamicin	0	[0.0-5.6]						68.8	26.6	4.7								
Ciprofloxacin	4.7	[1.0-13.1]	93.8	1.6			3.1	1.6										
Nalidixic acid	4.7	[1.0-13.1]									93.8	1.6		1.6	1.6		1.6	
Colistin	0	[0.0-5.6]							98.4	1.6								

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

In total, 55 out of 64 (85.9% [95% CI: 75.0-93.4%]) *E. coli* isolates from dog feed were susceptible to all antimicrobial agents tested for, three isolates were resistant to one antimicrobial, two to two antimicrobials, two to three antimicrobials and two to four antimicrobials. Resistance to tetracycline and sulfamethoxazole were the most frequently identified resistance determinants, followed by resistance to ampicillin, ciprofloxacin and nalidix acid. None of the isolates displayed any resistance to colistin, nor to the third generation cephalosporins cefotaxime or ceftazidime, indicating a prevalence below 5.6%. Resistance to quinolones (i.e. ciprofloxacin and/or nalidixic acid) was identified in three (4.7% [95% CI: 1.0–13.1%]) of the *E. coli* isolates.

Comparison to previous results from 2000-2002 is difficult due to changes made in methods as well as in sampling procedure. However, resistance to tetracycline and sulfamethoxazole seem to have been among the most frequently detected also in 2000-2002.

Cephalosporin resistant *Escherichia coli* from dog feed

A selective screening for *E. coli* resistant to third generation cephalosporins was performed on the 85 dog feed samples. In total, 15 (17.6% [95% CI:10.2-27.4%]) *E. coli* resistant to third generation cephalosporins were detected. Eight of these were resistant only to beta-lactams, i.e. ampicillin and the third generation cefalosporins cefotaxime and ceftazidime. In addition, there was some resistance observed for sulfamethoxazole, tetracycline and gentamicin.

All the isolates had a cephalosporin resistance profile corresponding to an AmpC phenotype. Genotyping showed that 14 isolates contained the bla_{CMY-2} gene and one had mutations in the promoter and attenuator regions of the chromosomally located *ampC* gene resulting in *ampC* overexpression (Table 15). Four of the isolates showed decreased susceptibility to the carbapenem ertapenem, with MIC values above the EUCAST cut-off value at 0.06 mg/L, i.e. MIC 0.12-0.25 mg/L. Ertapenem is known to have lower specificity for detection of carbapenemase-producing *Enterobacteriaceae* than the two other carbapenems imipenem or meropenem (Cohen *et al.* 2010). None of the isolates were resistant to imipenem or meropenem.

Quinolone resistant *Escherichia coli* from dog feed

Selective screening for quinolone resistant *E. coli* was performed on samples from 85 dog feed samples. One isolate per positive sample was susceptibility tested. Results are presented in the text and in Table 23.

TABLE 23. Antimicrobial resistance in isolates of quinolone resistant *Escherichia coli* from samples of dog feed (wet) (n=44) in 2016.

	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	25.0	[13.2-40.3]								75.0			2.3	2.3	4.5	15.9		
Tigecycline	0	[0.0-8.4]					95.5	4.5										
Chloramphenicol	4.5	[0.6-15.5]										95.5			2.3		2.3	
Ampicillin	27.3	[15.0-42.8]							6.8	13.6	52.3					27.3		
Cefotaxime	0	[0.0-8.0]					100											
Ceftazidime	0	[0.0-8.0]						100										
Meropenem	0	[0.0-8.0]		100														
Sulfamethoxazole	22.7	[11.5-37.8]										70.5	6.8					22.7
Trimethoprim	15.9	[6.6-30.1]					52.3	31.8							15.9			
Azithromycin	ND	ND								13.6	47.7	38.6						
Gentamicin	2.3	[0.1-12.0]						75.0	22.7						2.3			
Ciprofloxacin	100	[92.0-100.0]				13.6	63.6	2.3	11.4			2.3	6.8					
Nalidixic acid	93.1	[81.3-98.6]										4.5	2.3		4.5	29.5	59.1	
Colistin	2.3	[0.1-12.0]							95.5	2.3	2.3							

*Bold vertical lines denote epidemiological cut-off values for resistance. ND=cut-off not defined by EUCAST. 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

By use of the selective method, quinolone resistant *E. coli* were found in 51.8% [95% CI: 40.7-62.7%] of the dog feed samples. Among these, additional resistance to ampicillin, sulfamethoxazole, tetracycline, and trimethoprim was most commonly detected (Table 23).

None of the isolates showed additional resistance to third generation cephalosporins. One isolate showed, however, additional resistance toward colistin, and the plasmid-mediated colistin resistance gene *mcr-1* was identified (see separate text page 70).

Carbapenemase-producing *Enterobacteriaceae* from dog feed

A selective screening for carbapenemase-producing *Enterobacteriaceae* was performed on 85 dog feed samples. No carbapenemase-producing *Enterobacteriaceae* were detected.

ZOONOTIC AND NON-ZOONOTIC ENTEROPATHOGENIC BACTERIA

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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. In Norway, *Salmonella* isolates from control programmes concerning feed samples, animals and food products, as well as diagnostic samples from animals are monitored for antimicrobial resistance.

Regarding human isolates, all *Salmonella*, *Shigella* and *Yersinia enterocolitica* isolates are included, as well as a representative number of *Campylobacter* isolates. Sampling, laboratory methods and data processing are described in Appendix 4.

The NORM-VET results are interpreted according to the determined epidemiological cut-off values (ECOFFs) of the European Committee on Antimicrobial Susceptibility Testing (EUCAST), while NORM results on human isolates of enteropathogenic *Enterobacteriaceae* are interpreted according to the clinical breakpoints set by EUCAST. In absence of clinical breakpoints, ECOFFs as determined by EUCAST or national zone distribution evaluations are used to determine breakpoints. Multi-drug resistance (MDR) has been defined as non-susceptibility to three or more antimicrobial categories, in line with the 2011 ECDC/CDC joint definition.

SALMONELLA SPP.

Salmonella from animals

The situation regarding occurrence of *Salmonella* spp. in food production animals in Norway is very favourable as such animal populations are considered virtually free from *Salmonella* spp. To document and maintain this favourable situation, Norway runs an extensive surveillance programme that covers both live animals (cattle, pigs and poultry) and meat samples. 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 21 and in the text.

TABLE 24. Antimicrobial resistance in *Salmonella* spp. (n=20) from animals (wild birds=8, poultry=3, reptiles=3, cat=2, dog=2, tiger=1) and dog feed (n=1); *S.* Typhimurium (n=14) and other *Salmonella* spp. (n=6) in 2016.

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								20									
Tigecycline	0					20												
Chloramphenicol	0										20							
Ampicillin	0							18	2									
Cefotaxime	0					20												
Ceftazidime	0						20											
Meropenem	0			16	4													
Sulfamethoxazole	0										7	12	1					
Trimethoprim	0					19	1											
Azithromycin	ND								2	17	1							
Gentamicin	0						19	1										
Ciprofloxacin	0	5		15														
Nalidixic acid	0									20								
Colistin	0							19	1									

*Bold vertical lines denote epidemiological cut-off values for resistance. ND = cut-off not defined by EUCAST. White fields denote range of dilutions tested for each antimicrobial agent. MIC values higher than the highest concentration tested for 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 2016, a total of 20 *Salmonella* spp. isolates from animals and dog feed were susceptibility tested. The 14 isolates of *S.* Typhimurium included one each from eight wild birds, two dogs, two cats, and two chicken flocks, respectively. The remaining six isolates belonged to six different serovars; *S.* Bareilly from one poultry flock, *S.* Mbandaka from feed, *S.* München, *S.* Panama and *Salmonella* sp. serogruppe O:57 from reptiles, and *Salmonella* sp. (57 : k : enz15) from a tiger. All of the 20 isolates were fully susceptible to the tested antimicrobials.

Salmonella from human clinical specimens

In 2016, the National Reference Laboratory (NRL) for Enteropathogenic Bacteria at the Norwegian Institute of Public Health (NIPH) performed antimicrobial susceptibility testing on a total of 895 unique *Salmonella* isolates from human infections.
As indicated in Table 25, 20.6% were reported as acquired in Norway, 74.0% were acquired abroad, whereas the place of acquisition was unknown for 5.5% of the isolates.

All isolates were tested for resistance against four different antibiotic groups: beta-lactams (ampicillin, cefotaxime, ceftazidime and meropenem), fluoroquinolones (ciprofloxacin and pefloxacin), gentamicin, and trimethoprim-sulfamethoxazole. In addition, 326 of the 895 isolates were tested for nalidixic acid, azithromycin, tetracycline and chloramphenicol.

TABLE 25. Distribution of human isolates of *Salmonella* serovars (n=895) in 2016 according to place of acquisition.

	Place of acquisition		
	Norway	Abroad	Unknown
S. Typhimurium including *S. enterica* serovar 4,[5],12:i- (n=143)	43	91	9
S. Enteritidis (n=324)	49	259	16
S. Typhi (n=16)	0	15	1
S. Paratyphi (n=7)	0	4	3
Other *Salmonella* (n=402)	92	293	20
Total (n=895)	**184 (20.6%)**	**662 (74.0%)**	**49 (5.5%)**

The major serovars were *S.* Typhimurium (n=84) and its monophasic variant (n=59), with 143 isolates (16.0%) of all *Salmonella* isolates, and *S.* Enteritidis with 324 isolates (36.2%). The number of *S.* Typhi and *S.* Paratyphi isolates remained low. For 2016 their total number was sixteen and seven, respectively.

The results of the antimicrobial susceptibility testing for 2016 *Salmonella* isolates are presented in Tables 26-29, Figures 45-52, and in the text.

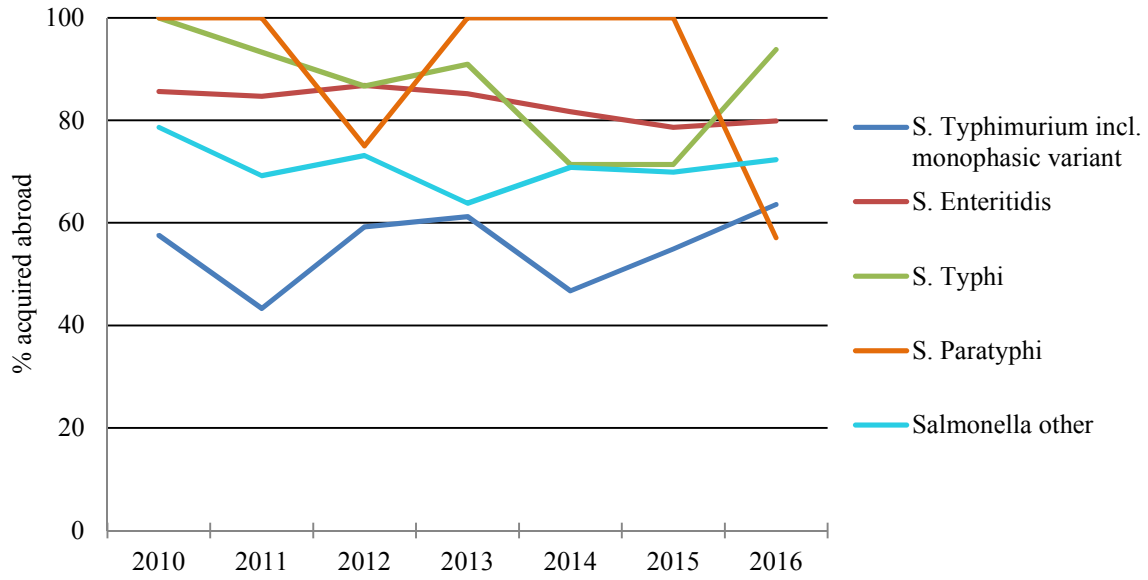

FIGURE 45. Proportions of unique *Salmonella* isolates acquired abroad, stratified by serovars from 2010- 2016.

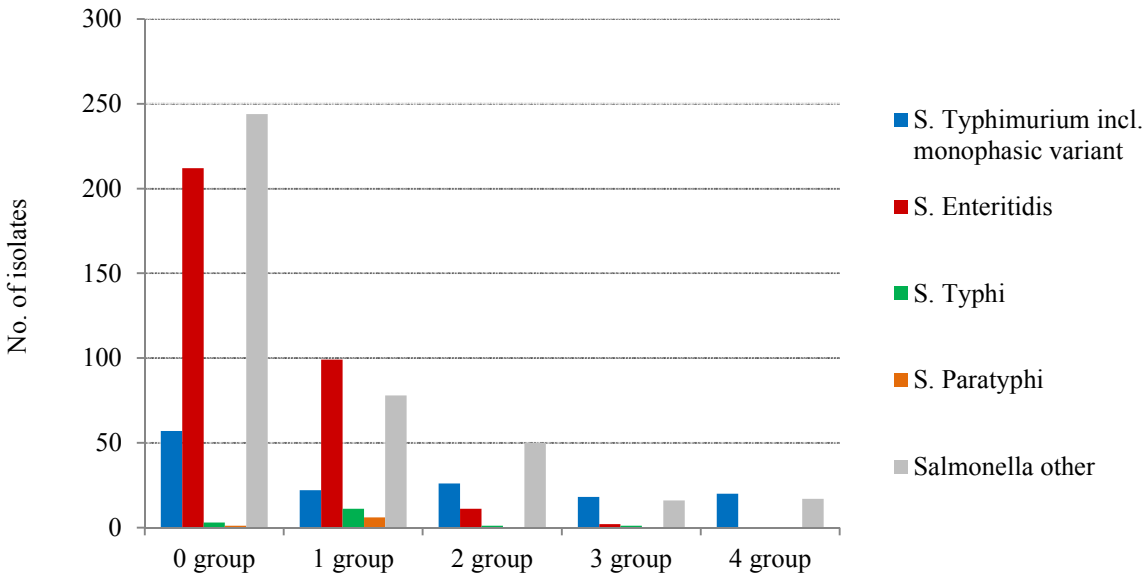

FIGURE 46. The number of antibiotic groups that *Salmonella* isolates in 2016 (n=895) were resistant to, stratified by serovars. The four antibiotic groups tested were; beta-lactams, aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole.

ANITMICROBIAL RESISTANCE IN BLOOD CULTURE ISOLATES OF SALMONELLA

A total of 66 isolates were recovered from blood cultures, representing 7% of all *Salmonella* infections. Four belong to *S.* Typhimurium and its monophasic variant, 18 *S.* Enteritidis (27.3%), 12 *S.* Typhi, five *S.* Paratyphi, and 27 (40.9%) to 19 different other *Salmonella* serovars (Figure 47). Most isolates from blood cultures were tested against seven groups of antibiotics. The number that each group of *Salmonella* was resistant to is shown in Figure 48. The most frequent serovar in blood cultures was *S.* Enteritidis followed by isolates from the "*Salmonella* other" group. Although most *S.* Enteritidis isolates were acquired abroad (79.7%), the level of resistance to the different antibiotic classes remained low.

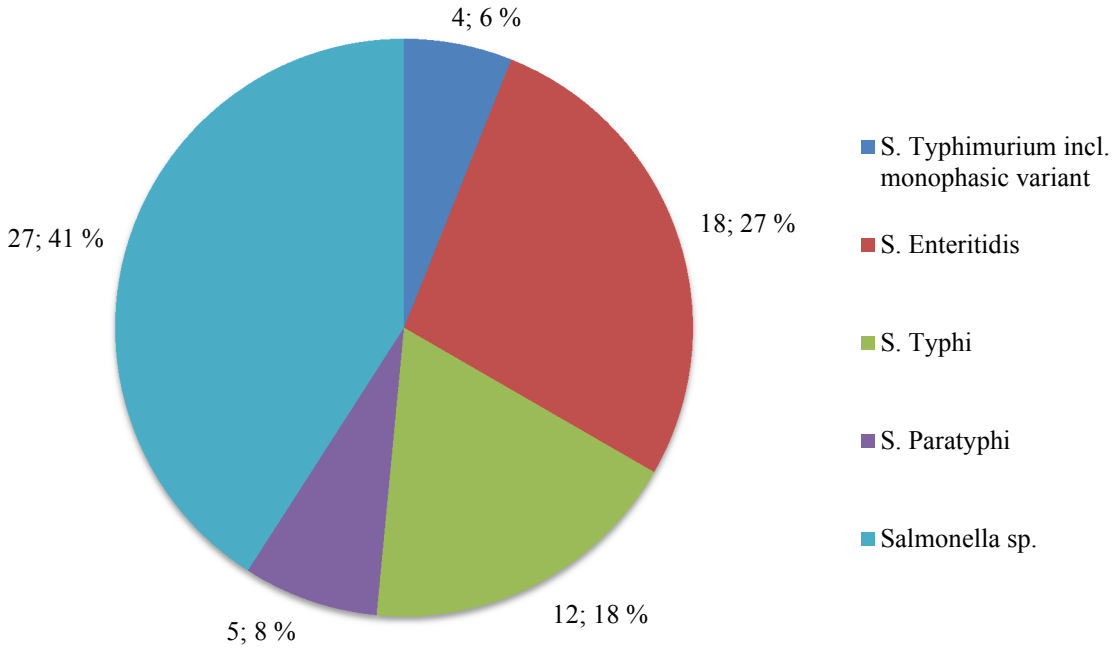

FIGURE 47. Distribution of blood culture isolates into different *Salmonella* serovars (n=66) in 2016.

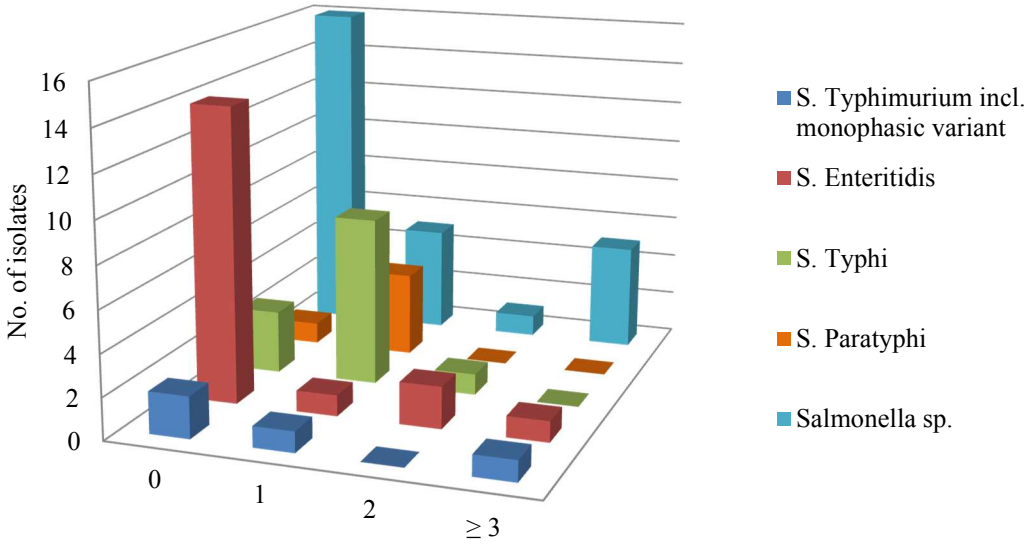

FIGURE 48. Antimicrobial resistance in *Salmonella* isolated from blood culture in 2016 tested against seven antibiotic groups. Displaying the number of isolates resistant to; none, one, two, and three or more antimicrobial groups. The seven antibiotic groups tested were; beta-lactams, aminoglycosides, fluoroquinolones, trimethoprim-sulfamethoxazole, macrolides, tetracycline and chloramphenicol.

RESISTANCE IN SALMONELLA IRRESPECTIVE OF SAMPLE MATERIAL

TABLE 26. Distribution (%) of antimicrobial susceptibility categories of human isolates of domestically acquired *Salmonella* Typhimurium-group (n=43) including *S. enterica* serovar 4,[5],12:i:- (n=13) in 2016. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	55.8	-	44.2
Cefotaxime	≤ 1	> 2	100.0	0.0	0.0
Ceftazidime	≤ 1	> 4	97.7	2.3	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin/Pefloxacin[1]	≥ 24	< 21	86.0	4.7	9.3
Nalidixic acid[2]*	≥ 16	< 16	95.7	-	4.3
Gentamicin	≤ 2	> 4	95.3	0.0	4.7
Azithromycin[3]*	≥ 12	<12	95.7	-	4.3
Tetracycline[3]*	≥ 17	< 17	30.4	-	69.6
Chloramphenicol*	≤ 8	> 8	78.3	-	21.7
Trimethoprim-sulfamethoxazole	≤ 2	> 4	100.0	0.0	0.0

[1] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. Susceptibility to ciprofloxacin is therefore inferred from pefloxacin disk diffusion susceptibility with breakpoints based on epidemiological cut-off values on national zone distribution evaluations in line with EUCAST recommendation. [2] Breakpoints based on EUCAST ECOFFs (accessed June 2017). [3] Epidemiological cut-off values based on national zone distribution evaluations. *Only tested in 23/43 isolates.

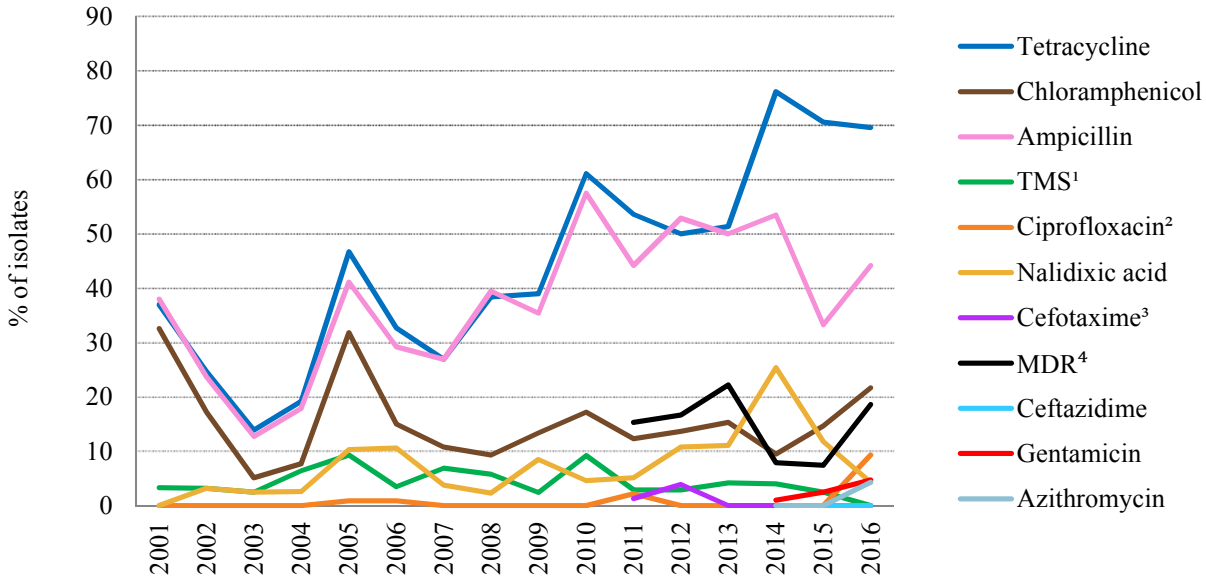

FIGURE 49. Percentage of resistance to various antimicrobial agents in the *Salmonella* Typhimurium-group including *S. enterica* serovar 4,[5],12:i:- from humans infected in Norway 2001-2016. [1]TMS; trimethoprim-sulfamethoxazole. [2]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion susceptibility in 2016. [3]Cefpodoxime was tested before 2014. [4]MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

TABLE 27. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Salmonella* Typhimurium-group (n=91) including *S. enterica* serovar 4,[5],12:i:- (n=42) acquired abroad in 2016. Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	47.3	-	52.7
Cefotaxime	≤ 1	> 2	94.5	0.0	5.5
Ceftazidime	≤ 1	> 4	94.5	1.1	4.4
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin/Pefloxacin[1]	≥ 24	< 21	74.7	12.1	13.2
Nalidixic acid[2]*	≥ 16	< 16	98.3	-	1.7
Gentamicin	≤ 2	> 4	92.3	0.0	7.7
Azithromycin[3]*	≥ 12	<12	96.6	-	3.4
Tetracycline[3]*	≥ 17	< 17	27.6	-	72.4
Chloramphenicol*	≤ 8	> 8	58.6	-	41.4
Trimethoprim-sulfamethoxazole	≤ 2	> 4	83.5	1.1	15.4

[1] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. Susceptibility to ciprofloxacin is therefore inferred from pefloxacin disk diffusion susceptibility with breakpoints based on epidemiological cut-off values on national zone distribution evaluations in line with EUCAST recommendation. [2] Breakpoints based on EUCAST ECOFFs (accessed June 2017). [3] Epidemiological cut-off values based on national zone distribution evaluations. *Only tested in 23/43 isolates.

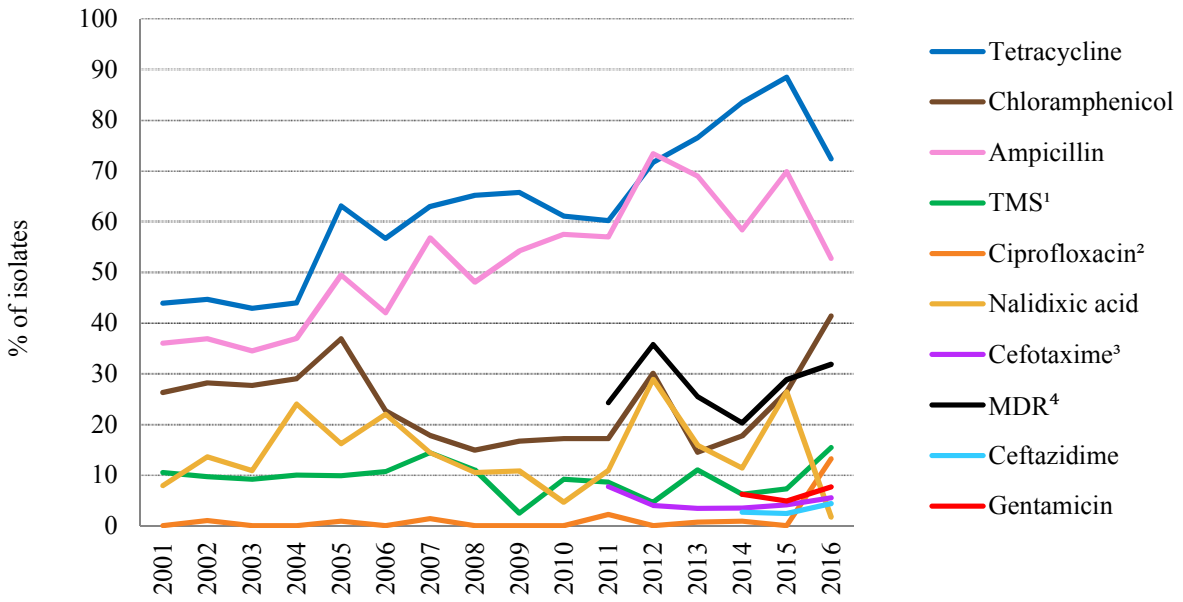

FIGURE 50. Percentage of resistance to various antimicrobial agents in *Salmonella* Typhimurium-group including *S. enterica* serovar 4,[5],12:i:- from humans infected outside Norway 2001-2016. [1]TMS; trimethoprim-sulfamethoxazole. [2]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion susceptibility in 2016. [3]Cefpodoxime was tested before 2014. [4]MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

TABLE 28. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Salmonella* Enteritidis (n=324), acquired during 2016, irrespective of place of acquisition (Norway (n=49); abroad (n=259); unknown (n=16)). Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	96.3	-	3.7
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
Ciprofloxacin/Pefloxacin[1]	≥ 24	< 21	66.0	8.0	25.9
Nalidixic acid[2]*	≥ 16	< 16	70.8	-	29.2
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin[3]*	≥ 12	<12	100.0	-	0.0
Tetracycline[3]*	≥ 17	< 17	96.9	-	3.1
Chloramphenicol*	≤ 8	> 8	100.0	-	0.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	99.4	0.0	0.6

[1] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. Susceptibility to ciprofloxacin is therefore inferred from pefloxacin disk diffusion susceptibility with breakpoints based on epidemiological cut-off values on national zone distribution evaluations in line with EUCAST recommendation. [2] Breakpoints based on EUCAST ECOFFs (accessed June 2017). [3] Epidemiological cut-off values based on national zone distribution evaluations. *Only tested in 96/324 isolates.

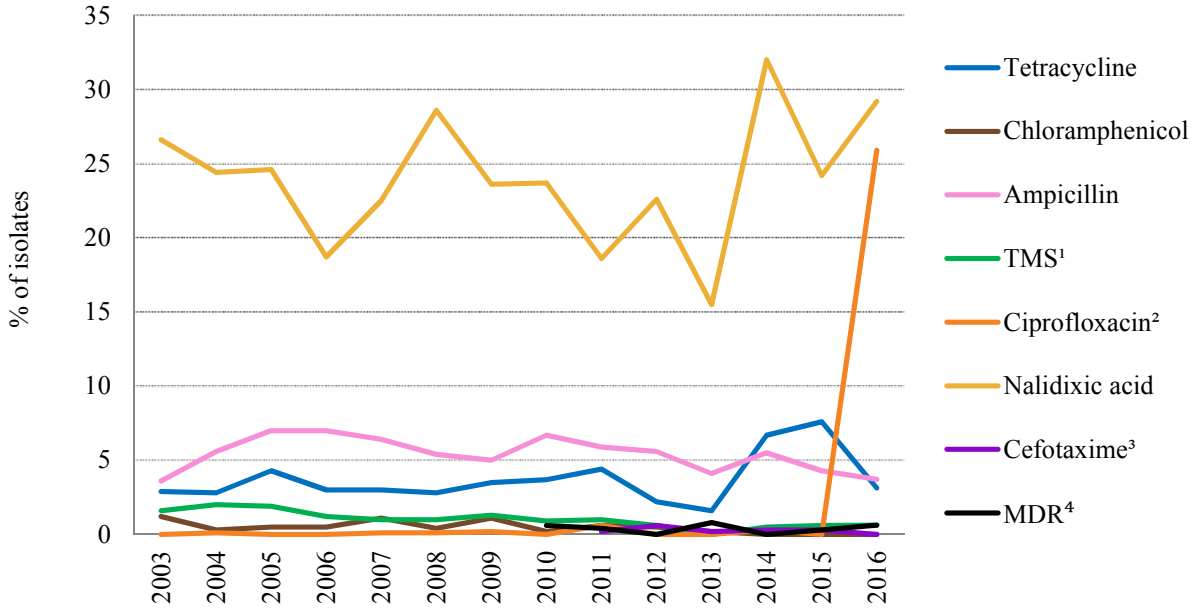

FIGURE 51. Percentage of resistance to various antimicrobial agents in *Salmonella* Enteritidis from humans irrespective of place of infection 2003-2016. [1]TMS; trimethoprim-sulfamethoxazole. [2]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion susceptibility in 2016. [3]Cefpodoxime was tested before 2014. [4]MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

TABLE 29. Distribution (%) of antimicrobial susceptibility categories on human isolates of *Salmonella* spp. including *S.* Paratyphi B variant Java, excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi (n=405), acquired in 2016, irrespective of place of acquisition (Norway (n=92); abroad (n=293); unknown (n=20)). Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	81.5	-	18.5
Cefotaxime	≤ 1	> 2	98.5	0.0	1.5
Ceftazidime	≤ 1	> 4	97.8	1.0	1.2
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin/Pefloxacin[1]	≥ 24	< 21	73.6	7.4	19.0
Nalidixic acid[2]*	≥ 19	< 19	85.3	-	14.7
Gentamicin	≤ 2	> 4	98.3	0.0	1.7
Azithromycin[2]*	≥ 12	<12	98.4	-	1.6
Tetracycline[2]*	≥ 17	< 17	60.5	-	39.5
Chloramphenicol*	≤ 8	> 8	86.8	-	13.2
Trimethoprim-sulfamethoxazole	≤ 2	> 4	88.9	0.0	11.1

[1] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. Susceptibility to ciprofloxacin is therefore inferred from pefloxacin disk diffusion susceptibility with breakpoints based on epidemiological cut-off values on national zone distribution evaluations in line with EUCAST recommendation. [2] Epidemiological cut-off values based on national zone distribution evaluations. *Only tested in 129/405 isolates.

