

ESBL_{CARBA}

KURS I RESISTENSBESTEMMELSE AV MIKROBER "AFA KURSET"
OSLO UNIVERSITETSSYKEHUS RIKSHOSPITALET, 16. NOVEMBER 2017

Ørjan Samuelsen, Prof., PhD
Nasjonalt kompetansetjeneste for påvisning av antibiotikaresistens (K-res)
Universitetssykehuset Nord-Norge
Avd. mikrobiologi og smittevern
9020 Tromsø
E-post: orjan.samuelsen@unn.no
<https://unn.no/fag-og-forskning/k-res>



Norwegian National Advisory Unit on
Detection of Antimicrobial Resistance

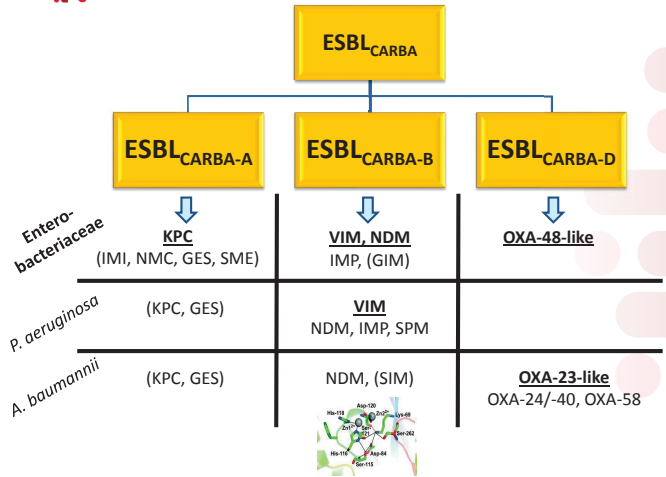
UNIVERSITETSSYKEHuset NORD-NORGE
DAVI-NORGA UNIVERSITEITABUOHCCIESSU



HELSE NORD



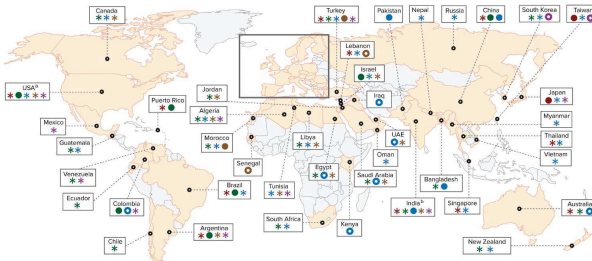
ESBL_{CARBA}



EPIDEMIOLOGI ESBL_{CARBA}

Enterobacteriaceae

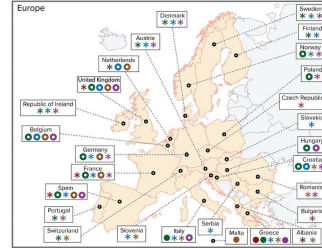
- ESBL_{CARBA}-A: KPC, ESBL_{CARBA}-B: NDM, VIM, IMP, ESBL_{CARBA}-D: OXA-48-like



- KPC: USA, Sør Amerika (Brazil, Argentina, Colombia), Israel, Kina
- NDM: India, Pakistan, Bangladesh, Kina
- IMP: Japan, Taiwan
- OXA-48-like: Tyrkia, Nord Afrika (Marocco)

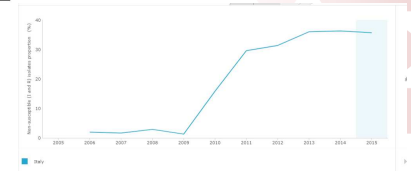
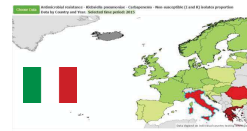
Logan LK. et al. *J. Infect. Dis.* 2017;215(5):S28-36

ESBL_{CARBA} EUROPA



Endemisk:

