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.

Carbapenemases are mainly found in Amber class A, B and D, with KPC, NDM and OXA-48 being the most frequently encountered in Enterobacteriaceae.

The emergence of carbapenemases in gram-negative bacteria, including Enterobacteriaceae, Pseudomonas and Acinetobacter species has become a major health crisis worldwide.

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. Rapid detection and identification of the carbapenemase genes is critical for the implementation of health control policies.

Confirmation of carbapenemase activity can be achieved with phenotypic tests such as combined disc methods and rapid colorimetric based tests.

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.

Adler et al (67) report that carbapenemase-producing K. pneumoniae exhibited a marked inoculum effect and were more resistant to the bactericidal effect of meropenem, suggesting that MIC measurement alone may not be suffitient in predicting efficacy.

Potron et al (4) confirms that multidrug resistance is quite common among non-fermentative gram-negative rods, in particular Pseudomonas aeruginosa and Acinetobacter baumannii. The authors detail the most important mechanisms of resistance in P. aeruginosa and A. baumannii and their most recent dissemination worlwide.

Logan LK et al (5) report that carbapenem-resistant Enterobacteriaceae (CRE) in US children showed a major increase during 1999-2012 (from 0.0% to 5.2 %) and the most substantial increases were in children 1 – 5 years of age. Among CRE isolates, the largest number (36.8%) were Enterobacter spp. from urine and from non-ICU patients.

Hargreaves et al (8) report the clonal dissemination of Enterobacter cloacae harboring KPC-3 in the upper Midwestern of USA.All strains possessed identical IncFIA-like plasmids and an additional Inc X3 plasmid. The spread of this clone is unprecedent for E. cloacae and highlights the importance of continued surveillance.

Humphries et al (9) report that in 2010 the CLSI revised the carbapenems breakpoints for Enterobacteriaceae. However, no manufacturer of commercial automated susceptibility testing systems has obtained FDA-clearence for these new standards, despite 5 years having passed. The authors mention that the Check-Point CPE for detection of CRE in rectal surveillance cultures had an unacceptable number of false-positives.

Harbarth et al (10) report that gene identification is becoming more accessible, but some commercially available tests are unable to discern between gene variants (OXA-48, 163 and 405). False positives can also be a problem as detected resistance genes may be present in non-pathogenic bacteria. Besides genes may be present but expressed poorly or not al all.

Al-Bayssari et al (11) report that several methods have been developed to rapid detect carbapenemases. One is the use of combined tablets (triple disk from Rosco Diagnostica) permitting the detection of KPC+MBL in the same strain. The Microarrays method, appear to be both time-consuming and expensive, while low-cost approaches like the rapid colorimetric tests (98024, CarbaNP) appear to be highly reliable in detecting carbapenemases.

Chea et al (6) report that when they used the CDC and Prevention CRE definition in their study, 21 % of K. pneumoniae carbapenemase-producing were misclassified as non-CP. The new definition requiring resistance to 1 carbapenem resulted in 55% false positive results and when adding the Hodge test (MHT) to the definition decreased false positives to 12 %. The MHT might falsely identify NDM-producing strains as non-CP and may falsely identify non-CP Enterobacter spp as CRE. The authors recommend adding resistance-mechanism testing in order to identify real carbapenemase production.

Bush K (7) in a Review reports the beta-lactamase-inhibitor (BLI) combinations, available today and in the near future, that are effective against multidrug- resistant Gram-negative pathogens. It contains the new generation of BLIs such as avibactam, relebactam, RPX7009 and their combinations.

Meletis (12) reports that carbapenem-resistant gram-negative nosocomial pathogens will continue to evolve accumulating more carbapenem-resistance mechanisms or more than one carbapenemase-encoding gene and untreatable infections could emerge.

Oikonomou et al (13) report the rapid dissemination in Central Greece of colistin and carbapenem resistant ST101 clone of Acinetobacter baumannii. Colistin resistance gradually increased and reached 21.1% in 2014.

Both the CLSI and EUCAST recommend reporting the susceptibility result against carbapenems”as found”, independent of the presence of a carbapenemase or not (3). New clinical data would potentially modify these recommendations, because the production of a carbapenemase also affects specific treatment advice. Detection of carbapenemases is not only for infection control purposes but for the establishment

of a suitable antimicrobial therapy (in many cases combining a carbapenem with other antibacterials) that should be guided by breakpoints values (Meropenem/Imipenem MICs <= 8 ug/ml)

Screening

Recently the EUCAST subcommittee for detection of resistance mechanisms proposed screening cut-off values for carbapenems. For bacteria producing OXA-48 beta-lactamases, temocillin was proposed as an indicator antibiotic with high sensitivity. The use of Temocillin 30 ug (zone below 12 mm) and Piperacillin tazobactam 100/10 ug (zone<16 mm) can significantly increase the sensitivity of the screening procedure for OXA-48 and similar.

Hartl et al (48) proposed resistance to temocillin combined with the meropenem double disk synergy test as an algorithm that could be introduced into diagnostic laboratories for the identification of carbapenemase production and differentiation of Amber Class A, B and D enzymes

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 >= 0.25 µg/ml) or Ertapenem (zone < 25 mm) on Mueller-Hinton Agar with McFarland 0.5 inoculum should be suspected of possessing carbapenemases. Ertapenem Neo-sensitabs is the most sensitive indicator, for possible carbapenemases. It is important to recognize small resistant colonies growing inside the Ertapenem zone.

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.

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-5	S	4-64	☐ 16	0.25-8	± wk	no	yes
	IMI-1 to IMI-6	S	S	☐ 64	4-32	+	no	yes
	KPC-2 to KPC-19	☐ 32	☐ 64	4☐16	4☐16	+ or wk	no	yes
	GES-2 to GES-24	☐ 32	16☐R	0.25☐16	0.5-16	+ or 0	no	yes
B Metallo-beta-lactamases	IMP 1-16-51	☐ 32	S☐R	0.5-128	0.25☐R	no	yes	no
	VIM 1-12-45	☐ 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
	NDM-1 to 16	R	S	R	R	no	yes	no
	IND-1 to 7							
	AIM,DIM,KHM							
D Oxacillinases	OXA 23-27-49	> 256	> 256	4-64	4-128	± wk	no	no
	OXA 40-143	S☐R	S☐R	2-64	0.25-64	wk	no	no
	OXA 54-55	S	S	4	0.25	wk	no	no
	OXA-48-162-181-204-232-244-245	S					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 in Enterobacteriaceae.

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

Phenylboronic 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).

Boyd et al (64) describe the isolation of 2 E. coli and 2 Serratia marcescens harboring GES-5 on plasmids persisting in a Hospital in Canada. GES-5, are Class A ESBLs that contains variants that hydrolyze carbapenems and consequently are carbapenemases. It appeared that the GES-5 plasmid had persisted in an environmental niche for at least 2 years in the hospital.

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.

Yilmaz et al (16) report oxacillinases (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.

Poirel et al (60) explain why phenotypic methods are useful for the detection of carbapenemases.The diagnostic techniques must be easy to perform and interpret.Routine laboratories cannot rely on complicated screening strategies that may provide difficult to interpret results (molecular methods).
Opposite to the phenotypic methods, the molecular techniques are expensive, they require significant expertise, correlation between gene identification and resistance is not always observed and by definition they do not identify the novel and emerging resistance genes.
In occasions, carbapenemase-producers showing low MICs towards carbapenems are difficult to detect, particularly when automatic methods are employed.

Oteo et al (61) describe the clonal dissemination of K pneumoniae ST11 and ST101 producing KPC-2 in Madrid. Two of the isolates coproduced KPC-2+OXA-48.
Prats et al (62) found that the combination of the Rosco KPC/MBL and OXA-48 Confirm kit with the modified Hodge test had a good sensitivity and specificity in the detection of carbapenemases in Enterobacteriaceae and non-fermenters.

Lombardi et al (65) describe the emergence of carbapenem-resistant K. pneumoniae in a cardiac surgery division. 35% were carbapenemase producers. 89 of 98 of the isolates were positive for KPC-2 and 9 of

98 were positive for VIM-1. The combined disk test: KPC/MBL Confirm kit from Rosco and PCR were used to detect the carbapenemase genes.

