Carbapenemases

Carbapenemases are beta-lactamases that significantly hydrolyze at least imipenem and/or meropenem. Carbapenemases involved in acquired resistance are of Ambler classes A, B and D. They may be plasmid or chromosomally encoded.
Because several of these carbapenemases confer only reduced susceptibility to carbapenems in Enterobacteriaceae, they may remain underestimated, because they are not detected in the laboratory. Acquired carbapenemases are increasingly reported worldwide and consequently it is important to be able to detect them in the laboratory.

For many isolates with carbapenemases, the MICs of carbapenems are around the susceptible breakpoint making resistance difficult to detect - particularly with automated systems. Therefore, special zone breakpoints are needed in first line screening

Enterobacteriaceae with reduced susceptibility to Imipenem 10 µg (zone < 23 mm or MIC > 1 µg/ml) or Meropenem 10 µg (inhibition zone < 25 mm or MIC > 0.5 µg/ml) or Ertapenem (zone ≤ 22 mm) on Mueller-Hinton Agar with McFarland 0.5 inoculum, should be suspected of possessing carbapenemases

P. aeruginosa with inhibition zones Imipenem 10 µg (< 22 mm) or Meropenem 10 µg (< 26 mm) should be suspected of possessing carbapenemase. Most isolates with KPC and GES enzymes are highly resistant to Ceftazidime. Ertapenem Neo-Sensitabs is the most sensitive indicator for possible carbapenemase, but in approximately 20% of cases other resistance mechanisms are involved (confirmation of carbapenemase with Modified Hodge Test is necessary). It is important to recognize small resistant colonies growing inside the Ertapenem disk zone.

Carbapenemases classification (1)

Ambler classification	Enzymes	MICs µg / ml				Inhibited by		
		3rd gen cepha	AZT	IMP	MRP	CLAV	EDTA	Boronic acid
A	NmcA	S	4	≥ 16	2-8	± wk	no	yes
	Sme-1 to Sme-3	S	4-64	≥ 16	0.25-8	± wk	no	yes
	IMI-1 to IMI-2	S	S	≥ 64	4-32	+	no	yes
	KPC-1 to KPC-4	≥ 32	≥ 64	4→16	4→16	+ or wk	no	yes
	GES-2 to GES-5	≥ 32	16→R	0.25→16	0.5-16	+ or 0	no	yes
B Metallo-beta-lactamases	IMP 1-16	≥ 32	S→R	0.5-128	0.25→R	no	yes	no
	VIM 1-12	≥ 64	S→R	1→R	0.5→R	no	yes	no
	SPM-1	≥ 256	4	R	R	no	yes	no
	GIM-1	16-32	8-16	> 8	> 8	no	yes	no
	SIM-1	≥ 256	128	8-16	16	no	yes	no
D Oxacillinases	OXA 23-27	> 256	> 256	4-64	4-128	± wk	no	no
	OXA 40-48	S→R	S→R	2-64	0.25-64	wk	no	no
	OXA 54-55	S	S	4	0.25	wk	no	no
	OXA-60	S	R	0.5	2	no	no	no
	OXA-58	4-128	≥ 32	3-32	2→64	no	no	no

wk = weak

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Detection of acquired carbapenemases Ambler classes A and D

Class A carbapenemases are penicillinases with greater activity against imipenem than meropenem and they also give resistance to penicillins, cephalosporins and aztreonam.

Boronic acid in an inhibitor of class A carbapenemases and consequently synergy with meropenem or imipenem, is the best method to detect these enzymes (26,27,28,22).

Clavulanate is an inhibitor of class A carbapenemases and therefore synergy with imipenem may be useful to detect these enzymes (1,2,3,4,5).

The KPC family of enzymes confer greater resistance to third gen cephalosporins than to carbapenems (3,5).