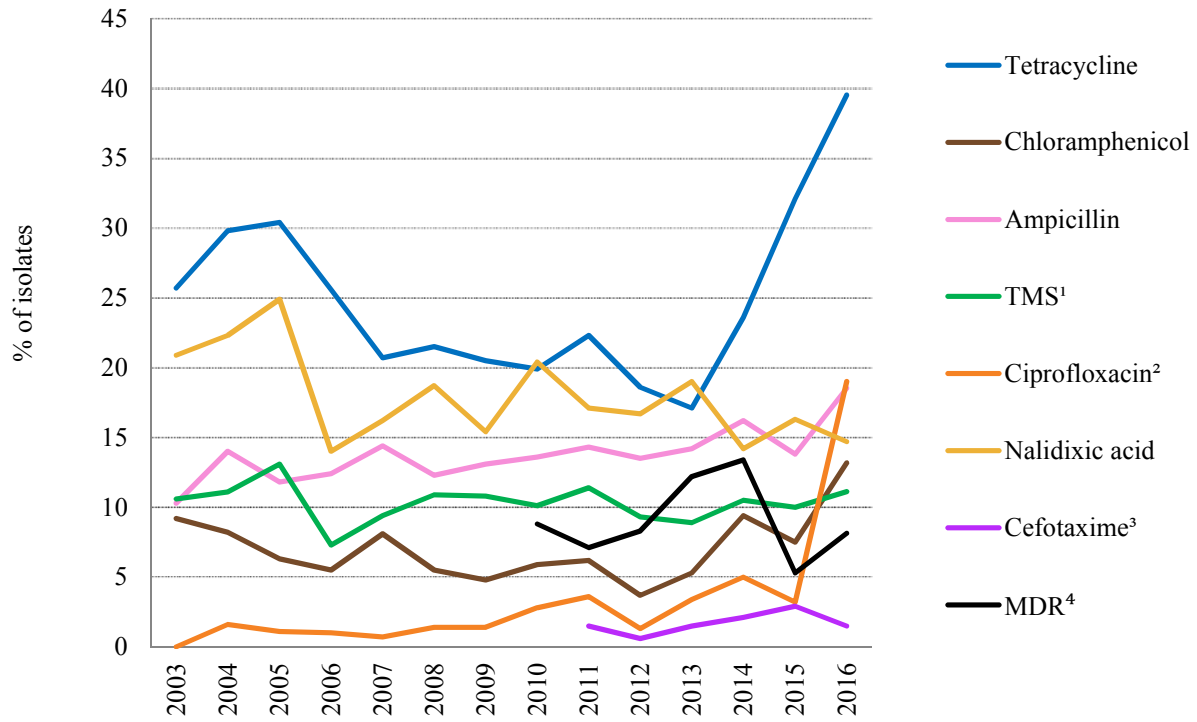

FIGURE 52. Percentage of resistance to various antimicrobial agents in *Salmonella* spp. including *S.* Paratyphi B variant Java; excluding *S.* Typhimurium, *S.* Enteritidis, *S.* Typhi and *S.* Paratyphi, from humans in 2003-2016. [1]TMS; trimethoprim-sulfamethoxazole. [2]Resistance to ciprofloxacin is inferred from pefloxacin disk diffusion susceptibility in 2016. [3]Cefpodoxime was tested before 2014. [4]MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

RESULTS AND COMMENTS

As previously recorded, the overall level of resistance was higher among *S.* Typhimurium and its monophasic variant group, and the "*Salmonella* other" group than *S.* Enteritidis (Figure 46). In previous years, ciprofloxacin resistance was inferred from disk diffusion susceptibility according to local breakpoints in the absence of breakpoints defined by EUCAST. Consequently low-level ciprofloxacin resistance was probably underestimated. In accordance with EUCAST guidelines, for *Salmonella* isolates in 2016 ciprofloxacin resistance was inferred from pefloxacin disk diffusion and therefore a jump in the prevalence of resistance was observed across all serogroups of this species. Breakpoints were set in accordance with locally observed pefloxacin zone distributions (Appendix 4).

For infections acquired in Norway of the *S.* Typhimurium and its monophasic variant group, resistance to ampicillin and tetracycline continued along their upward trend over the last ten years (Figure 49). However for infections acquired abroad, a slight reduction in resistance to both these drugs was observed (Figure 50). Within this group the monophasic variant accounted for 30% of all the domestically acquired infections and 46% of all the infections acquired abroad. The overall level of resistance to all antibiotics was much higher in the monophasic variant of *S.* Typhimurium.

Antibiotic resistance in *S.* Enteritidis isolates was stable over the years (Firgure 51). An increase in ciprofloxacin resistance followed the change in methodology, proposing an overall resistance to ciprofloxacin of 25.9% regardless of place of acquisition.

In the "*Salmonella* other" group, in addition to the jump in resistance prevalence of ciprofloxacin, a significant increase was observed in resistance to tetracycline (Figure 52). In this group, *Salmonella* Stanley, followed by *Salmonella enterica* subspecies enterica O:4 (monophasic variant) were the dominant serotypes, and accounted for 11.1% and 10.8% respectively. The *Salmonella enterica* subspecies enterica O:4 (monophasic variant) was the largest contributor of tetracycline resistance in this group (50%), and 82% of its tested isolates were resistant to tetracycline. The increasing trend of tetracycline resistance may be linked to a steady increase in tetracycline resistance observed in *S.* Stanley isolates, which recorded an increase in tetracycline resistance from 25% to 45% in isolates from 2015 and 2016, respectively.

Also an increasing trend of chloramphenicol resistance was observed among *S.* Typhimurium and its monophasic variant group, and the "*Salmonella* other" groups. This trend was especially profound in the *S.* Typhimurium monophasic variant where resistant isolates increased from 3.1% to 30.2% in 2015 and 2016, respectively.

A total of 11 isolates carried extended spectrum beta-lactamases (ESBL) among the *Salmonella* species in 2016. $ESBL_A$ was carried by four isolates of *S.* Typhimurium or its monophasic variant, and by one each of *S.* Agona, *S.* Saintpaul, *S.* Haifa, *S.* Newport, respectively. $ESBL_M$ was carried by three isolates of *S.* Typhimurium or its monophasic variant, and by one each of *S.* Anatum and *S.* Minnesota, respectively. Two *S.* Typhimurium or its monophasic variant isolates carried both $ESBL_A$ and $ESBL_M$.

CAMPYLOBACTER SPP.

Campylobacter jejuni from broilers

Caecal samples from a total of 160 broiler flocks identified as *Campylobacter* positive in the surveillance programme for *Campylobacter* spp. in broiler flocks in Norway in 2016, were examined. *C. jejuni* isolates were obtained from 141 of these (88.2%). From these 141 isolates, 114 were included for the susceptibility testing (71.3%). The results are presented in Table 30, Figure 53 and in the text.

TABLE 30. Antimicrobial resistance in *Campylobacter jejuni* (n=114) from broilers in 2016.

	Resistance		Distribution (n) of MIC values (mg/L)*												
Substance	(%) [95% CI]		0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256
Tetracycline	5.3	[2.0-11.1]				94.7			0.9		1.8		1.8	0.9	
Erythromycin	0	[0.0-3.1]					100								
Streptomycin	3.5	[1.0-8.7]			0.9	9.6	45.6	38.6	1.8			3.5			
Gentamicin	0	[0.0-3.1]		19.3	45.6	33.3	1.8								
Ciprofloxacin	10.5	[5.5-17.7]		88.6		0.9			0.9	8.8	0.9				
Nalidixic acid	10.5	[5.5-17.7]					0.9	22.8	55.3	10.5		0.9	4.4	5.3	

*Bold vertical lines denote microbiological cut-off values. 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.

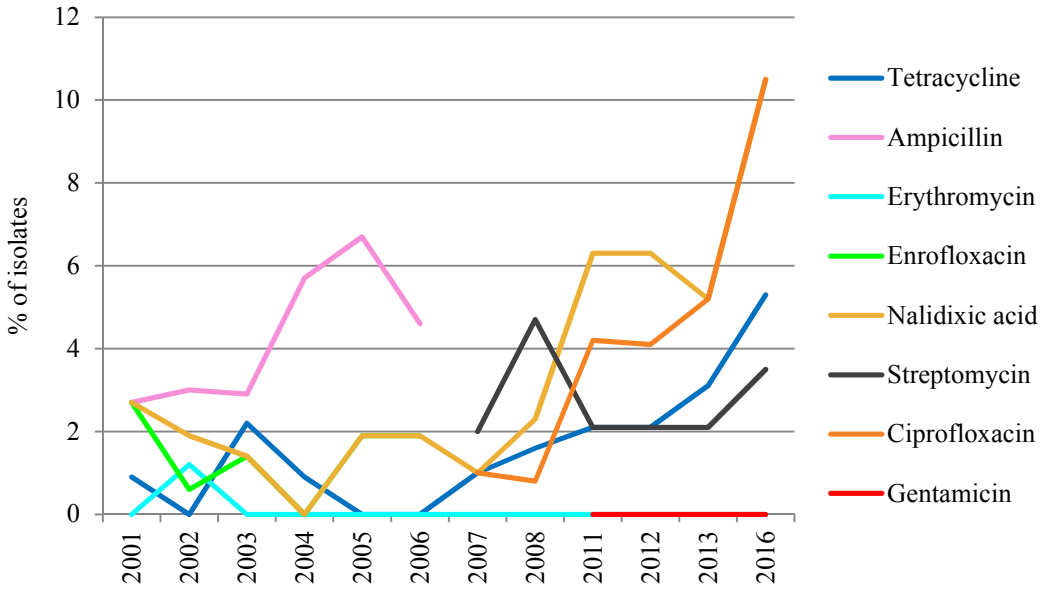

FIGURE 53. Prevalence of resistance to various antimicrobials in *Campylobacter jejuni* from Norwegian broilers 2001-2016. The cut-off values used in NORM-VET 2016 were applied.

RESULTS AND COMMENTS

The prevalence of antimicrobial resistance among *C. jejuni* isolates from Norwegian broilers is moderate. In total, 82.5% of the 114 isolates were susceptible to all anti-microbial agents included in the test panel. Resistance to one or two antimicrobial agents was detected in 7% and 7% of the isolates, respectively. In 3.5% of the isolates, resistance to three antimicrobial agents (quinolones and tetracycline) was detected. Resistance to the quinolones ciprofloxacin and nalidixic acid were the most frequently identified resistance determinants, followed by resistance to tetracycline and streptomycin.

The prevalence of resistance to ciprofloxacin and nalidixic acid seems to have increased over the last years from 1.0% in 2007, to 4.2% in 2011, to 5.2% in 2013, to 10.5% in 2016. However, these are statistically nonsignificant changes and further monitoring is needed to see if this is an upcoming trend. An increase in quinolone resistance in *C. jejuni* from broilers over a six years period (2008-14) has been shown in several of the countries reporting to EFSA (EFSA and ECDC Summary Report 2014). However, in an inter-national perspective, the occurrence of quinolone resistance in *C. jejuni* from Norwegian broilers is quite low, though the occurrence varies between countries reporting to EFSA with the Nordic countries having the lowest resistance levels.

Campylobacter spp. from human clinical cases

Of the 2,317 human campylobacteriosis cases registered in Norway in 2016, 48.1% were reported as acquired abroad. Based on epidemiological data from patients, the vast majority of cases were judged as sporadic. However, only a fraction of the isolates were forwarded to the National Reference Laboratory. Consequently, quality-assured species diagnoses, complete AMR data and molecular epidemiological data on *Campylobacter* isolates are lacking due to resource limitations. Outbreaks with less clear epidemiological links may very well have been overlooked, and the antimicrobial susceptibility testing results presented may therefore be underestimated or overestimated.

Susceptibility testing was performed on a total of 450 *C. jejuni* isolates from 152 patients infected in Norway, 277 infected abroad and 21 where the place of acquisition was unknown, as well as 35 *C. coli* isolates. The results for *C. jejuni* are presented in Tables 31-32, Figures 54-56, and in the text.

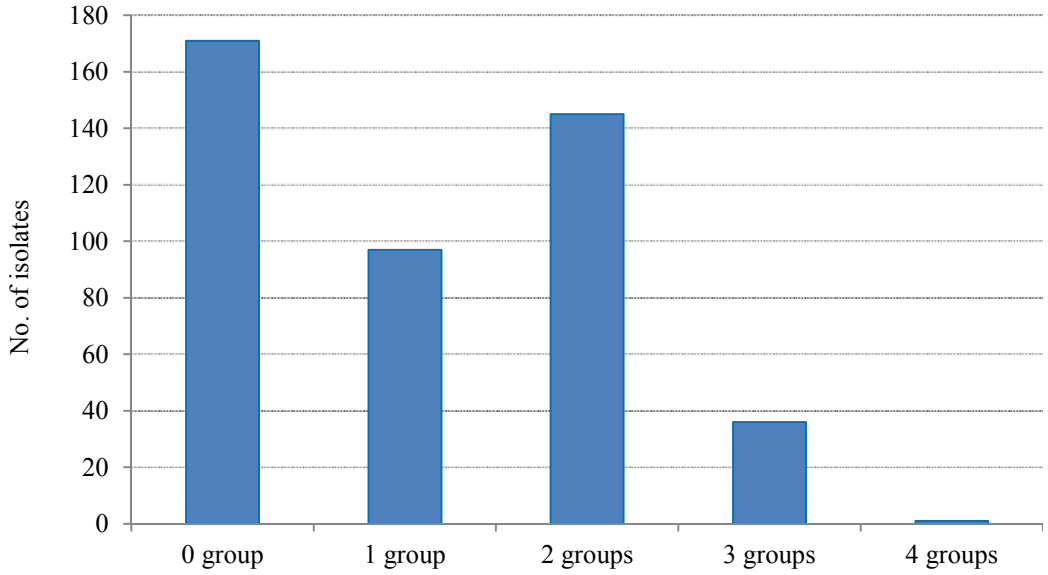

FIGURE 54. The numbers of antimicrobial groups *Campylobacter jejuni* isolates were resistant to in 2016. The four antibiotic groups tested were; tetracycline, fluoroquinolones, aminoglycosides and macrolides.

TABLE 31. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Campylobacter jejuni* from patients infected in Norway in 2016 (n=152).

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	80.3	-	19.7
Erythromycin	≤ 4	> 4	88.8	-	11.2
Gentamicin [1]	≤ 2	> 2	99.3	-	0.7
Nalidixic acid [1]	≤ 16	> 16	61.8	-	38.2
Ciprofloxacin	≤ 0.5	> 0.5	61.2	-	38.8

[1] Epidemiological cut-off values according to EUCAST (accessed June 2017).

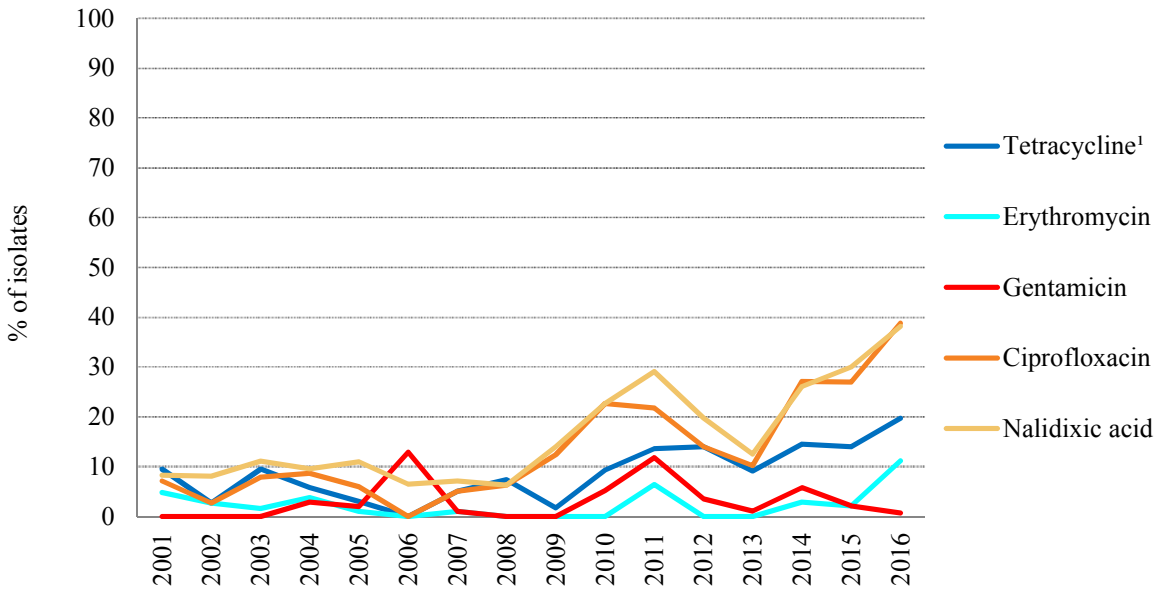

FIGURE 55. Percentage of resistance to various antimicrobial agents in *Campylobacter jejuni* isolated from humans infected in Norway 2001-2016. [1] Doxycycline was tested before 2006.

TABLE 32. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Campylobacter jejuni* from patients infected outside Norway in 2016 (n=277).

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Tetracycline	≤ 2	> 2	47.7	-	52.3
Erythromycin	≤ 4	> 4	84.5	-	15.5
Gentamicin [1]	≤ 2	> 2	99.3	-	0.7
Nalidixic acid [1]	≤ 16	> 16	34.3	-	65.7
Ciprofloxacin	≤ 0.5	> 0.5	35.4	-	64.6

[1] Epidemiological cut-off values according to EUCAST (accessed June 2017).

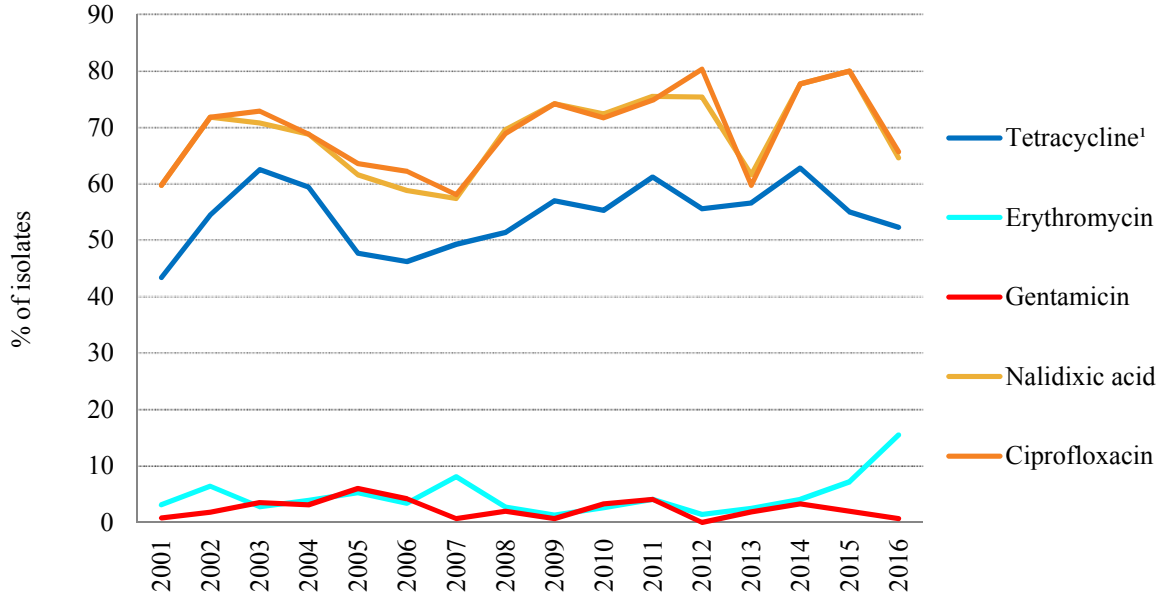

FIGURE 56. Percentage of resistance to various antimicrobial agents in *Campylobacter jejuni* isolated from humans infected outside Norway 2001-2016. [1] Doxycycline was tested before 2006.

RESULTS AND COMMENTS

The data show that resistance was more widespread among *C. jejuni* isolates recovered from patients infected abroad than from patients infected in Norway. Only 28.2% of the isolates from infections acquired abroad were susceptible to all antimicrobial agents tested compared to 55.9% of the isolates from patients infected in Norway. The main difference between the two groups was seen for ciprofloxacin, nalidixic acid and tetracycline. There was a statistically significant difference in levels of resistance between those isolates acquired abroad compared to those acquired in Norway ($p<0.001$ for both antimicrobial groups).

For isolates from patients infected in Norway it seemed to be an upward trend in resistance to all tested antibiotics save gentamicin. For infections acquired in Norway and acquired abroad, a significant increase was seen in resistance to macrolides (erythromycin). When acquired in Norway, resistance increased from 2.1% to 11.2%, and when acquired abroad resistance increased from 7.2% to 15.5%. Isolates resistant to erythromycin (n=63) were also more likely to be MDR (36/37).

Twenty-five of the 35 *C. coli* isolates were acquired abroad. Twenty-four of these were resistant to at least one of the antimicrobial agents tested.

Yersinia enterocolitica from human clinical cases

A total of 57 unique isolates of pathogenic *Yersinia enterocolitica* were analysed in 2016. Forty-two belonged to serogroup 3 including 14 acquired in Norway, 26 acquired abroad and two with an unknown place of acquisition. Fifteen isolates belonged to serogroup 9, of which eight were acquired in Norway, six acquired abroad and one isolate was acquired from an unknown location. No *Y. pseudotuberculosis* isolates were recovered in 2016. All *Y. enterocolitica* isolates were tested for susceptibility to four antimicrobial groups (beta-lactams, quinolones, aminoglycosides, and trimethoprim-sulfamethoxazole) whereas only thirteen isolates were tested against all seven groups. The results are presented in Table 33 and Figures 57-58.

The crude number of isolates was considered low, and judgements should consequently be more conservative regarding AMR results for *Y. enterocolitica* than for other enteropathogenic bacteria.

TABLE 33. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Yersinia enterocolitica* serogroups O:3 and O:9 from patients infected in 2016 (n=57). Sampling, laboratory methods, and data handling are described in Appendix 4. Distributions of zone diameters are available at www.antibiotikaresistens.no.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	0.0	-	100.0
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
Ciprofloxacin	≤ 0.25	> 0.5	91.2	0.0	8.8
Nalidixic acid [1]*	≥ 16	< 16	84.6	-	15.4
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [1]*	≥ 12	< 12	100.0	-	0.0
Tetracycline [1]*	≥ 17	< 17	92.3	-	7.7
Chloramphenicol *	≤ 8	> 8	92.3	-	7.7
Trimethoprim-sulfamethoxazole	≤ 2	> 4	98.2	0.0	1.8

[1] Epidemiological cut-off values based on zone distribution evaluations. *Only tested in 13/57 isolates.

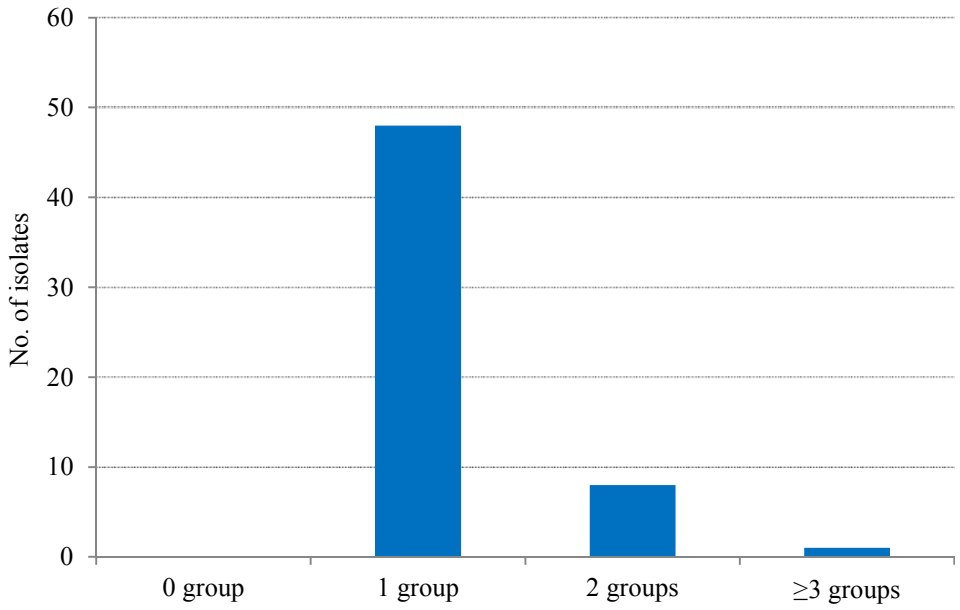

FIGURE 57. The numbers of antimicrobial groups that *Yersinia enterocolitica* isolates were resistant to in 2016.

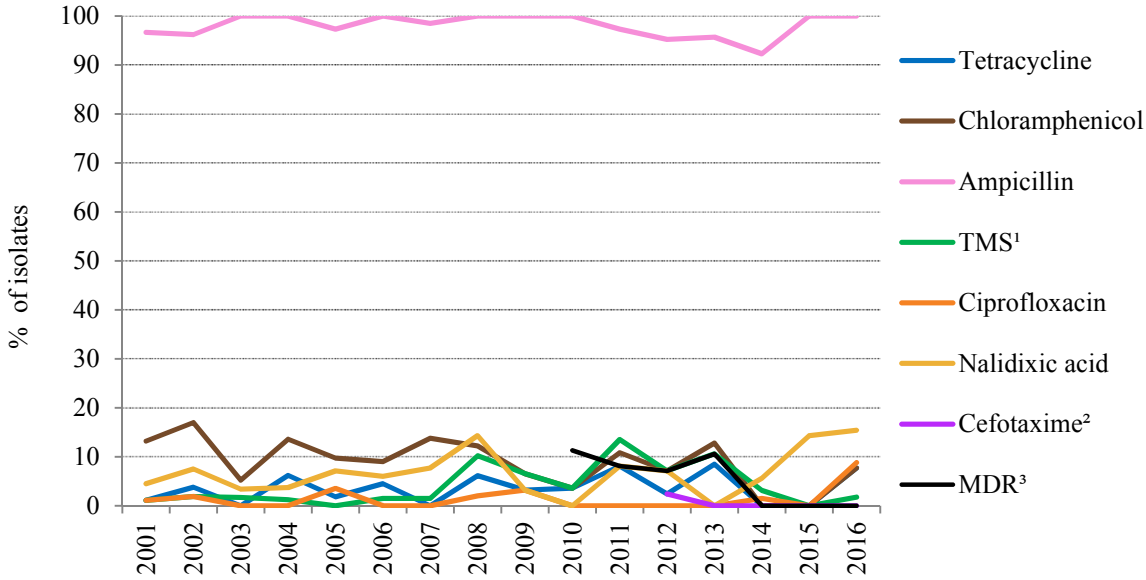

FIGURE 58. Prevalence of resistance to various antimicrobials in *Yersinia enterocolitica* from humans in Norway 2001-2016. [1] TMS; trimethoprim-sulfamethoxazole. [2] Cefpodoxime was tested before 2014. [3] MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

RESULTS AND COMMENTS

The prevalence of resistance to all antimicrobial agents appeared stable during the years 2001-2016. All isolates of pathogenic *Yersinia enterocolitica* expressed intrinsic resistance to ampicillin.

Shigella spp. from human clinical cases

In 2016, only 11 (13.3%) of the 83 unique isolates of *Shigella* were domestically acquired, and the prevalence of resistance presented in this report predominantly relates to isolates from infections acquired abroad. The species distribution of the 83 *Shigella* isolates that were tested for drug susceptibility was as follows: *S. sonnei* 55 (66.3%); *S. flexneri* 24 (28.9%); *S. boydii* 3 (3.6%); *S. dysenteriae* 1 (1.2%). The numbers of antimicrobial agents that *Shigella* isolates were resistant to are shown in Figure 59. All isolates were tested for resistance against four different antibiotic groups: beta-lactams (ampicillin, cefotaxime, ceftazidime, and meropenem), ciprofloxacin, gentamicin and trimethoprim-sulfamethoxazole. In addition, seventeen of the isolates were tested for nalidixic acid, azithromycin, tetracycline, and chloramphenicol. The results for *S. sonnei* and *S. flexneri* are presented in Table 34 and Figure 60, and in Table 35 and Figure 61, respectively.

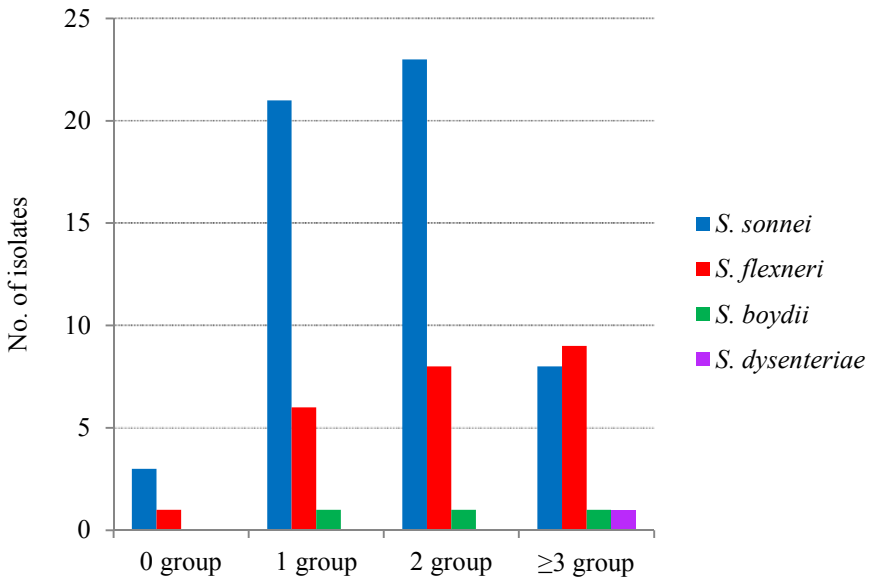

FIGURE 59. The number of antibiotic groups that *Shigella* isolates were resistant to stratified by species in 2016.

TABLE 34. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Shigella sonnei* in 2016 (n=55). Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	83.6	-	16.4
Cefotaxime	≤ 1	> 2	94.5	0.0	5.5
Ceftazidime	≤ 1	> 4	96.4	0.0	3.6
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Ciprofloxacin	≤ 0.25	> 0.5	58.2	9.1	32.7
Nalidixic acid [1]*	≥ 16	< 16	58.3	-	41.7
Gentamicin	≤ 2	> 4	98.2	0.0	1.8
Azithromycin [1]*	≥ 12	< 12	91.7	-	8.3
Tetracycline [1]*	≥ 17	< 17	16.7	-	83.3
Chloramphenicol *	≤ 8	> 8	100.0	-	0.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	10.9	5.5	83.6

[1] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 12/55 isolates.

