BIOMÉRIEUX

CARBAPENEM RESISTANCE

From diagnosis to outbreak management

PIONEERING DIAGNOSTICS

TABLE OF CONTENTS

We wish to thank Prof. Patrice Nordmann and Dr. Laurent Poirel for providing the content of this booklet and sharing their valuable knowledge on carbapenem resistance.

INTRODUCTION

Bacterial resistance to antibiotics has become a major public health issue worldwide. The reality of this threat was acknowledged in the WHO 2014 report (www.who.int/drug resistance/en) on antibiotic resistance.

Rising resistance is of particular concern for Gram-negative bacilli such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and Enterobacterales, the latter being the most important pathogens for mankind. Carbapenems are among the last resort antibiotics for treating infections due to these Gram-negative bacilli.[1]

Resistance to carbapenems in these species is related either to combined mechanisms of resistance (overproduction of broad-spectrum ß-lactamases together with efflux pumps and/or decreased outer membrane permeability) or production of carbapenem-hydrolyzing ß-lactamases, also known as carbapenemases.[2]

In Enterobacterales, carbapenemases represent the most important mechanism of resistance, since the carbapenemase genes are mostly plasmid-located, associated with multi- or pan-drug resistance and are highly transferable, at least within the Enterobacterales species, making them potentially responsible for outbreaks.[2,3] WHO has published a global priority list of pathogens to focus attention on the most significantly resistant pathogens and carbapenem-resistant Enterobacterales are top of the list.[4]

This booklet covers issues related to carbapenem-resistant Gram-negative bacilli (mostly carbapenemase producers in Enterobacterales), as well as their clinical relevance, detection, treatment and prevention.

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1 CARBAPENEM RESISTANCE

What are the mechanisms of resistance to carbapenems in Gram-negative bacilli?

Carbapenem resistance in Enterobacterales is related:

- either to a combination of decreased outer-membrane permeability/overproduction of efflux systems, with overproduction of ß-lactamases possessing very limited carbapenemase activity such as Ambler class C ß-lactamases [AmpC] or clavulanic-acid inhibited extended-spectrum ß-lactamases (ESBLs, mostly CTX-M),
- or to production of "true" carbapenemases.

Non-carbapenemase related mechanisms of carbapenem resistance are not transferred horizontally (from one bacterial strain to another).[2,5,6,7] If the resistance mechanism involves porin deficiency, this could theoretically impact bacterial fitness, contributing to a decreased rate of transmission. In addition, carbapenemase-encoding genes are usually associated with non-ß-lactam resistance genes, although carbapenem-resistant but non-carbapenemase-producing strains less frequently co-harbour such additional resistance determinants. These properties may explain why carbapenem-resistant isolates that do not produce carbapenemases are considered to be **less threatening to public health than carbapenemase producers**.[2,8,9] Non-carbapenemase related mechanisms of carbapenem resistance are mostly prevalent in Enterobacterales species that naturally produce an AmpC-type ß-lactamase, such as *Enterobacter* sp., *Serratia* sp. and *Morganella* sp.[2] or frequently associated with clavulanic acid inhibited expanded-spectrum ß-lactamases or plasmid-mediated AmpCs with extremely low-level carbapenemase activities.

Carbapenemase related mechanisms of carbapenem resistance, on the other hand, are mostly plasmid-encoded, making them **highly transferable**, at least within the Enterobacterales species, and therefore potentially responsible for outbreaks. They are also significantly **associated with multi- or pan-drug resistance to other antibiotic families**.[2,5]

CARBAPENEM RESISTANCE	
Decreased permeability/over-production of ß-lactamase with very low level carbapenemase activity	Not transferable → LOW RISK of transmission between patients
Carbapenemase	Transferable through plasmid → HIGH RISK of transmission from strain to strain

Currently, the spread of carbapenemase producers is the most important clinical issue in antibiotic resistance among Gram negatives, particularly in Enterobacterales.

The carbapenemases encountered among Enterobacterales differ from ESBLs in that they significantly hydrolyze carbapenems.[2] In most cases, the protein structure of the carbapenemases differs significantly from that of ESBLs with the notable exception of the GES and OXA-48 types, which gather enzymes with or without carbapenemase activity depending on specific point-mutant mutations.[2,5]

Carbapenemases belong to one of the three groups of ß-lactamases, namely **Ambler class A, B, and D groups**.[2,10] Differences between these carbapenemase enzymes are clinically significant, since their hydrolysis profile differs and susceptibility to novel antibiotics of the corresponding producers may vary **(Figure 1)**. Their species distribution and worldwide epidemiology is also different.[2,5]

→ Ambler class A ß-lactamases: "penicillinases" group (serine ß-lactamases)

This group includes "penicillinases" whose activity is partially inhibited *in vitro* by clavulanic acid and tazobactam, and well-inhibited by avibactam. The most widespread representative is KPC (*Klebsiella pneumoniae* carbapenemase),[11,12,13] but others have been identified, such as SME, NMC, IMI, GES.[11,14] These enzymes have a broad-spectrum activity similar to that of ESBLs, with an extended activity toward carbapenems. *In vitro,* their activity is partially inhibited by ß-lactamase inhibitors, such as clavulanic acid and tazobactam, and very significantly by avibactam. Avibactam is currently used in association with ceftazidime and will also be associated with aztreonam in the near future. Relebactam (imipenem/relebactam) and vaborbactam (meropenem/vaborbactam) significantly inhibit the activity of KPC enzymes.

→ Ambler class B ß-lactamases: metallo-beta lactamases

The second group of carbapenemases corresponds to the metallo-ß-lactamases (MBLs), including IMP, VIM, GIM, KHM and NDM ß-lactamases.[10,15-17] MBLs hydrolyze all ß-lactams except aztreonam. Their activity is not inhibited by clavulanic acid and tazobactam, nor by avibactam.

→ Ambler class D ß-lactamases: oxacillinases

The third group of carbapenemases comprises several (but not all!) oxacillinase OXA-48 derivatives and non-OXA-48 derivatives (OXA-23-like, OXA-40 and OXA-58).[18-21] They hydrolyze penicillins and 1st generation cephalosporins. They do not significantly hydrolyze 2nd and 3rd generation cephalosporins such as cefotaxime and ceftazidime. Finally, they do hydrolyze carbapenems, although at a low level. They are not inhibited by clinically-available ß-lactamase inhibitors, except OXA-48-like enzymes which are inhibited by avibactam but not by relebactam or vaborbactam.

None of the ß-lactamase inhibitors currently available on the market allows inhibition of all three carbapenemase groups (A, B, D).