Acquired carbapenemases Ambler class A and D

Ambler class.	Enzymes	MICs µg/ml PIP	3rd gen cepha	AZT	IMI	MRP	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-2-6	16?128	0.1-2	4-8	64	4-32	+	yes	E. asburiae	Plasmid
	KPC-1	> 128	? 32	> 64	16	16	+	yes	K. pneumoniae	Plasmid
	KPC-2-13	? 64	? 8	> 16	8?16	? 16	+	yes	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	(+ +)	yes	K.pneumonia Enterobacter E. coli, P. mira-bilis, Citrobacter	Plasmid
	KPC-4				R> 16	> 16	(+)	yes	Enterobacter	
	KPC-5-19							yes	Serratia	Plasmid
	FRI-1	R	S	R	R	R		yes		plasmid
	BCK-1	R	R	R	R	R	+	yes	K. pneumon.	plasmid
	VCC-1	R	S	R	R	R	(+)		Vibrio cholerae	
A	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-5-6	R	R	R	8-32	8-32	+	yes	P. aeruginosa, K.pneumoniae	Integron
	GES-11-14-18-24	R	> 256	> 256	4	8		yes	A. baumanni	plasmid
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-48-162-181-204-232-244-245.	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. baumannii	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

Ambler class.	Enzymes	PIP	3rd gen cepha	MICs µg/ml AZT	IMI	MRP	IMIPENEM + CLAV (synergy)	Mero-penem+ Boronic synergy	Organisms	Genetic Location
	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) in Enterobacteriaceae

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 Phenylboronic 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).
Interpretation (Double disk synergy test)

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

a) Negative metallo-beta-lactamases tests.
b) Positive synergy test between Phenylboronic Acid and the carbapenems (one or both).
c) Negative synergy test between Cloxacillin and the Carbapenems (11)
d) 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.
Rosco Diagnostica has introduced kit **98015**: KPC/MBL and OXA-48 confirm kit together with the Triple disk: Meropenem+ Boronic+Dipicolinic acid,permitting the detection in Enterobacteriaceae of KPC, MBLs and OXA-48 together with double enzymes KPC+MBL using the triple disk.

Clinically relevant carbapenemases (49)

	Class A (KPC)	MBL (VIM,IMP)	NDM	Oxacillinases
Klebsiella pneumoniae	+++	+++	++	+++ (OXA-48 or similar)
E. coli	+	+	++	++
P. mirabilis	+/0	+/0	-	+
Providencia spp	-	+/0	+/0	
K oxytoca	+/0	+/0	+/0	
S. marcescens	+/0	+		
Enterobacter spp	+/0	+	+/0	
Citrobacter freundii	+/0	+/0	+/0	
M. morganii		+/0	+/0	
Salmonella spp	+/0	-		
P. aeruginosa	+	+++		+
P putida	+	+/0		
Acinetobacter baumannii	-	++	+/0	+++ (OXA 23-40-58-143)
Acinetobacter spp	-	+	-	+

+++ = high prevalence,
++ = moderate prevalence (1-10%)
+ = low prevalence (< 1 case)
+/0 = isolated cases

Albiger at al (22) report the distribution of carbapenemase-producing Enterobacteriaceae (CPE) in Europe (May 2015). They noticed the rapid spread of OXA-48 and NDM producing Enterobacteriaceae. 13/38 countries reported inter-regional spread of or an endemic situation for CPE.OXA-48 and NDM were the most commonly found, followed by KPC and at a lower level VIM, while IMP was rare.

Zhao et al (73) report the dissemination of drug-resistant KPC-2 producing K. pneumoniae isolated from bloodstream infections in China. Most of the isolates co-carried ESBLs, particularly CTX-M-24 and SHV11.

Tomasso et al (68) report a large nosocomial outbreak of colistin-resistant, carbapenemase producing K. pneumoniae in Siena, Italy. The outbreak was attributable to the clonal expansion of a single mgrB deletion mutant from a KPC-3 producing Kl. pneumoniae.
Camargo et al (69) report the successfull treatment of carbapenemase producing (KPC) pandrug-resistant K. pneumoniae bacteremia using a combination of an intravenous CAZ-avibactam with ertapenem. Synergism in vitro was observed between a CAZ-avibactam disk and an ertapenem disc(ghost zone).

Anchordoqui et al (74) describe 3 cases of intrapatient transfer of the KPC-2 gene. E. coli and K. pneumoniae isolated from a rectal swab harboured both KPC-2. In a second case Enterobacter cloacae and K. pneumoniae from blood cultures harboured both KPC-2. In a third, case Citrobacter freundii and K. oxytoca isolated from skin possessed the KPC-2 gene. In conclusion, the authors document the horizontal dissemination of the KPC-2 gene from diverse Enterobacteriaceae clinical isolates, with different genetic backgrounds.

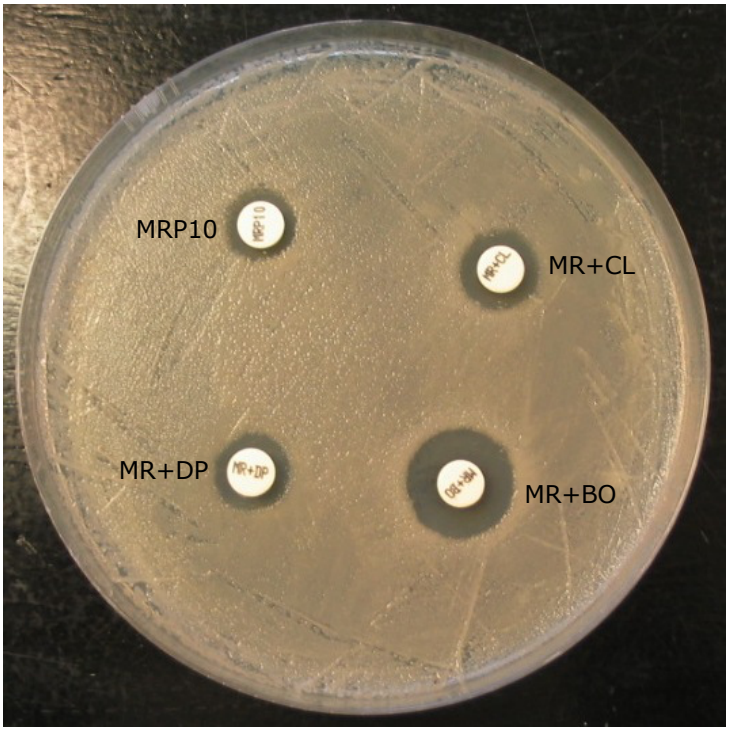
Fattouh et al (76) in Canada, indicates that the CLSI proposes screening of carbapenemases on isolates showing MIC >= 2 ug/ml against ertapenem or meropenem, while EUCAST recommends screening cutoff MIC >= 0.25 ug/ml for ertapenem and/or meropenem. They found that 14% of carbapenemase producers may go undetected when using the current CLSI recommendations and conclude that it would be important to detect the presence of carbapenemase genes even in isolates deemed "susceptible" to

carbapenems. In their study carbapenemase activily was confirmed in parallel with molecular detection using the KPC/MBL Confirmation kit from Rosco Diagnostica.

Detection of KPC-Beta-Lactamases

Combined disk tests. KPC + MBL Confirm kit (98006) and KPC/MBL and OXA-48 Confirm kit (98015) for Enterobacteriaceae

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



K.pneumoniae PHA3 CL5761 KPC positive

Interpretation of 98006 and 98015 (Enterobacteriaceae)

A Meropenem + Boronic inhibition zone ≥ 4 mm then Meropenem, Meropenem+DPA and Meropenem+Cloxacillin indicates a presence of a KPC enzyme (or other class A).
Meropenem+Phenyl Boronic and Meropenem+Cloxacillin inhibition zones ≥ 5 mm, than Meropenem and Meropenem+DPA indicates AmpC hyperproduction + porin loss, or efflux (30).
Negative synergy for all tests and Temocillin 30 ug zone < 12 mm indicates the presence of OXA-48.

A Meropenem +DPA inhibition zone ≥ 5 mm than Meropenem, indicates the presence of a metallo-β-lactamases (MBL).
For non-fermenters use kit: **98025**: KPC/MBL in Pseudomanas/Acinetobacter, version 2.

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 Phenyl 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.

Non-Fermenters: KPC/MBL in P. aeruginosa/Acinetobacter version 2(98025).

Al-Bayssari et al (70) describe the methods for detection of expanded-spectrum beta-lactamases in gram negatives in the 21st century. The authors describe the KPC/MBL and OXA-48 kit (98015) from Rosco, including an algorithm proposed by Miriagou for detecting the mentioned enzymes including the detection of co-produced MBL + KPC in the same strain.

Pantel et al (71) evaluated the performance of 2 rapid phenotypic tests for the detection of carbapenemases in Enterobacteriaceae: The Rapid CARB Screen with a sensitivity of 97.6% and specificity of 94.4 and the KPC/MBL and OXA-48 (98015) with a sensitivity of 98.8% and a specificity of 93.1%. The authors conclude that the 98015 kit provides a reliable phenotypic detection and results are obtained after 18 hours. Its interpretation is easy and the coexpression of several carbapenemases can be detected.

Willey et al (72) compare Temocillin Neo-Sensitabs (included in the kit 98015) against Temocillin paper discs from MAST as a marker for Class D and B carbapenemase producing Enterobacteriaceae. The authors mention that detecting temocillin resistance (TemR) enhance recognition of the hard to detect OXA-48-like or VIM-type carbapenemases.
Only the Temocillin Neo-Sensitabs showed high specificity (100%) while MAST specificity varied by lot of paper discs (71-82%) for excluding carbapenemase-negative isolates. The lower specificity may be explained by a deterioration of the inestable paper discs.