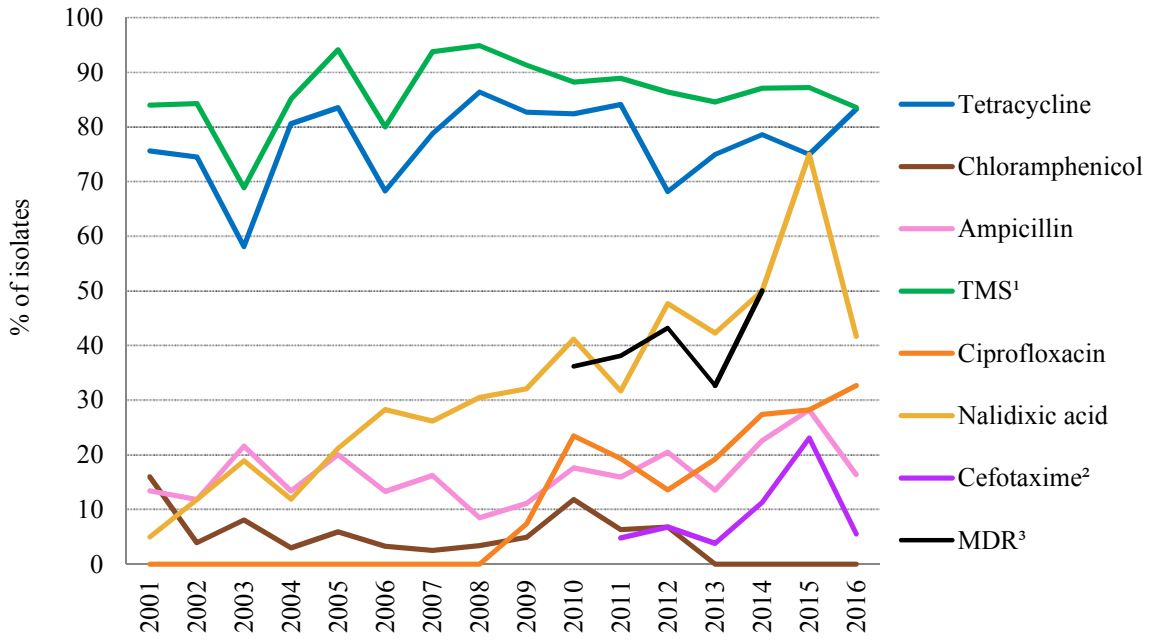

FIGURE 60. Prevalence of resistance to various antimicrobial agents in *Shigella sonnei* from humans in Norway 2001-2016. [1]TMS; trimethoprim-sulfamethoxazole. [2]Cefpodoxime was tested before 2014. [3]MDR was based on five antibiotic groups tested from 2011-2013 and seven groups tested from 2014 onwards.

TABLE 35. Distribution (%) of antimicrobial susceptibility categories of human isolates of *Shigella flexneri* in 2016 (n=24). Sampling, laboratory methods, and data handling are described in Appendix 4.

	Breakpoints (mg/L or mm)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 8	> 8	8.3	-	91.7
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
Ciprofloxacin	≤ 0.25	> 0.5	70.8	8.3	20.8
Nalidixic acid [1]*	≥ 16	< 16	100.0	-	0.0
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Azithromycin [1] *	≥ 12	< 12	80.0	-	20.0
Tetracycline [1] *	≥ 17	< 17	0.0	-	100.0
Chloramphenicol *	≤ 8	> 8	20.0	-	80.0
Trimethoprim-sulfamethoxazole	≤ 2	> 4	41.7	0.0	58.3

[1] Epidemiological cut-off values based on zone distribution evaluations. * Only tested in 5/24 isolates

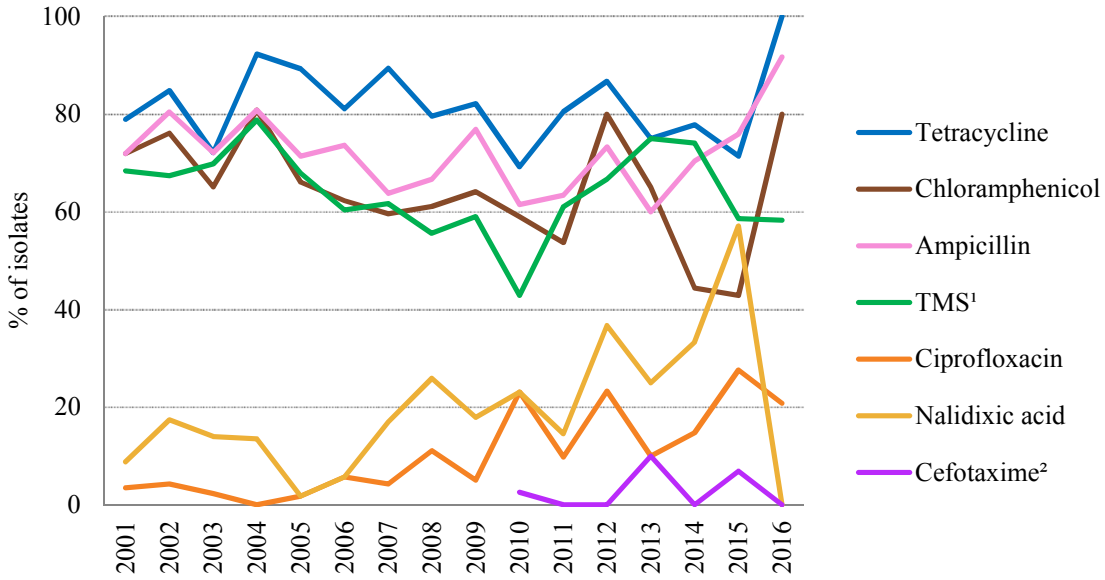

FIGURE 61. Prevalence of resistance to various antimicrobial agents in *Shigella flexneri* from humans in Norway 2001-2016. [1] TMS; trimethoprim-sulfamethoxazole. [2] Cefpodoxime was tested before 2014.

RESULTS AND COMMENTS

The resistance patterns for *S. sonnei* have been fairly stable during the period from 2001 to 2016. However resistance to the fluoroquinolones has been on an upwards trend since 2001 although a drop in resistance to nalidixic acid was observed in 2016, probably attributable to the low number of isolates tested. A similar observation for resistance to nalidixic acid and ciprofloxacin was recorded for *S. flexneri* isolates. The proportion of multi-drug resistance in both *S. sonnei* and *S. flexneri* (14.5% and 37.5%, respectively) was higher than in *Salmonella* as a whole (8.1%).

Three *Shigella sonnei* isolates (5.5%) were phenotypically $ESBL_A$ producers with inhibitory effect of clavulanic acid. $ESBL_M$ was carried by one *Shigella boydii* isolate.

HUMAN CLINICAL ISOLATES

Cecilie Torp Andersen, Elisabeth Astrup, Dominique Caugant, Petter Elstrøm, Hege Enger, Frode Width Gran, Aleksandra Jokovljev, Karin Rønning, Gunnar Skov Simonsen, Dagfinn Skaare, Martin Steinbakk and Didrik Vestrheim

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 36, 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 *Propionibacterium* 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 36. Number of blood culture isolates in 2016, proportion of all isolates, and proportion of isolates excluding possible skin contaminants (coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp.) 2012-2016. The table is based on data from the information systems of all laboratories in Norway.

Species	No. of isolates 2016	% of all isolates					% of all isolates excluding skin flora				
		2012	2013	2014	2015	2016	2012	2013	2014	2015	2016
Staphylococcus aureus	1,752	11.3	11.5	11.0	11.1	10.5	15.0	14.3	14.2	14.4	13.6
Coagulase negative staphylococci	3,434	22.5	17.4	20.4	21.1	20.7	-	-	-	-	-
Streptococcus pneumoniae	563	4.2	4.2	3.6	3.2	3.4	5.6	5.2	4.6	4.2	4.4
Streptococcus pyogenes	176	1.0	1.3	1.1	1.3	1.1	1.3	1.6	1.4	1.7	1.4
Streptococcus agalactiae	270	1.6	1.7	1.6	1.7	1.6	2.1	2.1	2.0	2.2	2.1
Beta-haemolytic streptococci group C and G	221	1.2	1.2	1.2	1.5	1.3	1.6	1.5	1.6	2.0	1.7
Viridans- and non-haemolytic streptococci	831	3.8	5.5	4.6	4.6	5.0	5.1	6.8	5.9	6.0	6.5
Enterococcus faecalis	598	4.0	4.1	3.8	3.1	3.6	5.3	5.1	5.0	4.0	4.6
Enterococcus faecium	241	1.5	1.8	1.6	1.4	1.4	2.0	2.2	2.1	1.8	1.9
Other Gram-positive aerobic and facultative anaerobic bacteria	544	3.1	3.3	3.5	3.6	3.3	1.9	2.0	2.0	2.3	2.3
Escherichia coli	4,153	23.9	24.4	24.4	24.8	24.9	31.4	30.4	31.5	32.4	32.2
Klebsiella spp.	1,178	6.5	6.8	7.0	6.9	7.1	8.6	8.4	9.0	9.1	9.2
Enterobacter spp.	281	1.9	1.9	1.9	1.7	1.7	2.5	2.4	2.5	2.3	2.2
Proteus spp.	274	1.3	1.7	1.6	1.6	1.6	1.8	2.1	2.1	2.1	2.1
Other *Enterobacteriaceae*	295	2.0	2.3	2.2	1.8	1.8	2.7	2.9	2.9	2.3	2.3
Pseudomonas spp.	260	1.7	1.7	1.8	1.7	1.6	2.2	2.1	2.3	2.2	2.0
Other Gram negative aerobic and facultative anaerobic bacteria	392	2.0	2.1	2.0	2.1	2.4	2.6	2.6	2.6	2.7	3.0
Bacteroides spp.	309	2.3	2.4	2.2	2.2	1.9	3.0	3.0	2.9	2.8	2.4
Other anaerobic bacteria	636	2.8	3.2	3.1	3.2	3.8	3.3	3.5	3.6	3.7	4.4
Yeasts	218	1.4	1.5	1.4	1.4	1.3	2.0	1.8	1.8	1.8	1.7
Total	**16,626**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**	**100.0**

As seen in Table 36 and Figure 62, aerobic and facultative Gram-positive and Gram-negative bacteria represented 51.9% and 41.1% 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 20.7%. This is at the same level as 21.1% in 2015, 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 *Propioni-bacterium* spp.) were excluded with 38.5% aerobic Gram-positives and 53.0% aerobic Gram-negatives.

Among aerobic Gram-positives, the prevalence of *S. pneumoniae* has steadily declined from 12.1% in 2005 to 4.4% in 2016 (skin contaminants excluded), following the introduction of the conjugate pneumococcal vaccine in the national childhood immunisation programme in June 2006. The proportions of other aerobic Gram-positives have remained stable over many years.

E. coli (32.2%) and other *Enterobacteriaceae* (15.8%) accounted for the vast majority of aerobic Gram-negative isolates, but the proportions have remained relatively unchanged over the years. *Pseudomonas* spp. (2.0%) 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 1.3% (1.7% 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* (0.9%/1.1%). However, a multitude of other species was also represented.

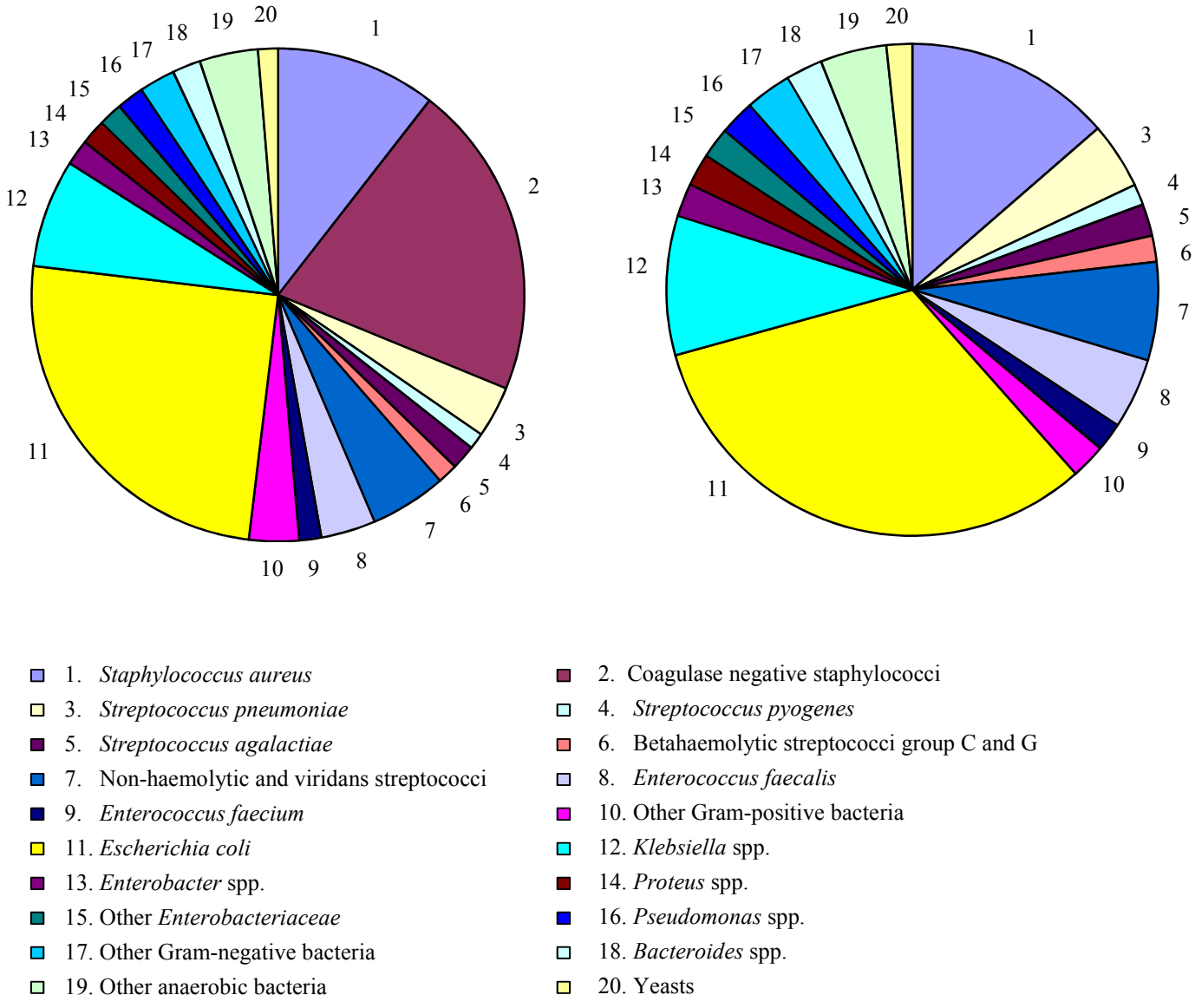

FIGURE 62. Distribution of all blood culture isolates (left, n=16,626) and blood culture isolates excluding common skin contaminants (right, n=12,868) such as coagulase negative staphylococci, *Micrococcus* spp., *Bacillus* spp., *Corynebacterium* spp. and *Propionibacterium* spp. Data for 2016 were retrieved from the information systems of all Norwegian laboratories.

Escherichia coli in blood cultures

TABLE 37. *Escherichia coli* blood culture isolates in 2016 (n=1,940). 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
Ampicillin	≤ 8	> 8	56.5	-	43.5
Amoxicillin-clavulanic acid	≤ 8	> 8	88.2	-	11.8
Piperacillin-tazobactam	≤ 8	> 16	94.3	3.8	1.9
Cefuroxime	≤ 8	> 8	90.6	-	9.4
Cefotaxime	≤ 1	> 2	93.8	0.2	6.0
Ceftazidime	≤ 1	> 4	93.9	1.2	4.9
Cefepime	≤ 1	> 4	93.3	2.8	3.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	93.3	0.4	6.3
Ciprofloxacin	≤ 0.25	> 0.5	82.9	4.5	12.6
Tigecycline	≤ 1	> 2	99.9	0.1	0.0
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	73.5	0.7	25.8
ESBL	Negative	Positive	94.2	-	5.8

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

NORM results are interpreted according to the breakpoints of the Norwegian Working Group for Antibiotics (NWGA) at the time of analysis, which correspond to EUCAST breakpoints.

The vast majority of isolates remained fully susceptible to broad-spectrum antimicrobial agents such as cefotaxime (93.8%), ceftazidime (93.9%), gentamicin (93.3%), cefepime (93.3%), piperacillin-tazobactam (94.3%), tigecycline (99.9%) and meropenem (100.0%) (Table 37). There were no significant changes in the prevalence of susceptibility for these agents from 2015 to 2016.

The prevalence of non-susceptibility (intermediate susceptibility and resistance) to gentamicin remained stable at 6.7% in 2016 compared to 6.4% in 2015 (Figure 63). However, the prevalence of gentamicin resistance is approximately eight times higher than at the turn of the century. A high proportion of gentamicin non-susceptible isolates (56/130, 43.1%) also produced ESBL enzymes. They were retrieved from 18 different laboratories across the country. The prevalence at individual laboratories varied due to relatively small numbers. When aggregated by region, the prevalence of gentamicin non-susceptibility was higher in the South-East (7.5%) compared to the North (6.7%), Middle (6.6%) and West (4.9%) regions.

The prevalence of non-suscpetibility to ciprofloxacin was 17.1% (4.5% I and 12.6% R). 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, and the results from previous years have been updated according to the 2017 breakpoint table. The apparent increase from 11.9% non-susceptibility in 2015 is only a relatively minor change from 16.6% to 17.1% when adjusting for the new breakpoints. The temporal association between ciprofloxacin non-susceptibility and ciprofloxacin usage is depicted in Figure 64. 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 sustained reduction of quinolone resistance rates. The resistance rates for ampicillin (43.5% in 2016, 44.1% in 2015) and trimethoprim-sulfamethoxazole (25.8% in 2016, 26.9% in 2015) are relatively stable.

Detection of extended spectrum beta-lactamases (ESBL) was based on zone diameters of cefotaxime and ceftazidime. All isolates with reduced susceptibility were further characterised by combination MIC gradient tests. A total of 113 isolates (5.8%) were reported as ESBL positive which is at the same level as in 2014 (5.8%) and 2015 (6.5%) (Figure 66). The isolates originated from 18 participating laboratories across the country. Estimates at laboratory level are uncertain due to small numbers. When aggregated at regional level there were only minor geographical differences in the prevalence of ESBL (South-East 5.4%, West 7.4%, Middle 5.2% and North 5.8%). All 113 ESBL isolates were non-susceptible to ampicillin and cefuroxime, and most of them were also non-susceptible to cefotaxime (n=111), ceftazidime (n=104) and cefepime (n=101). Many isolates were intermediately (n=26) or even fully susceptible (n=77) to piperacillin-tazobactam. Most displayed high level of co-resistance to ciprofloxacin (n=87), gentamicin (n=55) and/or trimethoprim-sulfamethoxazole (n=85). All were fully susceptible to tigecycline and meropenem. Sixteen additional isolates were reported as non-susceptible to cefotaxime (n=7) and/or ceftazidime (n=15) without being confirmed as ESBL producers.

One-hundred nine *E. coli* isolates with suspected ESBL production were molecularly characterised and revealed a predominance of CTX-M groups 1 (n=69) and 9 (n=32). The remaining eight isolates harboured derepressed chromosomally encoded AmpC (n=2), CMY (n=5) and TEM (n=1) beta-lactamases. No isolates with carbapenemase production were detected.

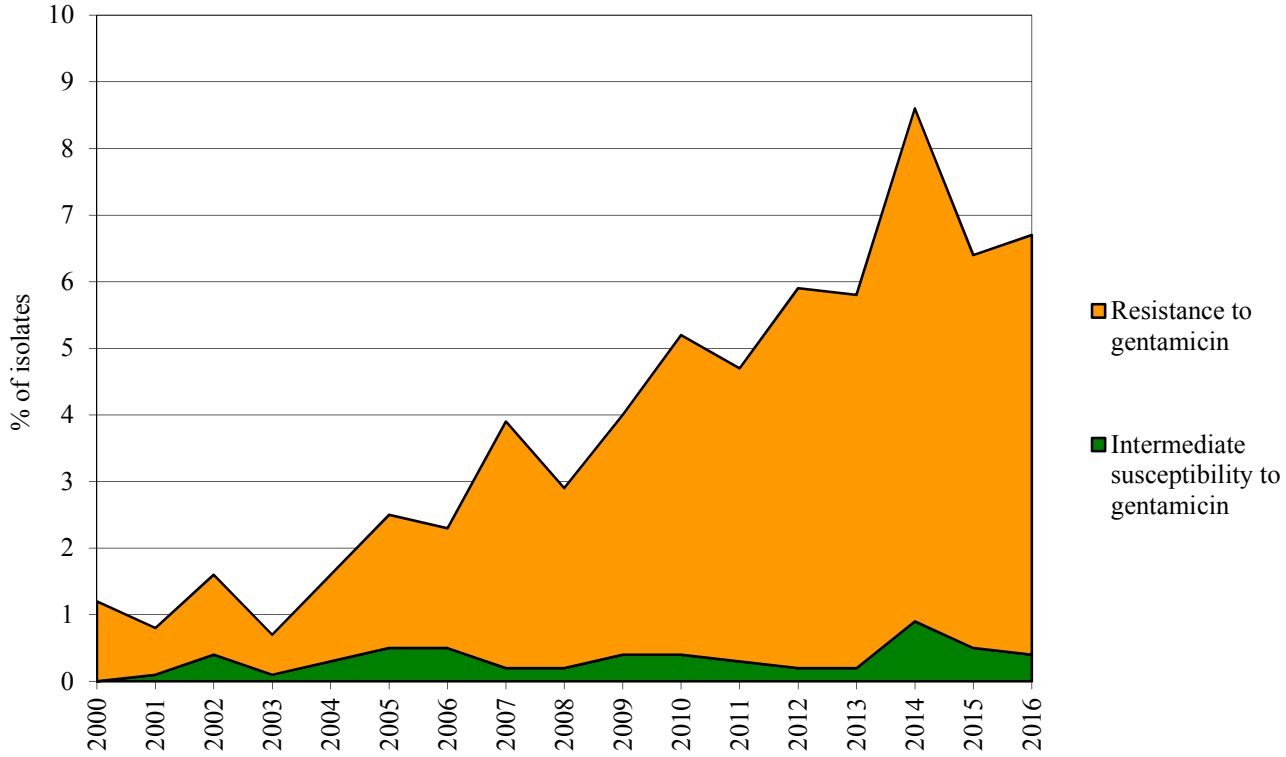

FIGURE 63. Prevalence of intermediate susceptibility and resistance to gentamicin in *Escherichia coli* blood culture isolates 2000-2016.

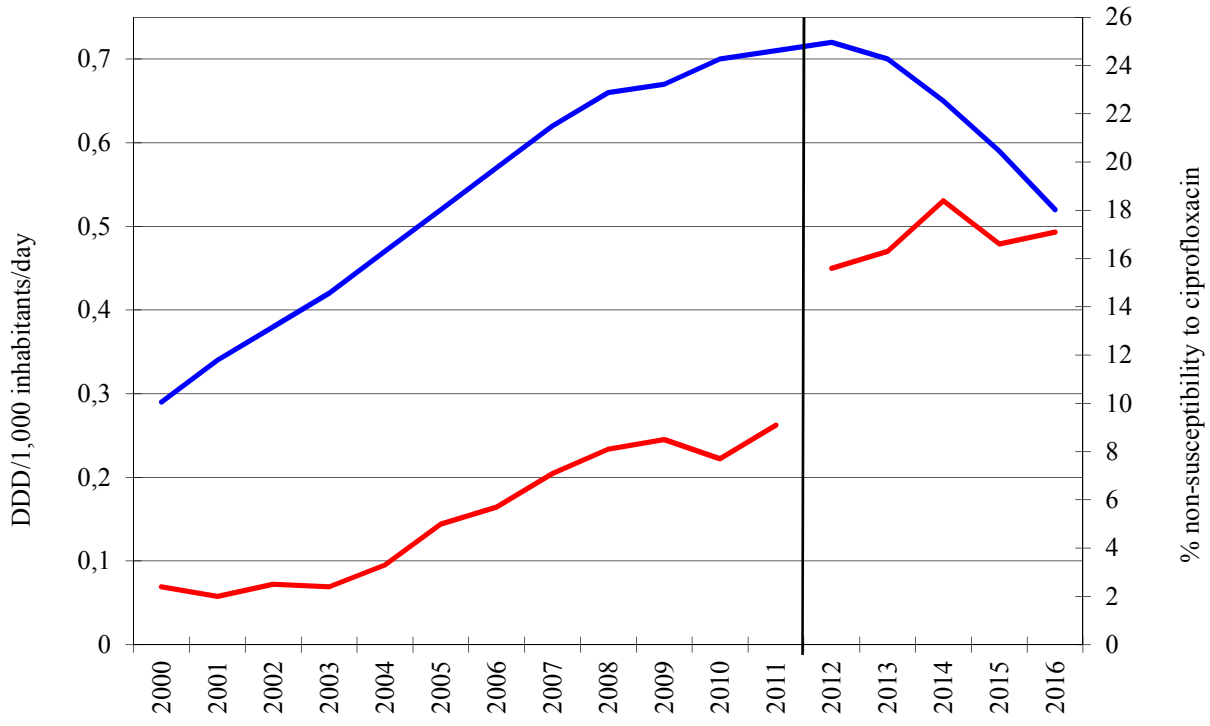

FIGURE 64. Usage of ciprofloxacin (blue) and prevalence of ciprofloxacin non-susceptibility in *Escherichia coli* blood culture isolates (red) as defined by MIC > 0.5 mg/L (2000-2011) and MIC > 0.25 mg/L (2012-2016). The breakpoint cannot be calibrated over the entire time period due to changes in susceptibility testing methodology in 2012.

Escherichia coli in urine

TABLE 38. *Escherichia coli* urinary tract isolates in 2016 (n=1,621). 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
Ampicillin	≤ 8	> 8	64.0	-	36.0
Mecillinam	≤ 8	> 8	94.1	-	5.9
Amoxicillin-clavulanic acid*	≤ 32	> 32	92.6	-	7.4
Cefuroxime	≤ 8	> 8	94.5	-	5.5
Cefotaxime	≤ 1	> 2	96.5	0.5	3.0
Ceftazidime	≤ 1	> 4	97.0	0.6	2.4
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	95.6	0.0	4.4
Ciprofloxacin	≤ 0.25	> 0.5	88.1	3.1	8.8
Nitrofurantoin	≤ 64	> 64	98.5	-	1.5
Trimethoprim	≤ 2	> 4	78.2	0.2	21.6
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	80.3	0.3	19.4
ESBL	Negative	Positive	97.0	-	3.0

*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 2016 is shown in Table 38 and the rates of resistance for 2000-2016 are shown in Figure 65.

The resistance rates among urinary tract isolates have remained relatively stable over the last ten years, but are 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 decreased over the last two years to around 20%. The prevalence of resistance to mecillinam is stable at 5.9% in 2016 compared to 5.1% in 2015 and 6.0% in 2014.

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 2016, 8.8% of isolates were resistant to ciprofloxacin in addition to 3.1% that were intermediately susceptibe. The corresponding rates for blood culture isolates were 4.5% intermediate susceptibility and 12.6% resistance in 2016. 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 7.4% in 2016 compared to 6.1% in 2015 and 7.9% in 2014. The breakpoint used (R < 32 mg/L) is only valid for uncomplicated urinary tract infections. Almost all isolates (98.5%) remained fully susceptible to nitrofurantoin.

Forty-nine isolates (3.0%) were reported as ESBL producers, which is at the same level as in 2015 (3.1%). As seen in Figure 66, the prevalence of *E. coli* ESBL is still lower in urine than in blood culture isolates (5.8%). The ESBL positive strains were isolated at 16 different laboratories in all parts of the country. Thirty-seven isolates were retrieved from samples submitted by general practitioners, while the others were found in hospitalised patients (n=16) and patients in nursing homes (n=6). The ESBL strains were all resistant to ampicillin and cefuroxime, as well as non-susceptible to cefotaxime (46/49) and ceftazidime (37/49). Most isolates were registered as *in vitro* susceptible to mecillinam (44/49). The clinical relevance of this finding is doubtful, since mecillinam is not stable for most beta-lactamases. Many of the ESBL isolates were non-susceptible to quinolones (34/49) and trimethoprim-sulfamethoxazole (27/49), but remained susceptible to nitrofurantoin (48/49) and gentamicin (33/49). All ESBL isolates were clinically susceptible to carbapenems.

Molecular characterisation of all fourty-nine isolates with phenotypical ESBL production revealed a predominance of CTX-M groups 1 (n=27) and 9 (n=18), or a combination of the two (n=1). A few isolates harboured derepressed, chromosomally encoded AmpC (n=2) enzymes or an unknown beta-lactamase (n=1). No isolates with carbapenemase production were detected.

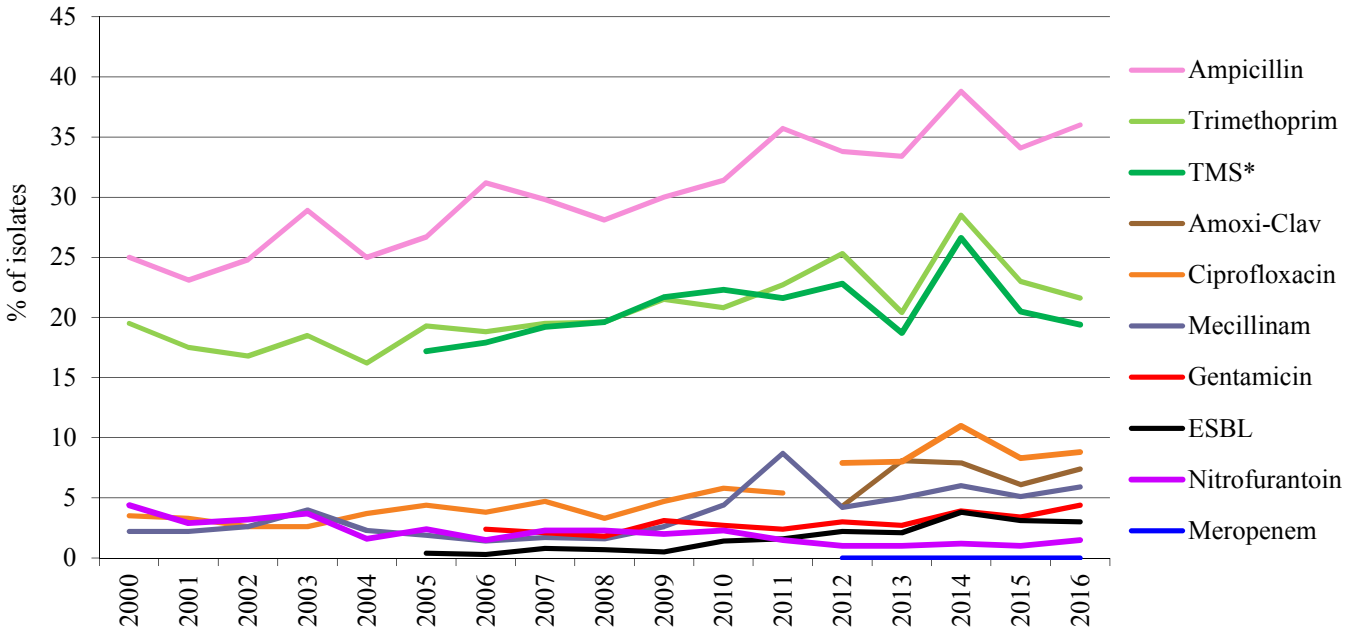

FIGURE 65. Prevalence of resistance to various antimicrobial agents in urinary tract *Escherichia coli* isolates 2000-2016 categorised according to the 2017 EUCAST guidelines. The breakpoint for ciprofloxacin resistance was changed from R > 1 mg/L to R > 0.5 mg/L in 2017. Data 2012-2016 have been recategorised according to the new breakpoint, but earlier results (2000-2011) cannot be calibrated due to changes in susceptibility testing methodology in 2012. *TMS=Trimethoprim-sulfamethoxazole.