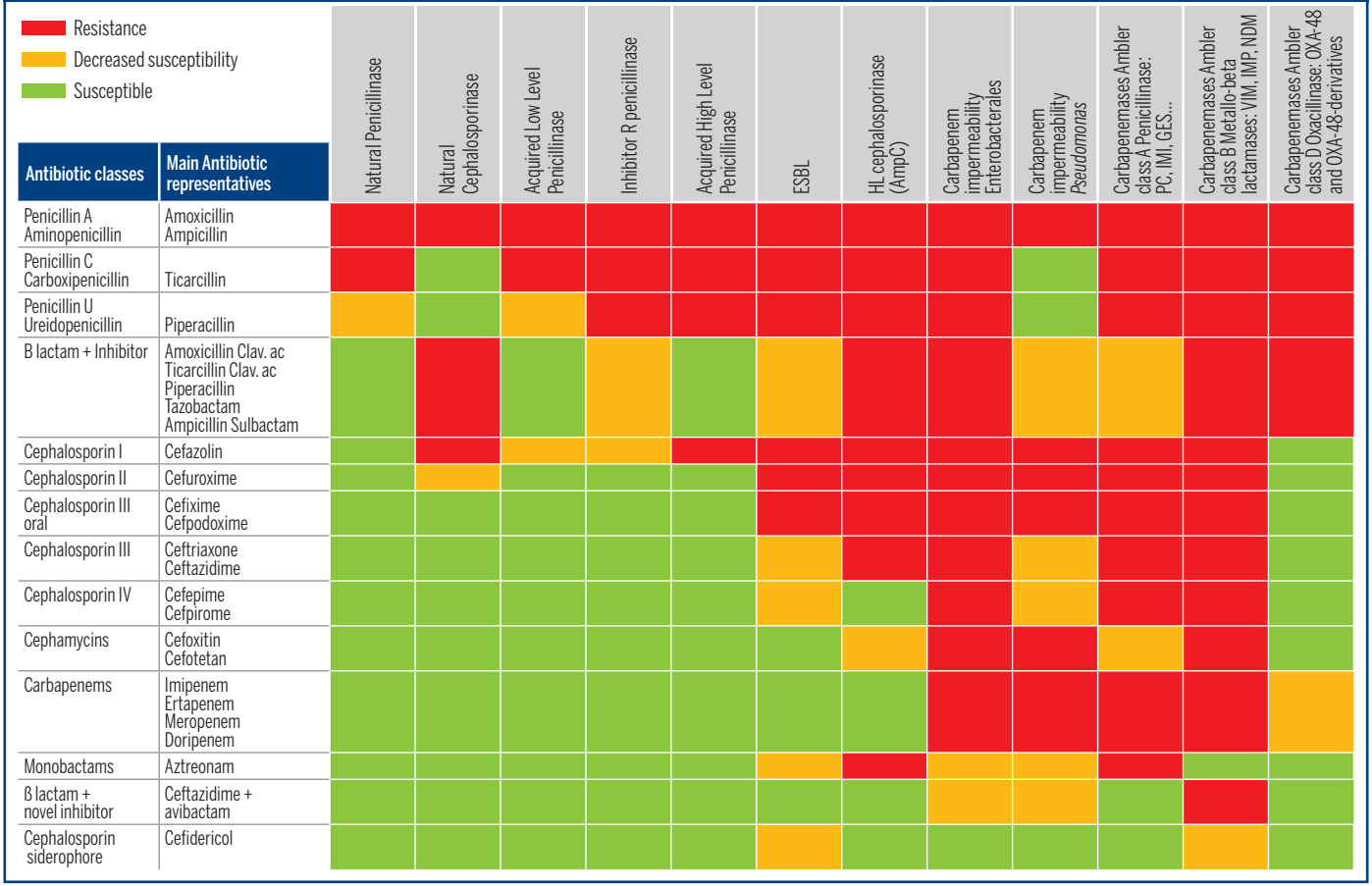
In ***Pseudomonas aeruginosa***, resistance to carbapenems is mostly due to decreased permeability to imipenem, associated with qualitative or quantitative changes of the porin OprD2.[22] Overexpression of the MexAB-OprM porin may lead to decreased susceptibility to meropenem.[22] However, carbapenemases have been also reported in *P. aeruginosa*.[23] They are mostly MBLs (VIM, IMP, SPM, SIM, NDM) or of the GES type and rarely of the KPC or OXA-48 types.[15,23]

In the healthcare-associated pathogen *Acinetobacter baumannii*, resistance to carbapenems is also extensively observed and is associated with different types of carbapenemases, such as those identified in Enterobacterales (NDM, IMP, VIM).[24] Several carbapenemases of the Ambler class D are almost totally exclusively identified in *A. baumannii*: OXA-23, OXA-40 and OXA-58 derivatives (but not OXA-48 derivatives) and are, by far, the most prevalent carbapenemases in that species.[21,24]

Those latter enzymes hydrolyze carbapenems at a low level and are not inhibited by commercially available ß lactamase inhibitors.[21] Most, if not all, carbapenem-resistant *A. baumannii* strains produce at least one of those carbapenemases, most often in association with a permeability defect and/or overexpression of genes encoding efflux pumps.[24] In rare cases, resistance to carbapenems results from the association of a permeability defect and overexpression of the naturally-occurring oxacillinases of *A. baumannii* with some carbapenemase activity (OXA-51-like).[24]

	CARBAPENEMASE	CARBAPENEM DECREASED PERMEABILITY
Enterobacterales	+++	++
P. aeruginosa	+	+++
A. baumannii	Frequently both simultaneously in the same strain	

Figure 1. Main resistance profiles observed in Gram-negatives.



2 EPIDEMIOLOGY

What is the extent of the spread of carbapenem-resistant bacilli worldwide ?

A Spread of carbapenem resistance by outer membrane permeability defect associated with over-production of a ß-lactamase

Carbapenem-resistant Enterobacterales isolates that do not produce a carbapenemase are mostly *K. pneumoniae* and *Enterobacter* sp. They usually express decreased outer membrane permeability associated with a plasmid-mediated ESBL of CTX-M-type enzyme (multicopies of the corresponding gene) or overexpression of a AmpC ß-lactamase, respectively. Although epidemiological data for these carbapenem-resistant isolates is limited, the prevalence rate appears to vary quite significantly from one country to another.[11,18] This combined mechanism is by far the most frequent mechanism of resistance to carbapenems observed in *Enterobacter* sp.[11,18]

K. pneumoniae	Decreased outer membrane permeability and plasmid-encoded CTX-M ß-lactamase
Enterobacter sp.	Decreased outer membrane permeability and overexpression of cephalosporinase

B Spread of carbapenemase producers

Data on the worldwide distribution of carbapenemase producers in Enterobacterales are well-known.[5,25]

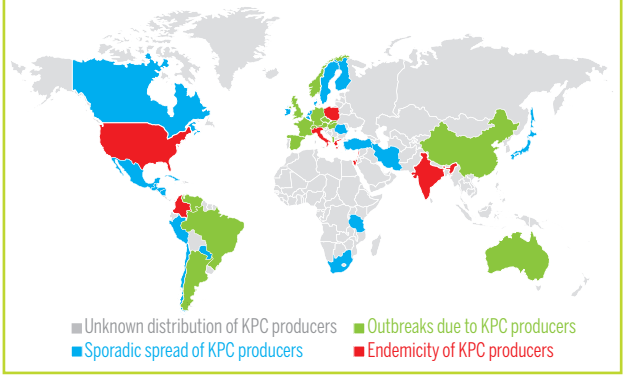
→ Class A: "penicillinases" group (serine ß-lactamases)

KPC enzymes are currently the most clinically significant enzymes among the class A carbapenemases worldwide.[26,27]

The **first KPC producer** (a KPC-2-positive *K. pneumoniae*) was identified in 1996 on the **Eastern coast of the USA.**[28] Within a few years, KPC producers were identified in almost all US states where they are now quite prevalent.[2] They have since spread worldwide and have been identified in many Gram-negative species, even though KPC enzymes are still mostly identified in *K. pneumoniae* **(Figure 2).**[2,11,12]

Figure 2: Geographical distribution of KPC producers

Adapted from Nordmann P, *et al. Clin Infect Dis.* 2019;69(Suppl 7):S521-S528; Bonomo R, *et al. Clin Infect Dis*. 2018;66(8):1290-1297; Nordmann P, *et al. Clin Microbiol Infect*. 2014;20(9):821-830 and Nordmann P, *et al. Emerg Infect Dis*. 2011;17(10):1791-1798. https://wwwnc.cdc.gov/eid/article/17/10/11-0655_article Reproduced with permission from Emerging Infectious Diseases (CDC).