KPC possessing *Enterobacter* spp. and *K. pneumoniae* were reported as falsely susceptible to carbapenems using automated systems (Vitek). MIC microdilution using standard inocula of 10^4 or 10^5 CFU/ml did not detect carbapenem resistance, while diffusion methods (E-test) using inocula of 10^8 CFU/ml detected resistance (5,7,12,18).

K. pneumoniae intermediate or resistant to ertapenem or meropenem should be considered resistant to all carbapenems (7). KPC possessing E.coli was identified in nine patients in New York. Three of the isolates possessed also ESBL: CTX M15 (19).

Pasteran et al (20) found that Boronic acid disks could be used to detect carbapenemases of type 2f (Class A) in Enterobacteriaceae. Class A producing strains showed synergy between Imipenem and Boronic acid disks (distance from edge to edge 6 mm). Strains showing zones of inhibition ≤21 mm with Imipenem 10 µg disks were screened with this test.

Carbapenemase IMI-2 is the first inducible and plasmid-encoded carbapenemase.

Please note that KPC detection may require screening multiple colonies, because carbapenemase susceptible strains may co-exist with resistant (21).

Class D carbapenemases correspond to the enzymes classified as OXA-types (oxacillinase activity). They hydrolyze imipenem and meropenem weakly and do not hydrolyze third gen cephalosporins and aztreonam (although MICs against the later drugs are often increased due to the presence of other beta-lactamases).
Clavulanate is a progressive inhibitor of most OXA carbapenemases, but not all. The synergy test (clavulanate and imipenem) may have value for the detection of these enzymes.

Clinicians should be aware of the potential for clinical failure (Class D, OXA-55 carbapenemase) when imipenem is used for treatment of serious infections caused by *S. algae* (9).

Fernandez et al report heteroresistance carbapenems in Ac. baumannii associated with Imipenems MIC 4-16 µg and the presence of OXA-58 (17).

Yilmaz et al (16) report oxacllinases (OXA-48) in 21 Enterobacteriaceae, mainly K.pneumoniae, but also in E.coli and Enterobacter cloacae/aerogenes in Turkey, and warns that oxacillinases (carbapenemases) are spreading in Enterobacteriaceae.

Castanheira et al (25) report the clonal dissemination of OXA-24 and OXA-58 producing A. baumannii in Houston, Texas.