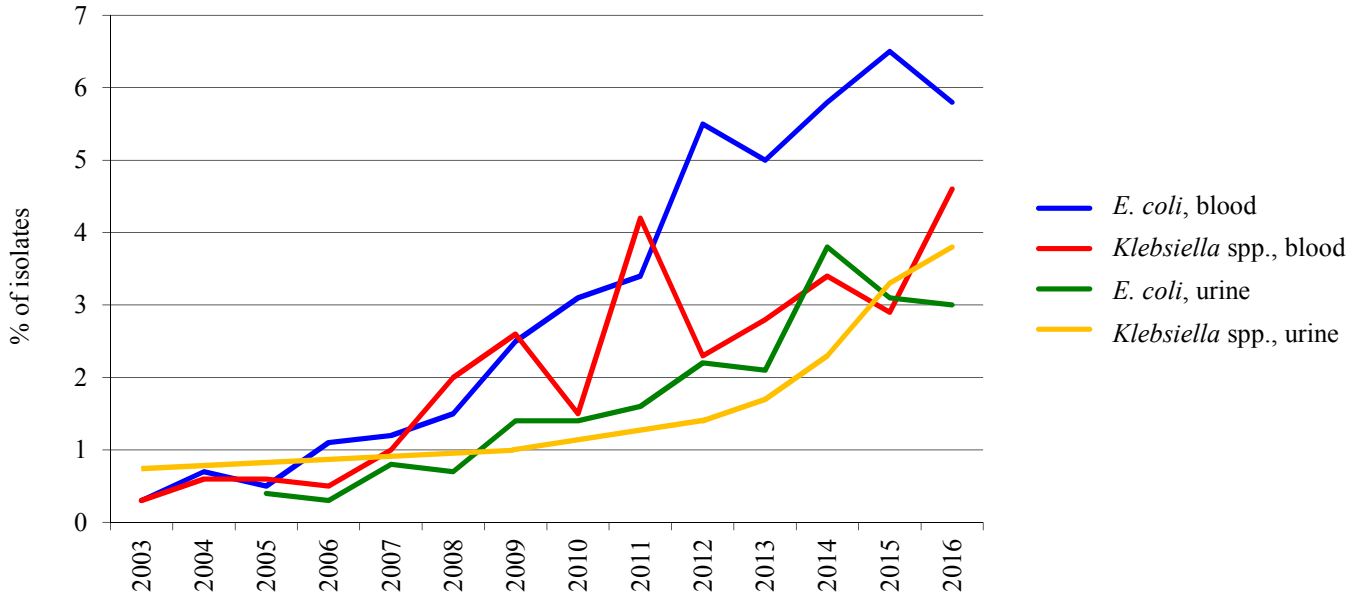

FIGURE 66. Prevalence of ESBL production among *Escherichia coli* and *Klebsiella* spp. isolates from blood and urine 2003-2016.

Fosfomycin – an old drug active against multidrug resistant bacteria

Fosfomycin, initially discovered in 1969, has recently gained new interest due to the increasing level of resistance [1]. Fosfomycin is a phosphonic acid derivative, has a low molecular weight and low protein binding. Fosfomycin is available as oral formulation in the form of fosfomycin trometamol or as fosfomycin disodium for intravenous use. According to the European Medicines Agency, fosfomycin is available through national authorisation in some 20 European countries for oral use, and in a handful of countries for intravenous use. Fosfomycin has recently been licensed for use in Norway.

Fosfomycin trometamol has its main indication for oral use in uncomplicated urinary tract infections (UTI), and primarily infections caused by *Escherichia coli*. The clinical efficacy in this indication is well documented [2]. Fosfomycin trometamol is rapidly absorbed after oral administration and converted to free acid with a bioavailability of approximately 40% after a 3 g dose [1, 2]. Fosfomycin is excreted unchanged in the urine by glomerular filtration and has an elimination half-life of approximately 4 h (range 2-8 h). A 3 g dose of fosfomycin trometamol can maintain a urinary concentration of >128 mg/L for 24-36 h and the fraction recovered in urine after 48 h is approximately 40% (range 32-43%) [2].

For fosfomycin disodium the most common daily dose is 3 g x 3 with a maximum dose of 5-8 g x 3. In critically ill patients, it is most commonly used in combination therapy [3,4]. In some countries fosfomycin has been used to treat patients with various infections including pneumonia and other lower respiratory infections, ear, nose, and throat infections, eye infections, osteomyelitis, meningitis, surgical infections, obstetric and gynaecological infections, arthritis, septicaemia, peritonitis, cervical lymphadenitis, diabetic foot infections and typhoid fever.

Fosfomycin is a broad-spectrum agent, with good activity against a variety of Gram-negative bacteria, being particularly effective against *E. coli* and some other *Enterobacteriaceae* (e.g. *Salmonella* spp., *Shigella* spp., *Enterobacter* spp., *Serratia* spp., *Citrobacter* spp., *Proteus mirabilis* and *Klebsiella pneumoniae*) [1,5]. Importantly, fosfomycin still retains good activity against multidrug resistant *Enterobacteriaceae* including ESBL- and carbapenemase-producing isolates [1,6]. Analysis of ESBL-producing *E. coli* from NORM 2010-2011 showed that all isolates were susceptible to fosfomycin [7]. Moreover, 85% of carbapenemase-producing *Enterobacteriaceae* identified in Norway 2007-2014 were susceptible to fosfomycin [8]. Activity against non-fermentative Gram-negative bacteria is limited and *Acinetobacter baumannii* and *Stenotrophomonas maltophilia* are considered intrinsically resistant [9]. Among Gram-positive organisms, fosfomycin is considered active against *Enterococcus* spp. and *Staphylococcus aureus* including VRE and MRSA and several coagulase-negative staphylococci such as *Staphylococcus epidermidis* [1, 5]. However, *Staphylococcus saprophyticus* and *Staphylococcus capitis* are intrinsically resistant to fosfomycin [9]. Fosfomycin is rapidly bactericidal and inhibits cell wall synthesis by irreversibly inhibiting enol-pyruvyl transferase (MurA), which catalyses the first step in the biosynthesis of peptidoglycan (formation of N-acetylmuramic acid) [1]. Fosfomycin enters the cells of susceptible bacteria by means of two different uptake systems: the constitutively active L-α-glycerophosphate transport system and the hexose-phosphate uptake system [1]. Fosfomycin is not active against Gram-negative anaerobic bacteria such as *Bacteroides* spp., but is active against *Peptostreptococcus* spp. and *Peptococcus* spp.

Resistance to fosfomycin is mainly associated with three key mechanisms: reduced uptake of fosfomycin due to inactivation or downregulation of transporters, mutations in *murA* resulting in reduced affinity of the enol-pyruvyl transferase for fosfomycin, and enzymatic modification of fosfomycin [1,5]. While reduced uptake and mutations in *murA* are due to chromosomal changes, the enzymatic modification is mainly due to enzymes associated with plasmids increasing the potential for rapid spread. The main concern is the plasmid-mediated spread of FosA and FosB among *Enterobacteriaceae* and Gram-positive bacteria (e.g. enterococci and staphylococci), respectively. FosA genes have now been identified on a variety of plasmids also harbouring other resistance determinants such as ESBLs and carbapenemases. *In vitro,* fosfomycin shows a relatively high mutant frequency rate for *E. coli* and *K. pneumoniae* [10, 11]. However, the *in vivo* relevance of this remains unresolved.

Currently, EUCAST has only set MIC breakpoints for *Enterobacteriaceae* (both intravenous and oral) and *Staphylococcus* spp. (only oral) at S ≤ 32/R > 32 mg/L [12]. For *Enterobacteriaceae* the oral breakpoint only relates to uncomplicated UTI. Disk diffusion breakpoints are only available for *E. coli* while for all other *Enterobacteriaceae* and *Staphylococcus* spp. the use of a MIC method is recommended. The reference method for fosfomycin MIC testing is currently agar dilution. Susceptibility testing of fosfomycin is associated with several issues and studies have shown discrepancies between different methods (see e.g. [11,13,14]. Irrespective of methods, glucose-6-phosphate is required either in the media (agar or broth) or in disks or gradient strips. One issue of agar-based methods including disk diffusion and gradient strips is the appearance of isolated scattered colonies within the inhibition zone. In terms of disk diffusion, isolated colonies within the inhibition zone shall be ignored and the outer zone edge should be read (Figure 67).

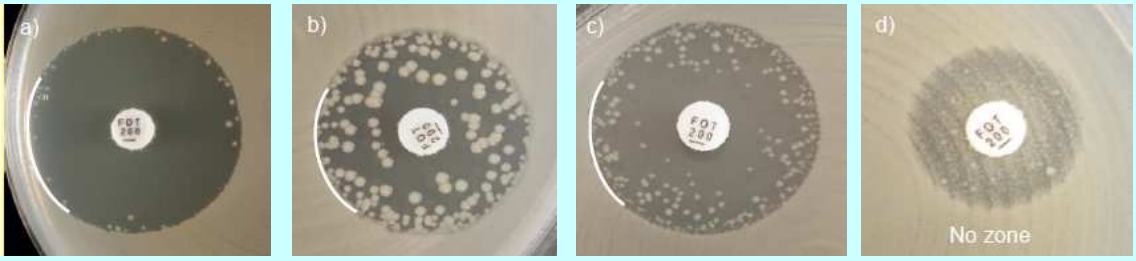

FIGURE 67. EUCAST guideline for interpreting inhibition zones in susceptibility testing of fosfomycin using disk diffusion (EUCAST Clinical Breakpoint Tables v. 7.1, [12]). Examples of inhibition zones for *Escherichia coli* with fosfomycin. a-c) Ignore all colonies and read the outer edge. d) Record as no inhibition zone.

For some microbes EUCAST states that there is insufficient evidence to set a breakpoint for intravenous fosomycin (e.g. *S. pneumoniae*, *H. influenzae* and *Moraxella*). EUCAST has neither set a PK/PD for oral nor intravenous fosfomycin. EUCAST has established epidemiological cutoff-values (ECOFFs) defining the perceived wild type for *E. coli* and *Proteus mirabilis* (ECOFF $\leq$ 8 mg/L) and *S. aureus* (ECOFF $\leq$ 32 mg/L).

In summary, fosfomycin is a broad-spectrum agent, with good activity against a variety of Gram-negative bacteria, being particularly effective against *E. coli* and some other *Enterobacteriaceae*. Fosfomycin trometamol has its main indication for oral use in uncomplicated UTI, and primarily infections caused by *Escherichia coli*. The clinical microbiological laboratories need to be aware of the challenges regarding susceptibility testing of fosfomycin.

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Klebsiella spp. in blood cultures

TABLE 39. *Klebsiella* spp. blood culture isolates in 2016 (n=855). 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
Amoxicillin-clavulanic acid	≤ 8	> 8	91.3	-	8.7
Piperacillin-tazobactam	≤ 8	> 16	88.2	7.7	4.1
Cefuroxime	≤ 8	> 8	88.8	-	11.2
Cefotaxime	≤ 1	> 2	94.9	0.4	4.7
Ceftazidime	≤ 1	> 4	93.6	1.6	4.8
Cefepime	≤ 1	> 4	92.4	3.9	3.7
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	96.5	0.1	3.4
Ciprofloxacin	≤ 0.25	> 0.5	84.5	4.3	11.2
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	88.0	1.1	10.9
ESBL	Negative	Positive	95.4	-	4.6

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 40. *Klebsiella pneumoniae* blood culture isolates in 2016 (n=671). 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
Amoxicillin-clavulanic acid	≤ 8	> 8	91.2	-	8.8
Piperacillin-tazobactam	≤ 8	> 16	87.3	8.9	3.7
Cefuroxime	≤ 8	> 8	88.4	-	11.6
Cefotaxime	≤ 1	> 2	95.2	0.1	4.4
Ceftazidime	≤ 1	> 4	93.1	1.5	5.4
Cefepime	≤ 1	> 4	92.4	3.7	3.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	96.0	0.1	3.9
Ciprofloxacin	≤ 0.25	> 0.5	81.2	5.1	13.7
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	86.3	1.2	12.5
ESBL	Negative	Positive	95.1	-	4.9

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 41. *Klebsiella oxytoca* blood culture isolates in 2016 (n=171). 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
Amoxicillin-clavulanic acid	≤ 8	> 8	93.0	-	7.0
Piperacillin-tazobactam	≤ 8	> 16	93.6	1.2	5.3
Cefuroxime	≤ 8	> 8	93.0	-	7.0
Cefotaxime	≤ 1	> 2	97.1	0.6	2.3
Ceftazidime	≤ 1	> 4	98.2	1.2	0.6
Cefepime	≤ 1	> 4	95.3	3.5	1.2
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	99.4	0.0	0.6
Ciprofloxacin	≤ 0.25	> 0.5	97.7	1.8	0.6
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	97.1	0.6	2.3
ESBL	Negative	Positive	99.4	-	0.6

*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 671 *K. pneumoniae* (78.5%), 171 *K. oxytoca* (20.0%), and 13 (1.5%) isolates not identified to the species level, giving a total of 855 *Klebsiella* spp. isolates (Tables 39-41). The breakpoint protocol of the Norwegian Working Group for Antibiotics (NWGA) has been in accordance with EUCAST since 2014.

The majority of *Klebsiella* spp. isolates remains susceptible to aminoglycosides. The prevalence of non-susceptibility increased from 1.0% in 2012 to 4.1% in 2014, but is now stable at 3.5% in 2016. *K. oxytoca* isolates are more often susceptible to aminoglycosides (99.4%) than *K. pneumoniae* isolates (96.0%). Aminoglycoside resistance in common *Enterobacteriaceae* species is a cause for great concern as these antimicrobials have traditionally been used in the empirical regimen for treatment of septiceamia 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 data from previous years have been updated according to the 2017 protocol, but results earlier than 2012 cannot be recategorised due to changes in susceptibility testing methodology. The overall prevalence of non-susceptibility to ciprofloxacin has been stable at 13-16% when taking into account the changes in breakpoints and interpretive criteria. The 15.5% non-susceptibility (4.3% intermediate suscepti-bility and 11.2% resistance) observed in 2016 is a slight increase from 13.1% in 2015. Non-susceptibility to ciprofloxacin is much more common in *K. pneumoniae* (18.8%) than in *K. oxytoca* (2.4%).

Non-susceptibility to trimethoprim-sulfamethoxazole remained stable at 12.0% in 2016 compared to 10.4% in 2015 and 11.6% in 2014. As for ciprofloxacin, the prevalence of resistance to trimethoprim-sulfamethoxazole was significantly lower in *K. oxytoca* (2.3%) than in *K. pneumoniae* (12.5%).

A comparison of ESBL rates and non-susceptibility to beta-lactam antibiotics between *Klebsiella* species is compli-cated by the diagnostic challenges of the chromosomal K1 beta-lactamase in *K. oxytoca*. Most *Klebsiella* spp. isolates were susceptible to cefotaxime (94.9%), ceftazidime (93.6%) and the beta-lactam/beta-lactamase inhibitor combination piperacillin-tazobactam (88.2%), see Figure 68. The rates of non-susceptibility to third generation cephalosporins were at the same level as in 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 increased from 3.4% in 2014 and 2.9% in 2015, to 4.6% 2016 (Figure 66). The 39 ESBL isolates originated from 12 different laboratories and were identified as *K. pneumoniae* (n=33, 4.9%), *K. oxytoca* (n=1) or *Klebsiella* spp. (n=5). The ESBL isolates were generally non-susceptible to cefuroxime (37/39), ceftazidime (38/39) and cefotaxime (38/39), and co-resistance was frequently seen for ciprofloxacin (19/39), trimethoprim-sulfa-methoxazole (29/39) and gentamicin (24/39). Many isolates were intermediately (19/39) or even fully (7/39) susceptible to piperacillin-tazobactam. All isolates were susceptible to meropenem.

Molecular characterisation of 33 isolates with a phenotypic ESBL profile at the Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-Res) confirmed the predominance of CTX-M groups 1 (n=24) and 9 (n=1). Three *K. pneumoniae* isolates were SHV hyperproducers whereas a single *K. oxytoca* isolate was a K1 hyper-producer. The remaining five *K. pneumoniae* strains harboured SHV (n=3) and TEM (n=2) beta-lactamases. No genetic determinants for carbapenemases were detected.

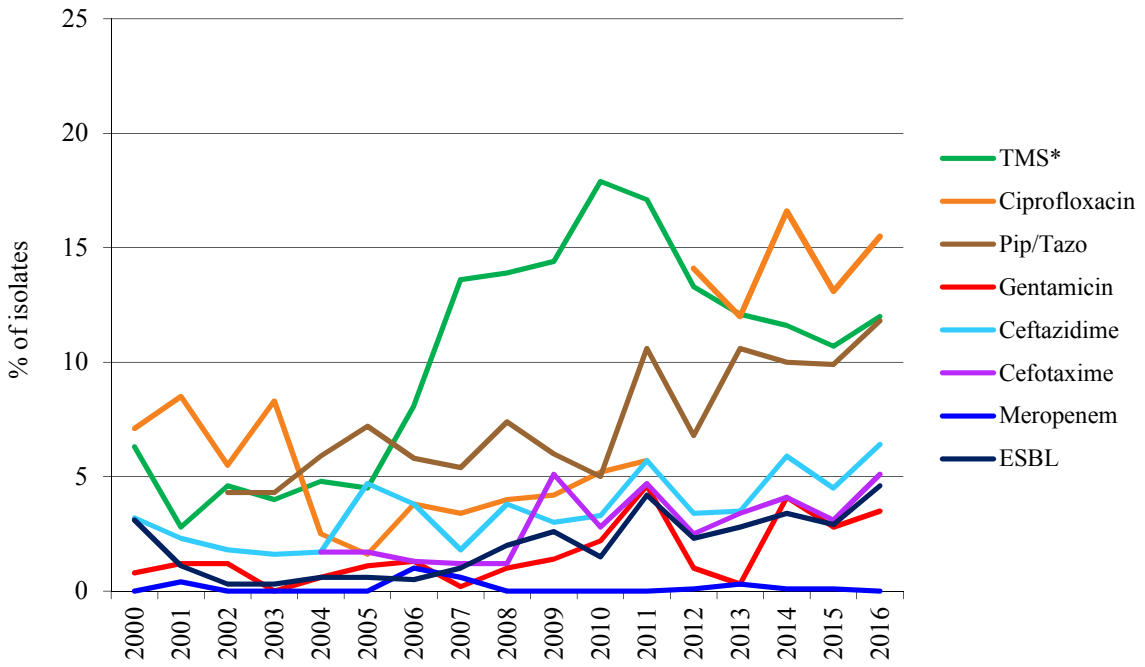

FIGURE 68. Prevalence of non-susceptibility to various antimicrobial agents in *Klebsiella* spp. blood culture isolates 2000-2016. The breakpoint for ciprofloxacin resistance was changed from R > 1 mg/L to R > 0.5 mg/L in 2017. Data 2012-2016 have been recategorised according to the new breakpoint, but earlier results (2000-2011) cannot be calibrated due to changes in methodology in 2012. *TMS=Trimethoprim-sulfamethoxazole.

Klebsiella spp. in urine

TABLE 42. *Klebsiella* spp. urinary tract isolates in 2016 (n=1,069). 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
Mecillinam	≤ 8	> 8	89.7	-	10.3
Amoxicillin-clavulanic acid*	≤ 32	> 32	93.1	-	6.9
Piperacillin-tazobactam	≤ 8	> 16	89.8	6.8	3.4
Cefuroxime	≤ 8	> 8	90.7	-	9.3
Cefotaxime	≤ 1	> 2	95.9	0.5	3.6
Ceftazidime	≤ 1	> 4	94.6	1.8	3.6
Cefepime	≤ 1	> 4	94.7	2.2	3.1
Meropenem	≤ 2	> 8	99.9	0.1	0.0
Gentamicin	≤ 2	> 4	97.1	0.5	2.4
Ciprofloxacin	≤ 0.25	> 0.5	86.1	4.8	9.1
Trimethoprim	≤ 2	> 4	81.2	2.2	16.6
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	86.7	0.6	12.7
ESBL	Negative	Positive	96.2	-	3.8

*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 2012-2015. 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. breakpoints for nitrofurantoin. The rates of resistance to urinary tract antibiotics were slightly lower in *Klebsiella* spp. than in *E. coli* isolates (Tables 42-44). A majority of isolates are still susceptible to gentamicin (97.1% compared to 97.2% in 2015). Among urinary tract *E. coli*, 95.6% were susceptible to gentamicin in 2016. When adjusting for changes in ciprofloxacin breakpoints, the rates of non-susceptibility in *Klebsiella* spp. have increased from 11.0% (7.8% intermediate susceptibility

and 3.2% resistance) in 2015, to 13.9% (4.8% intermediate susceptibility and 9.1% resistance) in 2016. The comparable rate for urinary tract *E. coli* in 2016 was 11.9% (3.1% intermediate susceptibility and 8.8% resistance). Susceptibility to trimethoprim (81.2% in 2016 compared to 79.9% in 2015) and trimethoprim-sulfamethoxazole (86.7% in 2016 compared to 84.8% in 2015) was higher than in *E. coli* (78.2% and 80.3%, respectively).

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 non-susceptibility to cefotaxime and/or ceftazidime and subsequent confirmatory ESBL MIC gradient tests. Fourty-one isolates were reported as ESBL positive of which 39 were *K. pneumoniae* and two were unspeciated. The 41 ESBL isolates were retrieved from 14 different laboratories and originated from general practices (n=19), hospitals (n=12), outpatient clinics (n=3) and nursing homes (n=7). The 3.8% ESBL rate (4.8% in *K. pneumoniae*) represented a further increase from 3.3% in 2015, and was at the same level as the 4.6% rate (4.9% in *K. pneumoniae*) found in blood culture isolates. The 41 ESBL isolates were generally non-susceptible to trimethoprim (n=37), trimethoprim-sulfamethoxazole (n=36) and ciprofloxacin (n=32), but many remained susceptible to gentamicin (n=23), mecillinam (n=34) and piperacillin-tazobactam (n=12). Molecular characterisation of 39 isolates with an ESBL phenotype confirmed the presence of CTX-M group 1 (n=31) and 9 (n=1), SHV-ESBL (n=4), DHA (n=1) and SHV hyperproduction (n=2). One CTX-M 1 positive isolate was non-susceptibile to meropenem and contained OXA-48.

TABLE 43. *Klebsiella pneumoniae* urinary tract isolates in 2016 (n=813). 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
Mecillinam	≤ 8	> 8	90.0	-	10.0
Amoxicillin-clavulanic acid*	≤ 32	> 32	94.1	-	5.9
Piperacillin-tazobactam	≤ 8	> 16	90.9	6.9	2.2
Cefuroxime	≤ 8	> 8	91.8	-	8.2
Cefotaxime	≤ 1	> 2	95.4	0.2	4.4
Ceftazidime	≤ 1	> 4	93.8	1.8	4.4
Cefepime	≤ 1	> 4	93.7	2.5	3.8
Meropenem	≤ 2	> 8	99.9	0.1	0.0
Gentamicin	≤ 2	> 4	96.4	0.5	3.1
Ciprofloxacin	≤ 0.25	> 0.5	85.3	5.5	9.2
Trimethoprim	≤ 2	> 4	79.3	2.1	18.6
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	84.9	0.5	14.6
ESBL	Negative	Positive	95.2	-	4.8

*Breakpoints for uncomplicated urinary tract infections. **Trimethoprim-sulfamethoxazole breakpoints are given for the trimethoprim component only.

TABLE 44. *Klebsiella oxytoca* urinary tract isolates in 2016 (n=150). 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
Mecillinam	≤ 8	> 8	88.7	-	11.3
Amoxicillin-clavulanic acid*	≤ 32	> 32	88.0	-	12.0
Piperacillin-tazobactam	≤ 8	> 16	88.6	2.7	8.7
Cefuroxime	≤ 8	> 8	86.0	-	14.0
Cefotaxime	≤ 1	> 2	98.7	1.3	0.0
Ceftazidime	≤ 1	> 4	99.3	0.7	0.0
Cefepime	≤ 1	> 4	98.0	2.0	0.0
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	100.0	0.0	0.0
Ciprofloxacin	≤ 0.25	> 0.5	96.0	1.3	2.7
Trimethoprim	≤ 2	> 4	92.0	0.7	7.3
Trimethoprim-sulfamethoxazole**	≤ 2	> 4	94.0	0.0	6.0
ESBL	Negative	Positive	100.0	-	0.0

*Breakpoints for uncomplicated urinary tract infections. **Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Temporal and regional trends in ESBL-prevalence in Norway

The worldwide increase in the occurrence of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* is of great concern. Continuous development of epidemiological knowledge is needed to improve the understanding and control of the ESBL problem. This is the first report on temporal and regional trends in ESBL-prevalence in Norway, using cefotaxime resistance as indicator of ESBL-production.
Routine data on cefotaxime resistance among *Escherichia coli* and *Klebsiella pneumoniae* blood culture isolates from primary diagnostic laboratories in Norway in 2011-16 were collected as part of the EARS-net (European Antimicrobial Resistance Surveillance Network) collaboration. Altogether 19 Norwegian laboratories reported data for at least one year within the time period; however, Haugesund Hospital in health region West Norway, started reporting only from 2014 and was excluded from the present analysis. Total number of *E. coli* and *K. pneumoniae* blood culture isolates tested for cefotaxime sensitivity and number of participating laboratories per year from each health region are presented in tables.

TABLE 45. Proportions of *Escherichia coli* isolates reported as cefotaxime resistant (resistant/total number of isolates tested) in Norway 2011-2016 by health region (South-East is shown as East and South, separately). Number of laboratories per region is given in brackets.

Region	2011	2012	2013	2014	2015	2016	*P-trend**
East	38/873 (5**)	45/872 (5)	47/806 (5)	50/893 (5)	47/775 (4)	38/811 (4)	*0.50****
South	23/586 (3)	25/634 (4)	36/658 (4)	34/683 (4)	33/706 (4)	36/763 (4)	*0.45****
West	7/204 (2)	41/541 (3)	36/556 (3)	45/634 (3)	45/624 (3)	47/678 (3)	*0.41****
Mid	6/411 (4)	10/392 (4)	26/474 (4)	28/508 (4)	29/499 (4)	32/576 (4)	*0.0004*
North	10/303 (2)	11/312 (2)	15/332 (2)	21/349 (2)	17/322 (2)	7/312 (2)	*0.86*
Norway	84/2377	132/2751	160/2826	178/3067	171/2926	160/3140	*0.0044****

*Chi-square test for slope. **Data from Oslo University Hospital Aker and Ullevål were merged. ***East: *P trend 0.49* when limited to the four laboratories with consistent reporting in 2011-16. South: *P trend 0.50* when limited to the three laboratories with consistent reporting in 2011-16. West: *P trend 0.053* when limited to the two laboratories with consistent reporting in 2011-16. Norway: *P trend 0.0021* when limited to laboratories with consistent reporting in 2011-16.

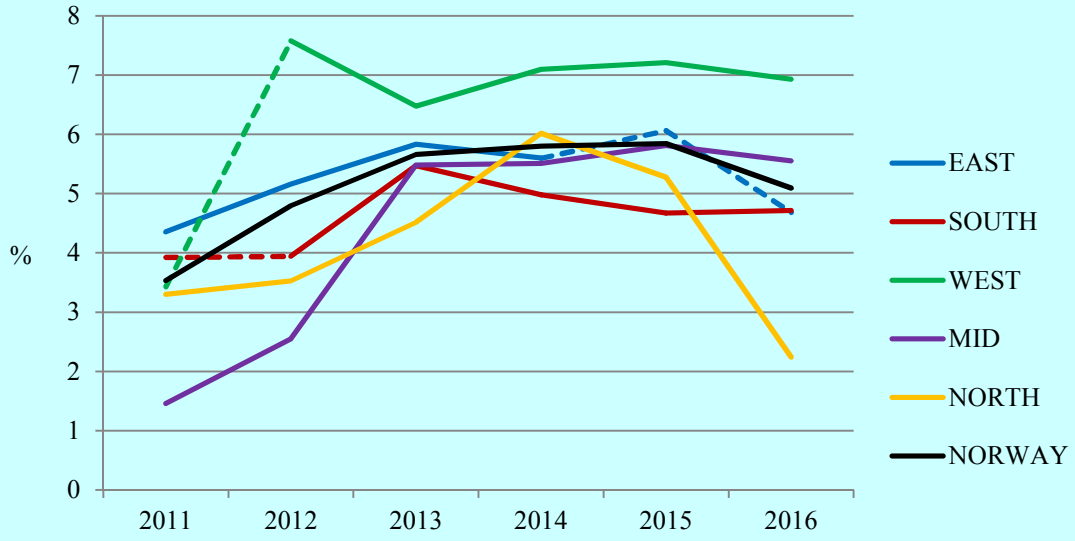

FIGURE 69. Proportion of *Escherichia coli* blood culture isolates reported as cefotaxime resistant in Norway 2011-2016 by health region. Dotted line indicates variation in number of reporting laboratories within a region.

There was a statistically significant increase in the overall prevalence of cefotaxime resistance in *E. coli* blood culture isolates in Norway from 3.5% in 2011 to 5.8% in 2014-15 and 5.1% in 2016 (*P for trend* 0.0044). While the highest prevalence was observed in health region West at about 7% during the last five years, the steepest increase in prevalence was observed in health region Mid; from 1.5 in 2011 to 5.8% in 2015; *P for trend* < 0.001). A drop in prevalence was observed in most regions in 2016. Data per hospital was not included in the primary analysis; however, when looking at the overall rate of cefotaxime resistance in *E. coli* blood culture isolates in 2011-16, almost every larger tertiary hospital in Norway was on the Top-20 list (> 5.5% for the total period).

Also for cefotaxime resistance in *K. pneumoniae* blood culture isolates, a statistically significant increase in the overall prevalence was observed; from 2.0% in 2011 to 6.3% in 2014 and 4.4% in 2016 (*P for trend* 0.019). Health region North had the highest prevalence during the study period and the largest increase in prevalence from 2.1% in 2011 to about 10% in 2014 and 2016 (*P for trend* 0.069). Moreover, there was a statistically significant increase in prevalence in health region South (*P for trend* 0.036). However, due to low number of observations per health region, careful interpretation of these data is required.

TABLE 46. Proportions of *Klebsiella pneumoniae* isolates reported as cefotaxime resistant (resistant/total number of isolates tested) in Norway 2011-2016 by health region (South-East is shown as East and South, separately). Number of laboratories per region is given in brackets.

Region	2011	2012	2013	2014	2015	2016	*P-trend**
East	4/143 (5**)	6/179 (5)	7/165 (4)	17/205 (5)	8/133 (3)	7/161 (4)	*0.16***
South	2/108 (3)	3/127 (3)	3/139 (3)	7/141 (3)	5/153 (3)	10/159 (3)	*0.036*
West	0/38 (2)	6/133 (3)	2/117 (3)	7/144 (3)	7/147 (3)	3/154 (3)	*0.95***
Mid	1/57 (3)	0/63 (3)	4/73 (3)	2/88 (3)	3/94 (3)	1/106 (3)	*0.92*
North	1/47 (2)	1/60 (2)	6/82 (2)	9/92 (2)	3/80 (2)	8/80 (2)	*0.075*
Norway	8/393	16/562	22/576	42/670	26/607	29/660	*0.019*

*Chi-square test for slope. **Data from Oslo University Hospital Aker and Ullevål were merged. ***East: *P trend 0.43* when limited to the three laboratories with consistent reporting in 2011-16. West: *P trend 0.12* when limited to the two laboratories with consistent reporting in 2011-16. Norway: *P trend 0.0066* when limited to the laboratories with consistent reporting in 2011-16.