In Latin America, KPC producers are endemic in some areas, such as Colombia and Argentina.[26] **In Europe**, KPC producers are found almost everywhere, most often linked to imports from endemic areas, namely Greece, Poland and Italy.

In South East Asia, the extent of the spread of KPC producers is not well known, even though China may face some endemic situations. **In India**, few reports on KPC-producing isolates exist, the most commonly identified carbapenemases being NDM and OXA-48-like enzymes (see below).

However, in Europe, many KPC-producing isolates have been identified in patients previously hospitalized in India or Pakistan.

One specific KPC-2- or KPC-3-producing *K. pneumoniae* clone (ST 258) has been extensively identified worldwide.[12,13]

Although NmcA was the very first sequenced carbapenemase identified in Enterobacterales in the 1990s,[26] other types of class A carbapenemases (NmcA, SME, IMI, GES) still have a local dissemination, with **GES-type ß-lactamases** having a more specific dissemination in **South America.**[5]

GES-type enzymes with carbapenemase activity are extensively reported in *P. aeruginosa*, in particular GES-5.[23]

KPC	
K. pneumoniae	+++
Enterobacter sp.	+
Other Enterobacterales	rare
P. aeruginosa	rare

Class B: metallo-beta lactamases

MBLs are intrinsic in many environmental and opportunistic bacterial species. However, since the early 1990s, they have also been identified as acquired enzymes, either in *Pseudomonas* sp., *Acinetobacter baumannii* or Enterobacterales.[15-17,29-33]

The most common MBLs identified in Enterobacterales include the VIM- and IMP- groups, together with the emerging NDM group, whereas others, such as GIM-1, SIM-1, SPM-1 or KHM-1, remain sporadic.[10,15,34,35-38]

Although reported worldwide, the **VIM producers** (mostly VIM-1) in Enterobacterales are highly prevalent in **Southern Europe, Eastern Europe and the Mediterranean geographical area**, whereas the **IMP producers** remain mostly located in **Asia**.[10,15]

One of the most clinically significant carbapenemase groups corresponds to NDM-type enzymes (New Delhi metallo-ß-lactamase) identified in 2009 in *K. pneumoniae* and *E. coli* isolates from a patient in Sweden previously hospitalized in India.[10,39] The main identified reservoir of **NDM-producing Enterobacterales** is the **Indian subcontinent (Pakistan, India, Sri Lanka, Bangladesh) (Figure 3)**.[10,29,40,41] These countries are experiencing endemicity of many different NDM producers. The spread of NDM producers has been not only extensively identified among patients from the Indian subcontinent but also from its soil.[17,42] The prevalence of human carriage in this region was estimated to be around 5 to 15 % in 2010-2015.[10,40,43]

Significant spread of NDM producers was first identified in the **United Kingdom (UK)** due to its close connections with India and Pakistan.[10,39] Subsequently, NDM producers in Enterobacterales have been reported almost worldwide, including many countries in Asia, Africa, Australia, America, and Europe **(Figure 3)**.[44]

Figure 3: Geographical distribution of NDM producers

Adapted from Nordmann P, *et al. Clin Infect Dis*. 2019;69(Suppl 7):S521-S528; Bonomo R, *et al. Clin Infect Dis*. 2018;66(8):1290-1297; Nordmann P, *et al. Clin Microbiol Infect*. 2014;20(9):821-830 and Nordmann P, *et al. Emerg Infect Dis*. 2011;17(10):1791-1798. https://wwwnc.cdc.gov/eid/article/17/10/11-0655_article
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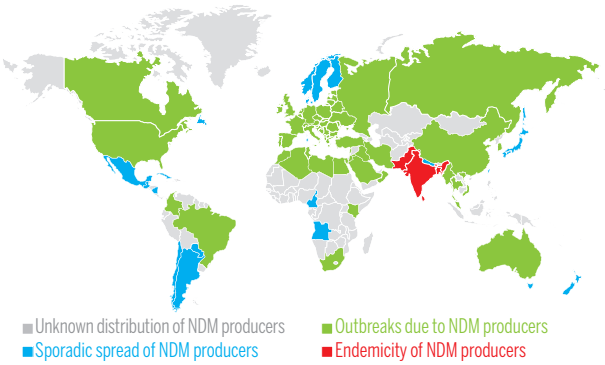

Another important source of NDM producers (or established **secondary reservoirs**) is made up of the **Balkan states**, the **Middle East** and **North Africa**.[10,45] While the spread of *K. pneumoniae* and *Enterobacter* sp. producing NDM enzymes is well-known, the spread of NDM producers in *E. coli* (NDM-5) has been recently highlighted in Europe,[46] including diffusion of this resistance trait among community-acquired strains.

METALLO ß-LACTAMASES	
Enterobacterales: *K. pneumoniae, Enterobacter* sp., *Proteus* sp. *E. coli*	NDM, VIM, IMP
P. aeruginosa *A. baumannii*	VIM, IMP, NDM (rare) NDM, IMP (rare)

Class D: oxacillinases

The **first OXA-48 producer** was identified in Paris from a *K. pneumoniae* isolate recovered from **Turkey** in 2003.[19] OXA-48 producers have since been extensively reported in Turkey, often being the source of healthcare-associated outbreaks, then in **North African countries** and more recently in the **Middle East** and **India**.[18,21,47-49]

OXA-48-like producers with signifiant carbapenemase activity are increasingly identified: OXA-162; OXA-181, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-436, OXA-484 and OXA-519.[32,50,51] They are identified mostly in *K. pneumoniae, E. coli, Enterobacter* sp and *Proteus* sp. Community-acquired enterobacterales expressing OXA-48-like enzymes are now extensively reported.

In Europe, they are becoming the most prevalent carbapenemases in many countries such as **France, Switzerland, Germany** and the **UK**.

OXA-48 producers are currently **more rarely identified** in **North and South America** than in other parts of the world **(Figure 4)**.[18,21,47]

Interestingly, several OXA-48-like enzymes, such as OXA-163, OXA-252, and OXA-405, do not possess carbapenemase activity.[18,21,47] **OXA-163** has been identified from Enterobacterales isolates recovered in **Argentina** and **Egypt** and differs from OXA-48 by a single amino-acid substitution together with a four amino-acid deletion. Its carbapenemase activity is almost undetectable, its substrate profile includes broad-spectrum cephalosporins and its activity is partially inhibited by clavulanic acid, giving it a resistance phenotype similar to that of an ESBL producer.[18,21,47]

OXACILLINASES WITH CARBAPENEMASE ACTIVITY	
Enterobacterales: *K. pneumoniae, E. coli,* *Enterobacter* sp., *Proteus* sp.	OXA-48-like
A. baumannii	OXA-23, OXA-40, OXA-58

Figure 4: Geographical distribution of OXA-48-like producers

Adapted from Nordmann P, *et al. Clin Infect Dis*. 2019;69(Suppl 7):S521-S528; Bonomo R, *et al. Clin Infect Dis*. 2018;66(8):1290-1297; Nordmann P, *et al. Clin Microbiol Infect*. 2014;20(9):821-830 and Nordmann P, *et al. Emerg Infect Dis*. 2011;17(10):1791-1798. https://wwwnc.cdc.gov/eid/article/17/10/11-0655_article
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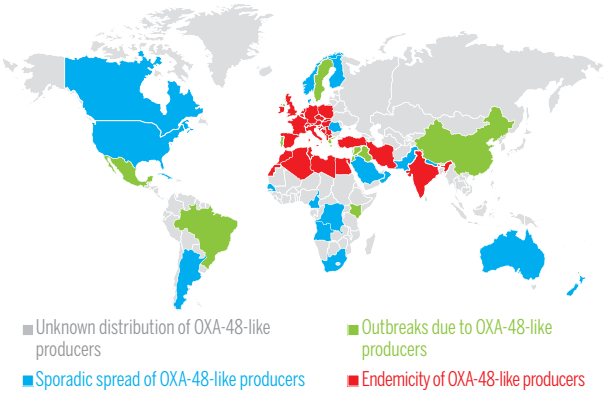