Acquired carbapenemases Ambler class A and D

Ambler class.	Enzymes	MICs µg/ml PIPER	3rd gen cepha	AZTRM	IMI	MEROP	IMIPENEM + CLAV (synergy)	Mero-penem+ Boronic synergy	Organisms	Genetic Location
A	NmcA	S	S (0.25-2)	4	≥ 16	2-8	± wk	yes	*E. cloacae*	Chromosomal
	Sme-1 to Sme-3	S	0.25-0.5	4-64	≥ 26	0.25-8	± wk	yes	*S.marcescens*	Chromosomal
	IMI-1	> 256	1-2	8	> 64	4	+	yes	*E. cloacae*	Chromosomal
	IMI-2	16→128	0.1-2	4-8	64	4-32	+	yes	*E. asburiae*	Plasmid
	KPC-1	> 128	≥ 32	> 64	16	16	+		*K. pneumoniae*	Plasmid
	KPC-2	≥ 64	≥ 8	> 16	8→16	≥ 16	+		*K. pneumoniae /oxytoca. Raoultella(39) Salmonella Enterobacter*	Plasmid
(11)	**KPC-2**	.	≥ 256	> 256	256	256	(+)	yes	*P. aeruginosa*	Plasmid/Chrom.
	KPC-3	256	256	> 256	> 4	> 4	(+ +)		*K.pneumoniae Enterobacter E. coli, P. mira-bilis, Citrobacter*	Plasmid
	KPC-4 KPC-5/6				> 16	> 16	(+)		*Enterobacter*	
	GES-2	128	≥ 32	16	4→16	4-16	+	yes	*E. cloacae P. aeruginosa*	Plasmid, integron
	GES-3	128	64→256	64	0.25	0.5	+	yes	*K. pneumoniae*	Plasmid
	GES-4	128	R	R	8	8	(+)	yes	*K. pneumoniae*	CEFOX R
	GES-5	R	R	R	8-32	8-32	+	yes	*P. aeruginosa, K.pneumoniae*	Integron
	GES-11	R	> 256	> 256	4	8		yes	*A. baumanni*	
D	**OXA-23** to **OXA-27**	> 256	> 256	> 256	4-64	4→128	± wk	no	*A. baumanni*	Chromosomal ± integron
	OXA-40	R	4→128	4→128	> 32	≥ 32	wk	no	*Ac.haemolyticus K. pneumoniae P. aeruginosa*	Plasmid
	OXA-48	8→R	S→R	S→R	2→64	0.25→64	wk	no	*K. pneumoniae* E.coli	Plasmid
	OXA-54	32	S	S	1	0.12	wk	no	*Sh. putrefaciens*	Not integron
	OXA-55	S	S	S	1-4	0.25	no wk	no	*Sh. algae* (9)	Chromosomal
	OXA-58	256	4-128	≥ 32	2-32	2→64	no	no	*A. baumanii*	Plasmid
	OXA-60	S	S	R	0.5	2	no	no	*R. pickettii*	Chromosomal
	OXA-62	S→R	S→R	S→R	2→64	64 →128	no	no	*Pandorea* (10) *pnomenusa*	Chromosomal
	OXA-23, **27**, 49 (subgroup 1)			>16	8→32	8→>32		no	*Ac. baumannii*	Plasmid (only 23)
D (8)	**OXA-24**, **25, 26, 40** (subgroup 2)	-	> 256		> 128	> 128	-	no	*Ac. baumannii*	Chromosomal
	OXA-51 + OXA-64-66, 68-71, 78-82-107 OXA-51-like (subgroup 3)		> 32		≥ 1	≥ 1		no	*Ac. baumannii*	Chromosomal plasmid
	OXA-58 (subgroup 4)	R	R	> 16	4/16			no	*Ac. baumannii*	Plasmid (only 58)
	OXA-143	R	FEP4	.	32	32	-	no	*Ac. baumannii*	Plasmid

Bold = involved in outbreaks

Procedure for KPC carbapenemases detection (Class A enzymes)

Isolates giving negative metallo-beta-lactamase tests, may produce other carbapenemases. The most current are KPC enzymes isolated from *Enterobacteriaceae (K. pneumoniae, E. coli, Enterobacter spp., P.mirabilis)* particularly *K. pneumoniae*, but also Sme, IMI, GES and Nunc A are found.
To detect these strains in rectal swab screening samples, direct plating on McConkey agar in the presence of Ertapenem Neo-Sensitabs and Imipenem Neo-Sensitabs may be useful.

Place one Boronic Acid Diatabs between one Ertapenem and one Imipenem Neo-Sensitabs (distance 6 mm from edge to edge).

Place one Cloxacillin Diatabs between Ertapenem and Imipenem Neo-Sensitabs - (6 mm from edge to edge).Perform Modified Hodge Test (MHT) with Ertapenem and Meropenem Neo-Sensitabs.