- Greece: KPC, VIM
- Italy: KPC
- Malta: OXA-48-like



EARS-Net database; Logan LK. et al. *J. Infect. Dis.* 2017;215(5):S28-36

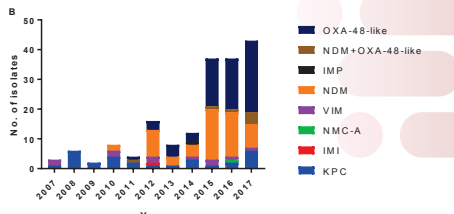
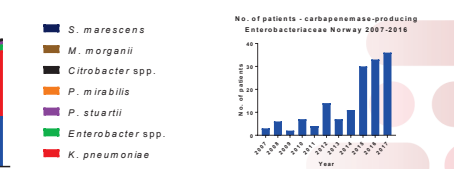
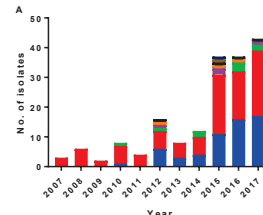
Occurrence of carbapenemase-producing *Klebsiella pneumoniae* and *Escherichia coli* in the European survey of carbapenemase-producing Enterobacteriaceae (EUSCAPE): a prospective, multinational study

Country	Enterobacteriaceae		<i>Klebsiella pneumoniae</i>		<i>Escherichia coli</i>	
	Number of patients	Number of isolates	Number of patients	Number of isolates	Number of patients	Number of isolates
Austria	8	4	11	4	0	0
Belgium	48	33	49	33	13	13
Denmark	6	5	6	5	0	0
France	48	27	52	27	3	3
Germany	5	5	3	3	0	0
Greece	26	2	9	2	0	0
Italy	9	0	1	0	0	0
Japan	9	0	0	0	0	0
Malta	0	0	0	0	0	0
Norway	0	0	0	0	0	0
Poland	20	10	20	10	0	0
Portugal	0	0	0	0	0	0
Romania	0	0	0	0	0	0
Spain	0	0	0	0	0	0
Sweden	0	0	0	0	0	0
Switzerland	0	0	0	0	0	0
Taiwan	0	0	0	0	0	0
Turkey	0	0	0	0	0	0
Ukraine	0	0	0	0	0	0
United Kingdom	0	0	0	0	0	0
USA	0	0	0	0	0	0
Total	173	83	189	83	13	13

- Structured survey 6 months 2013-2014
- Incidence/10 000 admissions:

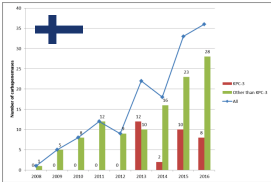
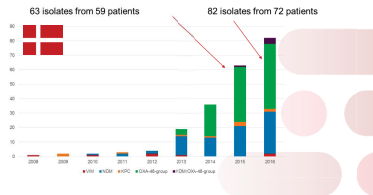
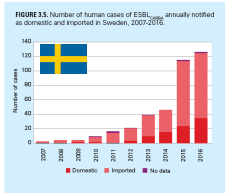
- Italy: 5.96
- Greece: 5.78
- Montenegro: 5.65
- Spain: 4.01
- Serbia: 3.0
- Norway: 0.02
- Denmark & Finland: 0
- Denmark & Sweden: Not calculated

ESBL_{CARBA} NORGE (midten av okt. 2017)



Grundmann H. et al. *Lancet Infect. Dis.* 2017;17:153-63

ESBL_{CARBA} NORDEN



6 isolater/4 pasienter (2015-2016)

0 isolater

Swedes-Swarm 2016; Personal communication: Anette Hammerum, Jari Jalava, Kristján Orrri Helgason & Shahin Gaini

ESBL_{CARBA} *P. aeruginosa*/*A. baumannii*

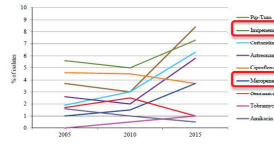
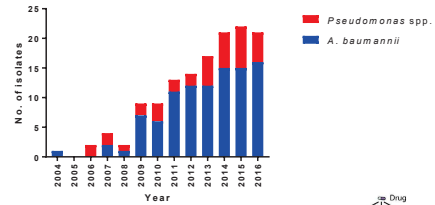
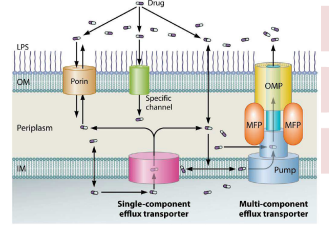


