

Challenges related to carbapenemase screening in Enterobacterales

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Today's agenda

- Challenges from WS 2022 → Changes in the ESBL_{CARBA} screening algorithm
- Challenges related to some carbapenemase screening
 - Screening agar -> CPE detection
 - Phenotypic detection with Combined Disc Test (CDT) and temocillin
 - Molecular confirmation of carbapenemase-production → NAAT/lateral-flow

Challenge 1 from WS 2022: Specificity of meropenem screening breakpoints depending on species

Algorithm for detection of carbapenemases

Meropenem <28 mm with disc-diffusion (or MIC >0.125 mg/L) in all *Enterobacterales*¹

Exception:
meropenem 25-27 mm AND
piperacillin-tazobaktam ≥ 20 mm:
no further testing¹

V 12.0

Can the exception be improved for especially *Enterobacter* spp?

2023 v 13.0, Revised flowchart ESBL_{CARBA}

Algorithm for detection of carbapenemases

Meropenem <28 mm (or MIC
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- *Enterobacter* spp. incl. *K. aerogenes*: meropenem 25-27 mm AND piperacillin-tazobactam ≥ 12 mm no further testing¹
- Other species: meropenem 25-27 mm AND piperacillin-tazobactam ≥ 20 mm: no further testing¹

V 13.0

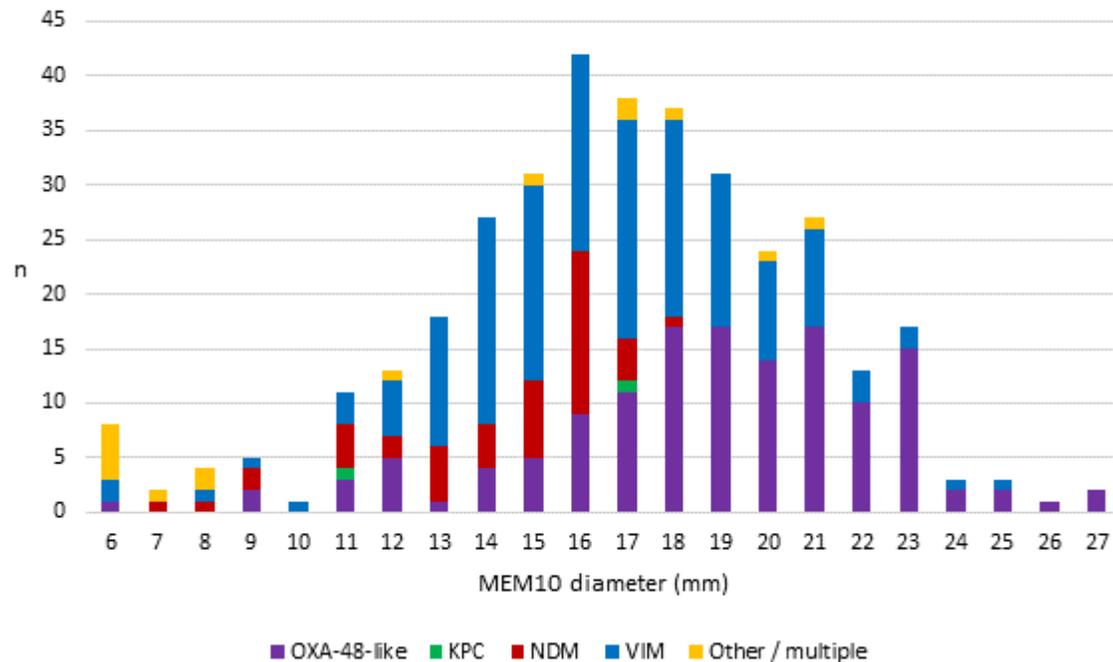


Purpose of the change: To reduce the workload in the laboratories regarding the most common species with derepressed AmpC-production as the reason for meropenem < 28 mm.

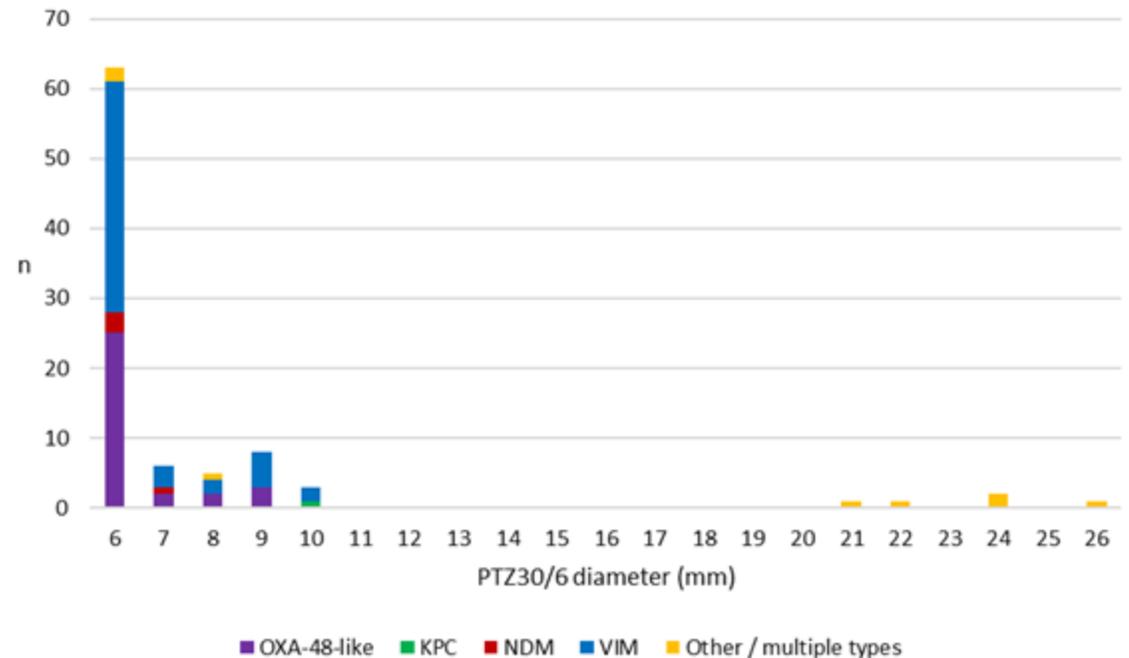
But at the same time reduce the risk of missing any OXA-48 positive isolates

Enterobacterales- Revised flowchart ESBL_{CARBA}

E. cloacae complex CPE (2015-2021; n=358)



E. cloacae complex CPE (2018-2021; n=90)



Background to the revised flowchart!

Pr. Dr. Te-Din Daniel Huang, MD, PhD

Chef de Laboratoire Associé / Biologie Clinique

National Reference Center for Antibiotic-Resistant Gram-Negative Bacilli

2023 v 13.0, Revised flowchart ESBL_{CARBA}

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Meropenem <28 mm (or MIC >0.125 mg/L) in all *Enterobacterales*¹

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V 13.0



The first exception does not seem to work for other *Enterobacterales* species like *Providencia* spp and *Proteus* spp. but data are very limited!

Challenge 2 from WS 2022: Revised temocillin cut-off for OXA-48?

¹ For laboratories using disk diffusion there is no added value in determining meropenem MIC regarding carbapenemase detection.