In *P. aeruginosa*, the most important carbapenem resistance mechanism is quantitative or qualitative modification of the OprD2 porin.[22] This porin allows special amino acids and imipenem to enter the cell. The prevalence rate of this resistance trait is stable, at least in **Europe**, ranging from 10 to 20%.[22] **KPC and MBLs** have been reported in *P. aeruginosa*, although the diffusion rate for KPC producers in *P. aeruginosa* is not well-known.[5,22,23,45] They are highly prevalent in the **northern part of South America** while **VIM producers** are extensively reported from **Southern Europe** and **IMP producers** in **Asia**. In *P. aeruginosa,* NDM producers remain rare[15] and OXA-like carbapenemases have rarely been identified (OXA-40, OXA-48, OXA-181 and OXA-198).[23]

In *A. baumannii*, the main resistance mechanism is production of carbapenem-hydrolyzing ß-lactamases of the OXA-type associated with outer membrane protein defects and/or overexpression of efflux systems.[24,25] The structures of those oxacillinases differ significantly from that of the OXA-48-like ß-lactamases.[20] **OXA-23 producers** are identified **worldwide** while OXA-40 and OXA-58 producers are less widely distributed.[20,25] **KPC** have also been rarely identified, whereas an increasing number of NDM producers is identified, at least in Europe and Asia. The prevalence rate of carbapenem resistance in *A. baumannii* varies from one country to another with a much **higher rate of resistance** (40-60%) in **Southern Europe, Middle East, Turkey, South America and Asia**.[25]

3 CLINICAL ASPECTS

What are the clinical aspects of infections due to carbapenem-resistant Gram negatives?

Infections caused by carbapenem-resistant Enterobacterales isolates include mostly **urinary tract infections, peritonitis, septicemia, pulmonary infections, soft tissue infections and device-associated infections**.[1,7] There is no gender preference and most of the cases are adults.[1,7]

The vast majority of infections are **urinary tract infections**, as observed for any Enterobacterales infection.

Both **hospital- and community-acquired infections** have been reported. No specific clinical manifestations have been associated with carbapenemase producers as compared to wild-type susceptible strains.[1,7]

All types of carbapenemase-producing Enterobacterales species are involved in infections, but ***K. pneumoniae*** and ***E. coli*** are the main sources of **hospital- and community-acquired infections**, respectively. *E. coli* that produce NDM or OXA-48-like producers are now identified worldwide.[3]

Carbapenem-resistant Enterobacterales isolates which are not carbapenemase producers have also been identified as sources of hospital-acquired infections (mostly *K. pneumoniae* and *Enterobacter* sp.).[45]

Like carbapenem-susceptible isolates, **carbapenem-resistant *P. aeruginosa* and *A. baumannii* isolates** are most often the source of **hospital-acquired infections** such as **septicemia, catheter-associated infections, pneumonia, wound infections, and urinary tract infections**.

No specific virulence factors are associated with carbapenemase producers, except in rare cases, such as that of hypervirulent *K. pneumoniae*.[52,53]

MAIN TYPES OF INFECTION[1,7]		
Urinary Tract	Peritonitis	Septicemia
Respiratory Tract	Soft Tissue / Wounds	Device-Associated

4 TREATMENT

How to treat infections due to carbapenem-resistant Gram-negative bacilli?

Most carbapenem-resistant Gram-negative bacilli are multi-resistant to non-ß-lactam antibiotics with the exception of imipenem-resistant *P. aeruginosa* isolates (OprD2 modification) which may remain susceptible to several broad-spectrum antibiotics.

No consensus exists for the optimal antibiotic regimen for treating infections due to carbapenemase producers in Enterobacterales.[1,9,54] However, several recent reports make practical proposals for treatment.[1,54,55]

Infected patients must be treated, but not carriers. Several studies report on the impact of extensive usage of carbapenem and other broad-spectrum antibiotics, such as third- and fourth-generation cephalosporins and fluoroquinolones, as factors for selection of carbapenem-resistant Gram negative bacilli.[9,38] An increased attributable mortality has been shown for infections due to carbapenemase producers compared to that due to carbapenem-susceptible strains.[9]

The **choice of the optimal antibiotic therapy** is based on the detailed analysis of the **antibiotic susceptibility testing results, the inhibitory activity of novel inhibitors** against specific carbapenemases and the **efficacy of novel molecules**. The **infection site** and the **diffusion of the antibiotics** at the infected site are also factors to consider for optimal antibiotic choice. In several cases, the antibiotic choice among "old molecules" remains limited to **colistin**, parenteral **fosfomycin, gentamicin, and tigecycline**.[8,22,55,56]

However, **novel inhibitors** are now on the market, namely the diazabicyclooctanes, **avibactam and relebactam**, along with the boronic acid derivative, **vaborbactam**.[42] Avibactam and relebactam inhibit the activity of KPC and OXA-48-like enzymes (relebactam is a weaker inhibitor of OXA-48 enzymes), while vaborbactam inhibits the activity of KPC enzymes only. None of the currently marketed inhibitors inhibit the activity of metallo-enzymes (NDM, IMP, VIM). Combinations of antibiotics that contain novel inhibitors are **ceftazidime/avibactam, meropenem/vaborbactam, imipenem/relebactam** and the combination currently in development **aztreonam/avibactam (Figure 5)**. This latter combination is theoretically active against NDM producers, since aztreonam is not hydrolyzed by metallo-ß-lactamases.

Figure 5. Theoretical activity of novel antibiotics against multidrug-resistant Gram negatives

Adapted from Yahav D, *et al. Clin Infect Dis*. 2021;72(11):1968-1974; Doi Y. *Clin Infect Dis*. 2019;69(Suppl 7):S565-S575; Jean S, *et al. Drugs* 2019;79(7):705-714.

	ESBL	KPC	MBL	AmpC	OXA	MDR-PA[2]	MDR-Ab[3]
Ceftazidime/ avibactam	+	+		+			
Aztreonam/ avibactam	+	+	+	+	+		
Meropenem/ vaborbactam	+	+		+			
Imipenem/cilastatin-relebactam	+	+		+		+	
Ceftolozane tazobactam	+			+		+	
Cefiderocol	+	+	+	+	+	+	+
Plazomycin	+	+	Variable[1]		+	Variable[1]	
Eravacycline	+	+	+	+	+		

1 Frequently inactive against strains that produce NDM-type metallo-ß-lactamases,
2 *Pseudomonas aeruginosa*, 3 *Acinetobacter baumannii*

Among the novel molecules, **cefiderocol** (a novel siderophore cephalosporin) and **eravacycline** (a novel tetracycline) have a broad spectrum of activity including most of the NDM producers.[57] Reduced susceptibility to cefiderocol has been reported recently for several NDM and PER (an ESBL) producers.[51] **Plazomycin** is a novel aminoglycoside which is not modified by aminoglycoside-modifying enzymes (acetylases, adenylases, phosphorylases).[57] However, in practice, its spectrum of activity does not include NDM producers since a high proportion of the NDM producers produce a 16S rRNA methylase that modifies the target of all aminoglycosides (including plazomycin), i.e. 16S RNA **(Figure 6)**.[58]

None of the available inhibitors inhibit the activity of OXA-type enzymes identified in *A. baumannii* (OXA-23, OXA-40, OXA-58). Cefiderocol and eravacycline have a large spectrum of activity that include many multidrug-resistant *A. baumannii* and *P. aeruginosa* strains.[57] In addition, the **association of ceftolozane/tazobactam** (ceftolozane is a derivative of ceftazidime) has been proposed for the treatment of infections due to multidrug-resistant *P. aeruginosa*.[57]

Figure 6. Theoretical activity of novel antibiotics against carbapenemase producers in Enterobacterales.