FIGURE 41. Prevalence of resistance to various antimicrobial agents in *Pseudomonas aeruginosa* blood culture isolates, 2005-2015. NORM/NORM-VET 2015



LI X-Z et al. Clin. Microbiol. Rev. 2015

"FERIE SUVENIRER"

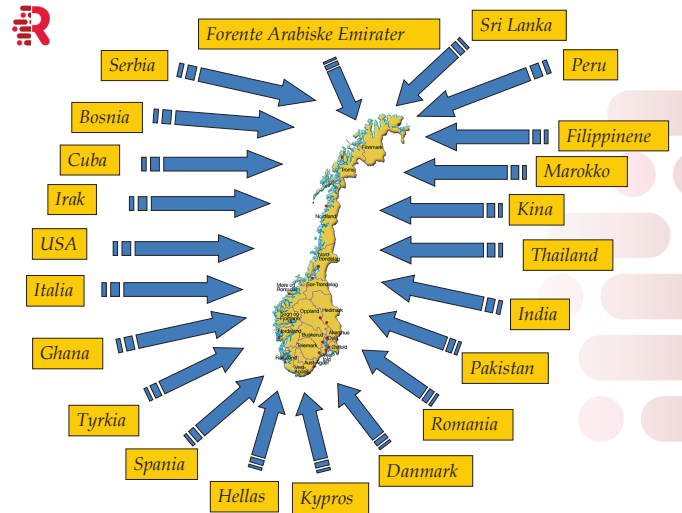
- Pasient innlagt sykehus 26 dager i Pakistan
- Overført til brannskadeavdeling i Norge



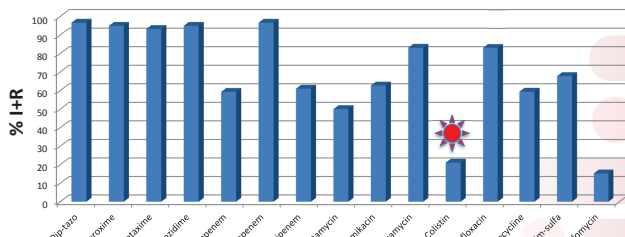
Lokalisasjon	Mikrobe	Resistensmekanisme
Blodkultur	<i>K. pneumoniae</i>	ESBL _{CARBA-D}
Fjernet SVK	<i>K. pneumoniae</i>	ESBL _{CARBA-D} + ESBL _A
	<i>P. mirabilis</i>	ESBL _{CARBA-B}
	<i>M. morgani</i>	ESBL _{CARBA-B} + ESBL _{CARBA-D}
	<i>A. baumannii</i>	ESBL _{CARBA-D}
Bronkial skyllevæske	<i>K. pneumoniae</i>	ESBL _{CARBA-D}
Sårsekret	<i>K. pneumoniae</i>	ESBL _{CARBA-D}
	<i>P. mirabilis</i>	ESBL _{CARBA-B}
	<i>E. cloacae</i>	ESBL _A
	<i>P. stuartii</i>	ESBL _{CARBA-B}
	<i>P. aeruginosa</i>	ESBL _{CARBA-B}
Screening avføring	<i>E. coli</i>	ESBL _{CARBA-D}

MRSA, VRE, *S. maltophilia*

Onarheim H. et al. Tidsskriftet 2016.