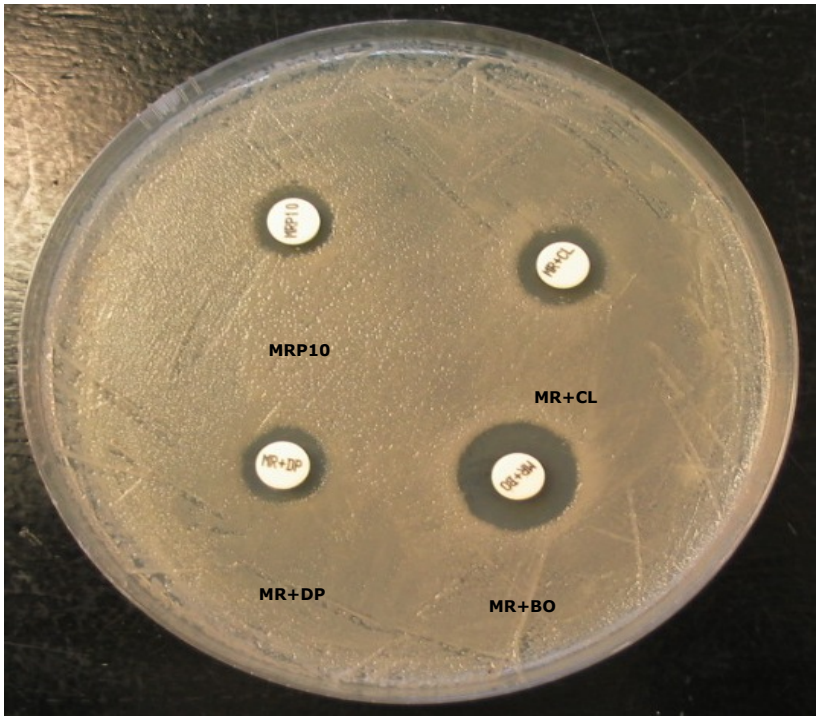
Interpretation (Double disk synergy test)

The following results will presumably indicate the presence of a KPC beta-lactamase:

a) Negative metallo-beta-lactamases tests.
b) Positive synergy test between Boronic Acid and the carbapenems (one or both).
c) Negative synergy test between Cloxacillin and the Carbapenems (11)
d) Positive synergy test between clavulanate (AMC) and carbapenems (one or both). Not always easy to see. Although isolates with ESBL + impermeability may give false positive results.
e) Positive Modified Hodge Test.
f) Sme, IMI, GES and Nunc A will show the same results as KPC, but the mentioned enzymes result in smaller zones around Imipenem compared to Ertapenem. With KPC enzymes zones around Imipenem and Ertapenem are similar.

Combined disk test. KPC + MBL Confirm ID kit

Apply Meropenem, Meropenem+DPA, Meropenem+Boronic, Meropenem+Cloxacillin on an inoculated plate.



K.pneumoniae PHA3 CL5761 KPC positive

Interpretation (combined test)

A Meropenem + Boronic inhibition zone ≥ 5 mm then Meropenem, Meropenem+DPA and Meropenem+Cloxacillin indicates a presence of a KPC enzyme (or other class A). Meropenem+Boronic and Meropenem+Cloxacillin inhibition zones ≥ 5 mm, than Meropenem and Meropenem+DPA indicates AmpC hyperproduction + porin loss, or efflux (30).

A Meropenem +DPA inhibition zone ≥ 5 mm than Meropenem, indicates the presence of a metallo-β-lactamases (MBL).

Please note

Test only ertapenem-resistant strains. Ertapenem susceptible strains may provide a false positive result with Boronic Acid.

Conclusion

Reduced susceptibility to ertapenem, synergy between Boronic Acid and the carbapenems, and no synergy between Cloxacillin and the carbapenems is clearly indicative of KPC enzyme being present (or other class A enzymes). Isolates producing high level AmpC + impermeability can be detected by synergy between Cloxacillin and the carbapenems (11). Isolates producing ESBL + impermeability will show synergy between AMC and the carbapenems or cephalosporins.

Procedure for Oxacillinase detection (Class D enzymes)

Strains producing oxacillinases will currently show zones of inhibition < 22 mm with Ertapenem and/or <25 mm with Meropenem Neo-Sensitabs. Most are resistant to Aztreonam. These enzymes are mainly found in Acinetobacter baumannii but also in Enterobacteriaceae (K. pneumoniae, Enterobacter) and P. aeruginosa although these are rare.