² NAAT (nucleic acid amplification test, e.g. PCR, LAMP)/lateral flow test should include the most common carbapenemases (OXA-48, NDM, VIM and KPC). If the test is negative, but the isolate is still suspected to be carbapenemase producing (e.g. due to laboratory or epidemiological reasons), consider additional testing for rarer carbapenemases (e.g. IMP, IMI, GES) or whole genome sequencing.

³ In rare cases highly resistant isolates with no synergy may harbor more than one carbapenemase.

⁴ High levels of resistance to temocillin (>128 mg/L, zone <12 mm) may indicate OXA-48. OXA-48 producing isolates are also resistant to piperacillin-tazobactam and amoxicillin-clavulanic acid.

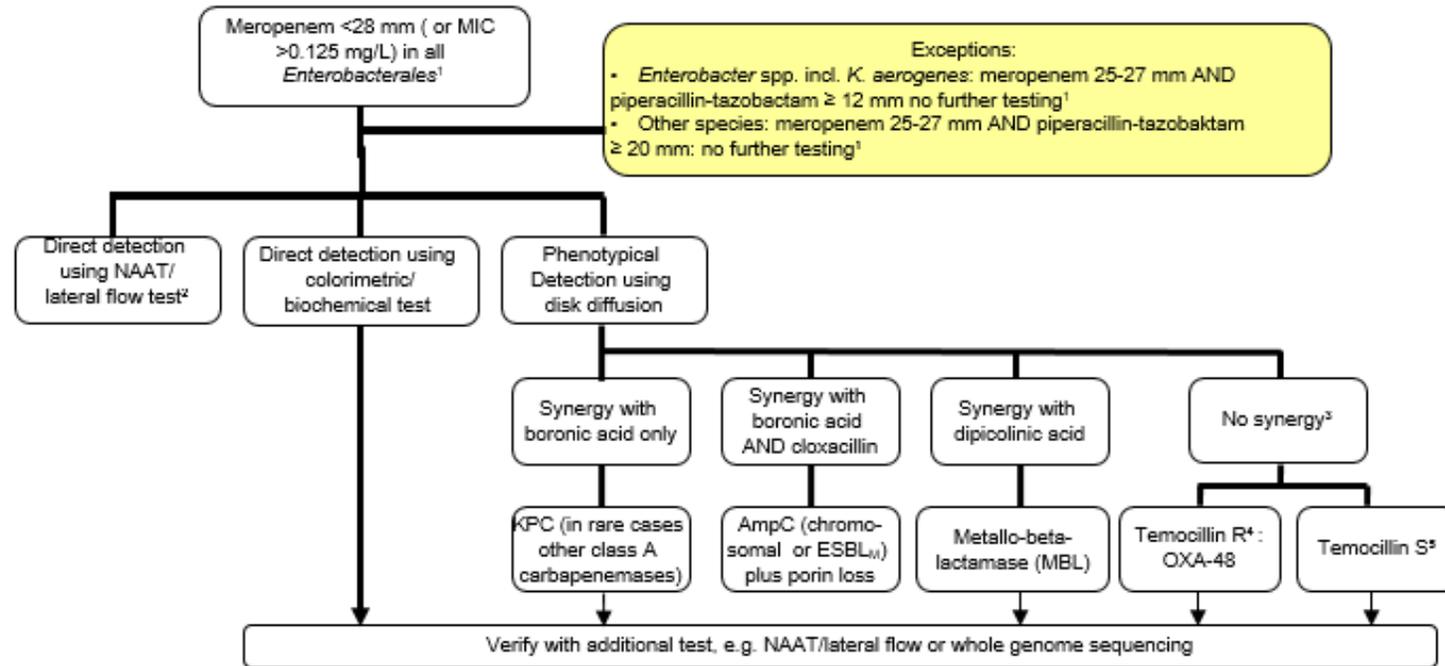
⁵ Some new OXA-48 variants (e.g. OXA-244) do not have resistance to temocillin. A phenotypical test using disc diffusion is not available to detect these variants and susceptibility to temocillin can not be used to rule out carbapenemase production



Can NordicAST provide a revised temocillin cut-off zone diameter that harmonize with manufacturers instruction for OXA-48 detection with CDT?

2023 v 13.0: Revised comments on Temocillin

Algorithm for detection of carbapenemases



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⁴ High levels of resistance to temocillin (>128 mg/L, zone <12 mm). may indicate OXA-48. **Follow the manufacturers instructions for temocillin interpretation if a commercial phenotypical test is used.**

⁵ Some new OXA-48 variants (e.g. OXA-244) do not cause resistance to temocillin. A phenotypical test using disc diffusion is not available to detect these variants and susceptibility to temocillin can not be used to rule out carbapenemase production. **All OXA-48 variants isolates are however resistant to piperacillin-tazobactam and amoxicillin-clavulanic acid.**

The OXA-244 challenge -a short reminder

- A variant of OXA-48 with a single Arg214Gln mutation
- 2012 first discovered on a plasmid of a *K. pneumoniae* isolate in Spain
- 2013 found in *E.coli* from Germany
- Since then increasing regional and national dissemination of OXA-244-producing *E. coli* in EU/EEA. Mainly ST38.
- 2020 Norway: a healthcare-associated outbreak involving 12 cases of OXA-244-producing *E. coli*
- ECDC rapid risk assessment report 2021:
 - The source and route of transmission in EU/EEA/UK is currently unclear, and there is a need for further investigation. The wide geographical dispersion of cases within countries, without cases being linked in place and time, indicates transmission in the community as the main mode of spread.
 - Adaptation of microbiology methods for detection is needed to prevent continuous unnoticed spread.

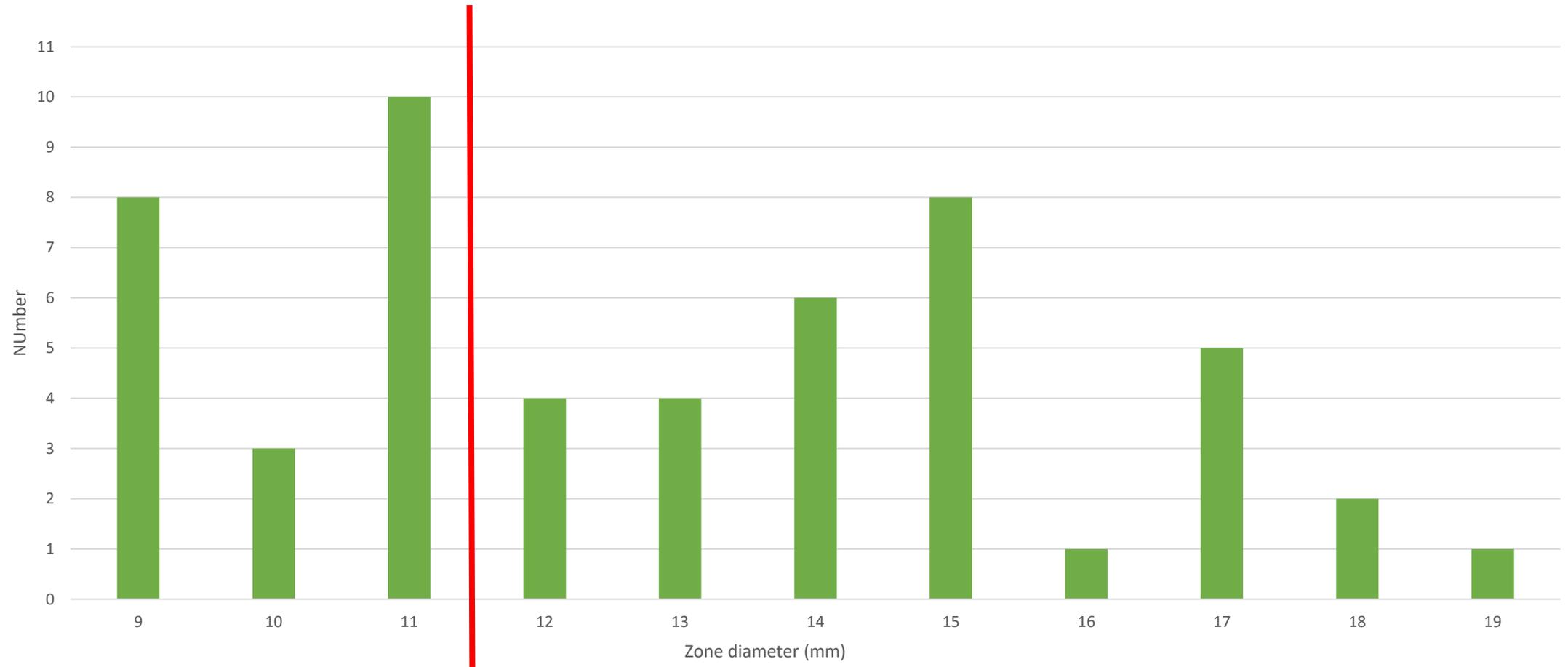
The OXA-244 challenge-example from Karolinska University laboratory