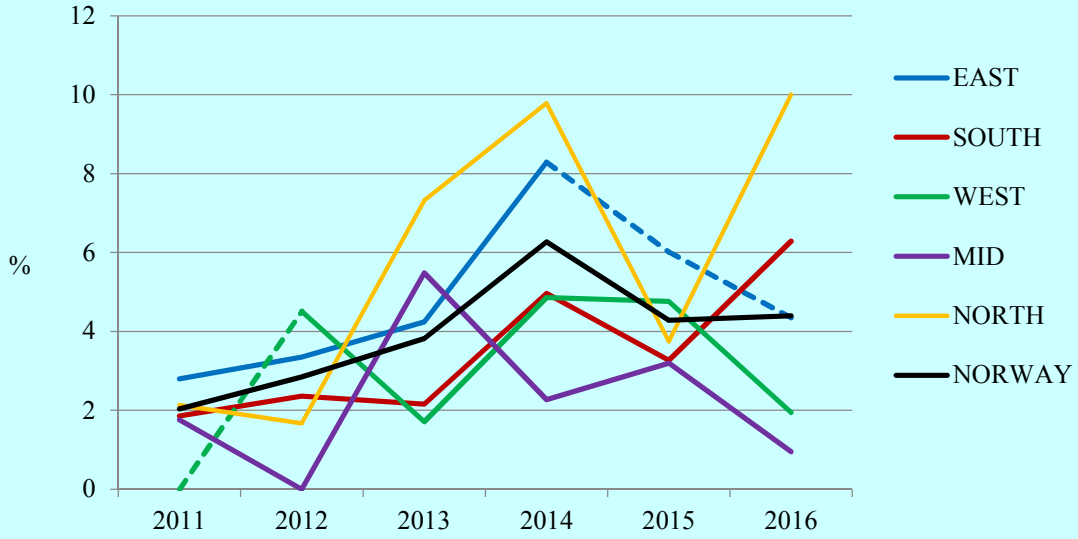

FIGURE 70. Proportion of *Klebsiella pneumoniae* blood culture isolates reported as cefotaxime resistant in Norway 2011-2016 by health region. Dotted line indicates variation in number of reporting laboratories within a region.

In conclusion, blood culture surveillance data show an increase in the prevalence of ESBL-positive *E. coli* and *K. pneumoniae* in Norway during 2011-2016. Data suggest regional differences in occurrence and spread of these bacteria over time. This should be further investigated in future studies including more detailed data on the microbes and risk factors.

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Resistance against empiric antibiotic combinations in the treatment of blood stream infections

Empiric treatment of blood stream infections (BSI) aims to cover as many likely causative microbial agents as possible, but coverage must be balanced against potential overuse of broad-spectrum antibiotics associated with development of antimicrobial resistance [1]. By combining narrow-spectrum beta-lactams with antimicrobials from other classes one may spare the use of critically important broad-spectrum beta-lactams, such as cefotaxime and meropenem [2], while still maintaining sufficient coverage. Thus it is of interest to show frequency of resistance to common antimicrobial drug combinations.

Table 47 shows frequency of resistance among important BSI microbes against common drugs and drug combinations used in Norwegian hospitals in the empirical treatment of sepsis [3]. Data are compiled from the regular NORM surveillance 2016 for invasive strains with the exception of MRSA. Due to a low number of invasive MRSA strains (n=12), data are shown for all MRSA strains regardless of origin. It should be noted that the invasive EBSL and MRSA strains also are included in their respective species statistics. Data on anaerobes, non-fermenting Gram-negative rods and other *Enterobacteriacea* mentioned have been collected from previous NORM reports. In the following, some of the frequently used drugs and drug combinations are commented with regards to different infection foci. One should bear in mind that these comments are based on national level surveillance data. Local epidemiology must always be taken into account when evaluating local treatment regimens.

TABLE 47. Resistance (%) to broad-spectrum antibiotics and antibiotic combinations among key pathogens.

Drugs and dosage		Proportion of invasive isolates resistant (%)								
		E.coli (n=1,940)	*Klebsiella* spp. (n=855)	*H. influenzae* (n=81)	*Enterococcus* spp. (n=616)	*S. pneumoniae* (n=594)	*S. aureus* (n=1,255)	*S. pyogenes* (n=187)	ESBL-A *E.coli* and *Klebsiella* spp. (n=152)	MRSA* (n=2,604)
Benzylpenicillin 3g x 4	Gentamicin 5-7 mg/kg x1	6.3	3.4	27.2	-	0.3	0.4	0	52.0	11.7
Benzylpenicillin 3g x 4	Ciprofloxacin 4-600mg x 2	13.0	11.2	0	-	0.3	5.7	0	76.3	19.3
Clindamycin 600-900 mg x 3-4	Gentamicin 5-7 mg/kg x1	6.3	3.4	100.0	100.0	4.2	0.1	2,1	52.0	2.7
Ampicillin 2g x 4	Gentamicin 5-7 mg/kg x1	6.1	3.4	18.5	22.1	0.3	0.4	0[4]	52.0	11.7
Piperacillin/tazobactam 4g x 3-4	Gentamicin 5-7 mg/kg x1	0.5	0.9	0[1]	22.1[2]	X	0.1	0[4]	8.6	11.7
Cefotaxime 2g x 3		6.0	4.7	0	100.0	0	1.0[3]	0[4]	100.0	100.0
Piperacillin/tazobactam 4g x 3-4		1.9	4.1	0[1]	22.1[2]	X	1.0[3]	0[4]	15.1	100.0
Meropenem 1g x 3		0	0	X	100.0	X	1.0[3]	0[4]	0	100.0

[1]Derived from results for amoxicillin-clavulanic acid. [2]Derived from ampicillin result. [3]Derived from cefoxitin result. [4] Derived from benzylpenicillin result. - No breakpoint/susceptibility testing not recommended. X No data available. *Includes MRSA isolates from all sources.

Ampicillin and gentamicin have good activity against *S.aureus*, *Streptococcus* spp., *Enterococcus* spp., *Enterobacteriaceae* and non-fermenting gram negative rods such as *Pseudomonas* spp. and *Acinetobacter* spp.. In addition, gentamicin has effect on a significant number of ESBL-A and MRSA isolates. Ampicillin´s coverage of *H. influenzae* is acceptable. The combination is well suited for urinary tract infections (UTI) and lower respiratory tract infections (LRTI) with a caveat for high prevalence of *H. influenzae*. It should also be noted that gentamicin has a weakness in infection foci where O_2 tension or pH-level is low, i.e abscess or empyema.

Benzylpenicillin covers *S. pneumoniae* very well. While wild-type *H.influenzae* most likely are susceptible, testing is methodlogically difficult and no breakpoint exists. An estimate based on beta-lactamase production and cefuroxime susceptibility suggests that 60-70 % of strains are susceptible to benzylpenicillin. As such, benzylpenicillin and gentamicin are a good option for both community acquired (CA) and hospital acquired (HA) LRTI with a caveat for high prevalence of *H. influenzae*. *Streptococcus* spp. and *S. aureus* coverage is excellent, making the drug combination a good option for skin and soft tissue infections (SSTI) as well. Note the aforementioned gentamicin-related weakness in some foci, leaving only 23.5% of *S. aureus* benzylpenicillin susceptible. This can be rectified by substituting benzylpenicillin with a beta-lactamase-stable penicillin, i.e dicloxacillin (with >99% *S. aureus* coverage).

Cefotaxime has, compared to ampicillin and gentamicin, equally good coverage against *E.coli*, *Klebsiella* and *S. aureus*. Its strength lies in superior coverage against *H. influenzae* and better coverage against *S. pneumoniae* with reduced susceptibility to benzylpenicillin, making it a good option in the treatment of CA-LRTI. Skin and soft tissue infection (SSTI) are also covered well, particularly in Gram-positive infections. Cefotaxim´s weakness lies in its lack of activity against non-fermenting gram-negative rods and its questionable effect on the AmpC-group of *Enterobacteriaceae* (*Enterobacter* spp., *Serratia* spp. etc)(4). This makes it less suitable for HA-LRTI compared to a gentamicin-based combination.

Metronidazol has excellent anaerobe coverage (97.7 % against Gram-negatives and 93.2% against Gram-positives), making it a good partner with ampicillin and gentamicin in the treatment of mixed abdominal infections. Piperacillin-tazobactam has an extensive coverage with low frequency of resistance, making it useful in treatment in a variety of clinical settings such as HA-LRTI, mixed SSTI and abdominal infections. Effect on a significant number of ESBL-A producing isolates can also make it a viable option when the antibiotic susceptibility profile has been determined. It should be noted a relatively high level of resistance in anaerobic Gram-negative rods (R = 18,7%; in particular in *Bacteroides* spp.) and no effect against MRSA and *Acinetobacter* spp.

In conclusion, gentamicin-based combinations provide good coverage in many clinical settings and thus represent powerful first line alternatives in BSI treatment permitting reduction in broad-spectrum beta-lactam use. As shown elsewhere in this report, resistance levels among important Gram-positive microbes constitutes a low risk in patient treatment. While resistance levels among Gram-negative microbes against primary regimes could be considered acceptable at the moment, the steadily increase of beta-lactam and gentamicin resistance is a source of great concern for the immediate future.

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Enterobacter spp. in blood cultures

TABLE 48. *Enterobacter* spp. blood culture isolates in 2016 (n=162). 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	≤ 8	> 16	78.4	5.6	16.0
Cefotaxime	≤ 1	> 2	75.3	1.9	22.8
Ceftazidime	≤ 1	> 4	70.4	7.4	22.2
Cefepime	≤ 1	> 4	83.4	11.7	4.9
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	96.3	0.6	3.1
Ciprofloxacin	≤ 0.25	> 0.5	85.8	4.3	9.9
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	90.1	0.6	9.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Enterobacter spp. blood culture isolates have previously been included in the NORM surveillance programme in 2008. The present survey covered all blood culture isolates in Norway in 2016 identified as *Enterobacter aerogenes*, *Enterobacter* species and *Enterobacter cloacae* complex (*Enterobacter cloacae*, *E. asburiae*, *E. hormaechei*, *E. kobei*, *E. nimipressuralis* and *E. ludwigii*). A total of 162 isolates were recovered including *E. cloacae* complex (n=120), *E. asburiae* (n=17) and *Enterobacter* spp. (n=19). All isolates are analysed as a single group.

Enterobacter wild-type strains contain a chromosomal AmpC beta-lactamase which is negatively regulated by the repressor AmpR. This mechanism is liable to escape mutants leading to high-level resistance to all penicillins and cephalosporins except cefpirome and cefepime. The spectrum of resistance may be expanded to include fourth generation cephalosporins as well as carbapenems when derepressed AmpC is combined with porin loss. Traditional beta-lactamase inhibitors are not active against AmpC enzymes.

Cephalosporins can be used in the treatment of systemic infections with susceptible *Enterobacter* strains, but derepressed AmpC mutants may arise during therapy and monotherapy is therefore not advisable. A primary objective of the *E. cloacae* surveillance protocol was to determine the prevalence of stable AmpC derepression in Norway. As seen in Table 48, 22.8% and 22.2% of the isolates were resistant to cefotaxime and ceftazidime, respectively. This is in accordance with international studies reporting 20-25% stably derepressed isolates in unselected materials, and it is also at the same level as in 2008 (cefotaxime 27.3% and ceftazidime 23.5%). As expected, the fourth generation cephalosporin cefepime was more active with 83.4% being fully susceptible. Eight isolates had meropenem zone diameters below the screening breakpoint (S ≥ 27 mm), but none of them were meropenem non-suscpetible by the clinical breakpoints and carbapenemase production was not detected.

Interestingly, 3.1% of the isolates were resistant to gentamicin and an additional 0.6% were intermediately susceptible. In 2008, all isolates were fully susceptible to this agent. High-level aminoglycoside resistance has thus emerged in *Enterobacter* spp. similarly to the development in other *Enterobacteriaceae*. The ciprofloxacin results cannot be compared to the 2008 survey as both breakpoints and methodology have been changed. A non-susceptibility rate of 14.2% is at the same level as in blood culture isolates of *E. coli* (17.1%) and *Klebsiella* spp (15.5%). There are only minor changes for trimethoprim-sulfamethoxazole with 90.1% susceptibility in 2016 compared to 91.7% in 2008.

Enterobacter spp. in urine

Enterobacter spp. urinary tract isolates were previously included in NORM in 2005. There was a higher proportion of *Enterobacter aerogenes* (40/158, 25.3%) in urine compared to blood cultures (10.5%). AmpC derepression was apparently lower in urinary tract *Enterobacter* spp. isolates (12-13%) than in systemic isolates (22-23%). A single isolate was confirmed as ESBL-A positive in addition to AmpC, but none were resistant to meropenem and carbapenemase production was not detected.

Mecillinam and nitrofurantoin are not suitable for treatment of *Enterobacter* urinary tract infections. Among the antibiotics traditionally used for this indication, resistance rates were lower for ciprofloxacin (2.5%), trimethoprim (13.3%) and trimethoprim-sulfamethoxazole (8.2%) than in the 2016 collection of *E. coli* isolates (8.8%, 21.6% and 19.4%, respectively). The results from 2005 are not comparable due to changes in breakpoints and methodology.

TABLE 49. *Enterobacter* spp. urinary tract isolates in 2016 (n=158). 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	≤ 8	> 16	87.4	4.4	8.2
Cefotaxime	≤ 1	> 2	86.0	1.3	12.7
Ceftazidime	≤ 1	> 4	84.1	3.2	12.7
Cefepime	≤ 1	> 4	93.1	4.4	2.5
Meropenem	≤ 2	> 8	100.0	0.0	0.0
Gentamicin	≤ 2	> 4	98.1	0.0	1.9
Ciprofloxacin	≤ 0.25	> 0.5	95.6	1.9	2.5
Trimethoprim	≤ 2	> 4	85.4	1.3	13.3
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	91.2	0.6	8.2

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Update on the ESBL$_{CARBA}$ situation in Norway

The World Health Organization defined in 2017 carbapenem resistant *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacteriaceae* as "critical" in terms of research, discovery and development of new antibiotics (1). A major contributor to carbapenem resistance is mobile carbapenemase genes encoding beta-lactamases that hydrolyse and confer resistance/reduced susceptibility to carbapenems (2,3). Depending on the substrate profile, carbapenemases also confer resistance to other beta-lactams such as penicillins and cephalosporins (2,3). Moreover, carbapenemase-producing isolates are frequently multi- or extremely-drug resistant. Consequently, the rapid global dissemination of carbapenemase genes among Gram-negative pathogens is a significant threat to patients and healthcare systems (4) and infections with carbapenemase-producing Gram-negative bacteria are associated with high mortality rates due to limited treatment options (3).

In Norway, carbapenemase-producing bacteria 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 2016. Isolates from the same patient are included if they were of different species and/or harboured different carbapenemases.

In 2016, thirty-seven carbapenemase-producing *Enterobacteriaceae* were identified (Figure 71) which is equal to the number of isolates identified in 2015. In total, 33 patients were affected in 2016 compared to 30 in 2015. In contrast to previous years when *K. pneumoniae* was the dominant species, the number of *E. coli* isolates (*n*=16) was equal to *K. pneumoniae* (*n*=16) in 2016 (Figure 71).

With respect to specific carbapenemase genes the dominance of $bla_{\text{OXA-48-like}}$ (*n*=17) and bla_{NDM} (*n*=15) continued in 2016 (Figure 72). Additionally, one isolate was also in 2016 found to harbour both bla_{NDM} and $bla_{\text{OXA-48-like}}$. The class A carbapenemase NMC-A was for the first time identified in Norway in 2016 in an *Enterobacter* spp. isolate. This illustrates the continued expansion of diversity with respect to carbapenemase genes circulating in the bacterial population.

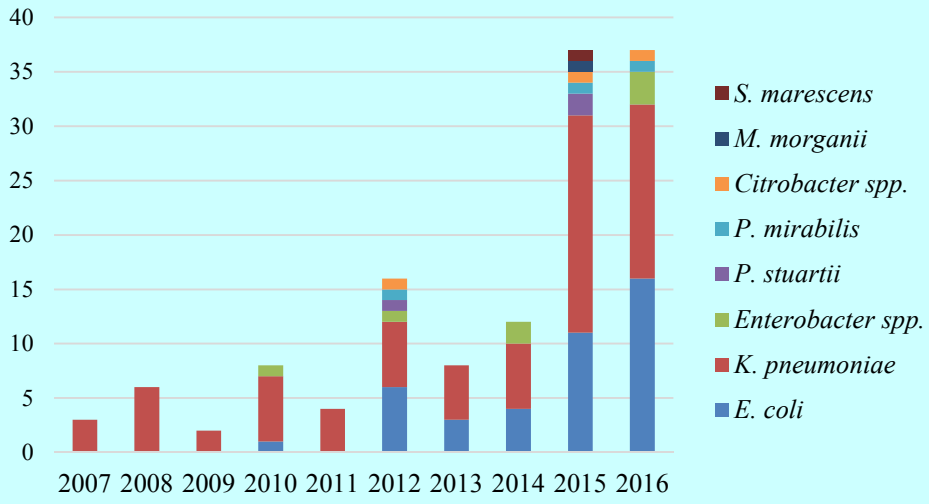

FIGURE 71. Carbapenemase-producing *Enterobacteriaceae* 2007-2016 according to species.

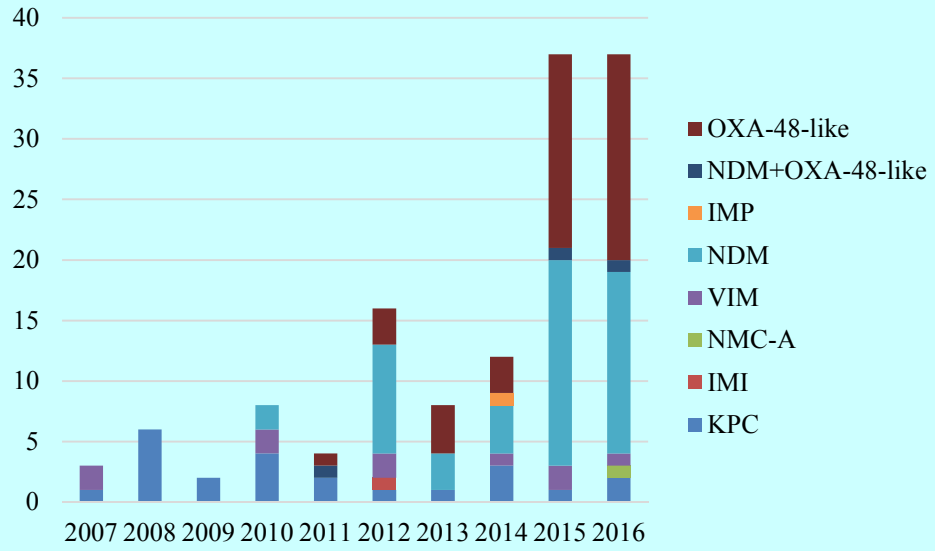

FIGURE 72. Carbapenemase-producing *Enterobacteriaceae* 2007-2016 according to carbapenemase variant.

In 2016, sixteen and five carbapenemase-producing *A. baumannii* and *Pseudomonas* spp. isolates were identified, respectively (Figure 73). This is comparable to 2014 and 2015 with no significant changes. As in previous years the dominant carbapenemase genes were $bla_{\text{OXA-23-like}}$ among *A. baumannii* (*n*=13) and bla_{VIM} among *Pseudomonas* spp. (*n*=4). $Bla_{\text{OXA-58-like}}$ and $bla_{\text{OXA-24-like}}$ were identified in two and one *A. baumannii* isolates, respectively. Bla_{IMP} was identified in one *P. aeruginosa* isolate.

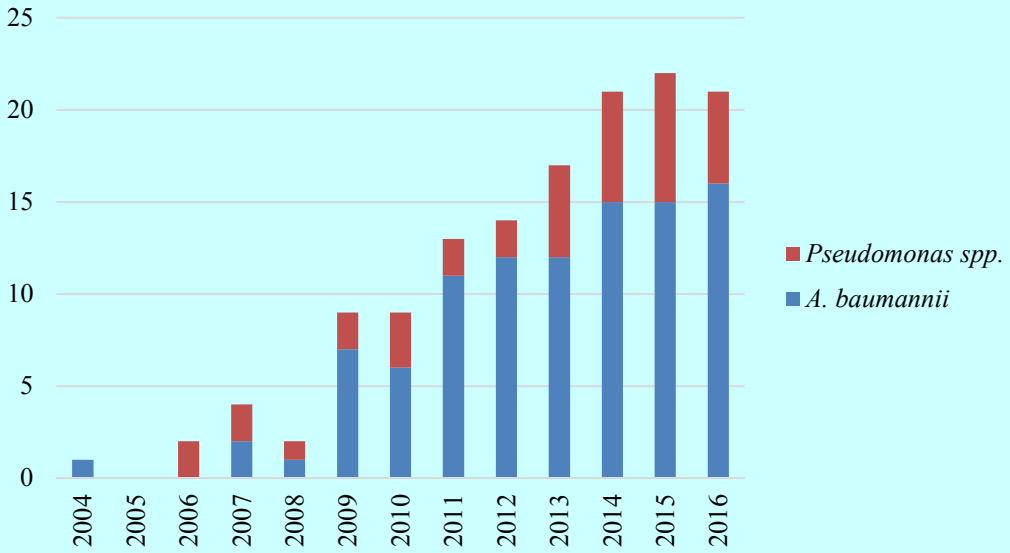

FIGURE 73. Identified carbapenemase-producing *Pseudomonas* spp. and *A. baumannii* in Norway 2004-2016.

In conclusion, the number of carbapenemase-producing isolates in Norway is still low. However, surveillance, antibiotic stewardship, strict infection control measures as well as high clinical and diagnostic awareness is important to maintain this situation.

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Haemophilus influenzae in blood cultures and cerebrospinal fluids

TABLE 50. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2016 (n=81). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Ampicillin	≤ 1	> 1	81.5	-	18.5
Amoxicillin-clavulanic acid	≤ 2	> 2	100.0	-	0.0
Cefuroxime	≤ 1	> 2	81.5	7.4	11.1
Cefotaxime	≤ 0.125	> 0.125	100.0	-	0.0
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.06	> 0.06	100.0	-	0.0
Chloramphenicol	≤ 2	> 2	97.5	-	2.5
Tetracycline	≤ 1	> 2	98.8	0.0	1.2
Trimethoprim-sulfamethoxazole*	≤ 0.5	> 1	88.9	1.2	9.9
Penicillin G (mm)	≥ 12	< 12	72.8	-	27.2
Cefaclor (mm)	≥ 23	< 23	74.1	-	25.9
Beta-lactamase	Negative	Positive	82.7	-	17.3

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 51. *Haemophilus influenzae* in blood cultures and cerebrospinal fluids in 2016 (n=81). Distribution (%) of MICs (mg/L) and zone diameters for penicillin G and cefaclor (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
Ampicillin						8.6	42.0	24.7	7.4			3.7	1.2	1.2		11.1
Amoxi-clav**						3.7	9.9	49.4	24.7	12.3						
Cefuroxime						1.2	3.7	21.0	55.6	7.4	4.9	2.5	3.7			
Cefotaxime	3.7	14.8	39.5	32.1	9.9											
Ceftriaxone	39.5	46.9	12.3	1.2												
Ciprofloxacin	7.4	43.2	48.1	1.2												
Chloramph.								11.1	75.3	11.1				1.2	1.2	
Tetracycline							19.8	77.8	1.2			1.2				
TMS***		1.2	17.3	48.1	13.6	4.9	2.5	1.2	1.2	1.2	1.2	1.2		6.2		

	< 11	11	12	13	14	15	16	17	18	19	20	21	22	23	24	≥ 25
Penicillin G	27.2		2.5	4.9	6.2	9.9	2.5	13.6	4.9	8.6	7.4	2.5	3.7	1.2	2.5	2.5
Cefaclor	9.9								1.2	1.2	2.5	2.5	8.6	1.2	9.9	63.0

*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. **Amoxi-clav=Amoxicillin-clavulanic acid. ***TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Systemic *H. influenzae* isolates were first included in the NORM programme in 2013. Resistance data are provided by the Reference Laboratory at the Norwegian Institute of Public Health on a yearly basis.

In 2016, 81 *H. influenzae* isolates were recovered from blood cultures (n=78), cerebrospinal fluids (n=2) and a pleural effusion. The latter was also isolated in a blood culture, but the rest all represented unique patients (Tables 50-51). Beta-lactamase production was detected in 17.3%, which is a slight increase from 11.5% in 2015 and 13.0% in 2014. A total of 14/81 isolates were resistant to ampicillin, and beta-lactamase production was detected in all of them. Four of these isolates were concomitantly non-susceptible to cefuroxime, thus indicating a combination of beta-lactamase production and chromosomal resistance mechanisms.

Cefuroxime MIC > 2 mg/L has been suggested as the most precise indicator for alterations in the wild type PBP3 sequence. Nine isolates (11.1%) displayed this phenotype compared to 8.3% in 2015 and 13.0% in 2014. Some of these isolates remained susceptible to ampicillin (5/9) and amoxicillin-clavulanic acid (9/9). All isolates were fully susceptible to cefotaxime and ceftriaxone.

Different substrates have been suggested for screening of beta-lactam resistance in *H. influenzae*. The penicillin G 1U disk (PCG1) successfully identified almost all ampicillin (15/15) and cefuroxime (7/9) resistant strains. Eight out of 67 (11.9%) beta-lactamase negative isolates were resistant to PCG1; one of these was resistant to ampicillin and seven were non-susceptible to cefuroxime.

The breakpoint for the cefaclor disk test is calibrated for beta-lactamase positive isolates. Eight out of 14 (57%) beta-lactamase positive isolates were resistant to cefaclor, and cefaclor correctly identified all four cefuroxime non-susceptible isolates in addition to four cefaclor borderline isolates (21-22 mm zone diameters) where cefuroxime resistance was not verified. The results illustrate the continuing challenges in defining the optimal algorithm for beta-lactam susceptibility testing in *H. influenzae*.

As previously seen in respiratory tract isolates, resistance to ciprofloxacin (0.0%), chloramphenicol (2.5%) and tetracycline (1.2%) was at very low levels. The 9.9% resistance rate to trimethoprim-sulfamethoxazole was at the same level as 10.1% and 11.5% in systemic isolates in 2014 and 2015, respectively.

Neisseria meningitidis in blood cultures and cerebrospinal fluids

TABLE 52. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2016 (n=22). 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	50.0	45.5	4.5
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Ciprofloxacin	≤ 0.03	> 0.03	100.0	-	0.0
Chloramphenicol	≤ 2	> 4	100.0	0.0	0.0
Rifampicin	≤ 0.25	> 0.25	100.0	-	0.0

TABLE 53. *Neisseria meningitidis* in blood cultures and cerebrospinal fluids in 2016 (n=22). 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				1	10	8	2	1								
Ceftriaxone	22															
Ciprofloxacin	1	21														
Chloramph.							2	7	11	2						
Rifampicin	4	9	5	2	2											
Azithromycin						2	3	6	9	2						
Tetracycline						1	13	7		1						
Sulfonamide								1	5	3	2	1		1		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.

RESULTS AND COMMENTS

N. meningitidis from blood cultures and cerebrospinal fluids were first included in NORM in 2013. As for systemic *H. influenzae* isolates, 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 52-53.

A total of 22 isolates were recovered from blood cultures (n=19), cerebrospinal fluids (n=2) and "other materials" (n=1). All isolates were from unique patients. The isolates belonged to serogroups B (n=9), Y (n=8) and W (n=5). We observed an increase in serogroup W isolates belonging to sequence type (ST) 11 (n=5), as has recently been reported elsewhere in Europe, but there were no known associations between the cases. Ten isolates displayed penicillin G MICs of 0.125-0.25 mg/L and were thus intermediately susceptible, whereas a single isolate (serogroup B) was resistant with an MIC of 0.5 mg/L. 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. Sulfonamide resistance has been widespread in *N. meningitidis* since the 1960ies. EUCAST has not defined clinical breakpoints for this agent, but the MIC distributions clearly demonstrate a high prevalence of acquired resistance among Norwegian isolates.

Neisseria gonorrhoeae

TABLE 54. *Neisseria gonorrhoeae* from all specimen types in 2016 (n=381). 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	> 1	2.9	66.1	31.0
Ceftriaxone	≤ 0.125	> 0.125	100.0	-	0.0
Cefixime	≤ 0.125	> 0.125	97.6	-	2.4
Azithromycin	≤ 0.25	> 0.5	64.1	24.4	11.5
Ciprofloxacin	≤ 0.03	> 0.06	51.4	0.0	48.6
Tetracycline	≤ 0.5	> 1	43.9	15.2	40.9
Spectinomycin	≤ 64	> 64	100.0	-	0.0
Beta-lactamase	Negative	Positive	76.4	-	23.6

TABLE 55. *Neisseria gonorrhoeae* from all specimen types in 2016 (n=381). 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	1.3	1.3	1.6	15.0	28.1	12.6	8.9	12.6	6.0	1.3	2.9	8.1		
Ceftriaxone	42.5	28.3	8.9	11.0	7.3	1.8										
Cefixime			74.3	9.4	6.6	7.3	2.1	0.3								
Azithromycin				3.4	9.7	15.5	35.4	24.4	6.8	1.0	0.8	0.8	1.0	0.8		0.3
Ciprofloxacin	35.4	13.9	1.8	0.3				0.5	5.2	9.4	8.1	6.0	3.7	15.5		
Tetracycline					0.5	2.6	11.5	29.1	15.2	12.9	2.6	3.1	8.9	10.2	1.8	1.3
Spectinomycin							0.3			0.3		27.6	71.1	0.8		

*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. **Pen G=Benzylpenicillin.

RESULTS AND COMMENTS

Neisseria gonorrhoeae was surveyed in NORM in 2003 and 2010. Oslo University Hospital analysed Norwegian gonococcal isolates 2013-2015, and from 2016, the Reference Laboratory at the Norwegian Institute of Public Health provides resistance data on *N. gonorrhoeae* on a yearly basis. All isolates from all specimen types were included in the survey, but only a single isolate was accepted from each patient. The microbiological data could not be linked to information from the Norwegian Surveillance System for Communicable Diseases (MSIS).

In 2016, a total of 381 isolates were available for analysis. The isolates were reported to originate from urethra (n=216), cervix uteri (n=60), anus (n=61), throat (n=16), eye (n=2) or "others/uknown" (n=26). A total of 301 isolates originated from men and 80 from women. 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 within the NORM protocol.

The results from susceptibility testing are presented in Tables 54-55. A majority of the isolates were intermediately susceptible (66.1% in 2016, 62.9% in 2015) or resistant (31.0% in 2016, 34.0% in 2015) to penicillin G. Ninety isolates (23.6%) produced beta-lactamase and were phenotypically non-susceptible to penicillin G. This is a further decrease from 32.4% in 2013, 30.6% in 2014 and 28.2% in 2015. Most beta-lactamase positive isolates (76/90, 84.4%) were also non-susceptible to ciprofloxacin. In addition, 33 isolates were resistant and 247 were intermediately susceptible to penicillin G in spite of being beta-lactamase negative, probably caused by alterations in penicillin binding proteins (PBPs) and/or reduced permeability through the outer cell membrane.