Adapted from Yahav D, *et al. Clin Infect Dis*. 2021;72(11):1968-1974; Doi Y. *Clin Infect Dis*. 2019;69(Suppl 7):S565-S575.

ANTIBIOTICS	CARBAPENEMASES
Ceftazidime/avibactam	KPC, OXA-48-like
Aztreonam/avibactam	KPC, OXA-48-like, NDM
Meropenem/vaborbactam	KPC
Imipenem/relebactam	KPC, OXA-48-like+/-
Cefiderocol	KPC, OXA-48-like, NDM
Eravacycline	KPC, OXA-48-like, NDM
Plazomycin	KPC, OXA-48-like

Treating infections due to carbapenemase producers in Enterobacterales

It has been proposed that, in case of low MIC values, carbapenems may be administered for treating carbapenemase producers at a high dosage and prolonged infusion regimen and preferably in association with an aminoglycoside or colistin.[1,49] However, most of those recommendations are based on studies performed with KPC and VIM producers and not with OXA-48 and NDM producers.

For severe infections due to carbapenemase producers, ceftazidime-avibactam, imipenem-relebactam meropenem/vaborbactam and cefiderocol have been proposed if active *in vitro*. For severe infections due to strains producing carbapenemases and being resistant to meropenem/vaborbactam, imipenem/relebactam or ceftazidime/avibactam, cefiderocol may be proposed, as well as the combination aztreonam/ceftazidime/avibactam for the metallo-enzymes.[54]

Except in the above-mentioned cases, combination therapy is not proposed for treating infections due to carbapenemase producers in Enterobacterales. The exact place of cefiderocol or eravacycline in the therapeutic arsenal remains debatable.

Treating infections due to carbapenem-resistant *P. aeruginosa* isolates

Treatment alternatives may include **broad-spectrum cephalosporin**, **aminoglycoside** and **fluoroquinolone** antibiotics to which many strains remain susceptible. A **combination of antibiotics** should be preferred to monotherapy, although this dogma has been debated.[59] Colistin and parenteral fosfomycin, or parenteral rifampicin may be included in the antibiotic combination for non-severe infections, provided that *P. aeruginosa* is naturally resistant to tigecycline and that no other therapeutic choice exists.[59] The combination ceftolozane/tazobactam has been proposed as first-line therapy for severe infections if the strain is susceptible to this combination.[57]

Treating infections due to carbapenem-resistant *A. baumannii*

If the strain is susceptible *in vitro*, the **combination of ampicillin and sulbactam** may be proposed for **hospital-acquired or ventilator-associated infections**. For infections due to sulbactam resistant strains **carbapenems**, **tigecycline and colistin** have been proposed, but the optimal antibiotic treatment for these infections remained unknown.[24,60] Combinations of antibiotics may be proposed for **severe infections only** (tigecycine, polymyxins, aminoglycosides, sulbactam combination). Carbapenem combination therapy using high-dose extended–infusion meropenem may be proposed if MIC <8 mg/L. The place of eravacycline and cefidericol in therapy is still debatable.[54] A few strains producing PER or NDM enzymes have been reported to be more resistant to cefiderocol.[51]

5 DIAGNOSIS

What are the criteria defining carbapenem resistance?

The relevant selection of suspicious isolates with reduced susceptibility to carbapenems is crucial for identification of carbapenemase-producing isolates.[35,61,62] Detection of carbapenemase-producing isolates in clinical specimens is first based on a careful analysis of susceptibility testing results. Recently, both the **CLSI (USA) and EUCAST (Europe) breakpoints** for carbapenems have been **significantly lowered** to allow **better detection of carbapenem-resistant isolates (Figure 7)**.

Figure 7. Breakpoints and MIC values of carbapenems for Enterobacterales, *P. aeruginosa* and *A. baumannii*.

Adapted from EUCAST, 2022; CLSI, 2022. Data accessible at www.clsi.org and www.eucast.org. Reprinted with permission from CLSI. Performance Standards for Antimicrobial Susceptibility Testing. 32nd ed. CLSI supplement M100. Clinical and Laboratory Standards Institute; 2022. EUCAST data is available free of charge and can be accessed from the EUCAST website at www.eucast.org

Enterobacterales	MIC BREAKPOINTS (mg/L)			
	EUCAST		CLSI®	
	S ≤	R>	S ≤	R≥
Doripenem	1	2	1	4
Ertapenem	0.5	0.5	0.5	2
Imipenem	2	4	1	4
Imipenem/relebactam	2	2	1/4	4/4
Meropenem	2	8	1	4
Meropenem/vaborbactam	8	8	4/8	16/8

P. aeruginosa	MIC BREAKPOINTS (mg/L)			
	EUCAST		CLSI®	
	S ≤	R>	S ≤	R≥
Doripenem	0.001	2	2	8
Imipenem	0.001	4	2	8
Imipenem/relebactam	2	2	2/4	8/4
Meropenem	2	8	2	8
Meropenem/vaborbactam	8	8		

A. baumannii	MIC BREAKPOINTS (mg/L)			
	EUCAST		CLSI®	
	S ≤	R>	S ≤	R≥
Doripenem	0.001	2	2	8
Imipenem	2	4	2	8
Imipenem/relebactam	2	2		
Meropenem	2	8	2	8

Why search for carbapenemase activity rather than carbapenem resistance?

The reasons for detecting **acquired carbapenemase genes** are multiple:

- As they are mostly **transmissible elements (plasmids)**, particularly in Enterobacterales, they can therefore **easily spread**.[63]
- All three main types of carbapenemase genes, namely ***bla*$_{KPC}$**, ***bla*$_{NDM}$** and ***bla*$_{OXA-48}$-like** genes, have the ability to **spread at least among Enterobacterales species**.
- The ***bla*$_{KPC}$** and ***bla*$_{NDM}$** genes, have been identified in Enterobacterales, *P. aeruginosa* and *A. baumannii*, showing their ability to **cross the species barrier**.
- Carbapenemase producers are also associated with other structurally-unrelated resistance traits that are also transmissible.

Therefore, identification of these multi- or even pan-drug resistant strains is important to prevent their spread and to guide the antibiotic therapy strategy.

By contrast, **resistance due to decreased permeability/efflux** is not transferable and does not have the same ability to spread among patients. Therefore, it does not require such stringent infection control measures. Furthermore, resistance through decreased permeability could theoretically **revert to susceptibility when antibiotic selection pressure stops**, while this is not the case for carbapenemase production.

How to detect carbapenemase producers as infectious agents?