- During 2018 to 2022 we detected 183 isolates with OXA-48

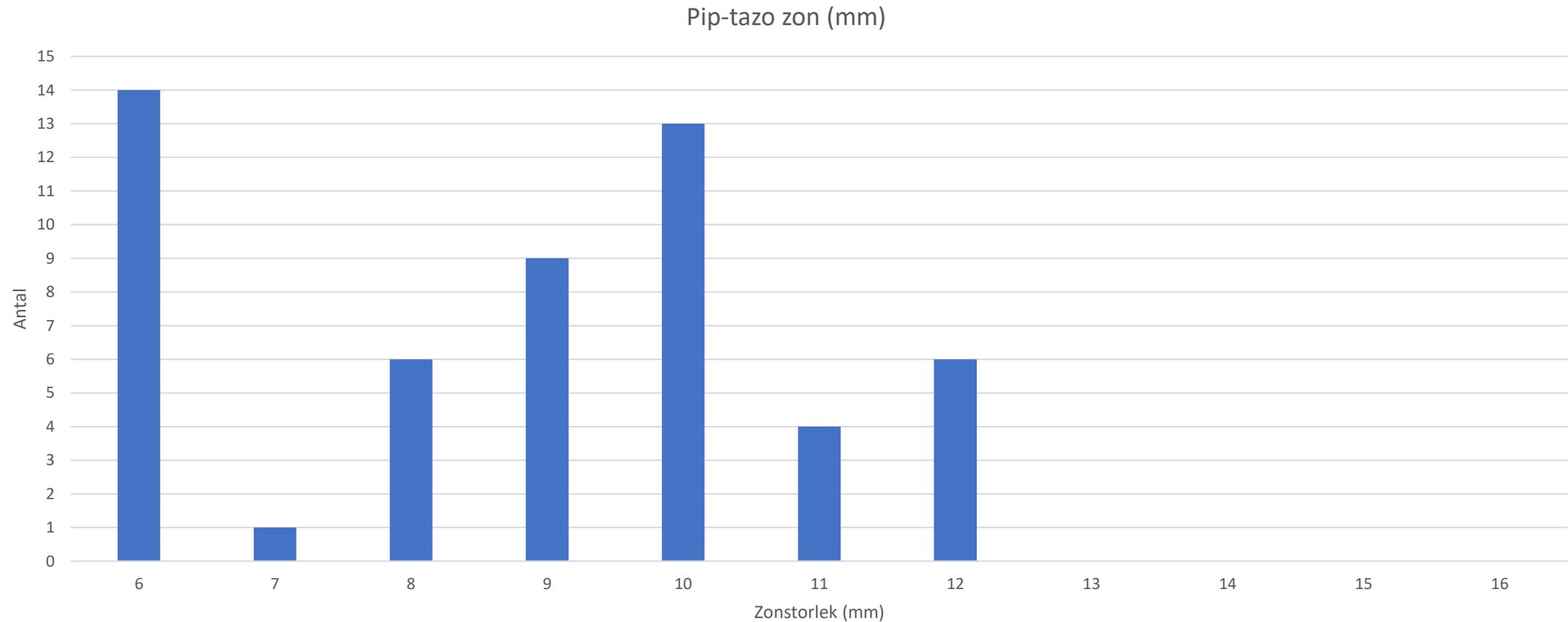
From March 2018 to December 2022	Number
OXA-244 positive isolates	64 (35%)
• Temocillin S (≥ 12 mm)	38 (60%)
• Temocillin R (<12 mm)	26 (40%)
• Piperacillin-tazobactam disk diffusion	All isolates ≤ 12 mm
• Ertapenem disk diffusion	All isolates below the R-breakpoint <25 mm
• ESBL positive isolates	2020-2021: 85% (16/19) ESBL-A: 74%, ESBL-M: 11%

OXA-244:

Temocillin zone diameters 2018-2022



OXA-244: Piperacillin-tazobactam zone diameters 2018-2022



Challenge: Screening agar

Screening for colonisation with CPE

NordicAST Nordic Committee on Antimicrobial Susceptibility Testing

NordicAST Organisation Methods Quality control Breakpoints and Dosages AST report panels FAQ National committees Workshops and Webinars Links RSS

Screening for carbapenemase-producing Enterobacteriaceae (CPE)

Metoddokument: CPE screening
Title: Screening for carbapenemase-producing Enterobacteriaceae (CPE)

Först publicerad: 2017-01-30 13:44
Updated: 2017-11-30 13:35
Owner: Kaisu Rantakokko-Jalava
Approved by: Dagfinn Skaare

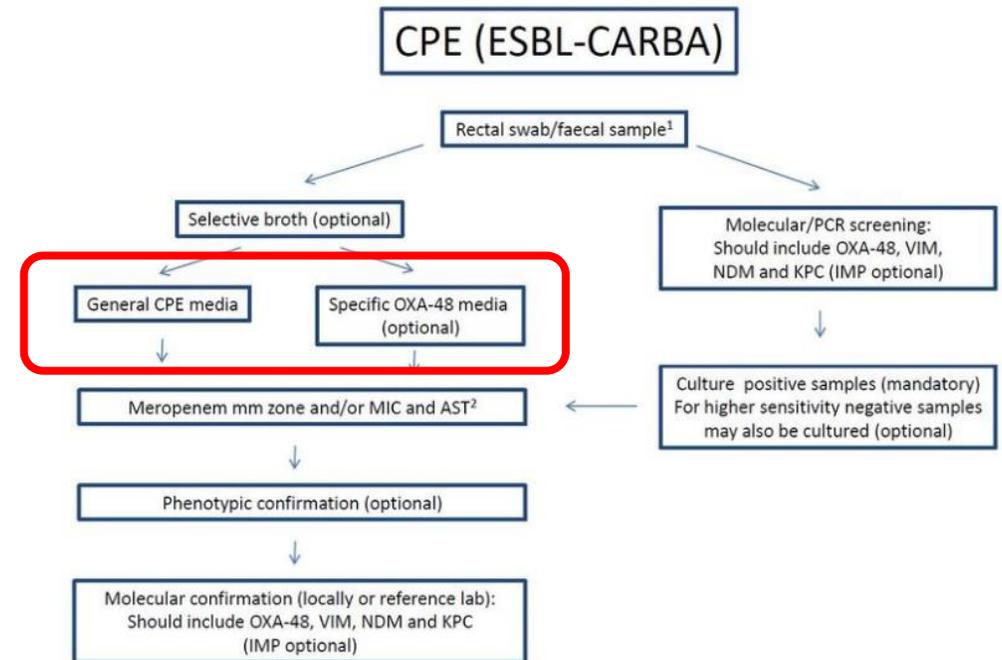
Edition: 1
Revision: 4

Scope of the document. This document gives recommendations on laboratory methods for screening for colonisation with carbapenemase-producing Enterobacteriaceae (CPE). Indications for screening, the number and interval of screening samples and the consequences of a positive or negative result are outwith the scope of this document and should be determined by local or national infection control policies.