Dortet et al (75) report the evaluation of an algorithm recommended by the CA-SFM (French Society of Microbiology) to screen for carbapenemases in Enterobacteriaceae.
Enterobacteriaceae showing the following inhibition zones should be reported as **no-carbapenemase producers.** The algorithm is the following:

	Ticarcillin + Clav	Temocillin 30 ug	Imipenem 10 ug
Inhibition zones	>= 15 mm	>= 15 mm	>= 22 mm

Procedure for Oxacillinase detection (Class D enzymes) in Enterobacteriaceae

Strains of Enterobacteriaceae producing oxacillinases (OXA-48 or similar) will currently show zones of inhibition < 22 mm with Ertapenem and/or <27 mm with Meropenem Neo-Sensitabs. Most are resistant to Aztreonam.

Interpretation for Enterobacteriaceae (**OXA-48 and similar**)

The following results will presumably indicate the presence of oxacillinases in Enterobacteriaceae:

a) Negative metallo-beta-lactamase tests.
b) Negative synergy test between Phenyl 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) Resistant to Temocillin 30 ug Neo-Sensitabs (zone < 12 mm) and resistant to Piperacillin + Tazobactam 100+10 ug(zone < 16 mm)or Piperacillin + Tazobactam 30 + 6 ug (Zone < 13 mm)(13).

Hammerum et al (66) describe a possible outbreak of carbapenem-resistant Acinetobacter baumannii in Denmark.Five ST2 isolates producing OXA-23, two ST1 isolates producing OXA-72 and one ST158-producing OXA-23. There was a spread between 2 patients staying in the same room, 2 patients hospitalized in the same ward and between 2 patients living in the same nursing home.

OXA-48. Oxacillinases (carbapenemases) in Enterobacteriaceae. KPC/MBL and OXA-48 Confirm kit (98015)

OXA-48 was discovered in a clinical K pneumoniae isolate from Istambul in 2001. This OXA-variant was plasmid encoded and had less than 50% aminoacid identity to the other OXA-members.
The first outbreak of carbapenem-resistant K. pneumonia isolates from Istambul, producing OXA-48 was reported by Carrer in 2008. They co-produced various beta-lactamases, particularly ESBLs. The identification of K. pneumoniae isolates harboring the worldwide spread CTX-M15 together with the OXA-48 carbapenemase is worrying.
Isolates of E. coli and K. pneumoniae possessing the OXA-48 carbapenemase are currently resistant to penicillins (including temocillin) and combinations with beta-lactamase inhibitors (including Piperacillin + tazobactam), show reduced susceptibility to carbapenems (particularly ertapenem) and show susceptibility to 3rd gen cephalosporins and aztreonam.
The co-production of an ESBL mask the zone of inhibition around 3rd gen cephalosporins and aztreonam discs, and consequently they may appear as they are resistant to all beta-lactams.

Cubero et al (21) report an infection in a tertiary hospital of K. pneumoniae co-producing OXA-48 and CTX-M-15 beta-lactamases. They remark the importance of using Temocillin 30 ug for detecting OXA-48 producing isolates.

An OXA-48 (or similar) producing E. coli or K. pneumoniae may be suspected when:
Zones of inhibition around ertapenem are reduced, while the isolate is susceptible to 3rd gen cephalosporins (zones around ceftazidime and cefotaxime larger than around imipenem/ertapenem) and the isolate is resistant to Temocillin 30 ug Neo-Sensitabs (zone < 12 mm) and resistant to Piperacillin + Tazobactam 100+10 ug (zone < 16 mm), or Piperacillin + Tazobactam 30 + 6 ug: Zone < 13 mm (13). When the isolate shows multiple resistance to all beta-lactams perform an ESBL test. If the ESBL test is positive, compare the zones around the Cefotaxime + Clavulanate against the zone around Imipenem 10

ug. If the zone around the clavulanate combinations is >= 5 mm larger than around Cefotaxime 30 ug and Imipenem 10 ug, the isolate produce an ESBL and most probably also OXA-48 (if temocillin resistant).

If the ESBL test is negative in the multiple resistant isolate, search for other mechanisms of resistance such as KPC or MBLs.
Diverse OXA-48 like beta lactamases (**11**) have been identified worldwide (OXA-162, OXA-181, OXA-162, OXA-204, OXA-244-OXA-245, OXA-232). OXA-162 and OXA-204 share the same hydrolytic properties as OXA-48.
OXA-181 possess a higher ability to hydrolyze carbapenems, while OXA-232 hydrolyze carbapenems less efficiently.
OXA-163 hydrolyze broad-spectrum cephalosporins and do not possess significant carbapenemase activity. The same is valid for OXA-405.They will show negative results with the carbapenemase colorimetric kits.

Decousser et al (12) report the failure of the Xpert Carba-R assay to detect carbapenem-resistant E. coli producing OXA-48-like beta -lactamases. The authors conclude that molecular diagnostic techniques in the current phase of their development should not be considered as Reference Standards for the detection of carbapenemase resistant Enterobacteriaceae carriers.

Barragan et al (14) found found in their hospital K. pneumoniae producing OXA 48 and CTX-M15 and being resistant to colistin.

Lopez-Urrutia et al (15) detected carbapenemases using the KPC/MBL and OXA48 Confirm kit from Rosco and conclude that it is necessary to use a phenotypic method to detect the different types of carbapenemases.

Ortega et al (16) looking at the results from the Vigilance Program from the Microbiology National Center, conclude that particularly OXA-48 in E. coli is increasing in Spain. The use of the Carba NP test showed negative results in 9.3 % of OXA-48 isolates.

Rosario-Quintana C et al (17) found that the OXA-48 has a great capacity of intrahospital dissemination. ESBLs were detected with the Rosco ESBL kit and the carbapenemases including OXA-48 using the KPC/MBL and OXA-48 kit from Rosco.

Branas P et al (19) report a study of the epidemiology of carbapenemase-producing K. pneumoniae in a tertiary care facility in Madrid from 2009-2014. Of 97 isolates, 59 harboured OXA-48, 37 harbouring VIM-1 and 1 isolate with KPC-2. There is an increasing trend in carbapenemase-producing isolates and the study highlight the establishment of OXA-48 and CTX-M-15 genes coproducing ST11 clone.

Dortet et al (20) conclude that OXA-48 type beta-lactamases are more diverse than expected. They are not all true carbapenemases as exemplified with OXA-405 and OXA-163 that are ESBLs, but belong to the OXA-48 type.
Temocillin-resistance is a good criterion for differenting OXA-48-type producers with carbapenemase activity (Temocillin resistant) from OXA-48-type without carbapenemase activity (OXA-163 and OXA-405) that are Temocillin susceptible.
Therefore, the first line screening of carbapenemase-producers in Enterobacteriaceae must be based on the biochemical detection of carbapenemase activity in clinical settings. The molecular techniques may overreport OXA-48-like producers as being all carbapenemases and conversely, may fail to detect carbapenemase producers related to totally novel or slightly structurally modified carbapenemase genes.

Summary detection of carbapenemases in Enterobacteriaceae: Kit 98015

Meropenem	MRP+DPA	MRP+BOR	MRP+Cloxa	Temocillin
Metallo-β-lactamases	Synergy	No synergy	No synergy	R
KPC	No synergy	Synergy	No synergy	S/V
AmpC impermeability	No synergy	Synergy	Synergy	S
Oxacillinases (OXA-48)	No synergy	No synergy	No synergy	R
OXA-48 + KPC	No synergy	Synergy	No synergy	R

Summary detection of carbapenemases in P. aeruginosa/Acinetobacter: Kit 98025

Imipenem	IMPBO	IMCX4	IMPDP	IMIED	
No Carbapenemases	No syn.	≥ 5 mm	No syn.	No syn.	P. aeruginosa
KPC	≥ 4 mm **and**	< 3 mm	No syn.	No syn.	P. aeruginosa
MBL	No syn.	No syn.	≥ 5 mm **and/or**	≥ 8 mm	P. aer./Acinetob.
Oxacillinases	No syn.	No syn.	No syn. **and**	4-7 mm	Acinetobacter.

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RAPID Detection of CARBAPENEMASE activity

Rapid identification of carbapenemase producers in gram-negatives is critical to limit their spread.
The optimal phenotypic methods are based on the hydrolysis of imipenem by bacterial colonies, which is detected by changes in pH value by indicators (phenol red or bromothymol blue).
These methods are described below.

Mirande et al (32) conclude that the detection of enzymatic carbapenem degradation by Maldi-tof MS lacks well-standardized protocols. KPC degrades antibiotics ver quickly, while it takes 90 min for other

enzymes. This enzymatic constraint limits the implementation of a standard protocol in routine microbiology laboratories.

Lutgring et al (36) report that reliable detection of carbapenemases in Enterobacteriaceae is the first step in comatting the emergence and spread of carbapenemase producers. The authors mention the Rosco Rapid CARB Screen and others as representatives of tests that can be used.