Any suspicion of carbapenemase activity should be based on the analysis of the **antibiotic susceptibility results**.[63] In a clinical laboratory, detection of any type of carbapenemase activity on a cultured isolate can be performed by using one of the following two methods:

➔ Mass spectrometry MALDI-TOF technology (4-5 hours)

Detection of carbapenemase activity is based on determining the modified spectrum of a carbapenem following contact with a lysate of the bacterial culture.[64,65] This technique requires the development and the validation of a specific protocol, a period of incubation time (3 to 5 hours), additional centrifugation steps, a MALDI-TOF instrument and trained personnel.[64]

➔ Rapid colorimetric detection of a pH change (0.15 min to 1 hour)

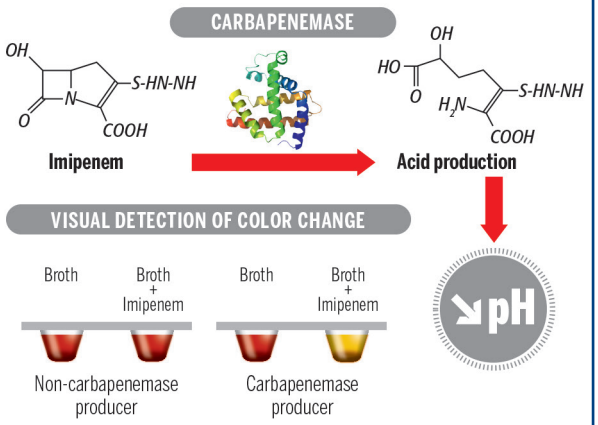
This type of test (RAPIDEC® CARBA NP or "lab-developed" Carba NP test) is based on detection of hydrolysis of the ß-lactam ring of a carbapenem molecule (imipenem).[65] Hydrolysis of the substrate (imipenem) acidifies the medium, changing the color of the pH indicator (phenol red solution). No reading device is required - the result can be read directly on the test **(Figure 8)**. The test is recommended by the CLSI since 2012.

Both techniques are **highly sensitive and specific** and **both detect carbapenem hydrolysis** and not a specific and limited number of resistance genes. They can detect any type of carbapenemase activity, including activity resulting from the spread and expression of novel carbapenemase genes, and results are available rapidly.[64-66] These techniques detect **carbapenemase activity in Enterobacterales** and ***P. aeruginosa***. For ***A. baumannii***, only a **home-test version of Carba NP** (CarbAcineto NP) is available.[67]

➔ Other techniques have been developed for detection of carbapenemase activity

- The detection of *in vivo* production of a carbapenemase using the **Modified-Hodge test** has been used for years.[68,69] This method should now be abandoned since it is both time-consuming (results obtained within 72 h) and lacks specificity and sensitivity.[68]
- The **carbapenem inactivation method (CIM)** is a simple, low-cost alternative to the Carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods.[70] It is based on immersion of a meropenem-containing drug susceptibility disk into a tube and suspending the microorganisms to be tested in water for 2 h at 35°C. After incubation, the disk is placed on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator (ATCC 29522) and further incubated for four hours at 35°C. If growth of the meropenem-susceptible indicator strain occurs, the meropenem in the disk is inactivated by the carbapenemase of the microorganism to be tested.
- **Modified versions of the CIM test** have been subsequently proposed such as **mCIM**.[69] These techniques are sensitive and specific, but are time-consuming (turnaround time for results in hours) and are homemade, limiting their practical use in routine microbiology.
- The **ß-Carba test** has been also developed based on color change of a chromogenic carbapenem degraded by carbapenemase activity. Although it is a rapid technique, it does not detect non-KPC carbapenemases (IMI, SME..) and many OXA-48 like producers.[71]

Figure 8: The principle of colorimetric detection of carbapenemase activity



In routine microbiology, colorimetric detection of carbapenemase activity is often associated with immunological detection of carbapenemases (see below) or molecular-based techniques, since both these techniques identify the most frequently encountered carbapenemases.

How to identify the carbapenemase type?

Determination of the exact carbapenemase type is currently required in three clinical situations.

- **For therapeutic purposes**: to select the antibiotic/inhibitor combination that will inhibit each type of carbapenemase.
- **During an ongoing outbreak**: to screen contact patients close to the source patient and to rapidly identify carriers of identical carbapenemase producers to prevent further spread.
- **For epidemiological purposes**: to monitor the spread of carbapenemase producers at the local, regional or national level.

Phenotypic detection of specific carbapenemases

1. Disk diffusion (ETEST®)

→ KPC

Phenotypic detection of the KPC enzyme is based on the inhibitory effects of boronic acid and its derivatives (phenyl-boronic and 3-aminophenylboronic acid).[63,72,73] **Boronic-based inhibition of KPC activity** is reliable at least with *K. pneumoniae* where it has been extensively evaluated, and when KPC is the only carbapenemase produced in a given clinical isolate.

→ MBL

Detection of MBL activity is based **on inhibition by MBL inhibitors**: EDTA, dipicolinic acid, 1.10 phenanthroline, mercaptopropionic acid, and mercaptoacetic acid.[15,63,72,73] These chelators inactivate MBLs by depriving them of Zn^{++} divalent ions.

The **double-disk synergy test and ETEST® MBL strip** with or without EDTA are based on the same principle.[15,64,71] The sensitivity of MBL detection has been improved by supplementing the culture media with zinc. Phenotypic detection of MBLs is reliable when dealing with Enterobacterales and *P. aeruginosa*, but not with *A. baumannii* for which false-positive results have been observed.

→ Oxacillinases

None of the above-mentioned tests can detect OXA-type carbapenemases in Enterobacterales or in *A. baumannii* since the enzymatic activity of OXA-type carbapenemase is not inhibited by clavulanic acid, tazobactam, sulbactam or zinc chelators.[21] Although the activity of oxacillinases of the OXA-48 type is inhibited by avibactam, disks containing imipenem or meropenem and avibactam are not commercially available.

High level resistance to temocillin and piperacillin-tazobactam in Enterobacterales exhibiting resistance or reduced susceptibility to a carbapenem may be predictive of the production of OXA-48 type carbapenemases since those enzymes confer resistance to temocillin.[74]

These techniques require an 18 h incubation time.

2. Biochemical analysis

The **extended Carba NP test** and the **Nitrospeed Carba NP test** have been designed to identify carbapenemase activity and types of carbapenemases using a combination of inhibitors.[66,75] The inhibitors used in the Nitrospeed Carba NP test are:

- avibactam and vaborbactam for class A carbapenemases;
- dipicolonic acid for class B enzymes;
- avibactam for class D carbapenemases.

The overall sensitivity and specificity of the Nitrospeed Carba NP test for the detection of all types of enzymes is 100% and 97%. Turnaround time to results is less than 60 min. None of these tests are currently commercially available.

3. Immunological detection

The **lateral flow technique** has been developed for identification of the main types of carbapenemases (KPC, NDM, VIM, IMP and OXA-48-like carbapenemases). This is an immunological-based assay for detecting epitopes for carbapenemase through an antigen-antibody reaction on a chromatographic paper. Turnaround time to results is 15 min. Both Carba 5 and Resist-5 OOKNV tests possess excellent specificity and sensitivity.[14,76,77]

Finally, very recently, a **rapid phenotypic method** for detection of carbapenem resistance in *A. baumanni* has been developed. The **Rapid Resalmipenem Acinetobacter NP test** is a culture-based test that allows this detection in less than 3 hours.[78] Its use may guide the implementation of a carbapenem- or non-carbapenem-based therapy for infections due to multidrug-resistant *A. baumannii*.

Genotypic characterization of carbapenemase genes[14,73]

Molecular techniques are mainly based on **PCR technology** and may be followed by sequencing of the entire coding region **(Figure 9)**. PCR-based methods include simplex, multiplex and real-time assays. Hybridization and microarrays may also be used.

A large number of commercially available tests are now available for molecular detection of the most common carbapenemases.[79]

Whole genome sequencing is increasingly used, through it remains labor- and time-intensive and is still not used in routine microbiology in many laboratories.

The results of molecular-based techniques are **highly reliable**. Several molecular techniques may also be used directly on clinical samples such as feces, although correlation between the molecular identification of a gene and carbapenemase production in clinically-relevant bacterial species has not yet been assessed.