Interpretation

The following results will presumably indicate the presence of oxacillinases:

a) Negative metallo-beta-lactamase tests.
b) Negative synergy test between Boronic acid/Cloxacillin and the carbapenems. (one or both).
c) Negative (or weak positive) synergy test between clavulanate (AMC) and carbapenems (one or both)
d) Positive Modified Hodge Test.

Summary detection of carbapenemases

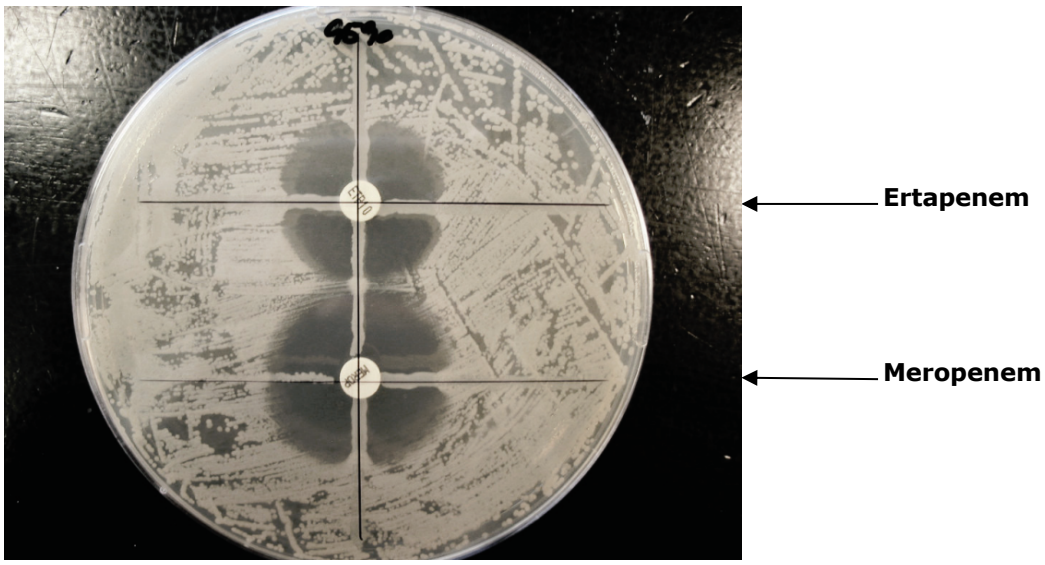
Meropenem	**MRP+DPA**	**MRP+BOR**	**MRP+Cloxa**
Metallo-β-lactamases	Synergy	No synergy	No synergy
KPC	No synergy	Synergy	No synergy
AmpC impermeability	No synergy	Synergy	Synergy
Oxacillinases	No synergy	No synergy	No synergy

Modified Hodge Test

Is used to determine if resistance to carbapenems is caused by a carbapenemase. A MH agar plate (or a McConkey plate) is inoculated with the susceptible strain E. coli ATCC 25922 (Mc Farland 0.5, diluted 1/10) as for disk diffusion.
When testing Enterobacteriaceae, one Ertapenem Neo-Sensitabs and one Meropenem Neo-Sensitabs are applied onto the plate approx.30 mm apart from each other. For non-fermenters one Imipenem Neo-Sensitabs and one Meropenem Neo-Sensitabs are applied.

A suspension of the microorganism to be tested for carbapenemase is adjusted to Mc Farland 0.5 standard and a loop is used to make a heavy streak passing through the two carbapenem disks. Two more streaks are placed perpendicularly making a cross.

Thereafter incubation for 18-24 hours at 35-37 C. Alteration in the shape (indentation) of the zones of inhibition around the test organism is considered indicitive of the presence of a carbapenemase (figure).