Pires et al (37) mention the causes of error that have been identificated in rapid colorimetric tests: the lack of standardization of inoculum and improper storage of test reagents (especially imipenem). The last problem does not exist when using the very stable Rosco Rapid colorimetric kits.

Rapid colorimetric (15 min-60 min) kits for detection of Carbapenemases.

No Lysis Buffer needed !!!

Neo-Rapid CARB (98024). (Enterobacteriaceae, Pseudomonas, Acinetobacter)

Add one 10 ul loop of the strain to be tested into 200 ul of 0.9 % NaCl, adjusted with 0.01N NaOH to pH 8.5.
Dilute 1.07 g Triton X-100 in 100 ml water and add 20 ul of this solution to the bacterial suspension.
Vortex for 1 minute and maintain at room temperature for 30 min.
Add 1 Imipenem (x2)+ Indicator Diatabs and close the tube.Vortex for 1-2 seconds to disintegrate the tablet.
Incubate the test at 35-37 degrees Celsius for 15 min, 30 min and 1 hour respectively.
The same process is repeated using the CARB negative Control Diatab.

Interpretation

A change of color from red to yellow (or orange-yellowish or light yellow) indicates a positive reaction, when the negative control remains red: carbapenemase positive.
If the reaction is positive after 15 min or 30 min, it is not necessary to incubate further.Longer incubation may result in false reactions.

Pasteran et al (35) found that the use of Triton X-100 at 0.1% (instead of lysis buffer) gave an enhanced detection of carbapenemase producers. Thefore we recommend this improved procedure now. This procedure is also useful to detect oxacillinases in Acinetobacter spp, in which case the use of 2x 10 ul loop of bacteria is recommended. Qun Yan et al (38) confirm the results from Pasteran using 0.1% Triton X-100, instead of extraction buffer.

In the ECCMID 2015 in Copenhagen, they were several presentations concerning the use of the Rapid CARB Screen kit.

Haldorson et al (16) compared the Carba NP and the Rosco Rapid CARB Screen using a collection of 99 Enterobacteriaceae and 39 P. aeruginosa isolates identified by PCR. Both tests showed a sensitivity of 96 % and a specificity of 100%.

Willey et al (18) compared the Rapidec Carba-NP, with Rosco Rapid CARB Screen, a modified Rosco CARB Screen and the Rosco CARBA Blue against 206 isolates, that previously have shown to be difficult to detect. The Rapidec Carba detected only 23% of the OXA enzymes and was associated with 12.9% false

positive. Most OXA-enzymes were detected with the 3 Rosco kits. The modified CARB Screen did not contain lysis buffer and performed well having the smallest amount of false positives.

Hombach et al (33) evaluated the Rapidec CARBA NP test (Bio-Merieux) and arrived at the following conclusions: when reading after 30 min incubation sensitivity was 49%. The test should strictly be read after 120 min incubation and the inoculum should be higher than recommended by the manufacturer.

Boran et al (22) tested 133 isolates the Rapid CARB Screen and found 98.7% sensitivity and 87.7% specificity and recommend the use of the Rapid CARB Screen in a carbapenemase detection algorithm for use in the routine laboratory.

De Sloovere et al (21) found that the combined positivity/negativity of both the Rapid CARB Screen and the Hodge test had a positive predictive value of 100% and a negative predictive value of 92%.

Hernandez-Cabeza et al (19) evaluated the Rosco Rapid CARB Screen kit for detecting carbapenemases in P. aeruginosa. The kit had a good performance, although they were some orange-yellow results that should be interpreted as positive. With the use of the new Neo-Rapid CARB Screen kit, the problem with orange color will be much less.

Morton et al (17) found that when combining the results of the Rosco Rapid CARB Screen kit and the modified Hodge test will detect the organisms with possible carbapenemases.

Lopez-Quintana B et al (25) used the Rosco Rapid CARB Screen to detect carbapenemases directly from blood cultures. They conclude that the Rosco kit is a rapid and reliable for the detection of gram-negative bacilli directly from positive hemocultures, with a sensitivity of 92% and specificity of 100%. It detected 24 out of 25 OXA-48 from E. coli E. coli, K. pneumoniae, E. aerogenes and S. marcescens.

Pitout (27) indicates the the ideal phenotypic confirmatory test should be able to **rapidly** detect different carbapenemases in Enterobacteriaceae, Pseudomonas spp and Acinetobacter spp. A commercial version of Carba NP test is available from Rosco Diagnostica (Neo-Rapid CARB 98024) and can be combined with temocillin resistance to ensure that all isolates with OXA-48 enzymes are detected.

Abdel Ghani S et al (29) tested 87 Class isolates, 40 Class B, 12 Class D (OXA-48 and OXA-181) and 50 non-carbapenemase producers comparing the Rosco Neo-Rapid CARB kit (98024) with the Carba NP and a modified Carba NP. The Rosco kit showed a **sensitivity of 99% and a specificity of 100%**. The authors conclude that the Rosco kit is the most convenient test to perform and its greater reagent stability is an advantage compared to Carba NP and modified Carba NP.

Abdel Ghani et al (31) recommend that when results are difficult to interpret with kit 98024 do as follows: 1) holding the tube vertical inspecting the bottom of the tablet for yellow color (positive) and 2) Compare test and Control tubes titled gently to horizontal and examine in bright light on a white background. If the result is unclear, repeat the test using a higher inoculum.

Bou Casals (34) critizises the comparative study of Dortet et al on rapid colorimetric tests. Dortet et al have a patent for NPCarba transferred to Bio-Merieux. Dortet et al have used an obsolete kit (98021) in their comparative study, while kit 98024: Neo-Rapid CARB kit has substituted kit 98021 more than 8

months ago. Bou Casals reports that kit 98024 contains twice as much imipenem as 98021 and uses much less lysis buffer than its forerunner and the kit has a shelf life of 2 years.

Rapid CARB Blue (98023) (Enterobacteriaceae, Pseudomonas; Acinetobacter)

No lysis buffer is needed for the tests
Add 3 x 10 ul loop of the strain to be tested to 1 ml of 0.9% NaCl sol at pH 8.5.Make a suspension of at least Mc Farland 4.
Take 200 ul of the suspension in a tube and Vortex the suspension for 1 minute and maintain at room temperature for 30 min.
Add 1 Imipenem (x2)+ Brthymol Blue Diatabs and close the tube. Vortex for 1-2 seconds to disintegrate the tablet.
Incubate the test tube at 35-37 degrees for 15 min, 30 min and 1 hour respectively.
The same process is repeated with using the CARB Blue Negative Control Diatab.

Interpretation

A change of color in the test tube, from blue to yellow indicates a positive reaction (carbapenemase present).
If the test is green/yellowish and the negative control is blue, means Carbapenemase positive.
If the test is yellow and the negative control is green, the result is carbapenemase positive.

At the ECCMID 2015 there were several presentations about the use of the Rapid CARB Blue kit.

Novais et al (12) compared the Rapid CARB Blue kit with the in-house Blue Carba test (developed by Peixe and Novais). The authors report very good sensitivity 94.5% and specificity (91.7%) in the detection of carbapenemase producers by the Rapid CARB Blue kit in a representative collection of carbapenemase and non-carbapenemase producing gram-negative isolates.

Novais et al (29) in a new evaluation of the recently launched Rapid CARB Blue kit, report a high sensitivity (93.3%) and specificity (100%).

Pasteran et al (13) tested both the Rapid CARB Blue and the in-house Blue Carba test. Pasteran cocludes that both products had equivalent performances for carbapenemase detection. For labs concerned with the widely disseminated KPC and NDM producers, the Rapid CARB Blue kit could be an accurate and cost-effective method to rapidly identify these carbapenemases.
Pasteran (22) studied the possibility of detecting carbapenemase from blood cultures using the Blue Carba test. He concludes that the Blue Carba test could be a rapid and cost-effective for detecting KPC and NDM producers directly from blood cultures.

Pires et al (23) compared Carba NP and Blue Carba in the detection of carbapenemases in Enterobacteriaceae. Both methods showed high sensitivity and specificity, but particularly the OXA-48 producers yielded stronger results with Blue Carba. The authors conclude also that the use of more experienced operators yield fewer mistakes (human errors cannot be excluded).

Pasteran et al 27) conclude that the Blue-Carba Test could be an accurate and cost-effective way to rapidly identify KPC, MBL producers and Class D OXA carbapenemase-producing Acinetobacter. For some mechanisms such as OXA-48-like producing Enterobacteriaceae, a negative result could require additional

tests such as the Modified Hodge Test in combination with Temocillin or Piperacillin+Tazobactam susceptibility results.