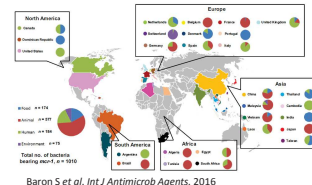
Resistensprofil karbapenemase-produserende Enterobacteriaceae Norge 2007-14



Samuelsen Ø et al. PLoS ONE in press

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study

Liu YY et al. Lancet Infect. Dis. 2016



Solheim M et al. Int J Antimicrob Agents. 2016

"IKKE BARE ESBL-CARBA"



- *E. coli* 5073 9822:
- *bla*_{NDM-1}, *bla*_{CMY-16-like}, *bla*_{CTX-M-15}, *bla*_{OXA-10}, *bla*_{TEM-1B}
- *aac(3)-IId-like*, *aadA1-like*, *aadA5*, *armA*, *strA-like*, *strB-like*
- *sul1*, *sul2*, *dfrA17*, *tet(A)-like*, *tet(B)*, *cmlA1-like*, *floR-like*, *mph(A)*, *mph(E)*, *ARR-3-like*, *msr(E)-like*

ESBL (ekstendert spektrum β -laktamase)

ESBL_{CARBA}



β -laktamgruppe	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Penicilliner (Ampicillin, Piperacillin)	✓	✓	✓
3. gen. cefalosporiner (Cefotaxime, Cefotaxidim)	(✓)	✓	-
4. gen. cefalosporiner (Cefepime)	(✓)	✓	-
Cefamyciner (Cefoxitin, Cefotetan)	✓	✓	-
Monobaktamer (Aztreonam)	(✓)	-	-
Karbapenemer (Meropenem, Imipenem, Ertapenem)	✓	✓	✓
β -laktamase inhibitorer	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Klavulansyre, Tazobaktam	(✓)	-	-
Borsyre	✓	-	-
Kloxacillin	-	-	-
Metall-kelatorer (EDTA/DPA)	-	✓	-
Avibactam	✓	-	(✓)

NB: VARIASJONER I AKTIVITETSSPEKTRUM

Antibiotika	MIC (mg/L)	
	<i>E. cloacae</i> IMI-9 (ESBL _{CARBA-A})	<i>E. asburiae</i> KPC-2 (ESBL _{CARBA-A})
Piperacillin-tazobactam	2 (S)	256 (R)
Ceftazidime	0,5 (S)	16 (R)
Cefotaxime	0,25 (S)	8 (R)
Cefuroxime	8 (S)	>256 (R)
Cefoxitin	>32 (R)	>32 (R)
Aztreonam	0,5 (S)	64 (R)
Meropenem	2 (S)	4 (I)

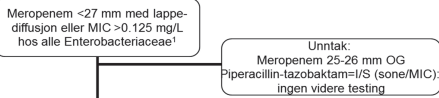
ESBL_{CARBA} ENTEROBACTERIACEAE

Enterobacteriaceae										
Enzym	Pen.	3-4 gen. ceph.			Aztr.			Karb.		
ESBL _{CARBA-A}	R	S	I	R	S	I	R	S	I	R
ESBL _{CARBA-B}	R	R			S			S	I	R
ESBL _{CARBA-D}	R	S			S			S	I	R

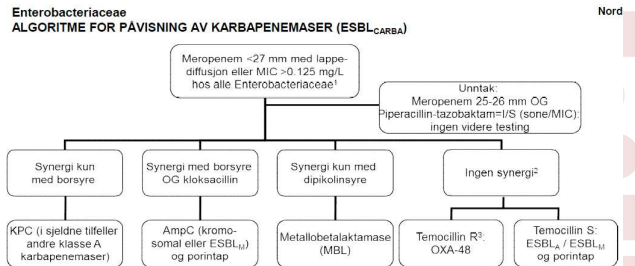
ESBL_{CARBA}-produserende Enterobacteriaceae ofte sensitive iflg. kliniske brytningspunkter!

Screeningsbrytningspunkt!

