BIOMÉRIEUX

CARBAPENEM RESISTANCE

From diagnosis to outbreak management

PIONEERING DIAGNOSTICS

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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 *Enterobacteriaceae*, the latter being the most important pathogens for mankind. Carbapenems are last resort antibiotics for treating infections due to these Gram-negative bacilli [31].

Resistance to carbapenems in these species is related either to combined mechanisms of resistance (overexpression of broad-spectrum ß-lactamases together with efflux pumps, impermeability) or expression of carbapenem-hydrolyzing ß-lactamases, known as carbapenemases [31].

In *Enterobacteriaceae*, carbapenemases represent the most important mechanism of resistance, since the carbapenemase genes are mostly plasmid-encoded, associated with multi- or pan-drug resistance and are highly transferable, at least within the enterobacterial species, making them potentially responsible for outbreaks [31, 36, 38].

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

Prof. Patrice Nordmann ■ **Dr. Laurent Poirel**

Emerging Antibiotic Resistance Unit, Medical and Molecular Microbiology,
INSERM European Laboratory (French National Institute for Health and Medical Research),
and National Reference Center for Emerging Antibiotic Resistance (Switzerland),
Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland

1 CARBAPENEM RESISTANCE

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

Carbapenem resistance in *Enterobacteriaceae* is related:

- either to a combination of decreased outer-membrane permeability with overexpression of ß-lactamases possessing limited carbapenemase activity (cephalosporinase [AmpC] or clavulanic-acid inhibited extended-spectrum ß-lactamase (ESBLs, mostly CTX-M)
- or to expression of true carbapenemases.

Non-carbapenemase related mechanisms of carbapenem resistance are not transferable [31, 36, 38]. In addition, if the resistance mechanism involves porin deficiency, this could significantly impact bacterial fitness, contributing to a decreased rate of transmission. These properties may explain why carbapenem-resistant isolates that do not produce carbapenemases are considered to be **less of a threat to public health than carbapenemase producers** [31]. Non-carbapenemase related mechanisms of carbapenem resistance are prevalent in enterobacterial species that naturally produce a cephalosporinase, such as *Enterobacter* sp. [31].

Carbapenemase related mechanisms of carbapenem resistance, on the other hand, are mostly plasmid-encoded, making them **highly transferable**, at least within the enterobacterial species, and therefore potentially responsible for outbreaks. They are also largely **associated with multi- or pan-drug resistance** [31, 36, 38].

CARBAPENEM RESISTANCE	
Carbapenem impermeability	Not transferable → LOW RISK of transmission between patients
Carbapenemase	Transferable through plasmid → HIGH RISK of transmission between patients

The carbapenemases encountered among *Enterobacteriaceae* differ from ESBLs in that they hydrolyze carbapenems efficiently [36]. In most cases, the protein structure of the carbapenemases differs significantly from that of ESBLs with the notable exception of several GES and OXA-48-type ß-lactamases which may have point-mutant analogues with ESBL activity [31, 36, 39].

Currently, the spread of carbapenemase producers is the most important clinical issue in antibiotic resistance in Gram negatives, particularly in *Enterobacteriaceae* [49].

Carbapenemases belong to one of the three groups of ß-lactamases, namely **Ambler class A, B, and D groups** [36]. Differences between these carbapenemase enzymes is clinically significant, since their hydrolysis profile differs **(Figure 1)**. Their species distribution and worldwide epidemiology is also different [31, 36].

→ Ambler class A ß-lactamases: penicillinases

This group includes "clavulanic-acid inhibited penicillinases". The most widespread representative is KPC (*Klebsiella pneumoniae* carbapenemase) [6, 29], but others have been identified, such as SME, NMC, IMI, GES... [36] These enzymes have a broad-spectrum activity similar to that of ESBLs, with an extended activity to carbapenems. Their activity is inhibited *in vitro* by clinically available ß-lactamase inhibitors such as clavulanic acid, tazobactam, and avibactam, in association with ceftazidime or aztreonam.

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

The second group is that of the metallo-ß-lactamases (MBLs), including IMP, VIM and NDM ß-lactamases [5, 35, 54]. MBLs hydrolyze all ß-lactams except aztreonam.

→ Ambler class D ß-lactamases: oxacillinases

The third group comprises several (but not all!) oxacillinase OXA-48 derivatives [42, 43]. 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, but are inhibited by avibactam.

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

In ***Pseudomonas aeruginosa***, resistance to carbapenems is mostly due to impermeability to imipenem, associated with qualitative or quantitative changes of the porin OprD2 [28]. Overexpression of the MexXY-OprM porin may lead to decreased susceptibility to meropenem. However, carbapenemases have been also reported in *P. aeruginosa*. They are mostly MBLs (VIM, IMP) [5].