K.pneumoniae KPC positive

Limitations:

- Not reliable for detection of SME from S. marcescens
- P. mirabilis swarming may give lecture problems

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Detection of acquired Metallo-beta-lactamases (MBL)

The worldwide spread of acquired metallo-beta-lactamases (MBL) in gram-negative aerobes is of great concern. MBL production in clinical isolates of key gram-negatives: *P. aeruginosa, E. cloacae, S. marcescens* and *K. pneumoniae* should be carefully monitored (5).

MLBs are classified into 5 major types: IMP, VIM, SPM, and GIM and SIM type enzymes. In Enterobacteriaceae only IMP and VIM enzymes have been found as yet.
MBLs hydrolyze most beta-lactams (carbapenems and large expectrum cephalosporins), except aztreonam. This phenotype of multiple beta-lactam resistance and aztreonam susceptibility may be helpful for identification of these strains in the laboratory. If the strain is resistant to aztreonam it may be due to additional resistance mechanisms (efflux, other beta-lactamases, ESBL etc.). Their expression is not inducible.

The MBL enzymes are resistant to beta-lactamase inhibitors and susceptible to chelating agents like EDTA (2-MPA) and Dipicolinic acid (DPA).

Early detection of MBL-producing microorganisms is essential to prevent dissemination of these organisms. The enclosed tables, including strains of Enterobacteriaceae and Non-fermenters producing MBLs, show that MPL-producers (particularly in Enterobacteriaceae) may show low MIC values against carbapenems making it difficult for the laboratory to detect MBL-positive isolates.

Suspicious isolates (resistant to ceftazidime showing no synergy between clavulanate and third gen. cephalosporins and possibly showing reduced susceptibility to carbapenems) should be tested for carbapenemase activity using Imipenem, Meropenem and EDTA and Dipicolinic acid tests.

The first metallo-beta-lactamase producing strain of *E. coli* (in Spain) has been detected in Barcelona, using Imipenem+EDTA Neo-Sensitabs and E-test (3,8). The first metallo-beta-lactamase producing strain of *K. pneumoniae* was found in France (4).

MBL- producing gram-negatives have now emerged in Australia (15).The resistance gene bla-IMP4 appears highly mobile, this outbreak involved 5 different gram-negative genera. Diagnostic laboratories in Australia and other countries must be now in high alert, because early detection may limit the wide dispersal of MBL-genes.

Kyegong (27) and Miriagou (28) showed the efficiency of Dipicolinic acid (DPA) to detect metallo-β-lactamases in Enterobacteriaceae and non-fermenters. Miriagou found that the DPA/Imipenem synergy test was positive for all VIM-producing isolates of Klebsiella/Enterobacter and P. mirabilis, while EDTA based tests could not identify VIM-producing P. mirabilis

Acquired Metallo-beta-lactamases
NON-FERMENTERS

MBL	3rd gen. Cepha MIC	AZT MIC µg/ml	IMP MIC µg/ml	MRP MIC µg/ml	Microorganisms	Genetic location
IMP 1-11	≥ 128	≤ 8/16	≥ 8	≥ 8	*Pseudomonas* spp. *Alcaligenes* spp. *Acinetobacter baumannii*	Chromosomal plasmid integron
IMP 12	≥ 128	32	32	128	*Pseudomonas putida*	
IMP 13-16	≥ 256	4-128	≥ 64	≥ 64	*Pseudomonas aeruginosa*	integron
VIM 1-3	R	S → R	2-128	1-128	*Achromobacter xylosoxidans* *Pseudomonas aeruginosa* *Pseudomonas putida* (VIM 2 and 4) *Acinetobacter baumannii*	Chromosomal plasmid integron
VIM 4-11	> 256	S → R	32-256	32-256	*Pseudomonas aeruginosa, A.baumannii*	
VIM 15-16	≥ 64	16-32	>128	≥128	*Pseudomonas aeruginosa*	integron
VIM-18	R	S → R	R	R	*Pseudomonas aeruginosa*	integron
SPM-1	≥ 256	4	R	R	*Pseudomonas aeruginosa*	Plasmid (**not** integron)
GIM-1	16 → 32	8-16	> 8	> 8	*Pseudomonas aeruginosa*	Integron
SIM-1	≥ 256	128	8-16	16	*Acinetobacter baumannii*	Integron
IND1-6	1-32-128	32-128	4-32-128	4-16-128	*Chryseobact indologenes*	Chromosomal (23)
AIM-1					*Pseudomonas aeruginosa*	