Meropenem	2	8	16	22	16
Meropenem (sone)	8/100	Note	18	27	Note



ALGORITME IDENTIFISERING ESBL_{CARBA} ENTEROBACTERIACEAE



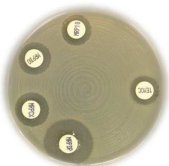
¹ For laboratorier som resistensbestemmer meropenem med diskdiffusjon tilfører det ingenting til karbapenemautredningen å gjøre MIC-bestemmelse.
² Sjeldne tilfeller kan høygradig resistente isolater med ingen synergi ha MBL og KPC i kombinasjon. Kombinasjonstabletter med både borsyre og dipikolsyre kan da benyttes, alternativt molekylarbiologiske metoder.
³ Høygradig temocillinresistens (MIC >128 mg/L eller sonestørrelse <12 mm med temocillin 30 μ g tapp) er en fenotypisk indikator på OXA-48. OXA-48-produserende isolater er også resistente mot piperacillin-tazobaktam og amokisillin-klavulansyre.

Synergi karbapenemer - hemmere

NordicAST v. 7.1

FENOTYPISKE METODER ESBL_{CARBA} ENTEROBACTERIACEAE

Kombinasjons tabletter:

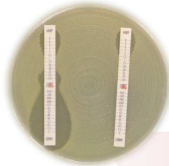


- Meropenem (MRP); Meropenem+borsyre (MRPBO); Meropenem+kloxacillin (MRPCX); Meropenem+DPA (MRPDP); Temocillin (TMO)

Algoritme:

Tablet	Meropenem + Piperacillin (MRP)	Meropenem + OXA (MRPBO)	Meropenem + Cloxacillin (MRPCX)	Temocillin (TMO)
MRP	2-8 mm	2-8 mm	2-8 mm	2-8 mm
MRPBO	2-8 mm	2-8 mm	2-8 mm	2-8 mm
MRPCX	2-8 mm	2-8 mm	2-8 mm	2-8 mm
TMO	2-8 mm	2-8 mm	2-8 mm	2-8 mm

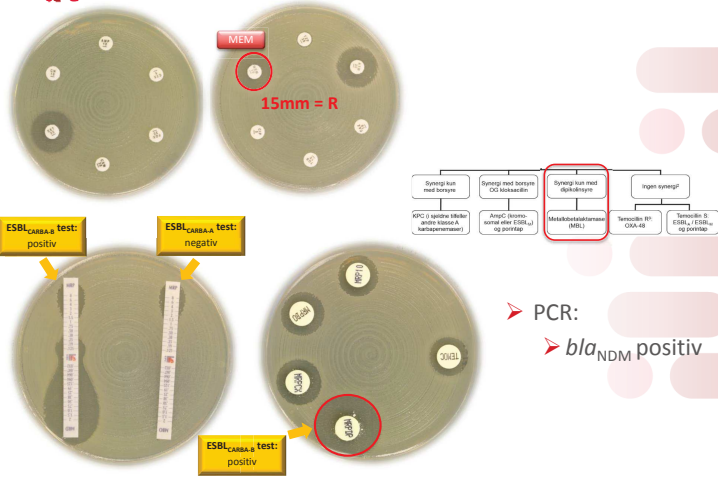
Kombinasjons gradient-tester:



- ESBL_{CARBA-A}:
 - Meropenem/meropenem+borsyre
 - Ertapenem/ertapenem+borsyre
- ESBL_{CARBA-B}:
 - Meropenem/meropenem+EDTA
- Positiv test:
 - Ratio ≥ 8
 - Fantomsoner

EKSEMPEL ESBL_{CARBA-A} (*K. pneumoniae*)

EKSEMPEL ESBL_{CARBA-B} (*E. coli*)