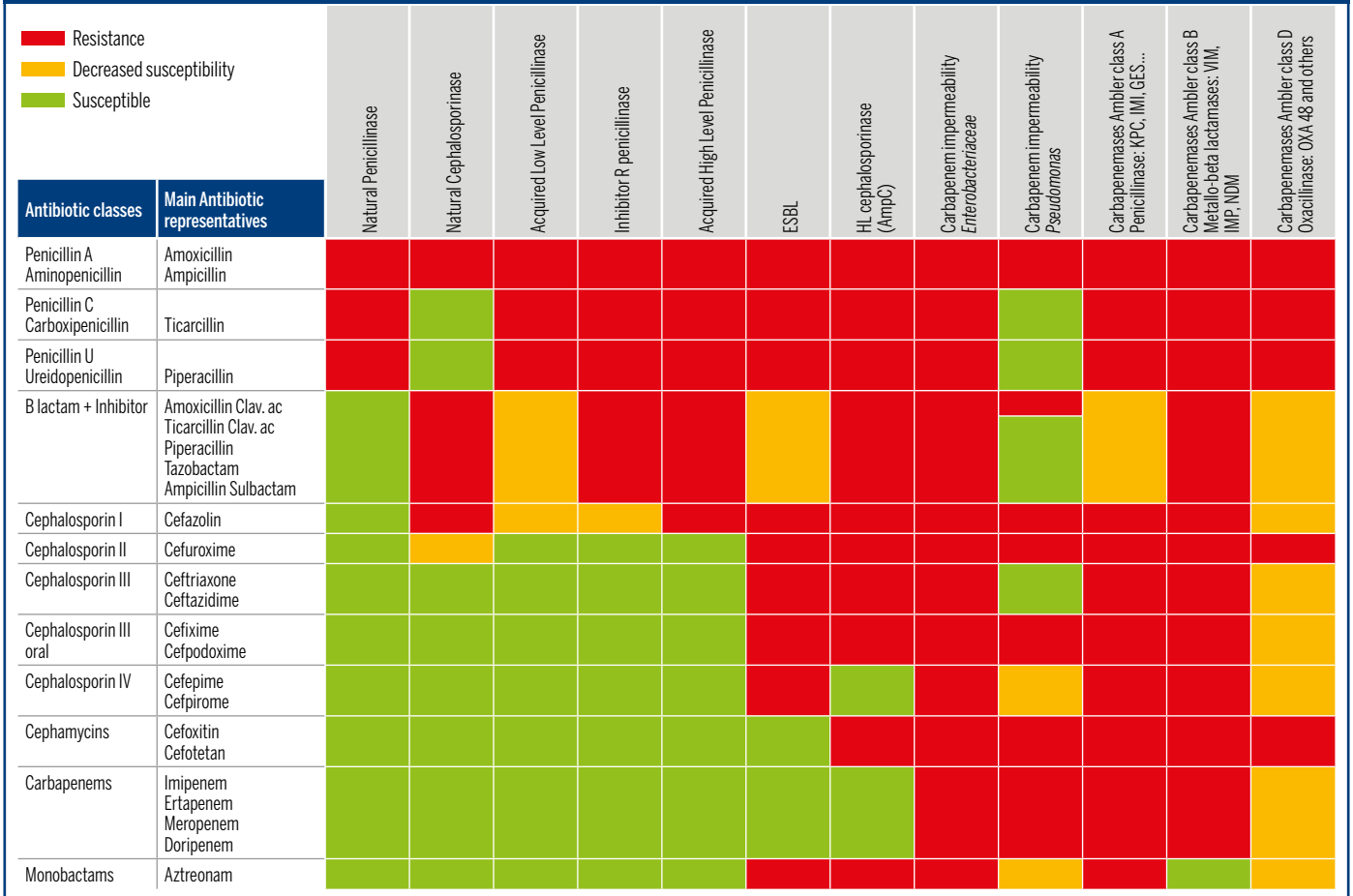
In the healthcare-associated pathogen *Acinetobacter baumannii*, resistance to carbapenems is also extensively observed and is associated with different types of carbapenemases such as those identified in *Enterobacteriaceae* (NDM, IMP, VIM) [2]. Several carbapenemases in the Ambler class D are specific to *A. baumannii*: OXA-23, OXA-40 and OXA-58 derivatives (but not OXA-48 derivatives !) [42].

CARBAPENEM RESISTANCE

These enzymes hydrolyze carbapenems at a low level and are not inhibited by commercially-available ß-lactamase inhibitors [42]. Most, if not all, carbapenem-resistant *A. baumannii* strains produce at least one carbapenemase which is often associated with a permeability defect and/or overexpression of efflux pumps [2].