No isolates were categorised as resistant to ceftriaxone. Ceftriaxone resistant isolates from Norway have previously been linked to treatment failure. Nine (2.4%) isolates were resistant to the oral cephalosporin cefixime compared to three isolates in 2015 and ten in 2014. Cefixime is no longer recommended for empirical treatment in Norway. The results confirm the emergence of cephalosporin resistant gonococci in Norway, which is of course extremely alarming from both a clinical and a public health perspective. The current European treatment guidelines consist of a combination of ceftriaxone and azithromycin. It should be noted that 35.9% of the isolates were categorised as non-susceptibilible to azithromycin including three of the nine cefixime resistant isolates.

Ciprofloxacin was previously used for empirical treatment of gonorrhoeae acquired outside South-East Asia. The prevalence of ciprofloxacin resistance persisted at a high level (48.6%) in spite of a significant reduction from 62.2% in 2015. Ciprofloxacin is consequently not a viable alternative except in cases where susceptibility has been documented. All strains were susceptible to the aminoglycoside spectinomycin.

Staphylococcus aureus in blood cultures

TABLE 56. *Staphylococcus aureus* blood culture isolates in 2016 (n=1,255). 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
Erythromycin	≤ 1	> 2	94.8	0.1	5.1
Clindamycin	≤ 0.25	> 0.5	98.1	0.6	1.4
Fusidic acid	≤ 1	> 1	95.5	-	4.5
Ciprofloxacin	≤ 1	> 1	93.1	-	6.9
Gentamicin	≤ 1	> 1	99.6	-	0.4
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	99.2	0.8	0.0
Tetracycline	≤ 1	> 2	96.6	0.2	3.3
Tigecycline	≤ 0.5	> 0.5	99.8	0.0	0.2
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.7	0.1	0.2
Beta-lactamase	Negative	Positive	27.3	-	72.7
Cefoxitin screen	≥ 22	< 22	99.0	-	1.0
MRSA (*mecA*)	Negative	Positive	99.0	-	1.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

Twelve methicillin resistant *S. aureus* (MRSA) isolates were detected in the NORM surveillance system in 2016, corresponding to a prevalence of 1.0% (Table 56). This is at the same level as in 2014 (0.8%) and 2015 (0.7%). The resistance phenotype was confirmed by *mecA* PCR in all cases. The isolates originated from nine different hospitals.

Laboratory screening for MRSA in NORM is performed using cefoxitin disks. All MRSA isolates had cefoxitin zone diameters below the screening breakpoint. Some MRSA isolates were concomitantly resistant to erythromycin (6/12), clindamycin (1/12), gentamicin (1/12), tetracycline (2/12) and/or ciprofloxacin (7/12). All MRSA isolates were susceptible to trimethoprim-sulfamethoxazole, linezolid, rifampicin, tigecycline and fucidic acid. The results from susceptibility testing of all Norwegian MRSA isolates are presented in Table 59 on page 119. 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 20 out of 1,753 (1.1%) *S. aureus* blood culture isolates were MRSA. None of the 15 *S. aureus* isolates recovered from cerebrospinal fluids were methicillin resistant, thus bringing the total number of systemic MRSA isolates to 20/1,768 (1.1%). This is unchanged from 2015.

Sixty-five *S. aureus* isolates (5.2%) were non-susceptible to erythromycin. This is a slight decrease from 5.5% in 2015. The macrolide resistance phenotypes of all isolates were determined by the double disk diffusion (DDD) test. Six isolates (9.2%) were constitutively MLS_B resistant, 46 (70.8%) were inducibly MLS_B resistant and 13 (20.0%) displayed efflux mediated M type resistance. These figures represent 0.5%, 3.7% and 1.0% of all *S. aureus* isolates from blood cultures, respectively. The distribution of MLS phenotypes was essentially unchanged from 2015 to 2016.
The prevalence of resistance to fusidic acid at 4.5% was comparable to 4.4 % in 2014 and 4.8% in 2015. The 6.9% prevalence of ciprofloxacin resistance is a marked increase from 2.3% in 2015, but this is partly due to a minor adjustment of the zone diameter of the breakpoint for resistance. There were no significant changes for gentamicin, rifampicin, tigecycline or trimethoprim-sulfamethoxazole. All isolates were susceptible to linezolid. The general test panel for *S. aureus* did not include vancomycin in 2016.
Figure 74 shows the prevalence of non-susceptibility to various antimicrobials. A total of 72.7% of the isolates were beta-lactamase positive which is unchanged from previous years. Beta-lactamase positive isolates were more likely to be resistant to ciprofloxacin (7.8%) and tetracycline (4.2%) compared to beta-lactamase negative isolates (4.4% and 0.9%, respectively). For the other antimicrobials there were only minor differences.

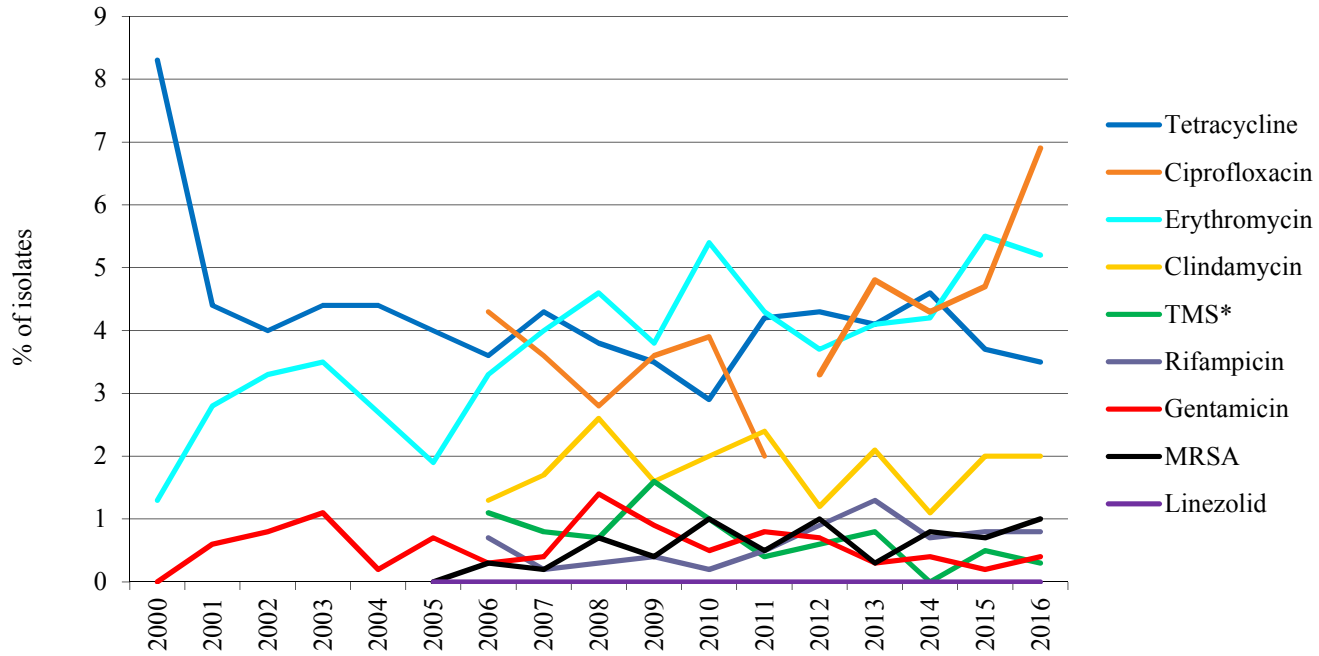


FIGURE 74. Prevalences of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* blood culture isolates 2000-2016. Doxycycline was replaced by tetracycline in 2006. The zone diameter of the breakpoint for ciprofloxacin resistance was adjusted in 2017. Data 2012-2016 have been recategorised, but earlier results (2000-2011) cannot be calibrated due to changes in methodology in 2012. *TMS=Trimethoprim-sulfamethoxazole.

Staphylococcus aureus in wound specimens

TABLE 57. *Staphylococcus aureus* isolates from wound specimens in 2016 (n=1,090). 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
Erythromycin	≤ 1	> 2	94.0	0.1	5.9
Clindamycin	≤ 0.25	> 0.5	98.1	0.6	1.3
Fusidic acid	≤ 1	> 1	92.7	-	7.3
Ciprofloxacin	≤ 1	> 1	95.3	-	4.7
Gentamicin	≤ 1	> 1	99.0	-	1.0
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.064	> 0.5	98.4	1.3	0.3
Tetracycline	≤ 1	> 2	94.5	0.3	5.2
Tigecycline	≤ 0.5	> 0.5	99.9	0.0	0.1
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	99.4	0.2	0.4
Beta-lactamase	Negative	Positive	23.5	-	76.5
Cefoxitin screen	≥ 22	< 22	98.4	-	1.6
MRSA (*mecA*)	Negative	Positive	98.4	-	1.6

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

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. Seventeen out of 1,090 (1.6%) isolates were confirmed as MRSA by *mecA* PCR. The prevalence was slightly higher than in 2014 (1.3%) and 2015 (1.2%). The MRSA isolates originated from patients admitted to hospitals (n=3), general practitioners (n=13) and a nursing home (n=1) in different parts of the country. Most MRSA isolates displayed reduced susceptibility or co-resistance to erythromycin (n=9), ciprofloxacin (n=8), tetracycline (n=3), gentamicin (n=5), trimethoprim-sulfamethoxazole (n=2) and fusidic acid (n=1) in different combinations. All MRSA isolates were phenotypically susceptible to clindamycin, rifampicin, tigecycline, trimethoprim-sulfamethoxazole and linezolid. 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 118).

The prevalence of resistance to fusidic acid in *S. aureus* wound isolates increased to 7.3% compared to 6.8% in 2015 (Table 57 and Figure 75). 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 significantly lower in blood culture isolates (4.5%).

For other antimicrobial agents such as trimethoprim-sulfamethoxazole, gentamicin, rifampicin, and tetracycline there were only minor changes from 2015 to 2016, and the prevalence of non-susceptibility was in general similar for blood culture isolates and isolates from wound specimens. All isolates were susceptible to linezolid.

Sixty-five (6.0%) isolates were non-susceptible to erythromycin which is a small increase from 5.0% in 2015. All these isolates were further examined for determination of resistance phenotype. The majority were inducibly (41/65, 63% of macrolide resistant isolates) or constitutively (10/65, 15% of macrolide 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 (14/65, 22% of macrolide resistant isolates) compatible with efflux mediated M type resistance. The findings are in accordance with the results from previous years.

A total of 76.5% of the isolates were beta-lactamase positive compared to 76.2% in 2015. Beta-lactamase positive isolates were more likely to be resistant to erythromycin (6.4%) and tetracycline (6.0%) compared to beta-lactamase negative isolates (4.3% and 2.7%, respectively). For the other antimicrobials there were only minor differences.

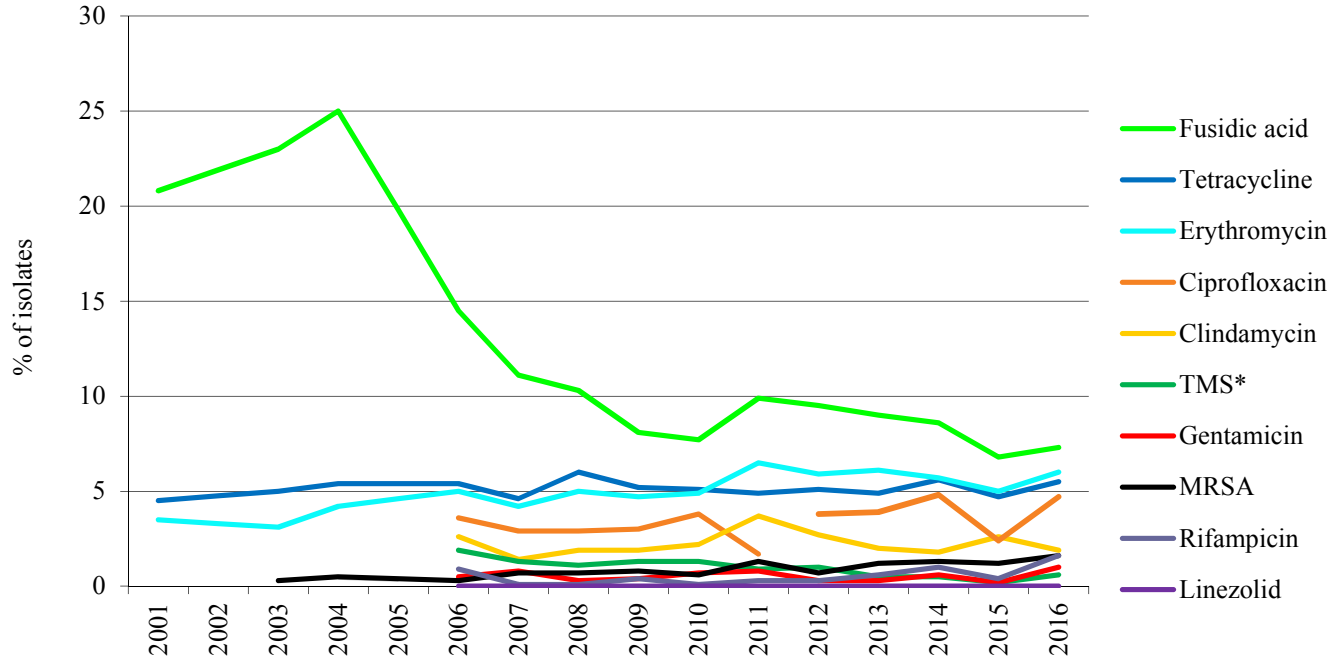


FIGURE 75. Prevalence of non-susceptibility to various antimicrobial agents among *Staphylococcus aureus* wound isolates 2001-2016. Doxycycline was replaced by tetracycline in 2006. The zone diameter of the breakpoint for ciprofloxacin resistance was adjusted in 2017. Data 2012-2016 have been recategorised, but earlier results (2000-2011) cannot be calibrated due to changes in methodology in 2012. *TMS=Trimethoprim-sulfamethoxazole.

Methicillin resistant *Staphylococcus aureus* (MRSA) infections in Norway 2016

Infections with methicillin resistant *Staphylococcus aureus* (MRSA) have been notifiable in Norway since 1995 and colonisation since 2005. In all, 2,538 cases of MRSA were reported in 2016 (49 per 100,000 person-years). Of these, 887 (35%) cases were clinical infections while 1,651 were registered colonised (1,594) or unknown status (57) (Figure 76).

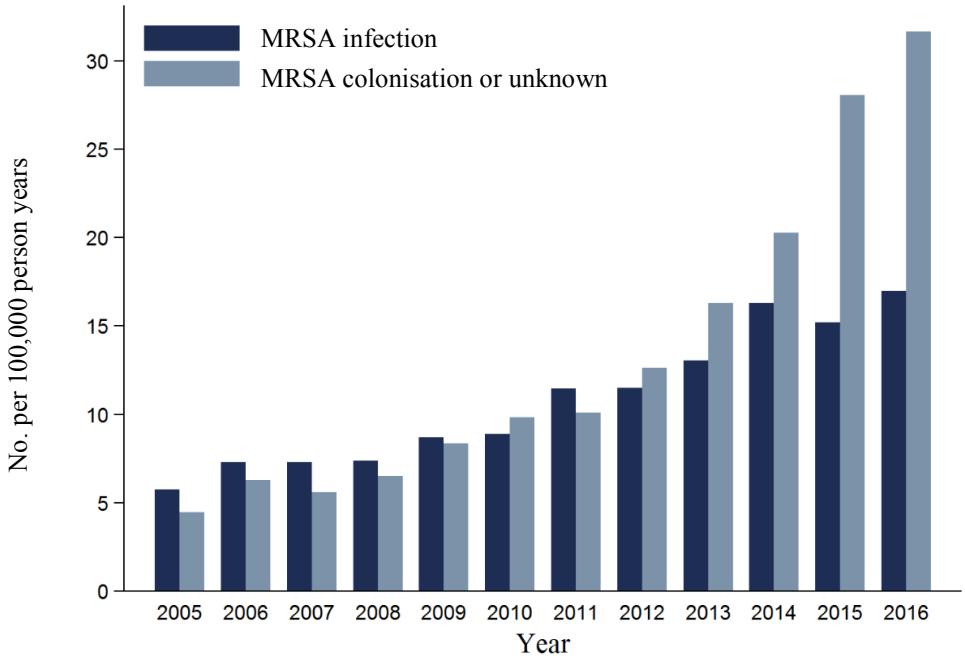


FIGURE 76. Number of MRSA cases per 100,000 person-years in Norway 2005-2016, by infection and colonisation.

The notification rate of MRSA increased by 14% from 2015 to 2016. However, the number of infections notified in 2016 was at the same level as over the last two years, 883 compared to 785 and 887.

The main objective of the Norwegian MRSA infection control measures is to prevent MRSA from becoming endemic in healthcare institutions. In line with this policy, the increase in MRSA notifications over the last few years is primarily seen in the group diagnosed by their general practitioners. In 2016, 524 (21%) of all persons notified with MRSA were inpatients at the time of diagnosis. Twenty-nine (1%) were residents in nursing homes and 1,929 (76%) were diagnosed in general practice (Figure 77). The detection rate in healthcare institutions remains relatively stable, with only a slight increase in hospital in-patients and a small decline in long-term care facilities. For 55 persons we lack information about place of diagnosis. Sixty-four of the reported MRSA cases in 2016 were found in healthcare workers.

The MRSA notifications in MSIS may reflect higher prevalence in the community, but also a more active search behaviour.

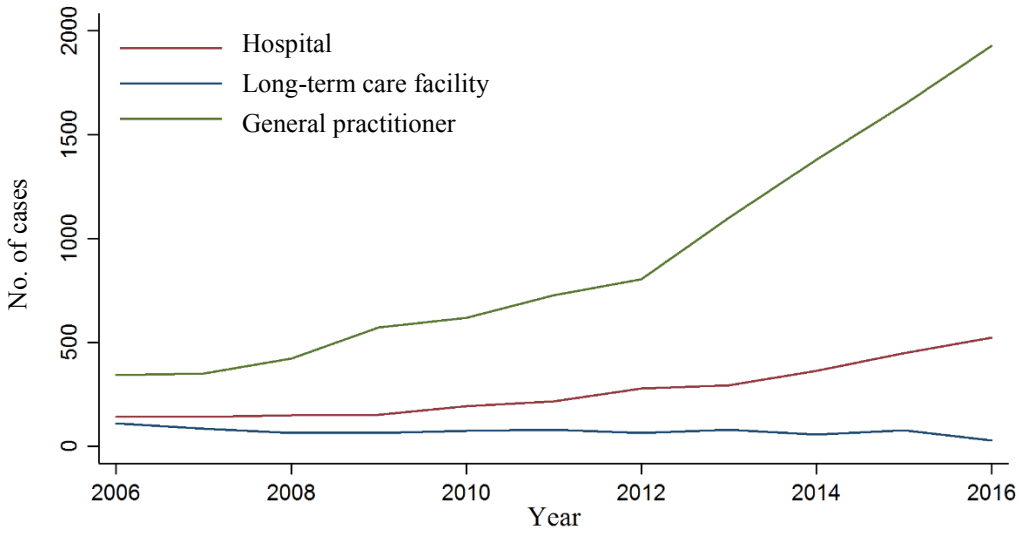


FIGURE 77. Reported cases of MRSA in Norway 2006 – 2016, by the type of healthcare setting where MRSA was diagnosed.

The numbers above reflect where the MRSA infection/ colonisation was diagnosed, not where it was acquired. Information on where MRSA was most likely acquired shows that the number infected in Norway has remained stable over the past ten years, while the overall increase in MRSA incidence is seen in the group infected outside Norway. Thus, the main source of MRSA is "import" rather than domestic transmission. The Norwegian MRSA measures seem to be effective so far in limiting domestic spread of MRSA in the healthcare sector, however, we must expect a further increase in MRSA coming in from abroad.

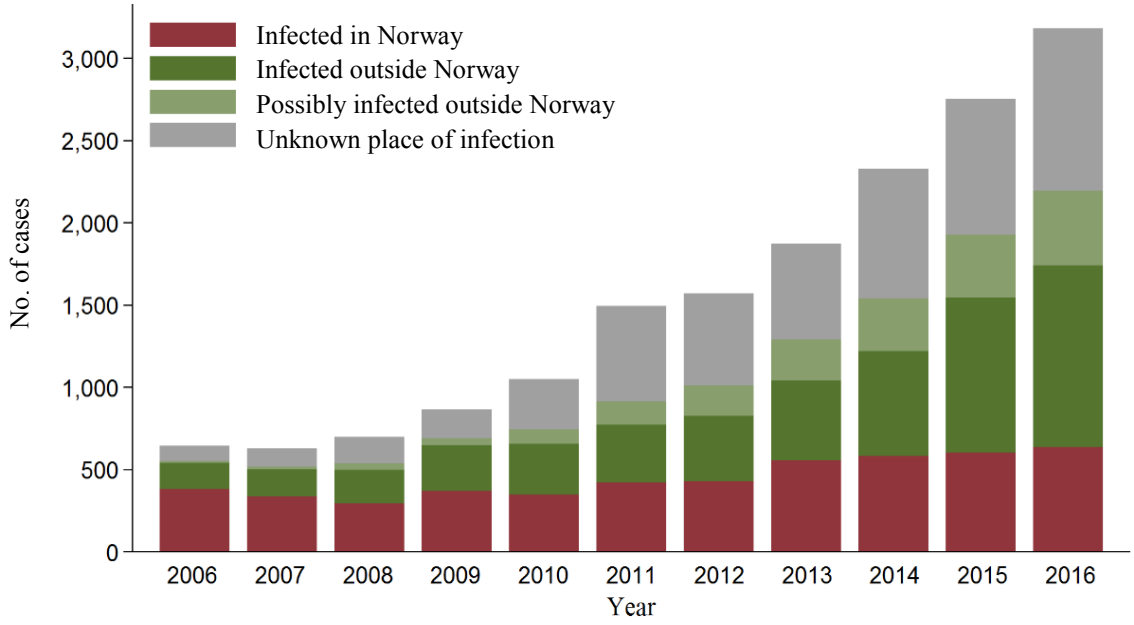


FIGURE 78. Reported cases of MRSA in Norway 2006 – 2016, by the place of infection.

Livestock associated MRSA (LA-MRSA) was first reported in Norwegian pig herds in 2013. Table 58 shows LA-MRSA diagnosed in humans, where the MRSA isolates are PVL negative and of the same *spa*-types as found in pigs. Of all the notifications of LA-MRSA, in the period from 2011 up to and including 2016, 21 (18%) were reported as clinical infections. LA-MRSA is still a small proportion of the total number of MRSA. Norway has implemented a national strategy for handling LA-MRSA in the swine population. This includes measures to avoid introduction into the pig farming units, as well as a surveillance program of the total swine population and a "search and destroy" policy at pig farm level for elimination of LA-MRSA from affected farms. So far these measures seem effective in preventing MRSA to establish in the pig production industry (see page 55).

TABLE 58. Number and proportion LA-MRSA of total number of MRSA.

Year	LA-MRSA* (%)	LA-MRSA infected in Norway (%)	All cases of MRSA
2011	5 (0.5)	3 (0.2)	1,059
2012	6 (0.5)	1 (0.1)	1,204
2013	43 (2.9)	42 (2.8)	1,483
2014	19 (1.0)	14 (0.7)	1,867
2015	29 (1.3)	20 (0.9)	2,234
2016	17 (0.7)	14 (0.5)	2,538

*LA-MRSA diagnosed in humans, where the isolates are PVL negative and of same *spa*-types and clonal complex (CC398 and CC1) as found in pigs in Norway.

The Reference Laboratory for MRSA, St. Olav University Hospital, Trondheim, received 2,613 MRSA isolates in 2016. 359 different *spa*-types were identified and the six most frequent were (*spa*-type, n (%)): t223, n=243 (9.3 %), t002, n=226 (8.7 %), t304, n=204 (7.8 %), t127, n=171 (6.5 %), t008, n=114 (4.4 %) and t019, n=104 (4.0 %). 171 *spa*-types were reported as single events. Based on *spa*-type, the isolates were grouped in corresponding MLST clonal complexes. 1,861 isolates (71.2 %) belonged to the six most prevalent clusters (CC, n (%)): CC22, n=459 (17.6 %), CC5, n=453 (17.3 %), CC1, n=317 (12.1 %), CC6, n=224 (8.6 %), CC30, n=221 (8.5 %), and CC8, n=186 (7.1%).
The Reference Laboratory found 33 LA-MRSA (CC398) in humans (*spa* t034 (n=27), t011 (n=4), t1451 (n=1)) and t1606 (n=1)). *Spa* t034 was the 15th most common *spa*-type in Norway in 2016. Two isolates were found positive for *mecC* (*spa*-type t528 and t1519).

Susceptibility testing was performed on 2,604 MRSA isolates with the EUCAST 2016 disk diffusion method and analysed with breakpoints from NordicAST 2016. The laboratory received 2,479 complete antibiograms, and 958 strains (38.6 %) were sensitive to all antibiotics tested except beta-lactams. The most resistant profiles were found within CC239. The highest proportions of resistance were found for erythromycin (29.2%) followed by tetracycline (25.8%) and ciprofloxacin (19.3%). 17.2% of the strains were clindamycin resistant, of which 54.1% were inducibly resistant by D-test. Low rates of resistance were found towards mupirocin (0.4%), rifampin (1.1%) and trimethoprim-sulfamethoxazole (2.5%). One isolate was resistant to linezolid and none were vancomycin resistant.

TABLE 59. Methicillin resistant *Staphylococcus aureus* (MRSA) isolates from human cases in 2016 (n=2,604). Distribution (%) of antimicrobial susceptibility categories.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Erythromycin	≤ 1	> 2	70.6	0.2	29.1
Clindamycin	≤ 0.25	> 0.5	81.2	1.6	17.2
Fusidic acid	≤ 1	> 1	88.1	-	11.9
Ciprofloxacin	≤ 1	> 1	80.7	-	19.3
Gentamicin	≤ 1	> 1	88.3	-	11.7
Linezolid	≤ 4	> 4	100.0	-	0.0
Rifampicin	≤ 0.06	> 0.5	98.0	0.9	1.1
Tetracycline	≤ 1	> 2	73.7	0.5	25.8
Trimethoprim-sulfamethoxazole*	≤ 2	> 4	95.3	2.2	2.5
Mupirocin	≤ 1	> 256	92.7	6.9	0.4
Vancomycin	≤ 4	> 4	100.0	-	0.0

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

Enterococcus spp. in blood cultures

TABLE 60. *Enterococcus* spp. blood culture isolates in 2016 (n=616). 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
Ampicillin	≤ 4	> 8	77.6	0.3	22.1
Imipenem	≤ 4	> 8	75.2	2.4	22.4
Gentamicin*	≤ 128	> 128	-	76.8	23.2
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.8	0.2	0.0
Vancomycin (any genotype)	≤ 4	> 4	98.0	-	2.0
Vancomycin (Van A or VanB)	Negative	Positive	99.5	-	0.5

*The wild type is defined as intermediately susceptible.

TABLE 61. *Enterococcus faecalis* blood culture isolates in 2016 (n=421). 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
Ampicillin	≤ 4	> 8	99.8	0.2	0.0
Imipenem	≤ 4	> 8	98.4	1.4	0.2
Gentamicin*	≤ 128	> 128	-	81.2	18.8
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	99.8	0.2	0.0
Vancomycin (VanA or VanB)	Negative	Positive	99.8	-	0.2

*The wild type is defined as intermediately susceptible.

TABLE 62. *Enterococcus faecium* blood culture isolates in 2016 (n=162). 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
Ampicillin	≤ 4	> 8	18.5	0.6	80.9
Imipenem	≤ 4	> 8	14.2	4.9	80.9
Gentamicin*	≤ 128	> 128	-	61.7	38.3
Linezolid	≤ 4	> 4	100.0	-	0.0
Tigecycline	≤ 0.25	> 0.5	100.0	0.0	0.0
Vancomycin (VanA or VanB)	Negative	Positive	98.8	-	1.2

*The wild type is defined as intermediately susceptible.

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 60. The surveillance in NORM 2016 included 421 (68.3%) *E. faecalis* isolates (63.8% in 2015), 162 (29.2%) *E. faecium* isolates (29.2% in 2015) and 33 (5.4%) unspeciated enterococcal isolates (7.0% in 2015). 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 2.6 in 2016 and 2.2 in 2015, which is comparable to previous years. The number of isolates not speciated to the genus level or identified as *E. faecalis* or *E. faecium* has generally decreased over the last five years. The panel of antimicrobial agents examined and the breakpoints for interpretation remained unchanged from 2015 to 2016.

E. faecalis was universally susceptible to ampicillin with a single exception (MIC 6 mg/L) (Table 61). The prevalence of resistance to ampicillin in *E. faecium* remained above 80% (80.9% in 2016, Table 62). As expected, the results for imipenem closely mirrored those for ampicillin.

The prevalence of high-level gentamicin resistance (HLGR) in *E. faecalis* was 18.8%, which is at the same level as 18.7% in 2014 and 18.1% in 2015 (Figure 79). The prevalence of HLGR in *E. faecium* decreased further from 40.8% in 2014 and 39.4% in 2015, to 38.3% in 2016. This is the lowest level recorded in a decade. All 62 HLGR *E. faecium* isolates were concomitantly resistant to ampicillin and imipenem. Conversely, 62 of 132 (47.0%) ampicillin non-susceptible *E. faecium* also displayed HLGR. These findings are similar to the results from previous years. The strong linkage between ampicillin resistance and HLGR may indicate the continuing presence of the internationally disseminated *E. faecium* clonal complex (CC) 17. 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. Eleven blood culture isolates were reported as vancomycin resistant in NORM 2016 (2.0%), but only three of these were confirmed by PCR to harbour transferable VanB resistance (two *E. faecium* and one *E. faecalis*). The remaining eight vancomycin resistant isolates were either *E. gallinarum* (n=5) or *E. casseliflavus* (n=3), which are inherently low-level resistant to vancomycin due to expression of the VanC ligase. All enterococcal isolates in NORM 2016 were fully susceptible to linezolid.

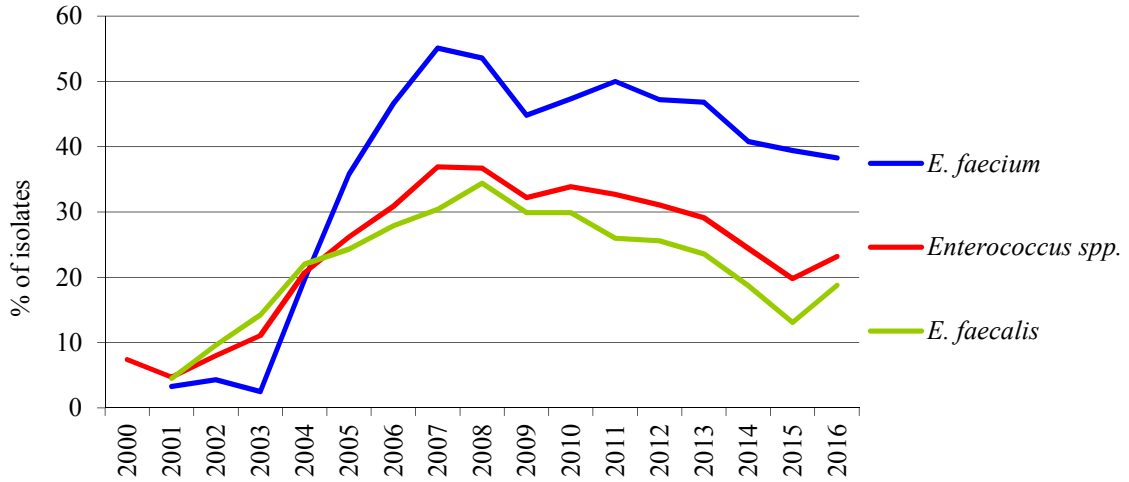


FIGURE 79. Prevalence of high-level resistance to gentamicin in blood culture isolates of *Enterococcus faecalis*, *E. faecium* and all enterococci combined during 2000-2016. The breakpoint was decreased from R ≥ 1,024 mg/L to R > 128 mg/L in 2004.