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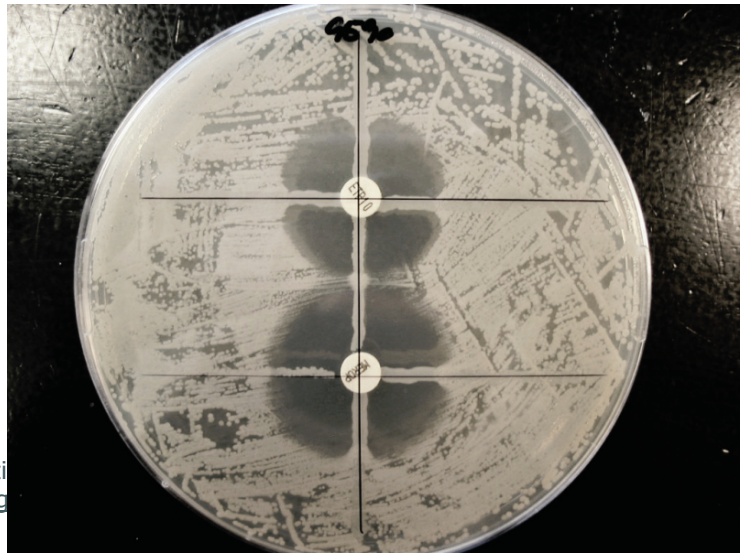
Modified Hodge Test

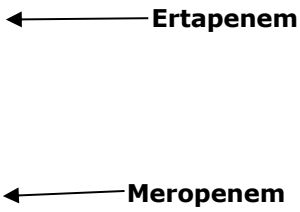
Is used to determine if resistance to carbapenems is caused by a carbapenemase. A MH agar plate (or a McConkey plate) is inoculated with the susceptible strain E. coli ATCC 25922 (Mc Farland 0.5, diluted 1/10) as for disk diffusion. Instead of Mac Conkey, MH agar supplemented with 20 mg/ml of bile can be used.
Pasteran et al found, when testing against P. aeruginosa, that replacing the indicator strain with K. pneumoniae ATCC 700603 led to an improved performance with 100% sensitivity and 97% specificity.
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 indicative of the presence of a carbapenemase (figure).

Takayama et al (63) found that in order to avoid false positives, the addition of 200 ug/ml cloxacillin to the MHA improves the reliability of the method. In fact, 5 isolates of Enterobacter cloacae that showed false positive results with MHA alone, were negative when using MHA+cloxacillin. Rosco Diagnostica has a Diagnostic Tablet: Imipenem + cloxacillin 4 mg (IMCX4) that can be used for the same purpose.

Use current MHA and perform the modified Hodge Test **using the IMCX4 Diagnostic tablet**, instead of Imipenem, Ertapenem or Meropenem. Imipenem 10 ug can be used in parallel as a control. The indentation will be clear around the IMCX4 tablet for the organisms producing carbapenemase and false positives (high production of AmpC) will be avoided. Indicator strain E. coli ATCC 25922.
The same procedure may be used for P. aeruginosa, where the strain recommended by Pasteran (K. pneumoniae ATCC 700603) is used as indicator strain.

Pasteran et al (77) found that the addition of Triton X-100 during the test improves the sensitivity of the test for the detection of NDM and other carbapenemases. The test is performed flooding the MHAgar plate with 50 ul of pure Triton-X-100 reagent (0.2 % in the MHA plate) and quickly distributed by streaking a swab over the entire plate.Meropenem is the best substrate, while the use of ertapenem may increase false positive results.





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 6 major types: IMP, VIM, SPM, GIM, SIM and NDM type enzymes. In Enterobacteriaceae: IMP, VIM, GIM and NDM enzymes have been found 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 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).

Schrøder Hansen et al (40) reports for the first time an NDM-5 outbreak in Europe.None of the patients had been travelling recently.The isolates belonged to a single clone.

Ruiz et al (41) describes the intrahospitalary dissemination of K. pneumoniae ST 437, producing carbapenemase NDM-7 in Madrid.

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.

Pesesky et al (62) sequenced 78 Enterobacteriaceae from Pakistan and the USA in order to characterize the genomic context of NDM-1 and K. pneumoniae carbapenemase (KPC). High similarities of the results indicate rapid spread of carbapenema resistance between strains.

Neves Andrade et al (63) conclude that efforts should be made for rapid microbiology detection and clinical and epidemiological measures to control infections caused by NDM producers and NDM-gene dissemination (for ex using simple tests to detect carbapenemases)

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.

Ameen et al (66) determined the frequency of imipenem-resistant MBL producing Pseudomonas isolates collected from clinical samples in a tertiary hospital of Karachi, Pakistan. They found that 35% of the

isolates were resistant to imipenem and MBL production was confirmed in 64.9% of the resistant isolates of Pseudomonas aeruginosa.

Pobiega et al (97) examined 21 imipenem-resistant P. aeruginosa from urine in Poland.42.8% were MBL-positive and VIM-2 was present in 14.2% of isolates.

Lange et al (98) out of 192 carbapenemase-producing E. coli isolates in Germany, found OXA-48 in 44.8%, VIM in 18.8 %, NDM-1 in 11.5 % and KPC-2 in 6.8 %

Seara et al (67) report the first global outbreak of NDM-7 producing K. pneumoniae in 3 different hospitals in Madrid. 7 patients were involved. Frequently transfer of aged or chronically ill patients between the facilities may have favoured the spread of the NDM-7 outbreak.

Hissong et al (68) studies different phenotypic methods for the detection of carbapenemases (MBL) in Bacteroides fragilis group. 5 isolates were cfiA positive. The authors recommend a combination of phenotypic methods (KPC/MBL in Pseudomonas/Acinetobacter kit) and the Carba NP.

Ank et al (72) report in Denmark, the identification of a multidrug-resistant Bacteroides fragilis recovered from blood of a patient that had been in vacation in Thailand. The isolate was MBL positive (Imipenem/Imipenem + EDTA synergism) and possessed nimE, cfiA and ermF genes corresponding to metronidazole, carbapenem and clindamycin resistance.

Neves Andrade et al (69) describes the detection of NDM in Brazil in Providencia rettgeri, NDM-1 in Morganella morganii, E. coli, K. pneumoniae, Acinetobacter baumannii and Citrobacter freundii. It seems like a rapid national expansion of NDM-encoding genes and NDM producers in Brazil. Efforts should be made for rapid microbiology detection using simple tests to detect carbapenemases.

Qing Yang et al (71) describes in China that from a total of 186 carbapenem-resistant Enterobacteriaceae, 90 isolates were identified as harboring the KPC-2 genes and 5 were NDM-1 positive. The authors report, for the first time, the endemic spread of Enterobacteriaceae spp with the NDM-1 gene in their hospital in Zhejiang. The isolates included E. cloacae, K. pneumoniae and E. coli, all of which belonged to the IncX3 -type plasmid.

Huang et al (74) report the presence of NDM-1 producing Citrobacter freundii, E. Coli and Acinetobacter baumannii from the urine and stool of a single patient in China. The case the broad host range of NDM-1 gene and its potential to spread between Enterobacteriaceae and A. baumannii.
Huang TW et al (82) report the transfer of NDM-1 plasmid among Acinetobacter spp. and suggest that these non-pathogenic Acinetobacter species may serve as a reservoir of this NDM-1 plasmid in the environment.

Giske CG (99) reports that old antimicrobials could be useful for treating infections by carbapenemase-producers. Recent data suggest that NDM and IMP producers are frequently susceptible to mecillinam, while KPC and VIM producers are resistant. Besides mecillinam is highly in vitro active against OXA-48 producers.

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**
IMP 12	☐ 128	32	32	128	Pseudomonas putida	**plasmid**
IMP 13-51	☐ 256	4-128	☐ 64	☐ 64	Pseudomonas aeruginosa	**integron** 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**
VIM 4-11	> 256	S ☐ R	32-256	32-256	Pseudomonas aeruginosa, A.baumannii	**integron**
VIM 15-16	☐ 64	16-32	>128	≥128	Pseudomonas aeruginosa	integron
VIM 18-45	R	S ☐ R	R	R	Pseudomonas aeruginosa	integron
SPM-1	☐ 256	4	R	R	Pseudomonas aeruginosa	Plasmid (**not** integron)
GIM-1-2	16 ☐ 32	8-16	> 8	> 8	P.aeruginosa,Enterobacter cloacae	Integron
SIM-1	☐ 256	128	8-16	16	Acinetobacter baumannii	Integron
IND1-7 DIM-1 FIM-1 TMB-1 POM-1 EBR-2	1-32-128	32-128	4-32-128	4-16-128	Chryseobact indologenes P. stutzeri P. aeruginosa P. aeruginosa Pseud otitidis Empedobacter falsenii	Chromosomal(23) Integron Chrom
AIM-1 NDM 1-16					Pseudomonas aeruginosa Pseud/Acinetobacter	Chrom/plasmid

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	Integron
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	Integron
IMP-4	256	·	3	6	Citrobacter youngae	Plasmid
IMP-6	> 128	0.25	2-8	64	E. coli,K. pneumoniae	
IMP-6	> 128	128	32	> 128	Serratia marcescens	
IMP 8-48	R	S ☐ R	0.5-8	0.25-4	Enterobacter cloacae, Klebsiella pneumoniae, S.marcescens,Enterobacter	
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 12-35	☐ 128	16	8	4	K. pneumoniae	Plasmid (16)
VIM-12	> 32	1	1	0.25	E. coli	Plasmid (22)
VIM-27					E. coli	Integron
GES7						
GIM-1					Enterobacter,E.coli,C. freundii	
KHM-1	R	0.25	2	4	C. freundii	Plasmid
SMB-1					Serratia marcescens	
NDM1-16	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).