Considerations for which screening agar to use



- Can we only use an ESBL agar for detection of CPE?
- Which CRE agar to use?



¹ CPE-screening must include a rectal swab or faecal sample. Other relevant sites may also be sampled.
² According to EUCAST/NordicAST recommendations.

Can we only use an ESBL agar for screening for CPE?

Class	Enzyme	β-lactam group					
		Penicillins	1-2. gen. cephalosporins	Cephamycins	Extended-spectrum cephalosporins	Aztreonam	Carbapenems
ESBL-CARBA-A	KPC	+	+	+/-	+	+	+
	IMI	+	+	-	-	+/-	+
	GES-variants	+	+	+/-	+	-	+
	FRI	+	+	-	+	+	+
ESBL-CARBA-B	NDM, VIM, IMP etc.	+	+	+	+	-	+
ESBL-CARBA-D	OXA-48-like,	+	+	+/-	-	-	+


2015-2021

Carbapenemase-variant	No. of <i>bla</i> _{CTX-M} positive isolates	No. of <i>bla</i> _{CTX-M} negative isolates	Total no. of isolates
OXA-48-like	145	53	198

 27% of CPE with OXA-48-like negative for *bla*_{CTX-M}

OXA-48-like CPE not co-producing an ESBL does not grow on ESBL agar

J Antimicrob Chemother 2020; **75**: 2120–2123
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Journal of
Antimicrobial
Chemotherapy

Screening of OXA-244 producers, a difficult-to-detect and emerging OXA-48 variant?

Cecile Emeraud¹⁻⁴, Laura Biez^{2,4}, Delphine Girlich^{2,4}, Agnès B. Jousset¹⁻⁴, Thierry Naas ¹⁻⁴,
Rémy A. Bonnin ²⁻⁴ and Laurent Dortet ^{1-4*}



- *E. coli* OXA-244, **ESBL-negative** ($n=18$) -> No growth on ESBL agar
- *E. coli* OXA-244, **ESBL-positive** ($n=81$) -> 95.1-96.3% grew on ESBL agar

➤ **Use of specific agar for CPE is required!**

Detection of CPE with KPCs, NDMs, VIMs

- In general CRE agars detect CPE with KPC, NDM, VIM

Table 2 Detection of the different agars stratified by carbapenemase

Carbapenemase	McCARB	McCARB-T	Brilliance CRE (Oxoid)	Chromatic CRE (Liofilchem)	chromID CARBA (bioMérieux)	ChromID OXA-48 (bioMérieux) ¹	Brilliance ESBL (Oxoid)	Chromatic ESBL (Liofilchem)	chromID ESBL (bioMérieux)
NDM [<i>n</i> = 9]	8 (88.9%)	8 (88.9%)	9 (100%)	9 (100%)	8 (88.9%)	1 (11.1%)	9 (100%)	9 (100%)	9 (100%)
OXA-48 [<i>n</i> = 20]	20 (100%)	20 (100%)	19 (95%)	20 (100%)	12 (60%)	20 (100%)	17 (85%)	17 (85%)	17 (85%)
KPC [<i>n</i> = 10]	10 (100%)	10 (100%)	10 (100%)	8 (80%)	9 (90%)	0 (0%)	10 (100%)	10 (100%)	10 (100%)
VIM (<i>n</i> = 20)	19 (95%)	19 (95%)	20 (100%)	18 (90%)	20 (100%)	3 (15%)	20 (100%)	20 (100%)	20 (100%)
GIM [<i>n</i> = 3]	3 (100%)	3 (100%)	3 (100%)	3 (100%)	3 (100%)	0 (0%)	3 (100%)	3 (100%)	3 (100%)
Multiple carbapenemases [<i>n</i> = 7]	7 (100%)	7 (100%)	7 (100%)	7 (100%)	7 (100%)	0 (0%)	7 (100%)	7 (100%)	7 (100%)

¹ This medium was developed to specifically detect OXA-48 but has been assessed for the detection of also other carbapenemases in this study. The number of isolates which were detected are displayed, the sensitivity is given in brackets

- Some variability between agars
- OXA-48 specific agar -> suppress growth of CPE with other carbapenemases

Detection of CPE with OXA-48

Table 1. Sensitivity of ChromID® CARBA SMART (bioMérieux), Brilliance™ CRE (Thermo Fisher) and mSuperCARBA™ (MAST Diagnostic) media

Microorganism	β-Lactamase content	MIC of temocillin (mg/L)	No. of strains	MIC of imipenem (mg/L)	Carba NP test +, n (%)	Growth on plate, n (%)					
						ChromID® CARBA SMART		mSuper CARBA™	Brilliance™		ChromID® BLSE
						CARBA side	OXA side		CRE	ESBL	
<i>E. coli</i>	OXA-244	≥512	1	4	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)
		32–256	18	0.25–2	10 (56)	0 (0)	0 (0)	17 (94)	8 (44)	0 (0)	0 (0)
	OXA-244 + ESBL	≥512	7	0.25–8	6 (86)	3 (43)	6 (86)	7 (100)	5 (71)	7 (100)	7 (100)
		32–256	47	0.25–16	22 (47)	0 (0)	0 (0)	47 (100)	17 (36)	47 (100)	47 (100)
	OXA-244 + case ^a	≥512	6	0.5–2	2 (33)	1 (17)	4 (67)	6 (100)	6 (100)	6 (100)	6 (100)
		32–256	15	0.25–4	3 (20)	0 (0)	1 (7)	15 (100)	13 (87)	12 (80)	13 (87)
OXA-244 + ESBL + case ^a	≥512	1	1	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	
	64	2	0.5	0 (0)	0 (0)	0 (0)	2 (100)	0 (0)	2 (100)	2 (100)	
total		97		43 (44)	5 (5)	13 (13)	96 (99)	51 (53)	75 (77)	76 (78)	
<i>K. pneumoniae</i>	OXA-244 + ESBL	512	1	0.25	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
<i>K. aerogenes</i>	OXA-244 + ESBL	64	1	0.5	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
<i>E. cloacae</i>	OXA-244	32	1	2	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)
<i>C. freundii</i>	OXA-244 + case ^a	64	1	0.5	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
Total			101		47 (47)	5 (5)	14 (14)	100 (99)	55 (54)	78 (77)	79 (78)
<i>K. pneumoniae</i>	OXA-232 + ESBL	≥512	20	2–128	18 (90)	19 (95)	17 (85)	20 (100)	20 (100)	20 (100)	20 (100)
		256	2	16–32	1 (50)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)
<i>C. freundii</i>	OXA-232 + ESBL	≥512	2	2–32	2 (100)	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)
		256	3	1–16	3 (100)	1 (33)	0 (0)	3 (100)	3 (100)	3 (100)	3 (100)
<i>E. coli</i>	OXA-232 + ESBL	≥512	2	2–4	2 (100)	1 (50)	2 (100)	2 (100)	2 (100)	2 (100)	2 (100)
<i>K. aerogenes</i>	OXA-232 + case ^a	128	1	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)
Total			30		27 (90)	25 (83)	21 (70)	30 (100)	30 (100)	30 (100)	30 (100)