The disadvantages of molecular techniques as screening techniques are their cost, expensive equipment, and for some techniques, the need for trained microbiologists.[5,62] However, molecular identification of carbapenemases can be concomitantly obtained with bacterial identification by using, for example, the **BIOFIRE® platform** in a syndromic approach.[80]

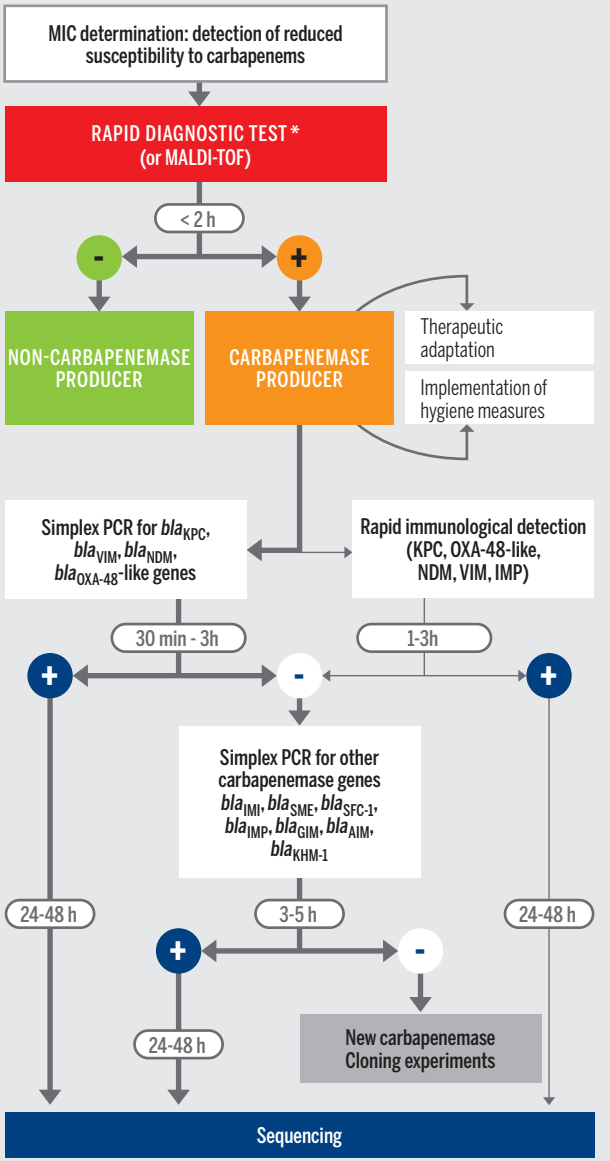
In addition, sequencing of the entire gene may be needed for several carbapenemase genes, such as the OXA-48 derivatives, in order to differentiate for example OXA-163 - which is a true ESBL without significant carbapenemase activity - from OXA-48, which is a true carbapenemase.[21]

Therefore, use of molecular-based screening of carbapenemases as a **first-line approach** may be currently limited to:

- **identification of carriers in an outbreak situation** by screening patients directly from stools;
- **for epidemiological purposes**.

Figure 9. Strategy for identification of carbapenemase producers from cultured Enterobacterales

Adapted from Dortet L, *et al. Antimicrob Agents Chemother.* 2014;58(4):2441-5. Reproduced with permission from the American Society for Microbiology.



* This rapid diagnostic test may also be performed directly from clinical samples.

6 SCREENING

Which patients should be screened for carriage of carbapenemase producers?

Detection of carriers is mandatory since they represent the invisible reservoirs for the further spread of carbapenemase producers. No worldwide consensus exists on the type of patient to screen.

Recommendations have been proposed for screening of carbapenemase producers in Enterobacterales.[43,61,69,81]

- **During an outbreak situation, patients in contact with the index patient should be screened.** In many cases, this screening includes at least all patients hospitalized in the same hospitalization unit. Patients transferred from abroad and patients hospitalized abroad within the year prior to the hospitalization should also be screened.
- Depending of the prevalence of carbapenemase producers in a country, **regular screening of at-risk patients**, such as those hospitalized in intensive care units (ICUs), in transplant units and immuno-compromised patients may be recommended.[61,69,81]

Screening of carbapenemase producers in *P. aeruginosa* and *A. baumannii* should include at least those patients hospitalized in the same hospitalization unit where the outbreak is occurring. Interestingly, carbapenemase producers in *A. baumannii* are always associated with multidrug resistance. **Carbapenemase production may therefore be considered as an indirect marker for multidrug resistance** in most cases.

Screening of non-carbapenemase related carbapenem-resistant Gram-negative bacilli: no specific recommendations have been established, however it appears logical to screen patients hospitalized in the same hospitalization unit where an outbreak has occurred.

PATIENTS AT RISK (MINIMUM LIST) JUSTIFYING SCREENING OF CARBAPENEMASES (Enterobacterales, *P. aeruginosa*, *A. baumannii*)

- Contact patients in case of an outbreak
- Patients directly transferred from any foreign hospital
- Patients hospitalized abroad within the year prior to hospital admission

How to screen carriers of carbapenem-resistant Gram negative bacilli?

Since the intestinal flora is the main reservoir of **Enterobacterales, rectal swabs and stools** are the most suitable clinical samples for performing screening of carbapenemase producers and carbapenem-resistant isolates **(Figure 10)**. In the case of ***P. aeruginosa*, environmental screening** may be also useful since water-borne sources of outbreak are often identified (e.g. sinks in ICUs). In the case of ***A. baumannii***, additional **skin or nasal swabs samples** may be useful for detection of carbapenem-resistant isolates.[24]

Direct identification of carbapenemases from clinical specimens

→ Molecular methods

Direct identification of several carbapenemase genes using molecular-based techniques is possible. Currently, molecular techniques are most recommended in an outbreak situation due to their cost **(Figure 11)**.[82] If molecular-based techniques are used, **identification of carbapenemase producers or carbapenem-resistant isolates by culture remains mandatory** in order to **compare the genotypes** of the strains in an outbreak situation and **determine the susceptibility pattern** to non-ß-lactam antibiotics **(Figure 11)**.

→ Phenotypic identification

MALDI-TOF or enzymatic tests may be used but are not feasible directly from stools due to the low level of carbapenemase activity.[83]

→ Culture methods

Clinical specimens can be plated on screening media, either directly, or after an enrichment step in broth containing imipenem 0.5-1 µg/mL or ertapenem 0.5 µg/mL.[69]

This **enrichment step** is particularly recommended **during an outbreak situation (Figure 11)**.[69,81] It may increase sensitivity, and consequently reduce the number of potential false-negative results by increasing the inoculum of the targeted strain. It has already been shown to **improve the detection of KPC producers in Enterobacterales**...[69] Its disadvantage is the additional time (12h - 24h) needed to detect carbapenemase production.

Specimens should be plated on **selective media, ideally chromogenic media** for ease of use and better specificity.[50,61,64,73,84,85] Some of these media may select carbapenem-resistant isolates and not specifically carbapenemase producers and are therefore less specific and less adapted to infection control needs. It is also important to be able to **screen for all carbapenemases, including OXA-48 type**, which is currently spreading at an increasing rate.

Among available screening media for Enterobacterales, the **SuperCarba medium**[60] and the **CHROMID® Carba Smart agar**[54,83] offer excellent screening performances.

Very recently, a screening culture medium intended to screen for carbapenem resistance in *P. aeruginosa* has been developed **(SuperCP Medium in industrial development)**.[27] It is highly selective for carbapenem-resistant *P. aeruginosa* strains and is able to detect OprD-defective as well as carbapenemase-producing strains.

The **CHROMagar Acinetobacter medium** can be used for detection of carbapenem-resistant *A. baumannii* isolates, although it shows variable sensitivity and specificity.[50]

Consequently, using chromogenic culture media for the screening of carbapenem-resistant strains, followed by phenotypic confirmation (colorimetric test) is currently an appropriate screening strategy for Enterobacterales.