	CARBAPENEMASE	CARBAPENEM IMPERMEABILITY
Enterobacteriaceae	+++	++
P. aeruginosa	+	+++
A. baumannii	Frequently both simultaneously	

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 impermeability

Carbapenem-resistant enterobacterial isolates that do not produce a carbapenemase are mostly *K. pneumoniae* and *Enterobacter* sp. They usually express decreased outer-membrane permeability associated with a CTX-M-type enzyme or overexpression of a cephalosporinase, respectively. Although epidemiological data for these carbapenem-resistant isolates is limited, the prevalence rate appears to vary quite significantly from one country to another (1-40%) [31, 38].

CARBAPENEM IMPERMEABILITY	
K. pneumoniae	Impermeability and CTX-Ms
Enterobacter sp.	Impermeability and cephalosporinases (AmpC)

B Spread of carbapenemase producers

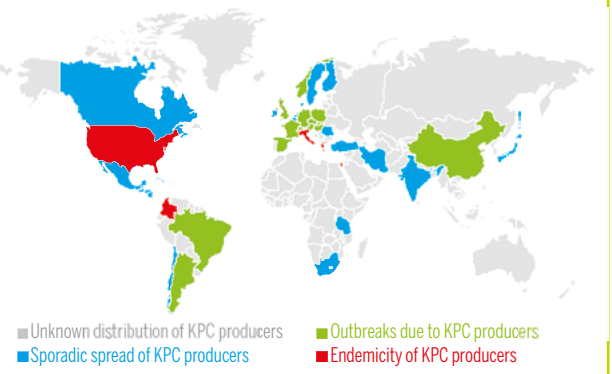
Data on the worldwide distribution of carbapenemase producers in *Enterobacteriaceae* are more well-known.

→ Class A: penicillinases

KPC enzymes are currently the most clinically-significant enzymes among the class A carbapenemases worldwide [29, 32].

The **first KPC producer** (a KPC-2-positive *K. pneumoniae*) was identified in 1996 on the **Eastern coast of the USA** [51]. Within a few years, KPC producers were identified in almost all US states where they are now quite prevalent [29]. They spread worldwide and have been identified in many Gram-negative species, even though KPC enzymes are still mostly identified in *K. pneumoniae* **(Figure 2)** [6, 29, 32].

Figure 2: Geographical distribution of KPC producers



Adapted from Nordmann P, Poirel L. Clin Microbiol Infect. 2014;20(9):821-3

In Latin America, KPC producers are endemic in some areas, such as Colombia and Argentina [25]. **In Europe**, KPC producers are found almost everywhere, most often linked to imports from endemic areas [29]. Greece and Italy are endemic areas in Europe. **In Israel**, the endemicity of KPC producers has been demonstrated with numerous healthcare-associated reports but also, noticeably, some community-acquired cases **(Figure 2)**.

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**, very few reports on KPC-producing isolates exist, the most commonly identified carbapenemases being NDM and OXA-48-like enzymes (see below).

One specific KPC-2- or KPC-3-producing *K. pneumoniae* clone (ST 258) has been extensively identified worldwide [6].

Although NmcA was the very first sequenced carbapenemase identified in *Enterobacteriaceae* in the 1990's [30], 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** [36].

KPC	
K. pneumoniae	+++
Enterobacter sp.	+
Other *Enterobacteriaceae*	rare
P. aeruginosa	rare

Class B: metallo-beta lactamases

MBLs are known to be intrinsic in many environmental and opportunistic bacterial species. However, since the early 1990's, they have also been identified as acquired enzymes, either in *Pseudomonas* or in *Enterobacteriaceae* [5, 20, 41, 53].

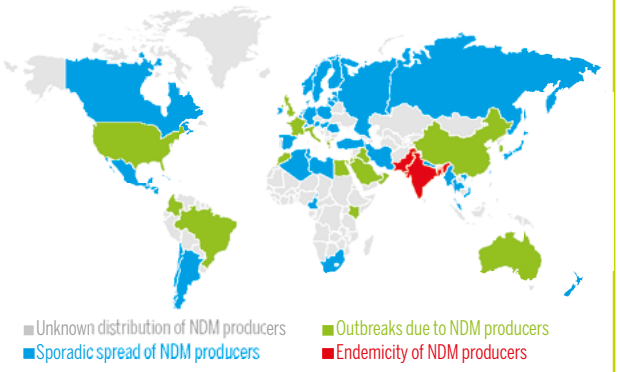
The most common MBLs identified in *Enterobacteriaceae* include the VIM- and IMP- groups, together with the emerging NDM group, whereas others, such as GIM-1, SIM-1, SPM-1 or KHM-1, remain sporadic [4, 25, 35, 46].