➤ Large variability in CPE agar to detect OXA-48-producers

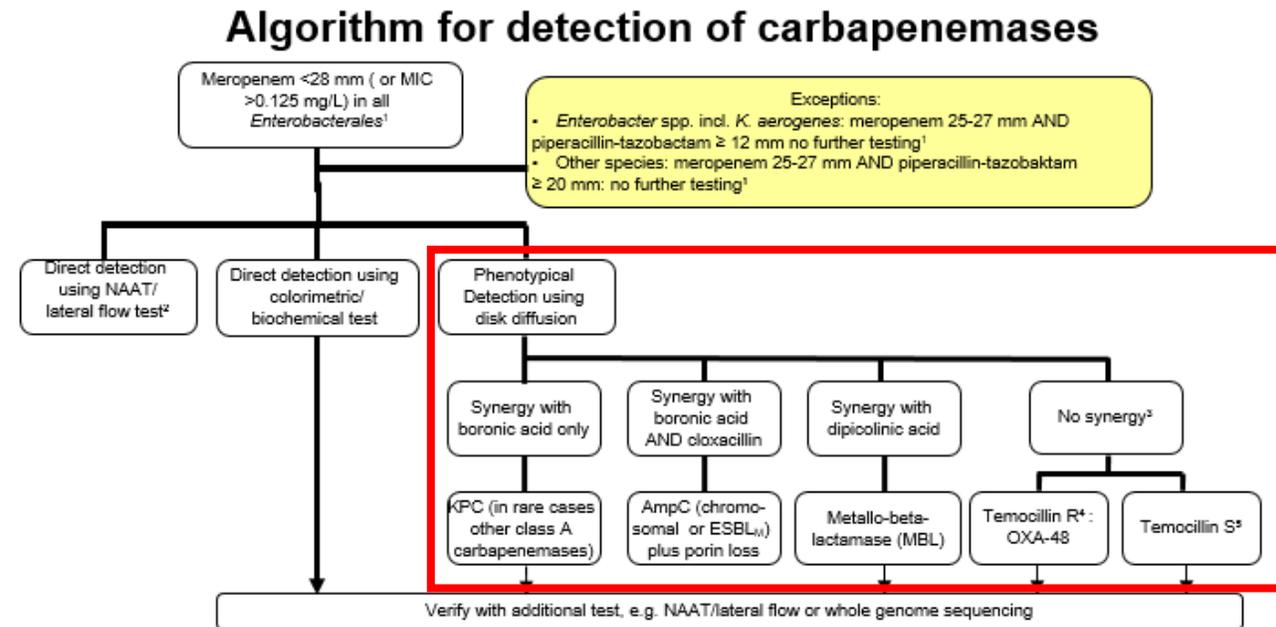
- Sensitivity:
 - ChromID CARBA: 14%
 - Brilliance CRE: 54%
 - mSuperCARBA: 99%

Considerations/recommendations

- Always include a CRE specific agar when screening for CPE colonisation! Do not rely only on an ESBL agar alone.
 - > Combination of both CRE + ESBL agar
- Make sure that your CRE agar detects OXA-48-producers including OXA-244
 - > Singel CRE agar or CRE agar + OXA-48 specific agar

Challenge: Phenotypic testing
with CDT

Phenotypic testing with CDT in the flowchart



¹ For laboratories using disk diffusion there is no added value in determining meropenem MIC regarding carbapenemase detection.

² NAAT (nucleic acid amplification test, e.g. PCR, LAMP)/lateral flow test should include the most common carbapenemases (OXA-48, NDM, VIM and KPC). If the test is negative, but the isolate is still suspected to be carbapenemase producing (e.g. due to laboratory or epidemiological reasons), consider additional testing for rarer carbapenemases (e.g. IMP, IMI, GES) or whole genome sequencing.

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Enterobacterales: Detection of carbapenemases

Metoddokument: CPE detection
Titel: Enterobacterales: Detection of carbapenemases

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Revision: 0

In this document we include nomenclature from 2009 for ESBL (with ESBL_{CARBA} is used as an alternative nomenclature to carba

Table 1: Overview of the most common acquired carbapenemases (ESBL_{CARBA}) in Enterobacterales

	Ambler Class A	Ambler Class B	Ambler Class C	Ambler Class D
Carbapenemase group	KPC (Klebsiella pneumoniae carbapenemase)	MBL (Metallo-beta-lactamase)	-	OXA (Carbapenem-hydrolysing class D betalactamases)
Important carbapenemase(s) within the group and main features	KPC Inactivates most beta-lactams including carbapenems IMI Chromosomal carbapenemase in E. cloacae, inactivates imipenem Rare: GES, NMC, SME	NDM VIM IMP Inactivate most beta-lactams except monobactams Rare: SPM	No carbapenemases in this Ambler class but AmpC+ porin loss can cause reduced susceptibility to carbapenems	A. OXA-48 group Often only slightly reduced susceptibility to carbapenems. Susceptible to cephalosporins. B. OXA-23, -24/-40, -58 Inactivate most beta-lactams including carbapenems
Common species (Rare species)	<i>Enterobacterales</i> (<i>P. aeruginosa</i>)	<i>Enterobacterales</i> <i>P. aeruginosa</i> (<i>A. baumannii</i> -group)	<i>Enterobacterales</i>	A. <i>Enterobacterales</i> B. <i>A. baumannii</i> -group
INHIBITED BY (used in phenotypical carbapenemase tests)	BORONIC ACID	DIPICOLINIC ACID and EDTA	CLOXACILLIN and BORONIC ACID	Inhibitor lacking (Temocillin ¹)

¹Temocillin >12 mm has been used as a phenotypical test to rule out OXA-48. Recently, new variants (e.g. OXA-244) have emerged which are not resistant to temocillin. Temocillin S can no longer be used to rule out carbapenemase production.