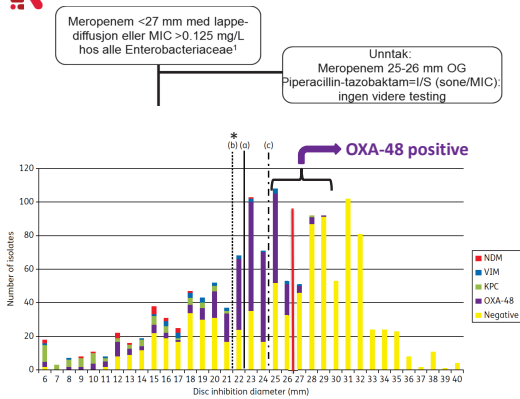


OXA-48 (ESBL_{CARBA-D}): EN UTFORDRING

Substrat	Aktivitet
Penicilliner	+
ES-Cefalosporiner	-
Monobaktam	-
Karapenemer	+

Enterobacteriaceae												
Penicilliner	3-4 gen. cefalosporiner			Aztreonam.	Karapenemer							
R	S	S	S	S	I	R						
β-laktamer (MIC mg/L)												
Ref.	Species	AMC	PIP	TZP	FOX	CXM	CAZ	CTX	AZT	MEM	IMP	ETP
50700924	<i>K. pneu.</i>	>256	>256	>256	2	8	0.25	0.5	0.032	0.25	1	1

SCREENINGSBRYTNINGSPUNKT



OXA-48:
Høygradig R mot TZP

EKSEMPEL ESBL_{CARBA-D} (*K. pneumoniae*)

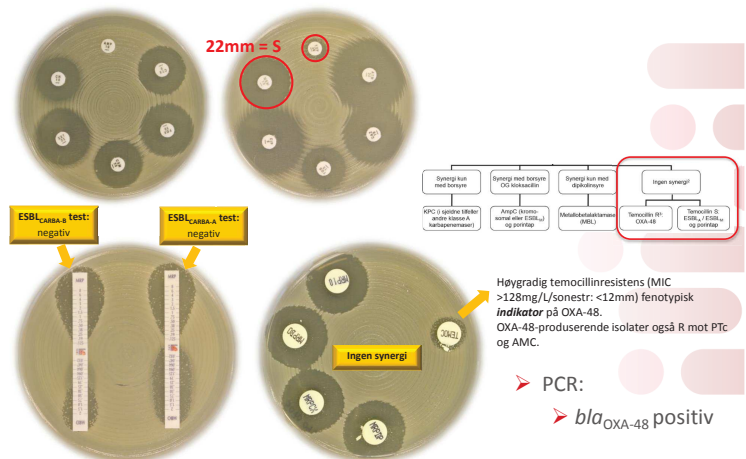


Figure 1. Distribution of meropenem 10 µg disc inhibition zone and of carbapenemase for Enterobacteriaceae isolates referred to the NRLs in 2012 (n=1354). (a) 2013 CLSI meropenem disc diffusion susceptibility zone diameter breakpoint (≥23 mm). (b) 2013 EUCAST meropenem susceptibility zone diameter breakpoint (≥22 mm). (c) 2013 EUCAST meropenem disc diffusion screening cut-off for the detection of CPE (≥25 mm).

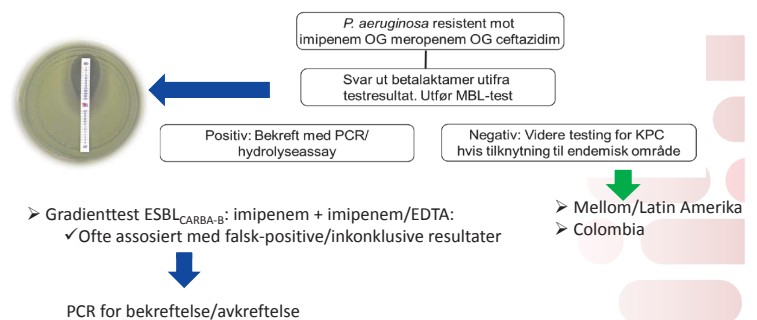
Huang T-D. et al. J. Antimicrob. Chemother. 2014

FENOTYPISKE METODER: NOEN "NB'er"!

- ESBL_A tester:
 - ESBL_{CARBA-A} + (ESBL_A): "falsk positiv" ESBL_A-test
 - ESBL_{CARBA-D} + ESBL_A: "falsk positiv" ESBL_A-test
- ESBL_{M-C} tester:
 - ESBL_{CARBA-A}: falsk positiv AmpC test (v/bruk av borsyre)
 - ESBL_{CARBA-B}: "falsk positiv" AmpC test
- ESBL_{CARBA} tester:
 - ESBL_{CARBA-A} + ESBL_{CARBA-B}: ingen syntergi

Positiv ESBL_A eller AmpC test utelukker ikke ESBL_{CARBA}!