Figure 10: Strategy for detecting carriers of carbapenemase producers in Enterobacterales OUTSIDE an outbreak situation

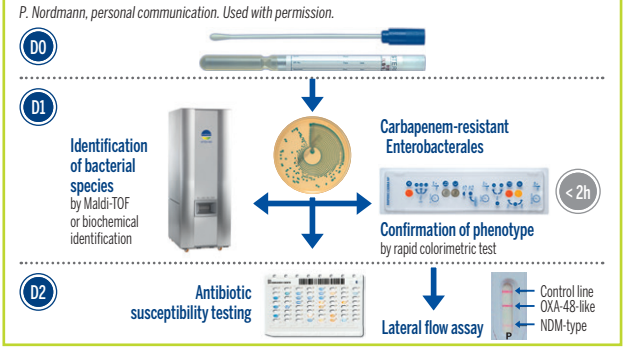
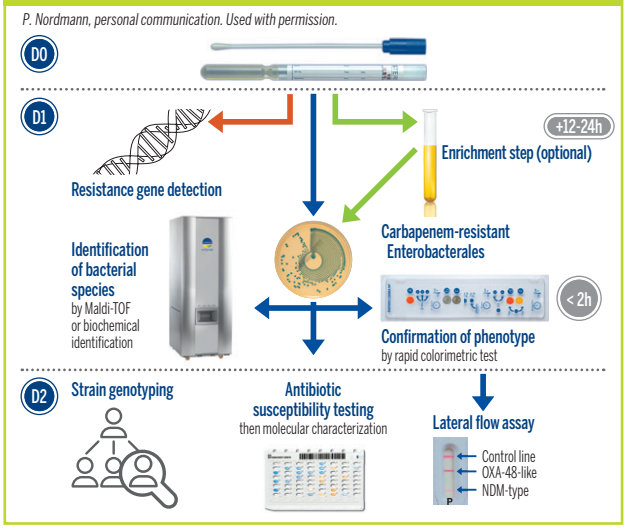


Figure 11: Strategy for detecting carriers of carbapenemase producers in Enterobacterales DURING an outbreak situation



7 INFECTION CONTROL AND PREVENTION

What infection control measures are recommended?

The implementation of screening and isolation measures is more effective if the **diagnosis of colonization is made at an early stage**.[87] Current CDC recommendations for preventing dissemination of carbapenemase producers in healthcare facilities have been published and mostly drawn from the experience of KPC outbreaks in Enterobacterales (www.cdc.gov).

These recommendations may also apply for the prevention of the spread of NDM or OXA-48 producers in Enterobacterales, since **person-to-person transmission** through the hands of nursing and medical staff is the main route of dissemination of these resistant bacteria. The role of the contaminated environment is probably less important.

Core prevention measures are based on standard precautions (hand hygiene) as well as contact precautions that apply to any multidrug-resistant bacteria.[87]

Contact precautions aim to prevent transmission **by minimizing the contamination of healthcare professionals** in contact with the patient or the patient's environment.

Adherence to contact precautions requires:

- **Appropriate use of gown and gloves** by healthcare staff for all interactions involving contact with the patient or the patient's environment.
- **Isolation of carrier patients** in single-patient rooms, or if not available, then cohorting of patients with the same carbapenemase producers.
- **Individual patient use** of non-critical medical equipment or disposable medical items (e.g., blood pressure cuffs, disposable stethoscopes).

In short-stay acute care hospitals or long-term hospitalization units, patients colonized or infected with carbapenemase producers should be placed on **contact precautions**.

INFECTION CONTROL AND PREVENTION

In long-term care settings (e.g., skilled nursing facilities, nursing homes), the use of contact precautions for residents is more complex and requires consideration of the potential impact of these interventions on their well-being and rehabilitation potential.[87]

In both acute and long-term care facilities

- To facilitate prompt implementation of contact precautions, **computerized surveillance** should be in place to identify patients with a history of colonization or infection by a carbapenemase producer on readmission.
- In addition to placing carbapenemase producer-colonized or -infected patients in **single-patient rooms**, **cohorting patients** together in the same ward should be considered.
- If feasible, there should be **dedicated staff** to exclusively care for patients with carbapenemase producers and therefore minimize the risk of transmission.

Similar recommendations can be applied to carbapenem-resistant Enterobacterales, *P. aeruginosa* and *A. baumannii*.[69,87]

The role of chlorhexidine bathing to interrupt transmission of carbapenemase producers is not established. Similarly, decontamination of the gut flora for carbapenemase producers remains unvalidated.

Although it is logical that decreased carbapenem consumption may lead to a decrease in the selection of carbapenem-resistant bacteria, stewardship of the usage of other broad-spectrum antibiotics may equally play a significant role in decreasing the selection pressure.[87]

SIX CORE MEASURES FOR PREVENTION OF CARBAPENEM-RESISTANT ENTEROBACTERALES IN ACUTE AND LONG-TERM CARE FACILITIES

1. Hand Hygiene
2. Contact Precautions
3. Patient and staff cohorting
4. Minimize use of invasive devices
5. Promote antimicrobial stewardship
6. Screening

For more information:
CDC 2015 CRE Toolkit update: https://www.cdc.gov/hai/pdfs/cre/CRE-guidance-508.pdf

CONCLUSION

Although rarely reported two decades ago, carbapenem-resistant Gram-negative bacilli are increasingly identified worldwide and now identified daily in routine microbiology. The future threat is the **evolution of these Gram-negative organisms from multiple resistance to pan-drug resistance**.

A well-demonstrated relationship between **antibiotic resistance and increased mortality** due to infection has been established.[9] Furthermore, aging populations, the development of intensive care, organ transplantations and anti-cancer treatments, as well as the extensive use of broad-spectrum antibiotics, are all factors leading to an increased number of immunosuppressed patients, who are ideal targets for infections due to carbapenem-resistant pathogens.[62]

These pathogens are now evolving from the status of strictly hospital-acquired to that of **community-acquired bacteria**. Taking in account the size of the reservoir of carbapenem-resistant bacteria and their worldwide location, **reversion of carbapenemase-resistant to susceptible isolates will likely not occur**, at least in Enterobacterales.

It is therefore essential to screen both carriers and infected patients with carbapenem-resistant bacteria.

This is the only way to **preserve the efficacy of the last resort antibiotics**, as well as the efficacy of novel marketed antibiotics, which remain rare.

LIST OF ABBREVIATIONS AND ACRONYMS

CDC	Centers for Disease Control and Prevention
CIM	Carbapenem inactivation method
CRE	Carbapenem-resistant Enterobacterales
CLSI	Clinical Laboratory Standards Institute
EDTA	Ethylene diamine tetra-acetic acid
ESBL	Extended spectrum ß-lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
GES	Guiana extended spectrum ß-lactamase
GIM	German imipenemase
ICU	Intensive care unit
IMI	Imipenemase
IMP	Imipenemase of IMP group
KPC	*Klebsiella pneumoniae* carbapenemase
KHM-1	Kyorin Health Science MBL-1
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-of-Flight
MBL	Metallo-beta-lactamase
MIC	Minimum inhibitory concentration
NDM	New Delhi metallo-ß-lactamase
NMC	Non-metallo-carbapenemase
OXA-48-like	Oxacillinase of type OXA-48
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
SIM	Seoul imipenemase
SME	*Serratia marcescens* enzyme
SPM	Sao Paulo metallo-ß-lactamase
VIM	Verona integron-encoded metallo-ß-lactamase
WHO	World Health Organization

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