CDT – Combined disc test

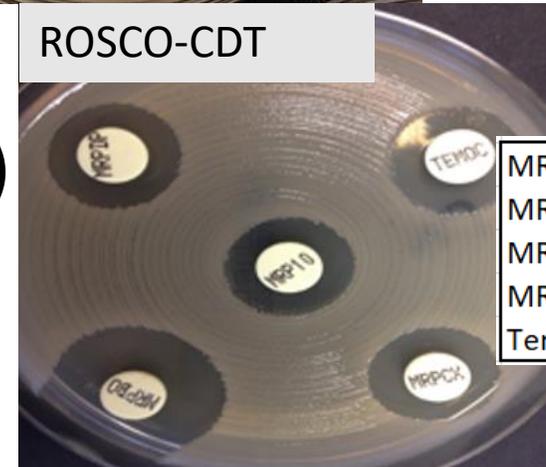
- Mastdiscs Combi Carba plus (MAST-CDT)



MAST-CDT

- | | |
|-----|----------------------------|
| A - | Penem |
| B - | Penem + MBL inhibitor |
| C - | Penem + KPC inhibitor |
| D - | Penem + AmpC inhibitor |
| E - | Temocillin + MBL inhibitor |

- Rosco KPC/MBL and OXA-48 Confirm kit (ROS-CDT)



ROSCO-CDT

- | | |
|-------|------------------|
| MRP | Meropenem(MEM) |
| MRPDP | MEM+dipikolin |
| MRPBO | MEM+ borsyra |
| MRPCX | MEM+ cloxacillin |
| Temo | Temocillin |

- Liofilchem KPC&MBL&OXA48 disc kit (LIO-CDT)

LIO-CDT

- | |
|------------------------------|
| Meropenem |
| Meropenem+EDTA |
| Meropenem+Phenylboronic acid |
| Meropenem+Cloxacillin |
| Temocillin |



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Systematic Comparison of Three Commercially Available Combination Disc Tests and the Zinc-Supplemented Carbapenem Inactivation Method (zCIM) for Carbapenemase Detection in *Enterobacterales* Isolates

Janko Sattler,^{a,b,c} Anne Brunke,^{a,b,c}  Axel Hamprecht^{a,b,c,d}

Conclusions:

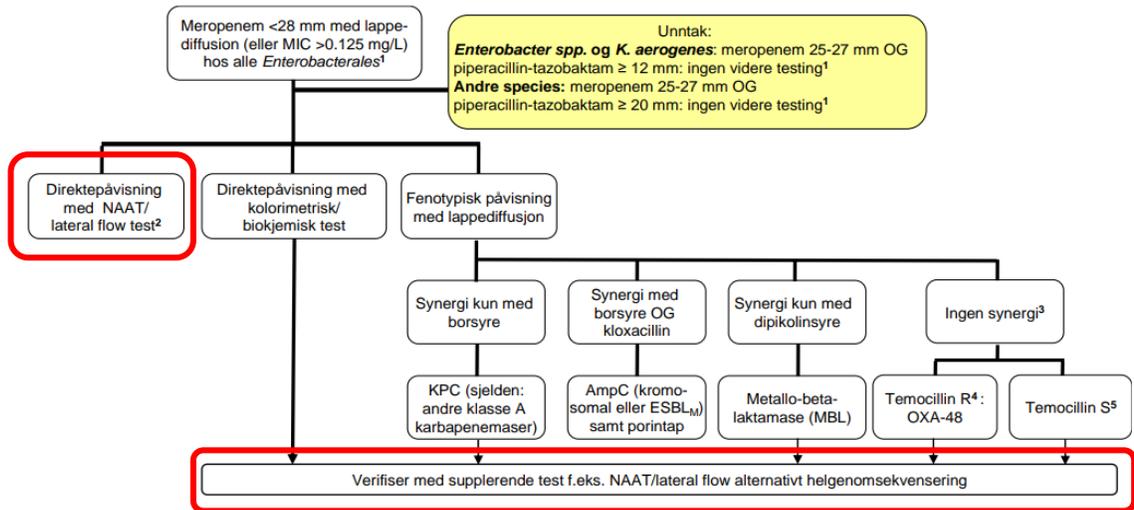
- ROSCO-CDT:
 - Class B: NDM+++ , IMP-
 - Class A:KPC +++
- LIO-CDT/MAST-CDT:
 - Class B: NDM/IMP+++
 - Class A: KPC-
- All of them: false negative when combination of MBL+ KPC

Conclusion and recommendations

- All CDT-test perform poorly if KPC+ MBL occur in the same isolate
- Know the weakness of your CDT-test and act on your knowledge
 - E.g: for ROSCO a negative test but with a tendency toward synergy for dipicolinic acid (3 mm) can be an IMP
- Temocillin S can never be used to rule out all OXA-48
 - In *E. coli*/*K. pneumoniae* with meropenem < 28 mm, use direct detection with NAAT/lateral flow to rule out OXA-244.
- Today CDT cannot be recommended as a stand-alone test to rule out CPE/ESBL-carba BUT perform well together with direct detection
- CDT can also be useful in detection of new carbapenemases
 - We only find what we are looking for with direct detection!

Challenge: Direct detection with
NAAT/Lateral flow in all labs?

Direct detection/verification of CPE



Is there available commercial tests suitable for all laboratories to perform direct detection/verification of CPE?

¹ For laboratorier som resistensbestemmer meropenem med lappediffusion tilfører det ingenting til karbapenemaseutredningen å gjøre MIC-bestemmelse.

² NAAT (nukleinsyre amplifiserende teknologi, f.eks. PCR, LAMP)/lateral flow test bør inkludere de epidemiologisk mest vanlige karbapenemasene (OXA-48, NDM, VIM og KPC). Dersom testen er negativ, men fortsatt mistanke om karbapenemaseproduksjon (f.eks. av laboratorieårsak eller epidemiologiske grunner), vurder supplerende test mtp. sjeldne karbapenemaser (f.eks. IMP, IMI, GES) eller helgenomsekvensering.

³ I sjeldne tilfeller kan høygradig resistente isolater med ingen synergi ha kombinasjoner av ulike karbapenemaser.

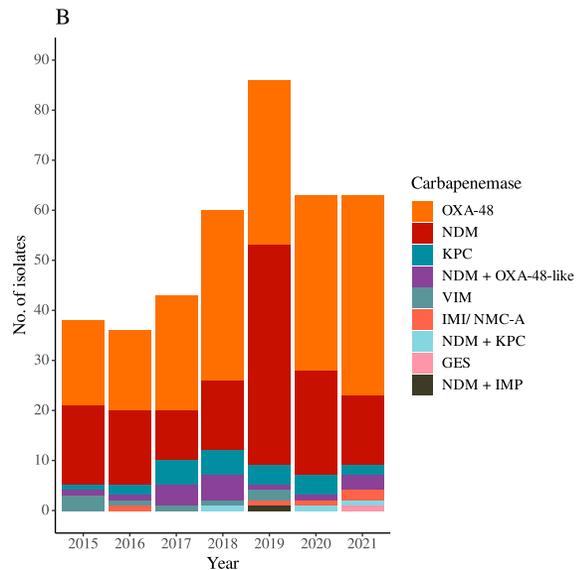
⁴ Høygradig temocillinresistens (>128 mg/L, sonediameter <12 mm) er en fenotypisk indikator på OXA-48. OXA-48 produserende isolater er også resistente mot piperacillin-tazobaktam og amoksisicillin-klavulansyre. Følg produsentens anbefalinger ved bruk av kommersielle metoder for tolkning av temocillin.

⁵ Noen OXA-48 varianter (f.eks. OXA-244) gir ikke høygradig temocillinresistens. Fenotypisk test med lappediffusion for påvisning av disse mangler, og temocillin følsomhet kan ikke bli brukt for å utelukke karbapenemaseproduksjon. Alle OXA-48, uansett variant, er resistente mot piperacillin-tazobaktam og amoksisicillin-klavulansyre.

Two reasons for direct detection/verification

- Epidemiology dominated by the «four big ones» - **NDM, OXA-48, KPC, VIM**

- A wide range of commercial methods are available



Commercial assays for the detection of acquired carbapenemases

- 98.5% of CPE harbor either OXA-48, NDM, VIM or KPC

Which method to choose?