ESBL_{CARBA} *Pseudomonas* spp.



➡ **MEROPENEM!!**

ESBL_{CARBA} *Acinetobacter* spp.

Karbapenemer	MIC-brytpunkt (mg/L)		Lappstørrelse (µg)	Zonbrytpunkt (mm)		Kommentarer
	S ≤	R >		S ≤	R <	
Doripenem	1	2	10	24	21	Brytningspunktene forutsetter dosering 1 g x 3 i v. (4 h infusjon)
Imipenem	2	5	10	23	17	
Meropenem	2	5	10	21	15	I Norge og Danmark anbefales at resistente isolater (meropenem MIC > 8) sendes til referanselaboratorum for molekylærbiologisk karbapenemkaraktisering. I Sverige anbefales at samtlige isolater eller resistente isolater sendes til Folkhälsomyndigheten for molekylærbiologisk karbapenemkaraktisering.

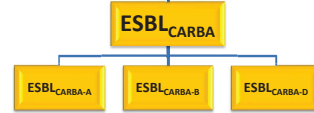


PCR ESBL_{CARBA-D}:
 ✓ OXA-23
 ✓ OXA-24/-40
 ✓ OXA-58

Gradienttest ESBL_{CARBA-B}: imipenem + imipenem/EDTA:
 ✓ Ofte assosiert med falsk-positive/inkonklusive resultater

PCR for bekreftelse/avkreftelse

ESBL (ekstendert spektrum β-laktamase)

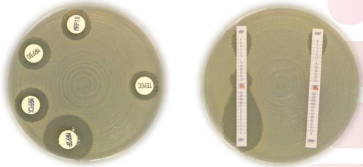


β-laktamgruppe	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Penicilliner (Ampicillin, Piperacillin)	✓	✓	✓
3. gen. cefalosporiner (Cefotaxime, Cefotaxidim)	(V)	✓	-
4. gen. cefalosporiner (Cefepime)	(V)	✓	-
Cefamyciner (Cefoxitin, Cefotetan)	✓	✓	-
Monobaktamer (Aztreonam)	(V)	-	-
Karbapenemer (Meropenem, Imipenem, Ertapenem)	✓	✓	✓
β-laktamase inhibitorer	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Klavulansyre, Tazobaktam	(V)	-	-
Borsyre	✓	-	-
Kloxacillin	-	-	-
Metall-kelatorer (EDTA/DPA)	-	✓	-
Avibactam	✓	-	(V)

OPPSUMMERING ENTEROBACTERIACEAE

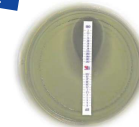
MEROPENEM, MEROPENEM, MEROPENEM

- ✓ MIC > 0,125mg/L eller disk diffusjon < 27mm
- ✓ (MEM: 25-26mm og PTz=I/S – ingen videre testing)



Positiv ESBL_A eller AmpC test utelukker ikke ESBL_{CARBA}!

OPPSUMMERING *Pseudomonas* & *Acinetobacter*

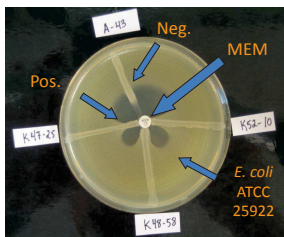


PCR ESBL_{CARBA-D}:
 ✓ OXA-23
 ✓ OXA-24/-40
 ✓ OXA-58

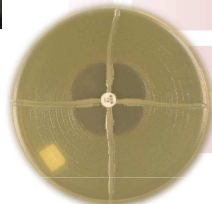
Gradienttest ESBL_{CARBA-B}: imipenem + imipenem/EDTA:

PCR for bekreftelse/avkreftelse

MODIFISERT HODGE TEST (MHT) - ENTEROBACTERIACEAE



- Subjektiv – kan være vanskelig å tolke
- Falsk positive resultater:
 - ESBL_A/ESBL_M (AmpC) + impermeabilitet
- Falsk negative resultater:
 - NDM-1

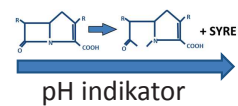


BIOKJEMISKE TESTER

RAPIDEC Carba NP, Rapid BLUE CARB, Neo-Rapid CARB...