Double disk synergy test

a) Enterobacteriaceae

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

b) Non-fermenters

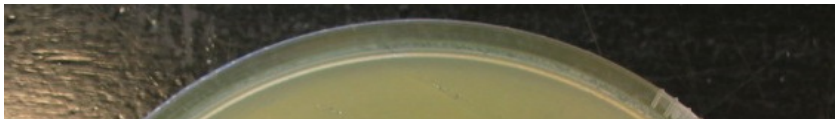
Apply one DPA Diatabs on the MH plate. Apply only one Imipenem Neo-Sensitabs 5 mm from the DPA (edge to edge). Apply Imipenem + EDTA (IM10E) on the inoculated MH plate.

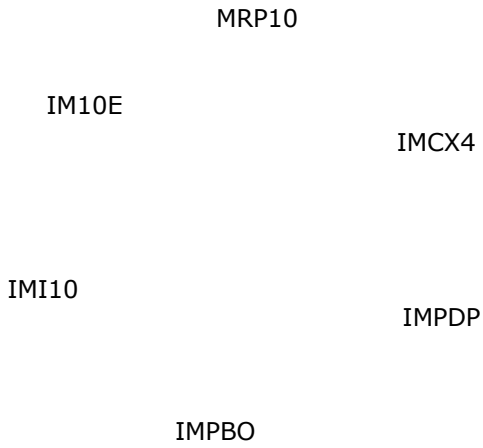
Heinrichs et al (67) evaluated several phenotypic methods for detecting carbapenemases in P. aeruginosa.Imipenem + DPA displayed high sensitivities (99%) and specificities (95%) for detecting MBL producing P. aeruginosa. Imipenem + Cloxacillin high showed a sensitivity of 97% and a specificity of 96% compared to 88% and 99% respectively for the Carba NP test. Both the Imipenem + DPA and Imipenem + Cloxacillin High are components of the Rosco kit: KPC/MBL in Pseudomonas and Acinetobacter, section 2. (98025).
Moraitu et al (68) reports a rapid test for identification of carbapenemase-producing bloodstream isolates. After gram-stain, direct susceptibility and phenotypic tests for carbapenemase activity were performed on each blood culture positive for gram-negative bacteria. Imipenem + Phenylboronic and
and Imipenem+EDTA were used to detect KPCs and MBLs respectively.
Matros et al (69) found that the introduction of routine tests for detection of MBL and other carbapenemases (KPC) is necessary. The authors used the Rosco kit KPC/MBL in Pseudomonas/Acinetobacter, version 2 (98025) and detected MBLs in 22 isolates out of 32 of P. aeruginosa.
Al-Sultan et al (77) detected carbapenem resistance in 69% of Acinetobacter baumannii in Saudi Arabia. The VIM gene was detected in 94% of isolets, while the OXA-23 like, genes were detected in 58%.

Combined disk test.

KPC and MBL Confirm ID kit (98006) Enterobacteriaceae
KPC/MBL and OXA-48 Confirm kit (**98015**) Enterobacteriaceae
KPC/MBL in Pseudomonas/Acinetobacter, Version 2 (**98025)** Non-fermenters
Total MBL Confirm kit (**98016**) Any species.

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



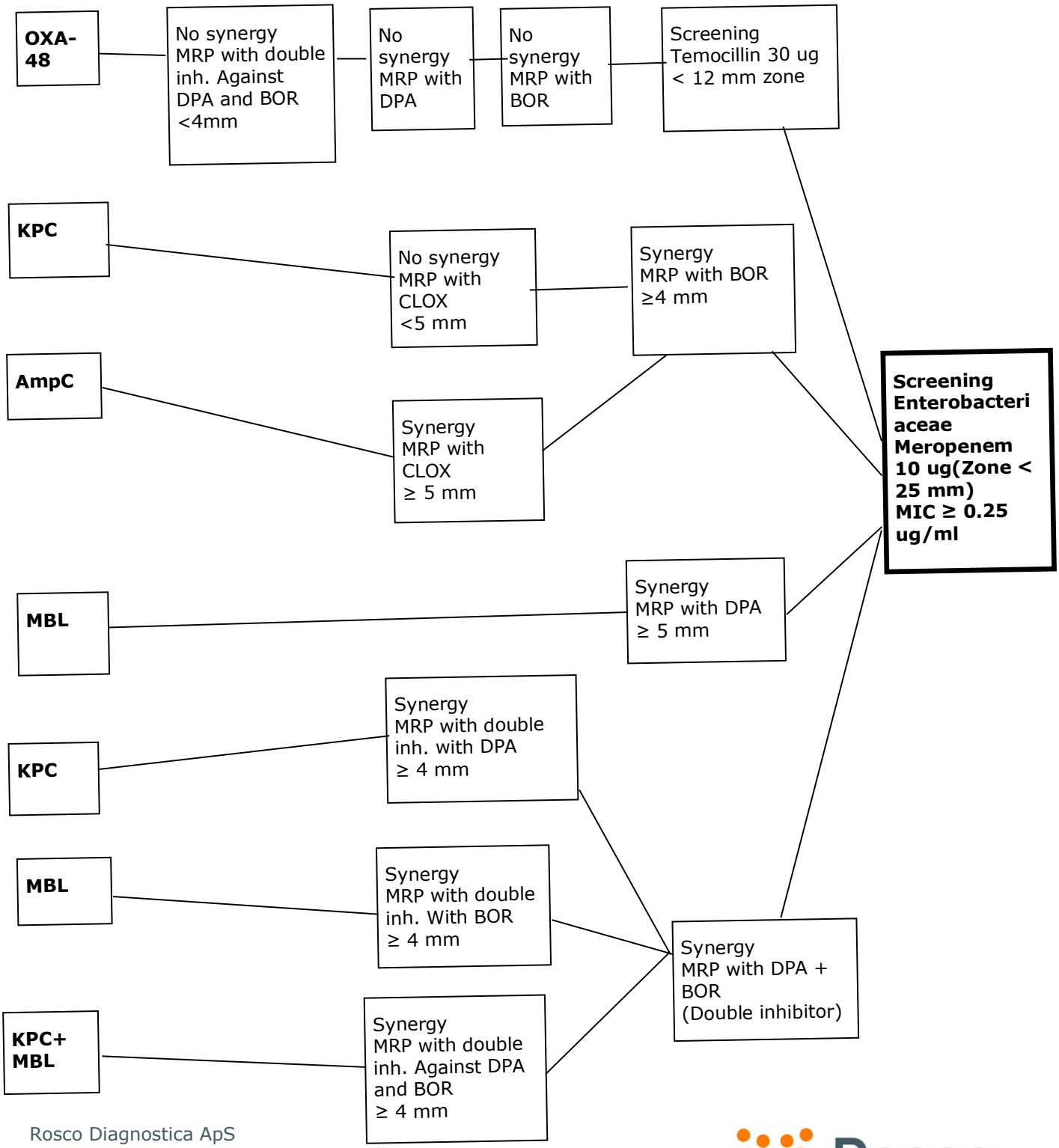


Total MBL Confirm kit (98016)

Composed of:
Meropenem 10 ug (MRP 10)
Dipicolinic acid (DPA)
Imipenem 10 ug (IMI10)
Imipenem + DPA (IM+DP)
Imipenem + EDTA (IM10E)

Interpretation:
Test only Ceftazidime resistant isolates.
1) **Enterobacteriaceae**: Synergism between MRP10 and DPA
   Synergism between IMI10 and DPA and/or IM10E >= 8mm end IMI10
2) **Pseudomonas /Acinetobacter**: Synergism IMI10 and DPA.
   IM+DP >= 5 mm end IMI10 and/or IM10E >= 8mm end IMI10
   Acinetobacter and oxacillinases: IM+DP <= 3 mm end IMI10 and IM10E : >=4 -7 mm end IMI10

Algorithm for interpretation of combined disk synergy tests to detect carbapenem-non-susceptible Enterobacteriaceae (38, 39, 40, 49, 59)



Coexistence of carbapenemases with other beta lactamases in the same isolate

Increasing reports show that double-carbapenemase-producing and even multiple carbapenemase-producing bacteria are emerging in some parts of the world,diminishing the already limited treatment options(80).

Detection of KPC and ESBL enzymes in the same strain of Enterocacteriaceae

Use kit 98015 and include Ticarcillin + Clavulanate (TCC) Neo-Sensitabs. Synergy between Meropenem and Meropenem + Boronic indicates the presence of a KPC enzyme.

Place TCC at approx. 10 mm from Menopenem + Boronic (edge to edge). Synergy (ghost zone) between the 2 tablets indicates the presence of an ESBL.