Streptococcus pneumoniae in blood cultures and cerebrospinal fluids

TABLE 63. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2016 (n=594). 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	> 2	94.6	5.1	0.3
Cefotaxime	≤ 0.5	> 2	99.3	0.7	0.0
Ceftriaxone	≤ 0.5	> 2	99.5	0.5	0.0
Erythromycin	≤ 0.25	> 0.5	94.4	0.0	5.6
Clindamycin	≤ 0.5	> 0.5	95.8	-	4.2
Tetracycline	≤ 1	> 2	93.1	1.5	5.4
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	89.2	2.9	7.9
Chloramphenicol	≤ 8	> 8	99.7	-	0.3
Oxacillin screen (mm)	≥ 20	< 20	89.9	-	10.1

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 64. *Streptococcus pneumoniae* in blood cultures and cerebrospinal fluids in 2016 (n=594). 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		0.2	56.9	35.4	2.2	0.7	3.0	0.2	0.3	0.8	0.3					
Cefotaxime		4.9	82.2	5.4	2.7	2.7	0.8	0.7	0.7							
Ceftriaxone		7.2	82.5	3.4	2.5	2.7	0.5	0.7	0.5							
Erythromycin					14.3	72.7	7.4		0.2	0.5	0.2	0.3	0.2			4.2
Clindamycin				0.2	12.3	70.2	12.8	0.3								4.2
Tetracycline					3.2	80.0	9.8		0.2	1.5	0.8	0.5	2.0	1.9	0.2	
TMS**					0.2	23.9	58.2	3.2	3.7	2.9	1.2	1.9	1.0	3.9		
Chloramph.						0.2				13.1	85.9	0.5	0.3			
Norfloxacin							0.2			2.2	24.9	66.0	5.7	0.8		0.2

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	10.1	1.3	0.7	1.5	1.7	3.4	16.5	14.1	15.5	16.3	9.1	8.4	1.2	0.2		

*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

The results are summarised in Tables 63-64 and Figures 80-81. All systemic *S. pneumoniae* isolates submitted to the National Reference Laboratory for Pneumococci at the Norwegian Institute of Public Health during 2016 were included in the surveillance protocol. Twenty-four strains were isolated from cerebrospinal fluids, and five of these were retrieved from patients who concomitantly had positive blood cultures. In addition, five isolates were recovered from a pleural effusion (n=1), synovial fluid or membrane (n=2), bronchial lavage (n=1) and an unknown location (n=1). Both blood culture isolates and isolates from other sterile sites were included from patients with positive cultures from more than one specimen type. Norwegian breakpoints for pneumococci are in accordance with EUCAST, and these remained unchanged in 2016. The results for penicillin G were interpreted according to the general breakpoints for pneumococci (S ≤ 0.06, R > 2 mg/L). The isolates from cerebrospinal fluids were in addition categorised according to penicillin G breakpoints for meningitis (R > 0.064).

A total of 5.1% (30/594) of *S. pneumoniae* isolates were intermediately susceptible to penicillin G (MIC 0.125-2 mg/L) and two isolates (0.3%) were classified as resistant (MIC 4 mg/L). The prevalence of non-susceptibility to penicillin G was at the same level as in 2014 (5.3%) and 2015 (7.5%). Two of the penicillin G intermediately susceptible strains were recovered from cerebrospinal fluids and were thus clinically resistant (MIC 0.25 mg/L). Four cefotaxime intermediate isolates from unique patients (three blood, one unknown specimen) were either resistant (n=2) or intermediate (n=2) to penicillin G, and three of them were also intermediately susceptible to ceftriaxone.

The oxacillin screening disk is often used to discriminate between penicillin susceptible and non-susceptible isolates. Among 32 penicillin G non-susceptible isolates, all were resistant to oxacillin. Conversely, 28/562 penicillin G susceptible isolates were oxacillin resistant. The sensitivity and specificity of the screening test was thus 100.0% and 95.0%, respectively. Many of the penicillin non-susceptible *S. pneumoniae* isolates were also non-susceptible to

erythromycin (18/32), tetracycline (18/32), trimethoprim-sulfamethoxazole (17/32) and/or chloramphenicol (1/32).
The prevalence of macrolide non-susceptibility remained stable at 5.6% compared to 4.8% in 2015. Most of these isolates (25/33, 76% of macrolide non-susceptible isolates, 4.3% of all isolates) were concomitantly high-level resistant to erythromycin and clindamycin, which is compatible with a constitutive MLS_B phenotype. The remaining eight isolates (24% of macrolide non-suscpetible isolates, 1.3% of all isolates) were either low-level resistant to erythromycin and susceptible to clindamycin as seen in the efflux-based M-type resistance (n=7), or inducibly resistant MLS_B (n=1). The distribution of MLS phenotypes was not significantly altered from 2015 to 2016. The results may suggest a continuing predominance of *erm*-mediated macrolide resistance as opposed to the *mef*-dominated peak 2002-2009 (Figure 81).
The 10.8% non-susceptibility to trimethoprim-sulfamethoxazole was at the same level as 11.7% % in 2015. The prevalence of non-susceptibility to tetracycline also remained stable at 6.9% in 2016 (Figure 80). The vast majority of isolates (99.7%) were susceptible to chloramphenicol, which was earlier used for empirical treatment of meningitis in Norway. The low prevalence of high-level norfloxacin resistance (Table 64) may reflect the very limited use of levofloxacin and moxifloxacin for respiratory tract infections in Norway.

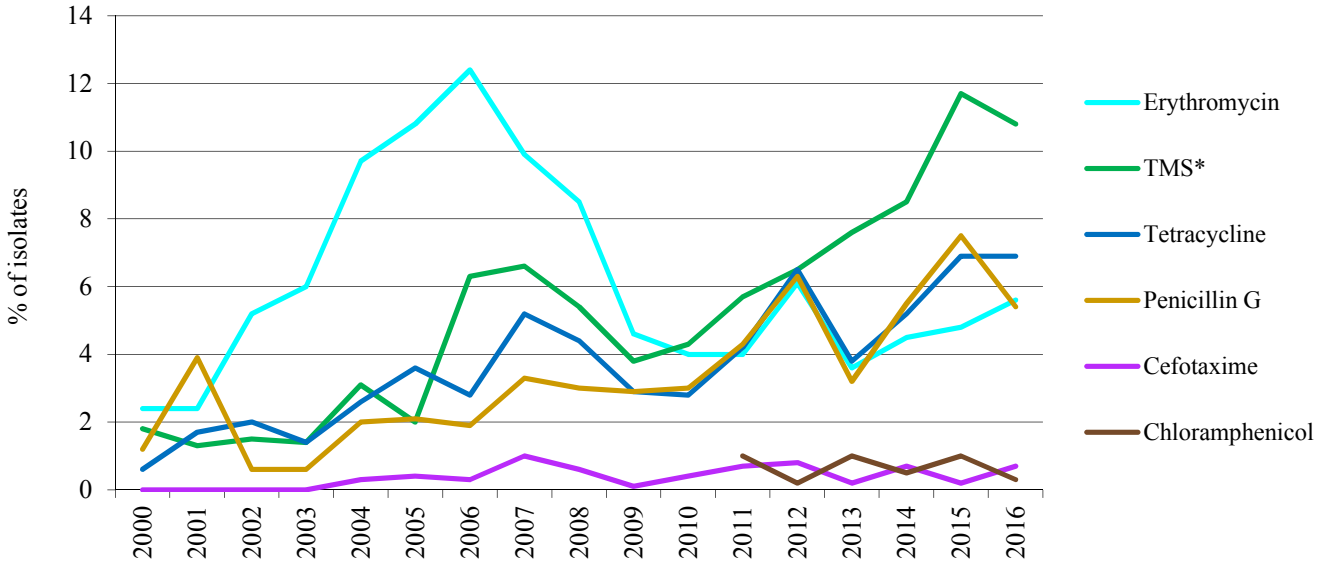


FIGURE 80. Prevalence (%) of non-susceptibility to various antimicrobial agents in *Streptococcus pneumoniae* blood culture isolates during 2000-2016. Doxycycline was substituted by tetracycline in 2005. All results are categorised according to the 2017 breakpoint protocol. *TMS=Trimethoprim-sulfamethoxazole.

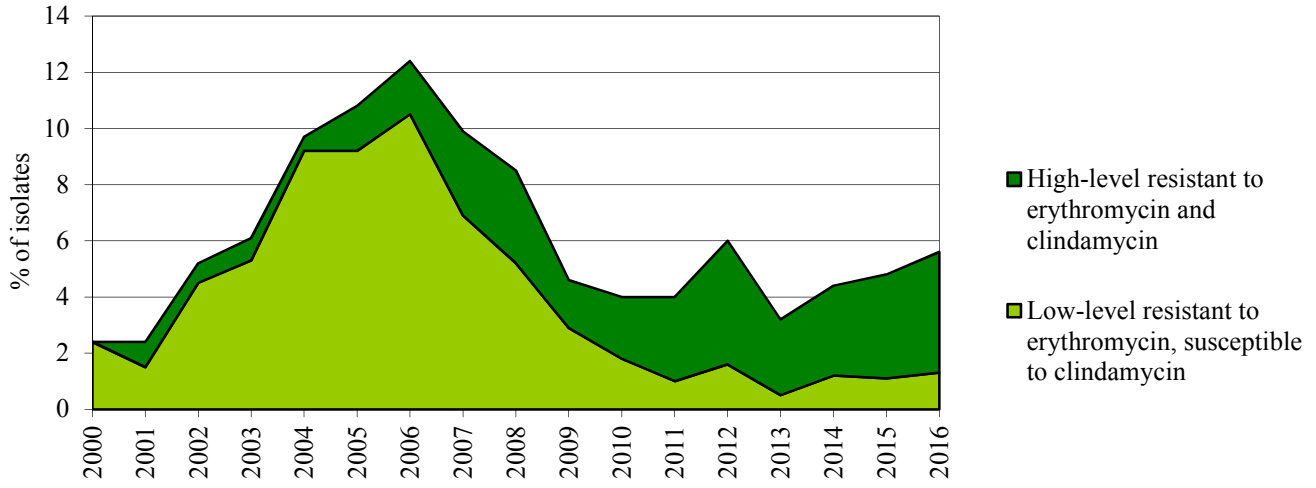


FIGURE 81. Prevalence (%) of non-susceptibility to erythromycin and clindamycin in *Streptococcus pneumoniae* blood culture isolates during 2000-2016.

Streptococcus pneumoniae in respiratory tract specimens

TABLE 65. *Streptococcus pneumoniae* in respiratory tract specimens in 2016 (n=376). 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	> 2	91.8	8.2	0.0
Cefotaxime	≤ 0.5	> 2	98.6	1.1	0.3
Ceftriaxone	≤ 0.5	> 2	99.4	0.3	0.3
Erythromycin	≤ 0.25	> 0.5	88.9	2.1	9.0
Clindamycin	≤ 0.5	> 0.5	93.4	-	6.6
Tetracycline	≤ 1	> 2	89.7	1.3	9.0
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	89.9	4.0	6.1
Chloramphenicol	≤ 8	> 8	99.2	-	0.8
Oxacillin screen (mm)	≥ 20	< 20	90.4	-	9.6

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 66. *Streptococcus pneumoniae* in respiratory tract specimens in 2016 (n=376). 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	3.7	35.1	42.6	9.0	1.3	2.4	3.2	1.1	0.8	0.8						
Cefotaxime	0.8	18.4	56.1	13.8	4.3	2.9	1.1	1.3	0.5	0.5			0.3			
Ceftriaxone	2.9	42.8	41.0	4.8	2.7	1.9	1.9	1.6	0.3					0.3		
Erythromycin			0.3	0.8	5.1	38.3	44.4	2.1		0.8	0.3	1.1	0.8	0.8	0.8	4.5
Clindamycin			0.3	1.9	6.6	31.1	46.5	6.9	0.5	0.8	1.1		0.3	0.5		3.5
Tetracycline		0.3	0.3	1.1	23.9	59.6	3.7	0.3	0.5	1.3	0.8	1.6	4.3	2.4		
TMS**			0.5	1.1	8.8	47.1	19.7	10.4	2.4	4.0	2.1	1.1	1.1	1.9		
Chloramph.			0.5	0.5	0.3			0.3	3.5	39.6	53.5	1.1	0.8			
Norfloxacin			0.3				0.8		0.3	3.7	21.5	48.1	23.1	1.1	0.3	0.8

	< 20	20	21	22	23	24	25	26	27	28	29	30	31	32	33	≥ 34
Oxacillin disk	9.6	1.3	0.5	2.1	1.9	2.4	7.4	7.7	11.2	17.3	8.2	16.0	4.3	3.7	2.7	3.7

*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

S. pneumoniae isolates from respiratory tract specimens were last surveyed in NORM in 2014. The rates of non-susceptibility to various antimicrobials are shown in Tables 65-66 and Figure 82.

The prevalence of non-susceptibility to penicillin G increased from 5.9% in 2014 to 8.2% in 2016. All the 31 isolates were intermediately susceptible to penicillin G, no penicillin resistant isolates were detected. Five penicillin non-susceptible isolates displayed reduced susceptibility (n=4, MIC 1-2 mg/L) or resistance (n=1, MIC 16 mg/L) to cefotaxime, and two of these were also intermediately susceptible (n=1, MIC 1 mg/L) or resistant (n=1, MIC 32 mg/L) to ceftriaxone. Thirty of the 31 penicillin G non-susceptible isolates were detected by the oxacillin screening test (sensitivity 96.8%), whereas six fully penicillin susceptible isolates were classified as oxacillin resistant (specificity 98.2%). Beta-lactam non-susceptible isolates were commonly cross-resistant to other antimicrobial agents such as erythromycin (16/31), trimethoprim-sulfamethoxazole (18/31) and tetracycline (18/31).

The rate of non-susceptibility to erythromycin was 11.1% in 2016 compared to 7.9% in 2014. Macrolide resistance was thus significantly more common in respiratory tract isolates than in isolates from blood and sterile sites (5.6%). The MLS fenotype of 41/42 isolates was determined by double disk diffusion. Twenty-four isolates (58% of erythromycin non-susceptible isolates, 6.4% of all isolates) displayed low-level M type resistance as opposed to 15 constitutively resistant (37% of erythromycin non-susceptible isolates, 4.1% of all isolates) and two inducibly resistant (5% of erythromycin non-susceptible isolates, 0.6% of all isolates) MLS_B isolates. A total of 16 isolates (4.3%) were concomitantly non-susceptible to penicillin G and erythromycin. This is a minor increase from 2.0% in 2014.

Tetracycline non-susceptibility remained unchanged at 10.3% in 2016 compared to 2014, whereas trimethoprim-sulfamethoxazole non-susceptibility has increased from 6.9% in 2014 to 10.1% in 2016.

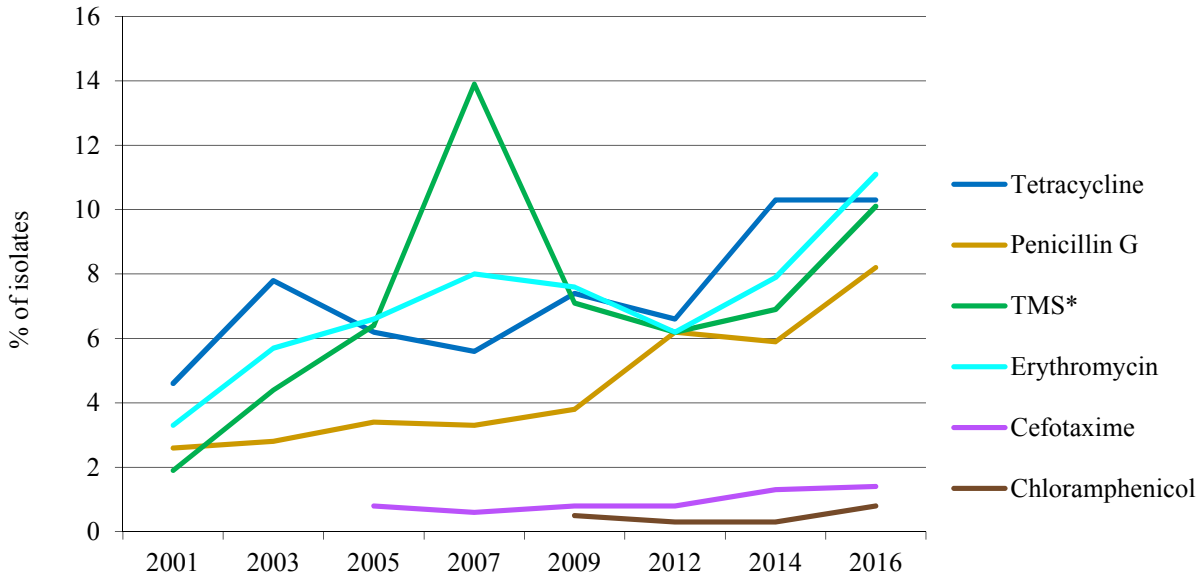


FIGURE 82. Prevalence of non-susceptibility to various antimicrobials in *Streptococcus pneumoniae* from respiratory tract samples 2001-2016. Doxycycline was replaced by tetracycline in 2005. *TMS=Trimethoprim-sulfamethoxazole. Please note that the x-axis is not to scale.

Streptococcus pyogenes in blood cultures

TABLE 67. *Streptococcus pyogenes* in blood cultures in 2016 (n=187). 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	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	96.3	0.0	3.7
Clindamycin	≤ 0.5	> 0.5	97.9	-	2.1
Tetracycline	≤ 1	> 2	83.5	0.5	16.0
Trimethoprim-sulfamethoxazole*	≤ 1	> 2	97.9	0.0	2.1

*Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

TABLE 68. *Streptococcus pyogenes* in blood cultures in 2016 (n=187). 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	4.3	93.6	1.6												
Erythromycin				0.5	39.0	56.1	0.5					0.5	1.1			2.1
Clindamycin				3.7	47.1	47.6										1.6
Tetracycline					14.4	65.8	3.2			0.5			3.7	9.6	2.7	
TMS**			0.5	0.5	16.6	43.9	31.6	3.7	1.1					2.1		

*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. **TMS=Trimethoprim-sulfamethoxazole. Breakpoints for the trimethoprim-sulfamethoxazole combination are given for the trimethoprim component only.

RESULTS AND COMMENTS

The Reference Laboratory at the Norwegian Institute of Public Health provides resistance data for systemic *S. pyogenes* isolates on a yearly basis. The Norwegian breakpoints for haemolytic streptococci are in accordance with EUCAST. All comparisons in this report are based on the 2017 recommendations.

As expected, all isolates were fully susceptible to penicillin G (Tables 67-68). The prevalence of resistance to erythromycin (3.7%) and clindamycin (2.1%) was unchanged from 2015 (3.5% and 2.0%, respectively). Three of the seven macrolide resistant isolates were also high-level resistant to clindamycin. The prevalence of tetracycline resistance has decreased from 20.0% in 2015 to 16.0% in 2015, whereas the prevalence of non-susceptibility to trimethoprim-sulfamethoxazole has remained stable at 2.1% in 2016 compared to 2.0% in 2015.

Streptococcus agalactiae in blood cultures and cerebrospinal fluids

TABLE 69. *Streptococcus agalactiae* isolates from sterile sites in 2016 (n=281). 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	≤ 0.25	> 0.25	100.0	-	0.0
Erythromycin	≤ 0.25	> 0.5	78.3	4.6	17.1
Clindamycin	≤ 0.5	> 0.5	87.6	-	12.4
Tetracycline	≤ 1	> 2	27.0	0.0	73.0
Vancomycin	≤ 2	> 2	100.0	-	0.0

TABLE 70. *Streptococcus agalactiae* isolates from sterile sites in 2016 (n=281). 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			0.4	15.2	83.6	1.8										
Erythromycin				0.4	4.6	18.9	54.4	4.6		1.1	1.4	4.6	2.5	1.1	0.4	6.0
Clindamycin				1.1	5.7	69.4	11.0	0.4	2.1	1.1	0.4	0.4	0.4		0.4	7.8
Tetracycline				0.4	16.0	8.2	1.4		0.7		1.1	4.3	32.0	31.3	4.3	
Vancomycin				0.7	0.7	23.1	71.9	3.6								
Gentamicin												0.7	6.1	44.8	45.6	2.8

*Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Calculation was performed on 281 strains isolated from unique patients. Strains isolated from different specimens collected at the same time from the same patient were excluded.

RESULTS AND COMMENTS

Streptococcus agalactiae (beta-haemolytic group B streptococci) has previously been included in NORM in 2006, 2009 and 2012. All systemic isolates in Norway are referred to the National Reference Laboratory at St. Olavs Hospital, Trondheim University Hospital, where confirmatory identification and susceptibility testing is performed. Since 2014, the reference laboratory has provided resistance data for all invasive *S. agalactiae* isolates on a yearly basis.

Relevant breakpoints have remained unchanged since 2009. A total of 281 strains isolated from invasive infections were included in 2016. Forty isolates originated from neonates and small children < 1 year of age (20 EOD and 20 LOD). Most isolates (91.1%) were recovered from blood cultures, but there were also 25 isolates from diverse specimens: cerebrospinal fluid (n=3), joint fluid (n=7), tissue biopsy (n=4), autopsy (n=1) and other specimens (n= 10).

As seen in Tables 69-70 there were no isolates with reduced susceptibility to penicillin G or vancomycin. Forty-eight isolates (17.1%) were resistant to erythromycin compared to 17.9% in 2015. In addition, 4.6% were only intermediately susceptible compared to 9.0% in 2015. The erythromycin non-susceptible isolates were analysed for MLS_B resistance phenotype. Twenty-nine displayed constitutive (n=21) or inducible (n=8) MLS_B resistance indicating the presence of an *erm* determinant. The remaining nineteen isolates had a resistance pattern in accordance with an efflux-mediated M phenotype encoded by a *mef* gene. Five isolates were recorded as clindamycin resistant (MIC 1-2 mg/L) in spite of erythromycin susceptibility (MIC 0.125-0.25 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 2.8% of the isolates. The prevalence of resistance to tetracycline (73%) was at the same high level as in 2015 (81.0%) with the majority of isolates displaying MIC values of 16-32 mg/L (Table 70).

Mycobacterium tuberculosis

A total of 298 cases of tuberculosis disease (TB) were reported to the Norwegian Surveillance System for Communicable Diseases (MSIS) in 2016. Of these, 33 cases were born in Norway. Two-hundred and fifty-five cases had TB for the first time, 20 cases had been treated with anti-TB drugs previously and one additional case had been diagnosed with TB but had not been treated. The rest, 22 cases, were categorised as uncertain.

Two hundred and twenty-eight cases were confirmed infections with *M. tuberculosis* complex by culture, and all isolates were susceptibility tested. The results are presented in Table 71. Cases are registered in MSIS the year in which the first culture positive test was taken. There were 11 MDR-TB cases. Four were co-resistant to pyrazinamide, two of them also ethambutol resistant, three were co-resistant to prothionamid and one to capreomycin. There were no isolates resistant to moxifloxacin or amikacin and consequently no XDR-TB cases, but the capreomycin resistant isolate was classified as pre-XDR. Nine of the MDR-TB cases had TB for the first time and two were categorised as uncertain.

TABLE 71. Antimicrobial susceptibility of 228 isolates of *Mycobacterium tuberculosis* complex (not *M. bovis* (BCG)) from human infections in 2016. Figures from 2015 are shown in parentheses.

			Resistance to antimicrobial agents (No. of isolates)				
Origin of birth	No. of cases	No. of isolates	Isoniazid	Rifampicin	Ethambutol	Pyrazinamid	MDR-TB*
Norway	33 (36)	22 (22)	2 (1)	2 (0)	0 (0)	0 (3)	2 (0)
Europe excl. Norway	39 (27)	34 (22)	3 (1)	2 (0)	2 (0)	7 (0)	2 (0)
Asia	104 (115)	80 (88)	4 (9)	3 (2)	0 (0)	2 (5)	3 (1)
Africa	120 (137)	90 (111)	10 (11)	4 (4)	0 (0)	6 (4)	4 (4)
America	1 (3)	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oceania	1 (0)	1 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total	298 (318)	228 (245)	19 (22)	11(6)	2 (0)	15[#] (12)	11 (5)
Proportion resistant isolates (%)			8.3 (9.0)	4.8 (2.4)	0.9 (0.0)	6.6 (5.0)	4.8 (2.0)

* 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).

[#] Three of these were *M. bovis* strains inherently resistant to pyrazinamid.

Candida spp. in blood cultures

TABLE 72. Antimicrobial susceptibility of *Candida albicans* blood culture isolates (n=137). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	100.0	0.0	0.0
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*/**	≤ 0.03	> 0.03	100.0	-	0.0
Micafungin*/**	≤ 0.016	> 0.016	100.0	-	0.0

* Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST. ** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin and micafungin are considered susceptible.

TABLE 73. *Candida albicans* blood culture isolates (n=137). 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					1.5	8.0	48.9	36.5	5.1								
Fluconazole					2.2	9.6	58.8	27.2	1.5	0.7							
Voriconazole	16.1	64.7	16.2	2.2	0.7												
Anidulafungin	72.8	25.7	1.5														
Micafungin	55.1	44.9															
Caspofungin**	0.7		0.7	8.8	53.7	28.7	5.9	1.5									

* Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. **There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin and micafungin are considered susceptible.

TABLE 74. Antimicrobial susceptibility of *Candida glabrata* blood culture isolates (n=35). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 0.002	> 32	0.0	82.8	17.2
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0
Micafungin*/**	≤ 0.03	> 0.03	100.0	-	0.0

* Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing - EUCAST. 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 75. *Candida glabrata* blood culture isolates (n=35). 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						14.3	11.4	60.0	14.3								
Fluconazole									2.9	5.7	22.9	37.1	11.4	2.9	2.9		14.3
Voriconazole**					11.4	37.1	22.9	11.4	2.9	2.9	2.9	8.6					
Anidulafungin	2.9	31.4	62.9	2.9													
Micafungin		31.4	62.9	5.7													
Caspofungin***				2.9	2.9	20.0	65.7	8.6									

* Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. **There is insufficient evidence that *C. glabrata* is a good target for therapy with voriconazole and no 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 76. Antimicrobial susceptibility of *Candida tropicalis* blood culture isolates (n=13). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	100.0	0.0	0.0
Voriconazole*	≤ 0.125	> 0.125	100.0	-	0.0
Anidulafungin*/**	≤ 0.06	> 0.06	100.0	-	0.0

* Recommended breakpoints by the European Committee on Antimicrobial Susceptibility Testing – EUCAST. ** There are no European breakpoints for caspofungin. Strains susceptible to anidulafungin are considered susceptible. There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no breakpoints are available. An MIC with comment without an accompanying S, I or R categorisation may be reported.

TABLE 77. *Candida tropicalis* blood culture isolates (n=13). 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	≥ 256
Ampho. B							2	3	8								
Fluconazole							4	8	1								
Voriconazole		1	3	4	5												
Anidulafungin		3	10														
Micafungin**			9	4													
Caspofungin***					1	5	7										

* Shaded areas in each row indicate susceptibility (light), intermediate susceptibility (medium) and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. **There is insufficient evidence that *C. tropicalis* is a good target for therapy with micafungin and no 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 are considered susceptible to caspofungin.

TABLE 78. Antimicrobial susceptibility of *Candida parapsilosis**** blood culture isolates (n=15). Sampling, laboratory methods, and data handling are described in Appendix 5.

	Breakpoints (mg/L)		Proportion of isolates (%)		
	Susceptible	Resistant	Susceptible	Intermediately susceptible	Resistant
Amphotericin B*	≤ 1	> 1	100.0	-	0.0
Fluconazole*	≤ 2	> 4	93.4	-	6.7
Voriconazole*	≤ 0.125	> 0.125	93.4	-	6.7
Anidulafungin*/**	≤ 0.002	> 4	0.0	100.0	0.0
Micafungin*/**	≤ 0.002	> 2	0.0	100.0	0.0

* Recommended breakpoints by the European Committee on antimicrobial susceptibility testing - EUCAST. ** There are no European breakpoints for caspofungin. Strains intermediate to anidulafungin and micafungin can be considered intermediate to caspofungin. ***The fluconazole resistant isolate was identified as *Candida orthopsilosis*, a species in to the *C. parapsilosis* complex.

TABLE 79. *Candida parapsilosis*** blood culture isolates (n=15). 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	≥ 256
Ampho. B							4	4	7								
Fluconazole						1	2	7	4								1
Voriconazole		4	4	5	1							1					
Anidulafungin							1	5	4	4	1						
Micafungin							1	8	6								
Caspofungin***								9	5		1						

* Shaded areas in each row indicate susceptibility (light), intermediate susceptibility and resistance (dark). Non-shaded rows indicate that breakpoints have not been defined. ** There are no European breakpoints for caspofungin. Strains intermediate to anidulafungin and micafungin can be considered intermediate to caspofungin. ***The fluconazole resistant isolate was identified as *Candida orthopsilosis*, a species in to the *C. parapsilosis* complex.

RESULTS AND COMMENTS

In 2016, 213 *Candida* isolates from bloodstream infections in 202 patients were referred to the National Mycology Reference Laboratory. In 2015 the Reference Laboratory received 210 isolates of nine different *Candida* species.

Candida albicans is by far the most common species causing candidemia in Norway (n=137, 64.3 %), but the proportion of non-albicans isolates (n=76, 35.7%) is increasing. Comparable rates in 2015 and 2014 were 33.8% and 30.5%, respectively. The proportion of *Candida glabrata* isolates is still low (n=35, 16.4 %) followed by *Candida parapsilosis* (n=15, 7.0%), *Candida tropicalis* (n= 13, 6.1%), *Candida dubliniensis* (n=6, 2.8%), *Candida krusei* (n=3, 1.4%) and single isolates of *C. auris, C. lusitaniae*, *C. rugosa* and *C. guilliermondii*. Six mixed infections with two or more *Candida* spp. and three persistent infections with the same species more than four weeks apart were observed in 2016.

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 micro-dilution method at Statens Serum Institut in Copenhagen. The results are presented in Tables 72-79.

One candidemia with a multiresistant *C. auris* was referred to the National Mycology Reference Laboratory in 2016. Due to its propensity to cause outbreaks and its antifungal resistance, *C. auris* poses a risk for patients in healthcare facilities. Difficulties with laboratory identification, combined with a lack of awareness of this new *Candida* species, might result in unnoticed outbreaks. In December 2016, the European Centre for Disease Prevention and Control identified a clear need to raise awareness in European healthcare facilities in order to adapt laboratory testing strategies and implement enhanced control measures to prevent hospital outbreaks (European Centre for Disease Prevention and Control. *Candida auris* in healthcare settings – Europe – 19 December 2016. Stockholm: ECDC; 2016). Nosocomial spread has not been identified in Norway.

Acquired antifungal resistance is rare in Norway and species identification predicts the susceptibility pattern of the *Candida* species isolated in Norway with high accuracy. All *C. albicans* isolates were susceptible to all drugs tested. All candidemia isolates were susceptible to amphotericin B, with exception of the *C. auris,* which was regarded as resistant (MIC 2 mg/L). Amphotericin B is not recommended treatment of *C. lusitaniae* infections (n=1) as *C. lusitaniae* has high MICs or develop resistance during treatment.