Rød farge

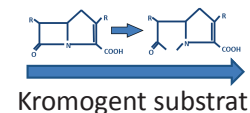


Gul farge

β-CARBA test

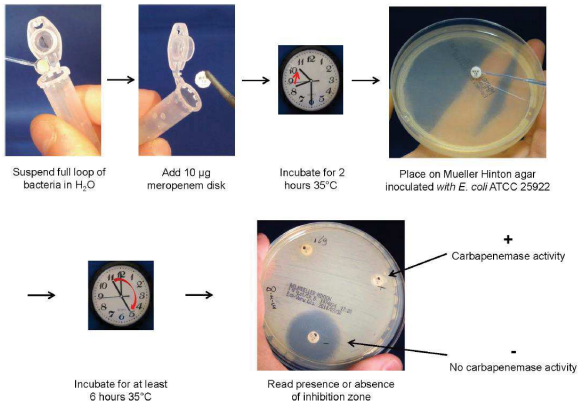


Gul farge



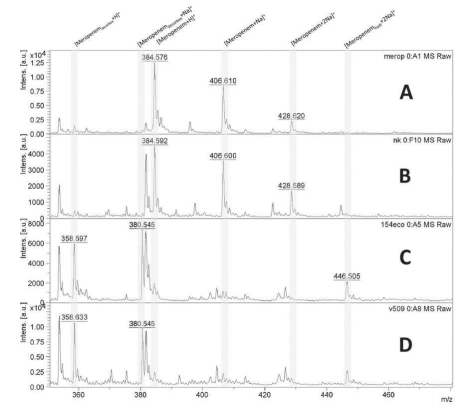
Rød farge

CARBAPENEM INACTIVATION METHOD (CIM)



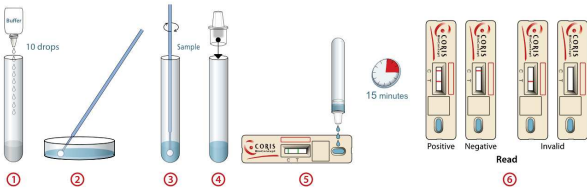
Van der Zwaluw, et al. PLoS ONE. 2015;10(3):e0123690

MALDI-TOF & ESBL_{CARBA}



K-SeT (NDM/OXA-48/KPC)

- Immunokromatografi



<https://www.youtube.com/watch?v=BbiX5-aWQ9w>

- “immunological capture of epitopes specific to the NDM/OXA-48/KPC enzyme using colloidal gold nanoparticles bound to a nitrocellulose membrane within a lateral flow device”

ESBL (ekstendert spektrum β-laktamase)



β-laktamgruppe	ESBL _A	ESBL _{M-C}	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Penicilliner (Ampicillin, Piperacillin)	√	√	√	√	√
3. gen. cefalosporiner (Cefotaxime, Cefotaxidim)	√	√	(√)	√	-
4. gen. cefalosporiner (Cefepime)	(√)	-	(√)	√	-
Cefamyciner (Cefoxitin, Cefotetan)	-	√	√	√	-
Monobaktamer (Aztreonam)	(√)	√	(√)	-	-
Karbapenemer (Meropenem, Imipenem, Ertapenem)	-	-	√	√	√
β-laktamase inhibitorer	ESBL _A	ESBL _{M-C}	ESBL _{CARBA-A}	ESBL _{CARBA-B}	ESBL _{CARBA-D}
Klavulansyre, Tazobaktam	√	-	(√)	-	-
Borsyre	-	√	√	-	-
Kloxacillin	-	√	-	-	-
Metall-kelatorer (EDTA/DPA)	-	-	-	√	-
Avibactam	√	√	√	-	(√)

NB: alltid unntak i substrat/inhibitor profil