- Coverage of carbapenemase genes – **NDM, OXA-48, KPC, VIM** + ...
- Throughput/turn-around time
- Cost
- Local workflow

Possible approaches

➤ NAATs integrated workflow – proprietary platform required

Assay	Assay coverage*	Additional equipment required	Workflow	Cost per isolate**	Equipment cost***						
 eazyplex® Superbug complete A/complete B/complete C/CRE Amplex	KPC, OXA-48-like, NDM, VIM	Heating block, centrifuge (for processing blood cultures)	Sample in buffer, heating 2 minutes at 99°C, processing in the GENIE®	£££	£££	 XPert® CARBA-R Cepheid	KPC, OXA-48-like, NDM, VIM, IMP-1-like	None	Swab/colonies in sample reagent, transfer of sample reagent to cartridge, processing of cartridge in GeneXpert®	££	£££-££££

➤ NAATs – PCR kits required and compatible PCR platforms

Table 2. NAATs – PCR kits required and compatible PCR platforms

Assay	Assay coverage*	Compatible platforms	Workflow	Cost per isolate**	Equipment cost***
AID carbapenemase AIDGmbH	KPC, OXA-48-like, NDM, VIM, IMP	Any cyclor platforms	DNA extraction from sample, PCR, reverse hybridisation	££	N/A
CARBAPLEX® Bruker	KPC, OXA-48-like, NDM, VIM, IMP	ABI 7500, BioRad CFX96, RotorGene Q, ABI QuantStudio 5, Bruker Hain Fluorocycler XT	DNA extraction from rectal swab, crude extract from pure culture, PCR using 2 mastemixes	££	N/A

Alternatively: in-house PCRs

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Short Communication

Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria

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➤ Immunochromatographic lateral flow assays

Table 5. List of immunochromatographic assays

Assay	Assay coverage*	Additional equipment required	Workflow	Cost per test**	Equipment cost***						
 O.K.N.V.I. RESIST-5 Coris BioConcept	KPC, OXA-48-like, NDM, VIM, IMP	Vortex	Colonies in lysis buffer, suspensions added in the strips, 2 strips required (KPC/OXA-48-like/NDM and VIM/IMP).	£	N/A	 NG-test® Carba-5 NG-Biotech	KPC, OXA-48-like, NDM, VIM, IMP	Vortex	Colonies in lysis buffer, suspensions added in the strips.	££	N/A

Evaluation of the different methods

Table 3. Performance of NAATs integrated workflow – proprietary platform required

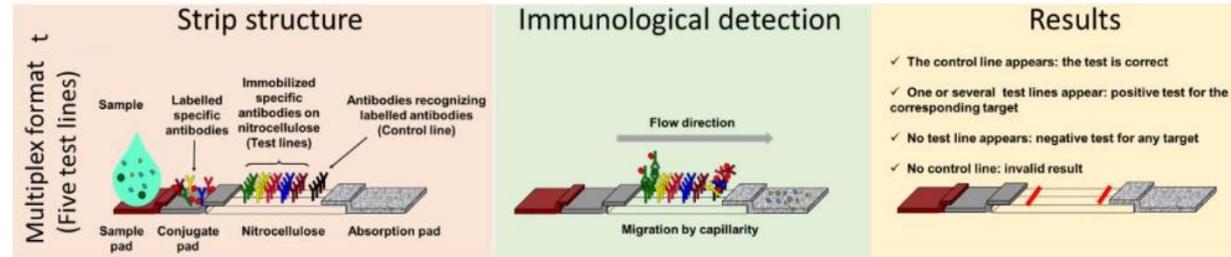
Assay	Assay coverage*	Throughput per run	Hands on time per sample	Sample to results#	Sample types validated by manufacturer	Performance characteristics (sample types tested)
eazyplex® Superbug complete A/complete B/CRE Amplex	KPC, OXA-48-like, NDM, VIM	8 to 12 samples	2 minutes	15 to 20 minutes	c, liquid swab, u, bc	From manufacturer: Sens 100% Spec 99.4% (c) ^{a*} From published data: (8) Sens 95.7% Spec 100% (c) (9) Sens 100% Spec 100% (c) (10) Sens 100% Spec 100% (c) (10) Sens 100% Spec 59% (cs)
ESBL 16-well CRE 16-well CRE EU 16-well AusDiagnostics	KPC, OXA-48-like, NDM, VIM, IMP	24 samples	5 minutes	Less than 4 hours	c	From manufacturer: Sens 98 to 100% Spec 99.5 to 100% (c) ^{a*} From published data: (11) Sens 100% Spec 100% (c) (12) Sens 100% Spec 99.4% (rs - requires prior DNA extraction)

Table 4. Performance of NAATs – PCR kits and compatible PCR platforms

Assay	Assay coverage*	Throughput per run	Hands on time per sample	Sample to results#	Sample types validated by manufacturer	Performance characteristics (sample types tested)
AID carbapenemase AIDGmbH	KPC, OXA-48-like, NDM, VIM, IMP	Depending on the platform	3 hours	4 hours	bc, u	From published data: (38) Sens 100% Spec 100% (c)
CARBAPLEX® Bruker	KPC, OXA-48-like, NDM, VIM, IMP	Depending on the platform	5 minutes	Less than 3 hours	c, rs	From published data: (39) Sens 96.2% Spec 90% (rs)
Check-Direct CPE Check-Points	KPC, OXA-48-like, VIM/NDM (VIM and NDM are not differentiated by the assay)	Up to 96 tests	5 minutes	2 hours	c, rs, prs	From manufacturer: Sens 100% Spec 100% (c) ^{a*} From published data: (8) Sens 100% Spec 100% (c) (40) Sens 100% Spec 94% (rs) (41) Sens 100% Spec 100% (c, rs) (42) Sens 100% Spec 88% (rs) (43) Sens 100% Spec 100% (c)

Immuno-chromatographic lateral flow assays

Principle

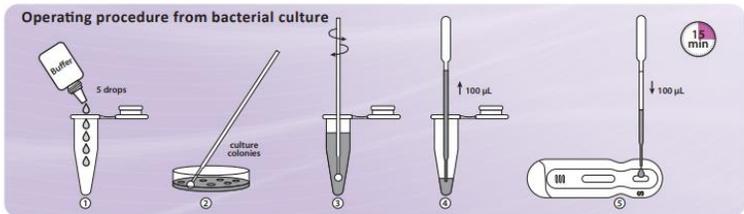


Boutal H. et al. *Diagnostics*. 2022

NG-Test CARBA 5



*Validated on: TSA, Mueller Hinton, ChromID® CARBA SMART, Drigalski (DRIG) CHROMagar™ mSuperCARBA™...etc