Fluconazol resistance was found in the *C. auris* (256 mg/L) isolate, one *C. orthopsilosis* (256 mg/L) isolate and one *C. rugosa* (8 mg/L) isolate. Otherwise, observed reduced susceptibility to fluconazole is due to intrinsic resistance in *C. krusei* (n=3). Breakpoints for fluconazole ($S \leq 0.002$; $R > 32$) in *C. glabrata* categorise the wild type as intermediately susceptible (n=29). 17% (n=6) of the *C. glabrata* isolates were categorised as resistant. There is still insufficient evidence that *C. glabrata* and *C. krusei* are good targets for therapy with voriconazole, and no breakpoints are set. EUCAST recommends reporting the voriconazole MIC value without S, I and R categorisation. Except from the fluconazole resistant *C. orthopsilosis*, all isolates with defined breakpoints were found susceptible to voriconazole. In 2015, isavuconazole has been added to the EUCAST breakpoint table for *Candida* spp., but there is insufficient evidence that *Candida* spp. is a good target for therapy with the drug and breakpoints have not been established.

Acquired echinocandin resistance was not found in 2016, and all *C. parapsilosis* (n=14) and *C. orthopsilosis* (n=1), which belongs to the *C. parapsilosis* species complex, belonged to the wild type and were categorised as intermediately susceptible to echinocandins.

Decreased susceptibility to different antifungal classes is common in some of the species not shown in the figures such as *C. guilliermondii* (n=1), a species without any breakpoints, but known to exhibit decreased susceptibility to amphotericin B, fluconazole and the echinocandins.

Appendix 1:
Collection of data on usage of antimicrobial agents in animals

Data sources. Collection of data
Veterinary Medicinal Products (VMPs)

Sales data,
In Norway, all veterinary medicinal products (VMPs) are prescription-only medicines, and have to be dispensed through pharmacies, which are supplied by drug wholesalers and/or by feed mills. Veterinarians are not allowed to dispense VMPs except in emergency situations in the field. In such cases the VMPs have to be sold at cost price. Premixes/medicated feeds are currently only used for farmed fish; partly due to the small size of livestock herds (terrestrial animals) in Norway. Group/flock treatment of livestock (terrestrial animals) with antibacterial agents is subjected to administration 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 medicated feed, to the Norwegian Institute of Public Health (NIPH).
The data on sales of each product presentation (name, form, strength and pack size) of the included VMPs were obtained from the NIPH, which collects the data from the wholesalers and feed mills

Prescription data
For 2015 and 2016, prescription data for farmed fish and broilers have been obtained from the Veterinary Prescription Register (VetReg) that was implemented by the Norwegian Food Safety Authority 1st January 2011. For farmed fish, the VetReg data provide information about, among others, fish species and production stage.

Ionophore coccidiostat feed additives
Until 2003, the Norwegian Agricultural Inspection Service was responsible for the collection of data on sales of antimicrobial growth promoters and coccidiostat feed additives, while from that time the Norwegian Food Safety Authority has been in charge of collecting such data. Data on the sales of the different active substances was obtained from these sources

Veterinary medicinal products included in the data set
The Anatomical Therapeutic Chemical vet (ATCvet) classification system was used to identify the veterinary medicinal products (http://www.whocc.no/atcvet) to be included in the data. The ATCvet codes for which the data were requested from the NIPH for terrestrial animals are shown in the table below, which is identical to the inclusion criteria by the European Surveillance of Veterinary Antimicrobial Consumption (ESVAC) (http://www.ema.europa.eu/ema/index.jsp?curl=pages/regulation/document_listing/document_listing_000302.jsp).

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] Solely sulfonamides

Antibacterial products sold on special exemption from market authorisation are included in the data. Dermatological preparations (QD) and preparations for sensory organs (QS) are not included in the data. Antibacterial human medicinal products (HMPs) are used in small animal practice and to a limited extent in food producing animal practice; however, data on sales of HMPs to animals are not included in this report as such sales cannot be separated from sales used in humans.

Data sources animal population data
Data on animal population, including farmed fish, were obtained from Statistics Norway (https://www.ssb.no).

Analysis and reporting of the data
The sales data for each product 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, were converted to the corresponding values for the active ingredient, here benzylpenicillin.
The sales data of antibacterial VMPs for terrestrial animals have been stratified into food producing animals and companion animals – i.e. by stratifying tablets, oral solution and oral paste approved for companion animals only; in addition dihydrostreptomycin tablets of pack size 10 pieces have been included. Sales of VMPs for food producing animals have been stratified into VMPs for treatment of individual food producing animals - bolus, oral paste injectables, intramammary preparations, intrauterine preparations and some tablets (dihydrostreptomycin pack size 20 and 100) and for group treatment (oral solution and oral powder). There is some use of injectable VMPs in companion animals thus the usage for this animal category is slightly underestimated and overestimated for food producing animals. However, it is thought that the stratified data give a valid picture of the development of the usage in companion and food producing animals.
For 2010-2016, a separate analysis normalising the sales for terrestrial food producing animals and farmed fish, respectively, by the population-at-risk – i.e. a population correction factor (PCU) - is provided. The animal categories included in the PCU as well as the calculation are identical to ESVAC and is described in detail in Annex 1 of the first ESVAC report (http://www.ema.europa.eu/docs/en_GB/document_library/Report/2011/09/WC500112309.pdf).

Appendix 2:
Collection of data on human usage of antimicrobial agents

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*) have analysed the data according to activity (admission and bed days).

Population statistics per 1 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 nationwide prescription database situated at the Norwegian Institute of Public Health. This database includes all prescriptions 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 2017 is used.

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 the antibacterials are as a main rule based on the use in infections of moderate severity. Some anti-bacterials 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), rifaximin (A07AA11) and oral and rectal metronidazole (P01AB01) are also included in some figures. For antifungals, only ATC-group J02 *antimycotics for systemic use* is included. Of the antimycobacterials (ATC J04), only rifampicin is included. The content of rifampicin has been calculated in plain products and in combinations and data is presented as total amount of rifampicin. 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 2017. 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 indicator bacteria *Escherichia coli* isolated from broiler and turkey flocks were from caecal samples collected at slaughter by the Norwegian Food Safety Authority (NFSA). From each flock 10 caecal samples were collected. A total of 185 pooled samples from broiler and 156 pooled samples from turkeys were included. Only one sample from each flock was included. In addition, 528 faecal samples from red fox (*Vulpes vulpes*) collected through the Norwegian monitoring programme for *Echinococcus multilocularis*, and 357 faecal samples from wild birds (various ducks and gulls) collected through the Norwegian surveillance programme for avian influenza in wild birds in 2016, were included. The samples were also used for selective isolation of *E. coli* resistant to third generation cephalosporins, quinolone resistant *E. coli* (wild animals), colistin (wild birds) and carbapenemase-producing *Enterobacteriaceae*, as well as for quantification of *E. coli* resistant to third generation cephalosporins (broiler and turkey). Samples of 175 broiler and 128 turkey meat were collected by the NFSA at retail in all regions of Norway following the specifications set by the European Food Safety Authorities (EFSA journal 2014;12(5):3686). Samples were taken without taking place of origin into consideration. At the same time 359 samples of seafood were collected. The seafood samples comprised shellfish such as blue mussels, scallops, oysters, scampi etc. of both domestic and imported origin. In addition, 179 samples of cheese were collected by the NFSA in connection with a survey of cheese on the Norwegian market. The cheese samples comprised both pasteurised and un-pasteurised cheeses of both domestically and imported origin. Only one sample from each production batch was included. All the food samples were analysed using selective isolation of *E. coli* resistant to third generation cephalosporins and carbapenemase-producing *Enterobacteriaceae*. In addition, *E. coli* as indicator bacteria and QREC were isolated from cheese and seafood. In addition, 391 batch samples, each of 10-25 individual bivalve molluscs, collected from 60 localities covering the Norwegian coast (Troms to Agder county) as part of the mandatory EU surveillance programme (854/2004/EC, 2004) and investigated at the National Institute of Nutrition and Seafood Research (NIFES), were included. Most of the bivalve samples were harvested from commercially active rearing localities, either by NFSA inspectors or by the commercial actors. Some samples were collected from positions established by NFSA for long-time reference monitoring purposes of shellfish safety. The samples were analysed for the indicator bacteria *E. coli* by the EU specified reference method followed by selective isolation of *E. coli* resistant to third generation cephalosporins, QREC and carbapenemase producing *Enterobacteriaceae.* Samples of dry feed for cattle and swine (155 samples) and wet feed for dogs (85 samples) were included. The samples were collected from all regions in Norway by the NFSA in connection with a surveillance programme in feed (cattle and swine feed) and a survey of wet feed. Only one sample per production batch was included. The feed samples were analysed for *E. coli* indicator bacteria, in addition to selective isolation of *E. coli* resistant to third generation cephalosporins, QREC and carbapenemase-producing *Enterobacteriaceae*.

Indicator isolates of *E. coli*

Sample material, i.e. caecal content from 10 broilers or turkeys per flock, and faecal samples from red fox or wild birds, were mixed and plated directly onto MacConkey agar and incubated at 41±0.5°C for 24h. For the meat, seafood, cheese, bivalve molluscs and feed samples, 10 µL of the enrichment broths were plated onto MacConkey agar. Typical colonies were subcultured on blood agar (Heart infusion agar, Difco) containing 5% bovine blood, and incubated at 37°C for 24h. Colonies were identified as *E. coli* by typical colony appearance and a positive indole reaction and verified by using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany).

Enrichment of samples

All samples were enriched prior to plating onto selective media. A total of 1±0.1 g caecal sample material was homogenised with 9 mL of buffered peptone water (BPW-ISO). Faecal samples on swabs were inoculated in 5 mL of BPW-ISO. A total of 25±0.5 g sample material of meat, seafood, cheese, bivalve molluscs, and feed were homogenised with 225 mL of BPW-ISO. Samples were incubated at 37±1°C for 20±2 h according to the protocol from the European Union Reference Laboratory for Antimicrobial Resistance version 3 (EURL-AR: http://www.eurl-ar.eu/233-protocols.htm). After incubation a loopful (10 µL) of enrichment broth was plated on selective media as described in the sections below.

***E. coli* resistant to third generation cephalosporins**

Aliquots of 10 µL from the overnight BPW-ISO broth from all caecal, faecal, meat, seafood, cheese, bivalve molluscs and feed samples were plated onto MacConkey agar (Difco) containing 1 mg/L cefotaxime and MacConkey agar containing 2 mg/L ceftazidime. The agar plates were incubated at 41±0.5°C for 24-48h. Presumptive cephalosporinase producing *E. coli* were subcultured on MacConkey agar containing 1 mg/L cefotaxime and blood agar, and confirmed as *E. coli* using MALDI-TOF MS (Bruker Daltonik GmbH, Bremen, Germany) before further tested for cephalosporinase production.

Quinolone resistant *E. coli*

Aliquots of 10 µL from the overnight BPW-ISO broth from caecal, faecal, meat, seafood, cheese, bivalve molluscs and feed samples were plated onto MacConkey agar containing 0.06 mg/L ciprofloxacin. Plates were incubated at 41±0.5°C for 24h and presumptive QREC were subcultured on blood agar and MacConkey agar containing 0.06 mg/L ciprofloxacin, and confirmed as *E. coli* using MALDI-TOF MS before further phenotypical testing.

Carbapenemase-producing *Enterobacteriaceae*

Aliquots of 10 µL from the overnight BPW-ISO broth from all caecal, faecal, meat, seafood, cheese, bivalve molluscs and feed samples were plated onto chromID™ CARBA and chromID™ OXA-48 agar (bioMérieux, Marcy l'Etoile, France). The plates were incubated at 37±1°C for 24-48 h. Presumptive carbapenemase-producing *Enterobacteriaceae* were subcultured on blood agar and confirmed using MALDI-TOF MS.

Colistin resistant *E. coli*
Aliquots of 10 µL from the overnight BPW-ISO broth from faecal content from wild birds was plated onto SuperPolymyxin agar (Oxoid) and incubated at 37±0.5°C for 20±2h (Nordmann *et al.* 2016). Presumptive positive colonies were selected, subcultured on blood agar and SuperPolymyxin agar, and confirmed as *E. coli* using MALDI-TOF MS.

Genotyping
For the presumptive cephalosporin resistant *E. coli*, PCR was performed for the identification of the genotypes $bla_{\text{CTX-M}}$, bla_{TEM}, bla_{SHV}, multiplex PCR to look for plasmid-mediated AmpC genes, or real-time PCR for the $bla_{\text{CMY-2}}$ gene (Pérez-Pérez et al. 2002, Hasman et al. 2005, Briñas et al. 2002, Schmidt et al. 2014). For isolates with an AmpC resistance profile where no plasmid mediated genes were detected, amplification of the promoter and attenuator regions of the chromosomal *ampC* gene was performed (Agersø et al. 2012, Peter-Getzlaff et al. 2011 Tracz et al. 2007). For the presumptive colistin resistant *E. coli*, PCR was performed for the identification of the genotypes *mcr-1* and *mcr-2* according to a protocol from the European Reference Laboratory on Antimicrobial Resistance (EURL-AR, Lyngby, Denmark, http://eurl-ar.eu/data/images/protocols/mcr-multiplex_pcr_protocol_v2_oct16.pdf). One isolate with unusual phenotypic profiles were subjected to whole genome sequencing at the EURL-AR .

Susceptibility testing
Isolates were tested for antimicrobial susceptibility using a broth microdilution method at NVI, Oslo. 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 18.06.2017) were used, except for azithromycin for *E. coli* for which cut-off values are not defined. See Appendix 6 for definitions of cut-off values.

Quality assurance systems
The following susceptible bacteria was included as quality control on a regular basis: *E. coli* ATCC 25922. 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 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 NVI as discrete values (MIC). Data management and analysis was performed in SAS-PC System® v 9.4 for Windows (SAS Institute Inc., Cary, NC, USA) and in R version 3.3.1 Copyright (C) 2016 The R Foundation for Statistical Computing Platform. The 95% confidence intervals were calculated by the exact binomial test using R version 3.3.0 for Windows (R Development Core Team, 2016).

Overview of antimicrobial groups and agents tested for in NORM-VET.

Antimicrobial group	Antimicrobial agents	*E. coli**	*Salmonella* spp.	*Campylobacter jejuni*
Tetracyclines	Tetracycline	X	X	X
	Tigecycline	X	X	
Amphenicols	Chloramphenicol	X	X	
Penicillins with extended spectrum	Ampicillin	X	X	
Second generation cephalosporins	Cefoxitin	(X)		
Third generation cephalosporins	Cefotaxime	X	X	
	Ceftazidime	X	X	
Fourth generation cephalosporins	Cefepime	(X)		
Carbapenems	Meropenem	X	X	
	Ertapenem	(X)		
	Imipenem and enzyme inhibitor	(X)		
Trimethoprim and derivatives	Trimethoprim	X	X	
Sulfonamides	Sulfamethoxazole	X	X	
Macrolides	Erythromycin			X
	Azithromycin	X	X	
Streptogramins	Quinupristin and dalfopristin			
Streptomycins	Streptomycin			X
Other aminoglycosides	Gentamicin	X	X	X
Fluoroquinolones	Ciprofloxacin	X	X	X
Other quinolones	Nalidixic acid	X	X	X
Polymyxins	Colistin	X	X	

*(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.

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

Salmonella
Samples from animals were collected according to 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 jejuni
Sample material from flocks identified as *Campylobacter* positive in the surveillance programme for *Campylobacter* spp. in broiler flocks in Norway in 2016, were included.
Caecal content from one broiler flock were plated directly onto mCCDA agar and incubated under microaerobic conditions at 41.5±0.5°C for 48h. Typical colonies were subcultured on blood agar and confirmed as *Campylobacter jejuni* using MALDI-TOF MS.

Susceptibility testing animal isolates
Isolates were tested for antimicrobial susceptibility using broth microdilution. MIC values were obtained using plates from Sensititre® (TREK Diagnostic LTD) with different panels depending on the bacteria to be tested.
For animal isolates, epidemiological cut-off values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 28.06.2017) were used, except for azithromycin and colistin for *Salmonella* spp. where EFSA recommended cut-off was used, and for azithromycin for which cut-off values are not defined. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme (see also Appendix 6).

Quality assurance systems NORM-VET
NVI have a quality assurance system according to the requirements of NS-EN ISO/IEC 17025. The participating laboratories at 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. The susceptibility data were stored as discrete values (MIC). Data management and analysis was performed in SAS-PC System® v 9.4 for Windows (SAS Institute Inc., Cary, NC, USA) and in R version 3.3.1 Copyright (C) 2016 The R Foundation for Statistical Computing Platform. The 95% confidence intervals were calculated by the exact binomial test using R version 3.3.0 for Windows (R Development Core Team, 2016).

NORM – enteropathogenic bacteria
Sampling strategy – humans

All human isolates of *Salmonella, Yersinia enterocolitica* and *Shigella* were obtained from clinical specimens. 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 human 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.

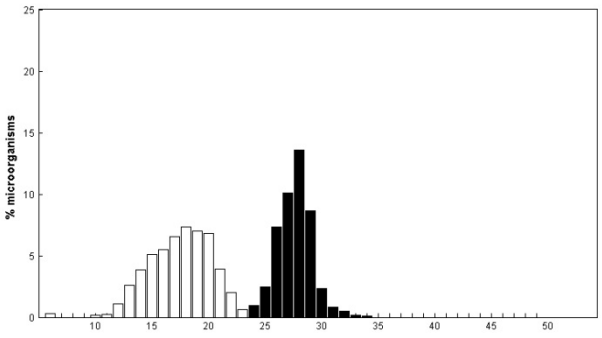
Identification of bacteria – human isolates
The reference analyses on human clinical isolates of enteropathogenic bacteria were performed according to conventional methods described in standard reference literature (e.g. Murray PR & al.: Manual of Clinical Microbiology, 8th edition ASM-Press, Washington 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 7.1 2017 were used if defined. In absence of clinical breakpoints, ECOFFs were used as determined by EUCAST or breakpoints were based on national zone distribution evaluations (e.g. nalidixic acid, azithromycin and tetracycline). Pefloxacin was used for inferred ciprofloxacin resistance in *Salmonella*, EUCAST breakpoints were adjusted in accordance with local epidemiological cut-off value (see figures below).
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.

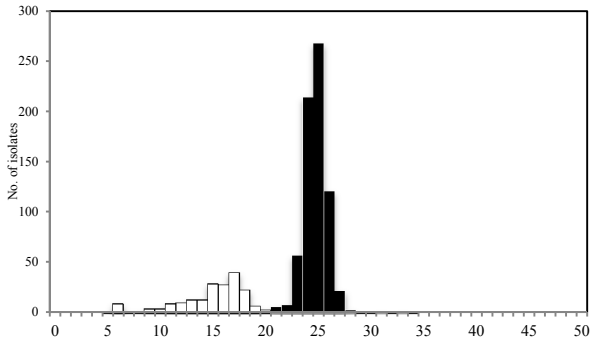
Quality assurance systems human isolates
The NRL for Enteropathogenic Bacteria at the NIPH is accredited according to the requirements of NS-EN ISO/IEC 17025. *E. coli* ATCC 25922 was used as quality control strain for AMR testing of non-fastidious *Enterobacteriaceae.* The NRL participated in the external 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.



EUCAST, zone distribution pefloxacin/*Salmonella* spp. based on 1257 observation; ECOFF at 24 mm (accessed June 2017).



NIPH zone distribution pefloxacin/*Salmonella* spp. based on 895 tested isolates; epidemiological cut-off set at 21 mm.

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. 2016 was the seventeenth 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 2016 were as follows: *E. coli* in blood cultures (6 months); *Klebsiella* spp., *Staphylococcus aureus, Enterococcus* spp. and *Enterobacter* spp. in blood cultures (9 months); *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Haemophilus influenzae*, *Neisseria meningitidis* and *Candida* spp. from blood cultures and cerebrospinal fluids (12 months); *S. aureus* from wound specimens (1 week); *S. pneumoniae* from respiratory tract samples (1 week); *E. coli* from urinary tract infections (3 days); *Klebsiella* spp. and *Enterobacter* spp. from urinary tract infections (3 weeks); *Mycobacterium tuberculosis* and *Neisseria gonorrhoeae* from all samples (12 months). *S. pneumoniae, S. pyogenes, H. influenzae* and *N. meningitidis* from blood cultures and cerebrospinal fluids, as well as *N. gonorrhoeae* from all clinical samples, were analysed at the the Norwegian Institute of Public Health in Oslo. *Candida* spp. isolates from blood cultures were analysed at Oslo University Hospital, Rikshospitalet. MRSA and *S. agalactiae* isolates were analysed at St. Olav University Hospital in Trondheim. ESBL-producing *Enterobacteriaceae* were genetically characterised at University Hospital of North Norway in Tromsø. *M. tuberculosis* isolates were analysed at the Norwegian Institute of Public Health and Oslo University Hospital (Ullevål and Rikshospitalet).

Susceptibility testing

E. coli, *Klebsiella* spp., *Enterococcus* spp., *S. aureus* and *Enterobacter* spp. isolates were examined according to the EUCAST disk diffusion standard 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 of NordicAST, which are harmonised with EUCAST. Beta-lactamase production in *S. aureus*, *H. influenzae* 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. pneumoniae*, *S. pyogenes*, *S. agalactiae*, *H. influenzae*, *N. meningitides,* 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 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 according to the instructions of the manufacturer (Liofilchem). ESBL positive strains from blood cultures were subjected to PCR and DNA sequencing for determination of ESBL genotype. *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. Erythromycin non-susceptible *S. pneumoniae*, *S. aureus, S. pyogenes* and *S. agalactiae* isolates were analysed for determination of MLS phenotype 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), *H. influenzae* ATCC 49247, *H. influenzae* NCTC 8468, *N. gonorrhoeae* CCUG 26213 / ATCC 49266, *N. gonorrhoeae* WHO L, *P. aeruginosa* ATCC 27853, *C. albicans* ATCC 90028.

Data processing

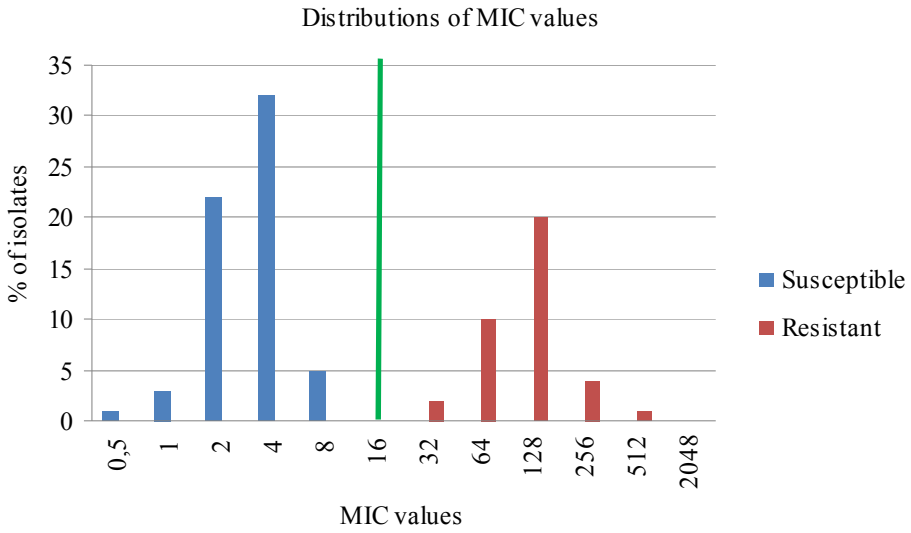
The specially designed web-based eNORM computer programme was used for registration and storage of patient data, sample data and resistance data. The results were further analysed by WHONET 5.6 with the aid of the BacLink programme, both developed by Dr. John Stelling. The distribution of microbial species 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 also 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 epidemiological cut-off values are presented at the web page: http://www.srga.org/Eucastwt/eucastdefinitions.htm.

The terms and usage of these two ways of classification of resistance are further explained below. The epidemiological breakpoint would normally be lower for MIC values and higher for disk diameters than the clinical breakpoins. However this is not always the case.



Epidemiological cut-off values

The epidemiological cut-off values may indicate emerging resistance in the bacterial populations. Based on the distribution of the minimum inhibitory concentration (MIC) 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 epidemiological cut-off 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 epidemiological cut-off values recommended by EUCAST. However, for some combinations of bacteria and antimicrobial agents these were not applicable to our data. In these cases epidemiological cut-off values defined on the basis of the actual MIC distributions obtained in the NORM-VET programme were used.

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 2013 by EFSA Journal 2015; 13(2):4036 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 values recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, accessed 28.06.2017) were used. For additional antimicrobial agents not defined in the EUCAST recommendations, cut-off values were defined on the basis of the actual MIC distributions obtained in the NORM-VET programme.

Antimicrobials	Resistant MIC (mg/L)	*Salmonella* spp.	*Escherichia coli*	*Campylobacter coli*
Ampicillin	> 8	■	■	
Azithromycin*	ND	X	X	
Cefotaxime	> 0.25		■	
	> 0.5	■		
Ceftazidime	> 0.5		■	
	> 2	■		
Chloramphenicol	> 16	■	■	
Ciprofloxacin	> 0.06	■	■	
	> 0.5			■
Colistin	> 2		■	
	ND	X		
Erythromycin	> 4			■

Antimicrobials	Resistant MIC (mg/L)	*Salmonella* spp.	*Escherichia coli*	*Campylobacter coli*
Gentamicin	> 2	■	■	■
Meropenem	> 0.125	■	■	
Nalidixic acid	> 16	■	■	■
Streptomycin	> 4			■
Sulfamethoxazole	> 64		■	
	> 256	●		
Tetracycline	> 1			■
	> 8	■	■	
Tigecycline	> 0.5	■	■	
	> 2	#		
Trimethoprim	> 2	■	■	

■ Cut-off values recommended by EUCAST. *Cut-off not defined (ND) by EUCAST. ● Cut-off defined by the MIC distributions obtained in NORM-VET.
Cut-off defined by EFSA

Appendix 8: Breakpoints NORM

NORM data are categorised according to the breakpoints of the Norwegian Reference Group on Antibiotic Susceptibility Testing (NWGA) which are harmonised with EUCAST breakpoints. NWGA breakpoints are available at www.antibiotikaresistens.no.

Antimicrobials	MIC (mg/L)		*Escherichia coli*	*Klebsiella* spp.	*Enterobacter* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
	S	R																			
Amphotericin B	≤ 1	> 1																■	■	■	■
Ampicillin	≤ 1	> 1								■											
	≤ 4	> 8												■							
	≤ 8	> 8	■			■	■	■													
Amoxi-Clav*	≤ 2	> 2								■											
	≤ 8	> 8	■	■																	
	≤ 32	> 32	■	■																	
Anidulafungin	≤ 0.002	> 4																			■
	≤ 0.03	> 0.03																■			
	≤ 0.06	> 0.06																	■	■	
Azithromycin	≤ 0.25	> 0.5										■									
						■[2]	■[2]	■[2]													
Cefaclor										■[1]											
Cefepime	≤ 1	> 4	■	■	■																
Cefixime	≤ 0.125	> 0.125										■									
Cefoxitin													■[1]								
Cefotaxime	≤ 0.125	> 0.125								■											
	≤ 0.5	> 2													■						
	≤ 1	> 2	■	■	■	■	■	■													
Ceftazidime	≤ 1	> 4	■	■	■	■	■	■													
Ceftriaxone	≤ 0.125	> 0.125								■	■	■									
	≤ 0.5	> 2													■						
Cefuroxime	≤ 1	> 2								■											
	≤ 8	> 8	■	■																	
Chloramphenicol	≤ 2	> 2								■											
	≤ 2	> 4									■										
	≤ 8	> 8				■	■	■							■						
Ciprofloxacin	≤ 0.03	> 0.03									■										
	≤ 0.03	> 0.06										■									
	≤ 0.06	> 0.06								■											
	≤ 0.25	> 0.5	■	■	■		■	■													
	≤ 0.5	> 0.5							■												
	≤ 1	> 1											■								
						■[3]															
Clindamycin	≤ 0.25	> 0.5											■								
	≤ 0.5	> 0.5													■	■	■				
Erythromycin	≤ 0.25	> 0.5													■	■	■				
	≤ 1	> 2											■								
	≤ 4	> 4							■												

Antimicrobials	MIC (mg/L) S	MIC (mg/L) R	*Escherichia coli*	*Klebsiella* spp.	*Enterobacter* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
Fluconazole	≤ 0.002	> 32																	■		
	≤ 2	> 4																■		■	■
Fusidic acid	≤ 1	> 1											■								
Gentamicin	≤ 1	> 1											■								
	≤ 2	> 2							■[1]												
	≤ 2	> 4	■	■	■	■	■	■													
	≤ 128	> 128												■							
Imipenem	≤ 4	> 8												■							
Linezolid	≤ 4	> 4											■	■							
Mecillinam	≤ 8	> 8	■	■																	
Meropenem	≤ 2	> 8	■	■	■	■	■	■													
Micafungin	≤ 0.002	> 2																			■
	≤ 0.016	> 0.016																■			
	≤ 0.03	> 0.03																	■		
Mupirocin	≤ 1	> 256											■								
Nalidixic acid						■[1]	■[1]	■[1]	■[1]												
Nitrofurantoin	≤ 64	> 64	■																		
Oxacillin															■[1]						
Penicillin G	≤ 0.06	> 0.25									■										
	≤ 0.06	> 1										■									
	≤ 0.06	> 2													■						
	≤ 0.25	> 0.25														■	■				
										■[1]											
Pip-Tazo**	≤ 8	> 16	■	■	■																
Rifampicin	≤ 0.06	> 0.5											■								
	≤ 0.25	> 0.25									■										
Spectinomycin	≤ 64	> 64										■									
Tetracycline	≤ 0.5	> 1										■									
	≤ 1	> 2								■			■		■	■	■				
	≤ 2	> 2							■												
						■[2]	■[2]	■[2]													
Tigecycline	≤ 0.25	> 0.5												■							
	≤ 0.5	> 0.5											■								
	≤ 1	> 2	■																		
Trimethoprim	≤ 2	> 4	■	■	■																

Antimicrobials	MIC (mg/L) S	MIC (mg/L) R	*Escherichia coli*	*Klebsiella* spp.	*Enterobacter* spp.	*Salmonella* spp.	*Yersinia enterocolitica*	*Shigella* spp.	*Campylobacter* spp.	*Haemophilus influenzae*	*Neisseria meningitidis*	*Neisseria gonorrhoeae*	*Staphylococcus aureus*	*Enterococcus* spp.	*Streptococcus pneumoniae*	*Streptococcus pyogenes*	*Streptococcus agalactiae*	*Candida albicans*	*Candida glabrata*	*Candida tropicalis*	*Candida parapsilosis*
TMS***	≤ 0.5	> 1								■											
	≤ 1	> 2													■	■					
	≤ 2	> 4	■	■	■	■	■	■					■								
Vancomycin	≤ 2	> 2															■				
	≤ 4	> 4												■							
Voriconazole	≤ 0.125	> 0.125																■		■	■

[1] Epidemiological cut-off value based on the wild type distribution by EUCAST. [2] Epidemiological cut-off values based on national zone distribution evaluations. [3] Low-level resistance against ciprofloxacin is underestimated using breakpoints based on ciprofloxacin disk diffusion; EUCAST emphasises evidence of poor clinical response in systemic infections with low-level ciprofloxacin resistant *Salmonella*. Susceptibility to ciprofloxacin is therefore inferred from pefloxacin disk diffusion susceptibility with breakpoints based on epidemiological cut-off values on national zone distribution evaluations in line with EUCAST recommendation. * Amoxi-Clav= Amoxicillin-Clavulanic acid. ** Pip-Tazo=Piperacillin-Tazobactam. *** TMS Trimethoprim-sulfamethoxazole. Breakpoints for the combination are given for the trimethoprim component only.

Appendix 9: References used in this report

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