MBL are not inhibited by clavulanate, but are inhibited by EDTA or DPA

Acquired Metallo-beta-lactamases
ENTEROBACTERIACEAE

MBL	3rd gen. Cepha MIC	AZT MIC µg/ml	IMP MIC µg/ml	MRP MIC µg/ml	Microorganisms	Genetic location
IMP-1	≥ 32	< 0.5	2	0.5	*E. coli*	
IMP-1	≥ 32	0.5 → R	4-128	4-128	*S. marcescens, K. pneumoniae, K. oxytoca, E. cloacae / E. aerogenes, Cit. freundii, P. rettgeri, M. morganii,*	Integron
IMP-3	64	0.5	1	·	*Shigella flexneri*	
IMP-4	256	·	3	6	*Citrobacter youngae*	Plasmid
IMP-6	> 128	0.25	2-8	64	*E. coli*	
IMP-6	> 128	128	32	> 128	*Serratia marcescens*	
IMP-8	R	S → R	0.5-8	0.25-4	*Enterobacter cloacae, Klebsiella pneumoniae, S.marcescens*	
VIM-1	R	8-128	8-32	2-32	*E. coli, P. mirabilis (integron) C. koseri, K. oxytoca*	Plasmid
VIM-1	16-128	S → R	1-64	1-32	*Klebsiella pneumoniae, E. cloacae*	Plasmid (integron)
VIM-2	≥ 32	S → R	≥ 1	0.5 → > 2	*Citrobacter freundii / E.cloacae*	Plasmid
VIM-2	≥ 128	32	16-64	8-64	*Serratia marcescens, P. rettgerii*	Integron
VIM-2	8	16	4	0.1 (S)	*Klebsiella oxytoca*	Plasmid (integron)
VIM-4	≥ 32	4 → R	2-4	0.5-1	*K. pneumoniae / E. cloacae*	Plasmid
VIM-12	≥ 128	16	8	4	*K. pneumoniae*	Plasmid (16)
VIM-12	> 32	1	1	0.25	*E. coli*	Plasmid (22)
VIM-2 + GES7					*E. coli*	Integron
KHM-1	R	0.25	2	4	*C. freundii*	Plasmid
NDM-1	R				*K. pneumoniae, E.coli, C.freundii (31,33)*	Plasmid

MBL are not inhibited by clavulanate, but are inhibited by EDTA or DPA.

Procedure for metallo-beta-lactamase (MBL) detection

Some resistance profiles may suggest MBL production, for example:

a) *Pseudomonas aeruginosa*, *Pseudomonas*. spp. and *Acinetobacter* spp.
All isolates non-susceptible to carbapenems and resistant to either ticarcillin, ticarcillin+clavulanate or ceftazidime should be tested for MBL production.

b) *Enterobacteriaceae*
For *E. coli*, *Klebsiella* spp., *P. mirabilis*, *Salmonella* spp. and *Shigella* spp.: All carbapenem S-I-R isolates that are resistant to cefoxitin and amoxicillin+clavulanate and are non-susceptible to ceftazidime (inhibition zone < 18 mm) should be tested for MBL production. In all other cases all isolates are non-susceptible to carbepenems (18).

Enterobacteriaceae

Apply one Dipicolinic Acid Diatabs (DPA) on an inoculated Mueller Hinton (MH) plate. Apply one Meroper Neo-Sensitabs and one Ertapenem Neo-Sensitabs onto the plate on either side of the DPA, 5mm from DPA (edge to edge). Apply Imipenem 10 µg + EDTA (IM10E) on an inoculated MH plate. Apply Imipenem 10 µg Neo-Sensitabs.