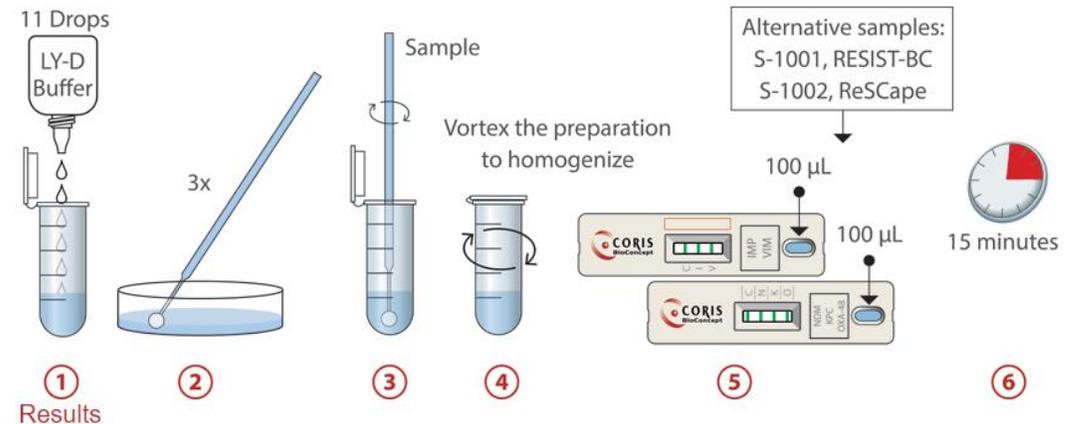


For professional in vitro diagnostic use only

KPC
OXA-48-like
NDM
VIM
IMP

O.K.N.V.I. RESIST-5

Procedure



Performance

Table 6. Performance of immunochromatographic assays

Assay	Assay coverage*	Throughput per cartridge	Hands on time per sample	Sample to results#	Sample types validated by manufacturer	Sensitivity/specificity (manufacturer)	Sensitivity/specificity (published data)
O.K.N.V.I. RESIST-5 Coris BioConcept	KPC, OXA-48-like, NDM, VIM, IMP	1 sample	Less than 5 minutes to 30 minutes depending of sample types to be tested	20 minutes to less than 4 hours depending of sample types to be tested	c, bc, rs	100%/100% (c)	93.8-98.4%/93.3-100% (c)
NG-test® Carba-5 NG-Biotech	KPC, OXA-48-like, NDM, VIM, IMP	1 sample	Less than 5 minutes to 20 minutes depending of sample types to be tested	20 minutes to 30 minutes depending of sample types to be tested	c, bc	100%/100% (c) 100%100% (bc)	96.3-100%/95.3-100% (c) 90-100%/100% (bc) 94.1%/100% (urine) 92.3%/100% (rs)

c: bacterial colonies; bc: blood culture; u: urine; rs: rectal swab

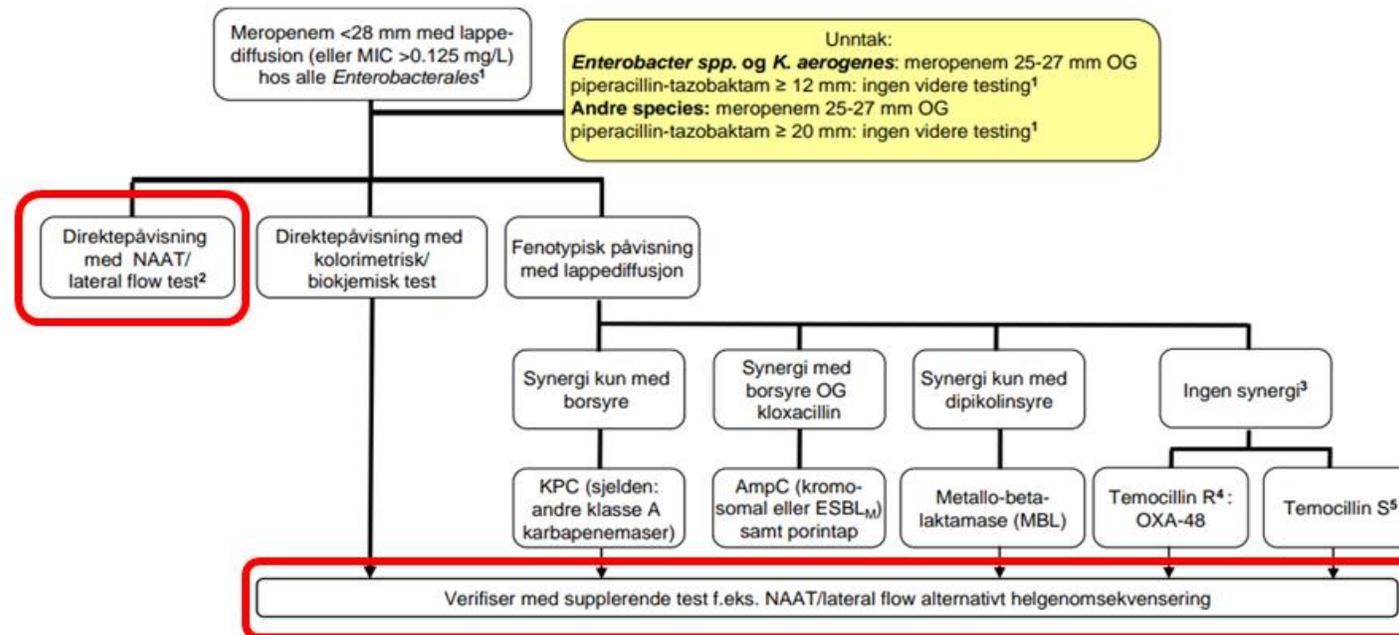
Limitations

- Antibody affinity and specificity
 - Diversity within carbapenemase families -> not all variants detected
 - Mutations in the epitope
 - Distinguish between carbapenemase & non-carbapenemase variants?
 - OXA-48 vs. OXA-163?
 - KPC carbapenemases vs. KPC non-carbapenemases (ceftazidime-avibactam resistant)?
- Do not detect rare carbapenemases (IMI, GES, FRI, SME, etc)

~156 KPC-variants
~82 VIM-variants
~55 NDM-variants
~99 IMP-variants
~many OXA-48 variants

Conclusion

- Commercial tests are available for detection of the most prevalent carbapenemases
-> possibility for rapid local clarification with respect to infection control



¹ For laboratorier som resistensbestemmer meropenem med lappediffusion tilfører det ingenting til karbapenemaseutredningen å gjøre MIC-bestemmelse.

² NAAT (nukleinsyre amplifiserende teknologi, f.eks. PCR, LAMP)/lateral flow test bør inkludere de epidemiologisk mest vanlige karbapenemasene (OXA-48, NDM, VIM og KPC). Dersom testen er negativ, men fortsatt mistanke om karbapenemaseproduksjon (f.eks. av laboratorieårsak eller epidemiologiske grunner), vurder supplerende test mtp. sjeldne karbapenemaser (f.eks. IMP, IMI, GES) eller helgenomsekvensering.

³ I sjeldne tilfeller kan høygradig resistente isolater med ingen synergi ha kombinasjoner av ulike karbapenemaser.

⁴ Høygradig temocillinresistens (>128 mg/L, sonediameter <12 mm) er en fenotypisk indikator på OXA-48. OXA-48 produserende isolater er også resistente mot piperacillin-tazobaktam og amoksisicillin-klavulansyre. Følg produsentens anbefalinger ved bruk av kommersielle metoder for tolkning av temocillin.

⁵ Noen OXA-48 varianter (f.eks. OXA-244) gir ikke høygradig temocillinresistens. Fenotypisk test med lappediffusjon for påvisning av disse mangler, og temocillin følsomhet kan ikke bli brukt for å utelukke karbapenemaseproduksjon. Alle OXA-48, uansett variant, er resistente mot piperacillin-tazobaktam og amoksisicillin-klavulansyre.

Main conclusions/recommendations

- Revised exception in flowchart reduces workload – risk of missing CPE limited.
- Temocillin as indicator for OXA-48 is unreliable
- CDT:
 - Cannot be used as a standalone test -> verification required.
 - Challenges with IMP and double carbapenemase-producers
- Screening agar -> always include CRE specific agar(s) that also detect OXA-48 producers
- Commercial tests (e.g. NAATs/lateral-flow) is now available for all labs