Li Wang et al (81) report the coexistence of KPC and CTX-M-55 in Klebsiella pneumoniae. Simillar coexsistences have been reported in several countries.

	MER+BOR - 10mm - TCC	
MER10	Syn	ghost zone (Syn)

Detection of KPC and MBL enzymes in the same strain of Enterobacteriaceae (Triple disk 68912).

The spread of double carbapenemase producers has compromised the performance of the chelator-based and boronate-based tests described previously.

Miriagou et al (49) introduced the double inhibitor disk (triple disk) for detecting double carbapenemase producers.
She described an algorithm,for the detection of KPC,MBL ,KPC+MBL and OXA-48 carbapenemases in Enterobacteriaceae.This algorithm has been recommended by Hraback et al (59) and it is used in several laboratories worldwide.We present the algorithm in the next page.
Apply Meropenem 10 ug, Meropenem + Phenylboronic, Meropenem + Dipicolinic (kit **98015**) and Meropenem + Phenylboronic+DPA (Triple disk ref **68912**) on an inoculated MH plate.

Interpretation:

Zone around Meropenem+Phenylboronic+DPA disk >= 4 mm larger than zone around Meropenem + DPA and zone around Meropenem + Phenylboronic indicates the presence of a **double enzyme**: MBL and KPC.
Silva et al (44) describes an outbreak caused by KPC-2 and IMP-10-producing Serratia marcescens isolates in a Brazilian teaching hospital. This is the first time that a clonal strain with the co-production of

IMP-10 and KPC-2 appeared in S. marcescens isolates causing an outbreak associated with high mortality in the ICU at a Brazilian teaching hospital.
Silva Pereira et al (45) showed in their study the co-production of 2 important carbapenemases KPC-2 and NDM-1 associated with mobile genetic elements of worldwide epidemiological importance, in Enterobacter hormaechei.
Feng et al (78) describe the coproduction of KPC-2 and NDM-1 in Citrobacter freundii.
Wu et al (79) describe the emergence of Enterobacter cloacae producing both KPC and NDM carbapenemases.
Kashikar et al (83) describe the presence of KPC-3 carbapenemase in Indiana (USA), with the co-production of either VIM or NDM metallo-beta-lactamases.
Wenjing Wu et al (84) describe an Enterobacter cloacae producing both KPC and NDM carbapenemases in China. The coexistence of 2 carbapenemase genes on separate plasmids will probably mediate the spread of antimicrobial resistance genes.
Zheng B et al (89): present the first description of the coexistance of KPC-2 and IMP-4 from the genus Raoultella. This study reinforces the idea odf a rapid dissemination of the IMP-4 and KPC-2 genes in clinical isolates of Enterobacteriaceae in China.
Feng et al (93) report the coexistence of KPC-2 and NDM-1 in 2 different plasmids in a clinical isolate of Citrobacter freundii in a Chinese Teaching Hospital.
Smita (100) reported in Jaipur (India) that 6.6% of K. pneumoniae isolates co-produced both MBL and KPC.
Solanki et al (101) reported the presence of NDM-1 in 44 /70 isolates of K. peumoniae,while NDM-1 + KPC were present in 14/70 isolates.

MER10 ug	MER+BOR	MER+DPA	MER+BOR+DPA
	Syn	Syn	(Triple disc) ≥ 4 mm

Detection of MBL and ESBL on the same strain.

It is not uncommon that some microorganisms co-express both an ESBL and a MBL.
In these cases, ESBL production is masked by the MBL and is difficult to detect directly.
In Enterobacteriaceae co-expression of both MBL and ESBL can be detected by a disc approximation test using Aztreonam 30 ug Neo-Sensitabs and Amoxycillin + Clav 20 + 10 ug Neo-Sensitabs. The 2 Neo-sensitabs are placed 10 to 15 mm apart (edge to edge).
Aztreonam is hydrolyzed by the ESBL but not by the MBL. The Clavulanate from the Amox+Clav disk blocks the activity of the ESBL resulting in a ghost zone or keyhole between the 2 disks, in the case of a positive ESBL production.
The MBL is detected as usual using Meropenem 10 ug and Meropenem + DPA Neo-Sensitabs (Zone enlargement >= 5 mm). Use **kit 98006 or kit 98015** for Enterobacteriaceae Chevet et al (46) detected a Klebsiella pneumoniae isolate co-producing a VIM metallo-beta-lactamase and an ESBL. They conclude

that the presence of synergism between Aztreonam and Amox+Clav, together with multiresistance to beta-lactams must indicate the presence of an ESBL + MBL.
Papagiannitsis et al (47) report a transmissible plasmid encoding VEB-1 and VIM-1 in an isolate of Proteus mirabilis.
Dimude et al (48) tested Enterobacter cloacae isolates from Egypt and Edinburgh and found a linked carriage of VIM-4 and CTX-M-14 on the plasmid, resulting in resistance to all beta-lactamas and limited treatment options.
Douka et al (59) describe an outbreak of a pandrug resistant Providencia stuartii involving 15 critically ill patients in a Greek intensive care unit. All isolates harboured the VIM-1 as well as the SHV-5 genes.
Toleman et al (60) found NDM-1 in 62% of gram-negative bacteria from environmental waters in Dhaka, Bangladesh. Most co-produced CTX-M-15.
Branas et al (73) report the copresence of VIM-1 and CTX-M-15 in K. pneumoniae, in a tertiary hospital in Madrid.
Datta et al (75) describe the use of MH Agar added of 200 mg/L dipicolinic acid in order to neutralize the MBLs of the sample. Using this medium and the CAZ+ Clav+Cloxa compared to CAZ+Cloxa (Rosco kit 98019) it was possible to detect ESBLs and AmpCs in the presence of NDM-1 producing Enterobacteriaceae and Acinetobacter spp.
Zhou et al (76) describe in a Chinese teaching hospital, an outbreak of multidrug-resistant KPC-2 producing K pneumoniae coproducing CTX-M-65, as well as other resistance genes.Both horizontal gene transfer and clonal spread were responsible for this outbreak.
Baraniak et al (85) describe the largest NDM outbreak in a non-endemic country (Poland) being an alarming phenomenon. 374 cases of infection/colonization with NDM-positive Enterobacteriaceae. The early isolates also co-produces CTX-M-15.

In non-fermenters (P. aeruginosa, Acinetobacter) and P. mirabilis: Ticarcillin + Clavulanate 75 + 10 ug is used as the source of clavulanate and Aztreonam 30 ug are used for the ESBL test.
For the MBL test use: Imipenem 10 ug and Imipenem + DPA Neo-Sensitabs. Use **kit 98025**
Glupczynski et al (49) found that BEL enzymes were produced in 80% of P. aeruginosa isolates with evidence of ESBL production. BEL or PER ESBLs co-existed with VIM carbapenemases in 15 isolates.
Yakupogullari et al (50) report a multidrug-resistant P. aeruginosa. MICs of imipenem and meropenem were > 128 ug/ml.ESBL detection was performed by a synergy test using aztreonam and ticarcillin+clavulanate.

AZT 10-15 mm AMC		MER10 ug MER+DPA
Syn		≥ 5 mm

Detection of MBL and OXA-48 in the same strain.

In Enterobacteriaceae co-expression of MBL and OXA-48 can be detected by disk approximation test using Aztreonam 30 ug and Temocillin 30 ug Neo-Sensitabs. The 2 Neo-Sensitabs are placed approx 10mm apart (edge to edge).
Aztreonam is not hydrolyzed by MBLs, while Temocillin is resistant to both MBLs and OXA-48. Aztreonam protects Temocillin from MBLs and consequently a ghost zone will be formed between the 2 disks, while

the Temocillin Neo-Sensitabs will show no zone on the opposite side to the Aztreonam disk. This indicates the possible presence of an OXA-48 enzyme.

The MBL is detected using Imipenem 10 ug and Imipenem + DPA Neo-Sensitabs, or Meropenem 10 ug and Meropenem + DPA (**kit 98015 or kit 98025**).