Non-fermenters

Apply one DPA Diatabs on the MH plate. Apply one Imipenem Neo-Sensitabs and one Meropenem Neo-Sensitabs at either side of the DPA, 5 mm from the DPA (edge to edge). Apply Imipenem + EDTA (IM10E) on the inoculated MH plate. Apply one Imipenem Neo-Sensitabs.

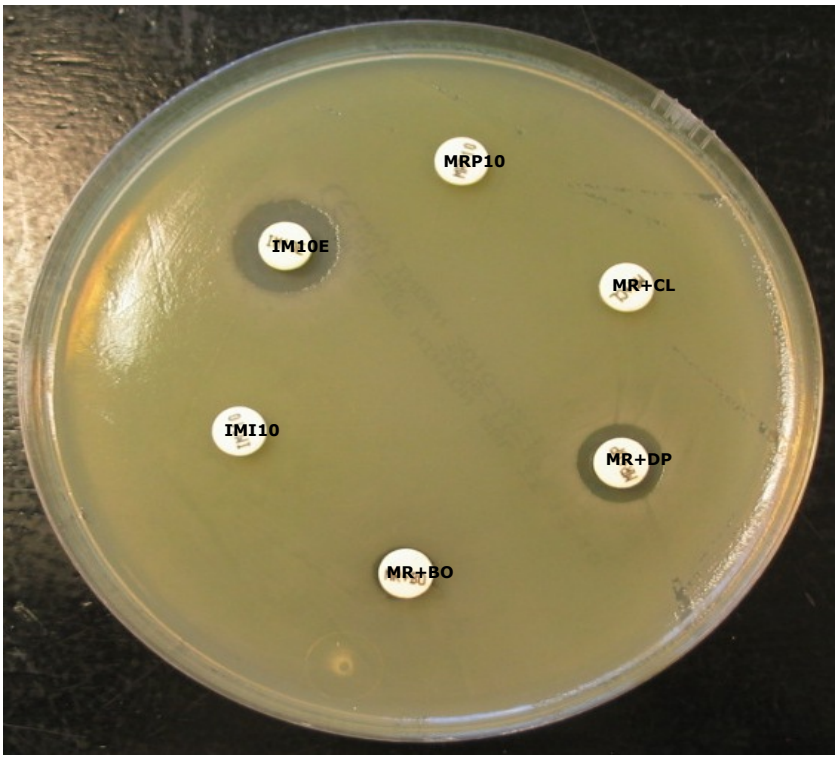
Interpretation (Double disk synergy test)

The use of two chelating agents EDTA and DPA will enhance the detection of metallo-ß-lactamases (MBL) in the clinical laboratory. A key hole or ghost zone between carbapenems (one or more) and Dipicolinic Acid indicates the presence of an MBL.
• An Imipenem + EDTA (10+750 µg) zone 7mm larger than Imipenem 10 µg indicates the presence of a Metallo-Beta-Lactamase.

A key hole or ghost zone between carbapenems (one or more) and Dipicolinic Acid indicates the presence of a MBL.

Combined disk test. KPC and MBL Confirm ID kit

Apply Meropenem, Meropenem+DPA on an inoculated MH plate. Interpretation: A Meropenem+DPA inhibition zone ≥ 5 mm than Meropenem alone indicates the presence of a metallo-beta-lactamase.



P.aeruginosa FN 8173 metallo-β-lactamase positive

Please note:

Most MH agar brands contain physiological levels of Zn++ions and should be used for carbapenem testing. Iso-Sensitest agar has low levels of zinc ions and may give false susceptibility results for carbapenems in the presence of MBL. Strains of Acinetobacter baumannii producing certain oxacillinases may give a false positive metallo-ß-lactamase test result.

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