Kilic et al (43) found the first Klebsiella pneumoniae isolate co-producing OXA-48 and NDM-1 in Turkey.
Castanheira et al (51) report the early dissemination of NDM-1 and OXA-181 (OXA-48 like) producing Enterobacteriaceae in Indian hospitals in 2006.
Poirel et al (52) report the occurrence of OXA-48 and VIM-1 carbapenemase-producing Enterobacteriaceae in Egypt.
Doi et al (53) report the co-production of NDM-1 and OXA-232 (OXA-48 like) by Klebsiella pneumoniae in the USA. They conclude that high-level resistance to amikacin and gentamicin can serve as a clue for suspecting potential NDM-1-producing isolates in clinical diagnostic laboratories.
Bousquet et al (54) et al reports the first case in a french hospital of a multidrug-resistant NDM-1 and OXA-232 (OXA-48 like) carrying Klebsiella pneumoniae.
Sun Young Cho et al (61) detected K. pneumoniae co-producing NDM-5 and oxacillinase OXA-181 (an OXA-48 like) beta-lactamases in South Korea in 2014.
Haciseyitoglu et al (86) describe an interhospital spread of carbapenemase producing K. pneumoniae producing OXA-48 and NDM carbapenemases that started in 2011 in Turkey.
Kazi M et al (94) describe the co-presence of NDM-1 and OXA 48 /181 as well as NDM-1 and VIM in Enterobacteriaceae in Mumbai, India.
Anandan et al (95) describe the co-production of OXA-48 and NDM in E. coli and K. pneumoniae, corresponding to 12.5% of the isolates.
Khajuria et al (102) report the emergence of E. coli co-producing NDM-1 and OXA-48 in 55% of urinary isolates in atertiary care Center in India.
Anandan et al (103) report 12.5% of K. pneumoniae/E. coli from blood stream infections co-produced NDM-1+ OXA 181. The Xpert Carba R missed to detect OXA-48 like carbapenemases, because only 4 of 10 variants of OXA-48 like were included in the Xpert Carba R.
Lyman et al (106) mention that CDC received reports of 52 Enterobacteriaceae isolates producing OXA-48 from 2010-2015 in the US 12% of the isolates co-produced NDM and OXA-48 carbapenemases. The CDC recommends determining the mechanisms of resistance for any carbapenem-resistant Enterobacteriaceae.

AZT – 10 mm – TEMO	MER10	MER+DPA
ghost zone		≥ 5 mm

Detection of MBL, OXA-48 and ESBL in the same strain of Enterobacteriaceae

In Enterobacteriaceae, we use the disk approximation test.Aztreonam 30 ug Neo-sensitabs is placed between Temocillin 30 ug and Amoxycillin + Clavulanate 20 + 10 ug Neo-Sensitabs. The distance between Temocillin and Aztreonam is 10 mm (edge to edge) and the distance between Aztreonam and Amoxycillin + Clav is 10 to 15 mm.
Besides use the **kit 98015**: KPC, MBL and OXA-48 confirm kit from Rosco, that contains Temocillin 30 ug.
If the isolate shows the following: a ghost zone between Temocillin and Aztreonam (OXA-48) as well as Synergism between Aztreonam and Amoxycillin + Clav (ESBL) and finally a zone of inhibition around

Meropenem+DPA >= 5mm larger than Meropenem 10 ug (MBL) than the isolate co-produces MBL+OXA-48+ESBL.
Seifert et al (55) report the emergence of Klebsiella pneumoniae co-producing NDM-1, OXA-48, CTX-M-15, CMY-16, QnrA in Switzerland, resistant to all antibiotics except colistin.
Aggoune Khinache et al (56) report the co-expression of NDM-1, OXA-48, CTX-m-15 and SHV 11 lactamases in Klebsiella pneumoniae from Algeria.
Meryern Iraz et al (81) report combinations of beta-lactamases including NDM-1, OXA-48 and CTX-M in the same strain of Klebsiella pneumoniae in Turkey. Of the 37 isolates tested 76% possessed OXA-48 + ESBL and there was a high prevalende of NDM-1 (19%) being a large increase in Turkey of the NDM-gene.
Bathoorn et al (90) report (august 2015) the isolation of a NDM-5 producing K. pneumoniae ST16, isolated from a Dutch patient in a long-term care facility without recent travel history abroad. The NDM-5 gene was accompanied of a CTX-M-15 (ESBL) and was clonally related to isolates detected in 4 patients in Denmark in 2014.
Torres-Gonzalez et al (91) report an outbreak caused by a NDM-1-harboring plasmid spread through different bacterial species (E. cloacae, E. coli,K. pneumoniae), in a tertiary care hospital in Mexico City. Besides NDM-1, the isolates harbored an ESBL (CTX-M-15).
Dortet et al (92) report the dissemination of carbapenemase-producing Enterobacteriaceae and P. aeruginosa in Romania. NDM-1 and OXA-48 were most common in Enterobacteriaceae and VIM-2 in P. aeruginosa. Besides NDM-1 or OXA-48 the isolates harbored ESBLs (CTX-M-15)
Hammerum et al (104) report in Denmark the detection of K. pneumoniae co-producing NDM-7 and OXA-181 and CTX-M-15 in a patient that was hospitalized in Goa. Besides, the same patient also possessed E. coli producing NDM-5 and A. baumannii producing OXA-23. GeneXpert did not detect the OXA-181 gene.The study highlights the importance of screening for carbapenemase-producing bacteria from patients travelling to countries with a high occurrence of carbapenemase-producing bacteria.

TEMO – 10 mm – AZT – 10-15 mm – AMC		MER10	MER+DPA
ghost zone	Syn		≥ 5 mm

Detection of oxacillinases and MBL in the same strain of Acinetobacter

Use the **kit 98025**: KPC/MBL in Pseudomonas/Acinetobacter as well as Aztreonam 30 ug Neo-Sensitabs. Apply Imipenem 10 ug Neo-ASensitabs and Aztreonam 30 ug Neo-Sensitabs at 10 mm from each other (edge to edge). Besides the remaining tablets from the 98025 kit on the inoculated MH agar plate.
An increase of 4 – 7 mm in the inhibition zone around IMI+EDTA compared to IMP10 ug and no synergy with Imipenem+DPA (compared to Imipenem 10 ug), indicates the presence of an **oxacillinase.**
An increase of >= 8 mm in the zone around IMI+EDTA compared to Imipenem 10 ug and/or an increase of >= 5 mm in the zone around Imipenem+DPA as compared to IMP 10 ug and synergism between Aztreonam and Imipenem indicates the presence of a **MBL**.
An increase >= 8 mm in the zone around IMI+EDTA, and/or an increase >= 5 mm in the zone around Imipenem+DPA as well as no-synergism between Aztreonam and Imipenem indicates the presence of a **MBL and an oxacillinase.**
Bosnjak et al (57) describes for the first time an Acinetobacter guillouiae co-producing NDM-1 and OXA-58 in Croatia.
Kumarasamy K et al (58) describes the coexistance of OXA-23 with NDM-1 and armA in clinical isolates of Acinetobacter baumannii from India.
Bedenic et al (65) describes 11 isolates of A. baumannii co-producing OXA-23 and VIM metallo-beta-lactamase in a nursing home. The E-test and combined disk test using EDTA gave false positive in 7 of

18 isolates. For testing MBLs in Acinetobacter is best to use the Imipenem/DPA combination, because the Imipenem/EDTA combination may give false MBLs positives.
Yao Sun et al (96) detected the co-production of OXA-23 and IMP genes in 7.8% of A. baumannii in Wenzhou, China.
Jin-Gui Cao et al (105) report for the first time a carbapenem-resistant Acinetobacter soli coharboring NDM-1 and OXA-58 genes in China.The authors indicate the importance of accurate epidemiological investigation of non-A. baumannii species.
Yu-Feng et al (107) report the ability of A. johnsonii to harbor 9 plasmids (including NDM-1, OXA-58 and PER-1) and suggest that this species could generate various platforms to mediate dissemination of resistance.

IMP – 10 mm – AZT	IM+ED	IM+DPA	Enzymes
No synergy	≥ 4-7 mm	No synergy	Oxacillinase

Synergy	≥ 8 mm **and**	≥ 5 mm	MBL
No synergy	≥ 8 mm **and**	≥ 5 mm	MBL+Oxacillinase

Detection of oxacillinases and Class A carbapenemases in the same strain of Acinetobacter

Hammoudi D et al (64) report the preponderance of OXA-23 and GES-11 co-producing isolates of A. baumannii in Lebanon.These resistance traits appear to spread via both bacterial epidemics and horizontal transfer.
To detect them Use the **kit 98025**: KPC/MBL in Pseudomonas/Acinetobacter as well as Ticarcillin+Clavulanate Neo-Sensitabs (TTC) and Boronic acid Diatabs (BORON).
Place the Imipenem 10 ug of the kit in between TTC (distance 5-8 mm between edges) and BORON (5 mm distance between edges). Use the remaining of the kit 98025.
Synergy (ghost zone) between Imipenem 10 ug and TTC and between Imipenem 10 ug and BORON indicates the presence of a Class A carbapebnemase (**KPC or GES**).
An increase of 4 – 7 mm in the inhibition zone around IMI+EDTA compared to IMP 10 and no synergy with Imipenem + DPA (compared to IMP 10) indicates the presence of an **oxacillinase.**
Cicek et al (87) report the presence of OXA-23 and GES-11(or GES-22) in extensively drug-resistant Acinetobacter baumannii in Turkey.) & % of the strains were MDR.
Cherkaoui et al (88) report the co-production of OXA-23 and GES-11 in multidrug-resistant Acinetobacter baumannii in Switzerland.

TCC - 5-8 mm – IMP – 5 mm – BORON		IMP10	IMI+ED	IMP+DP	Enzymes
ghost zone	ghost zone		Syn	No Syn	Class A (KPC or GES)
			4-7 mm	No Syn	Oxacillinase

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