Although reported worldwide, the **VIM producers** in *Enterobacteriaceae* are highly prevalent in **Southern Europe and the Mediterranean region**, whereas the **IMP producers** remain mostly located in **Asia** [5, 35, 51].

One of the most clinically-significant carbapenemases is NDM-1 (New Delhi metallo-ß-lactamase) identified coincidentally in 2009 in *K. pneumoniae* and *E. coli* isolates from a patient in Sweden previously hospitalized in India [22, 35]. The main identified reservoir of **NDM-producing *Enterobacteriaceae*** is the **Indian subcontinent (Pakistan, India, Sri Lanka) (Figure 3)** [12, 35]. These countries are experiencing multiple on-going outbreaks of different NDM producers [39]. The spread of NDM producers has been not only extensively identified among patients from the Indian subcontinent but also from its soil [54]. The prevalence of carriage in this region is estimated at 5 to 15 % [7, 37].

Significant spread of NDM producers has also been identified in the **United Kingdom (UK)** due to its close connections with India and Pakistan [21, 35]. Subsequently, NDM producers in *Enterobacteriaceae* have been reported almost worldwide, including many countries in Asia, Africa, Australia, America, and Europe **(Figure 3)** [3].

Figure 3: Geographical distribution of NDM producers



Adapted from Nordmann P, Poirel L. Clin Microbiol Infect. 2014;20(9):821-3

Another particularly important source of NDM producers (or established **secondary reservoir**) is made up of the **Balkan states**, the **Arabic peninsula** and **North Africa (Figure 3)** [33, 35].

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

Class D: oxacillinases

The **first identified OXA-48 producer** was a *K. pneumoniae* isolate recovered from **Turkey** in 2003 [40]. 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, 43].

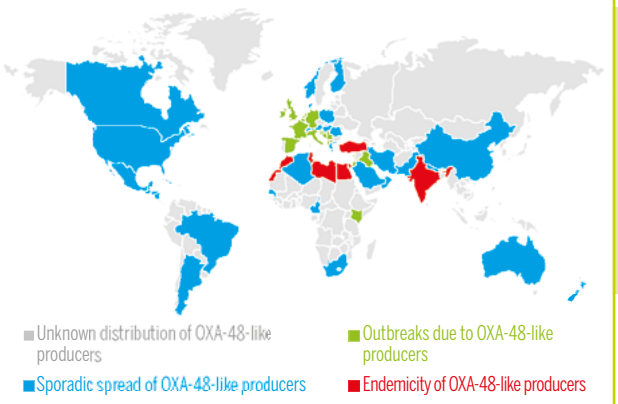
In Europe, it is becoming the most prevalent carbapenemase in many countries such as **France** and the **UK**.

OXA-48 producers are currently **rarely identified** in **North and South America (Figure 4)** [23, 43].

Interestingly, an atypical OXA-48-like enzyme, **OXA-163**, has been identified from enterobacterial isolates recovered in **Argentina** and **Egypt** [43]. OXA-163 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 [43].

OXACILLINASES WITH CARBAPENEMASE ACTIVITY	
Enterobacteriaceae: *K. pneumoniae*	OXA-48+++,
A. baumannii	OXA-23+++

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



Adapted from Nordmann P, Poirel L. Clin Microbiol Infect. 2014;20(9):821-3

In *P. aeruginosa*, the most important carbapenem resistance mechanism is quantitative or qualitative modification of the OprD2 porin [28]. The prevalence rate of this resistance trait is stable at least in **Europe** ranging from 15 to 20% [28]. **KPC and MBLs** have been reported in *P. aeruginosa*, although the diffusion rate for KPC producers in *P. aeruginosa* is not well-known [33]. 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**. NDM producers in *P. aeruginosa* remain rare [5].

In *A. baumannii*, the main resistance mechanism is production of carbapenem-hydrolyzing ß-lactamases. **OXA-23 producers** are identified **worldwide** while OXA-40 and OXA-58 producers are less widely distributed [2, 42]. The structure of these oxacillinases is significantly different from OXA-48 enzymes from *Enterobacteriacae* [42]. KPC and MBL producers have been also identified. 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** [2].

3 CLINICAL ASPECTS

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

Infections caused by carbapenem-resistant enterobacterial isolates include **urinary tract infections, peritonitis, septicemia, pulmonary infections, soft tissue infections and device-associated infections** [15, 49]. There is no gender preference and most of the cases are adults [15, 49].

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

Both **hospital- and community-acquired infections** have been reported. No specific clinical manifestations have been associated to carbapenemase producers as compared to wild-type susceptible strains [15, 49].

All types of carbapenemase-producing enterobacterial species are involved in infections, but ***K. pneumoniae*** and ***E. coli*** are the main sources of **hospital- and community-acquired infections**, respectively.

SOURCES OF HOSPITAL- AND COMMUNITY-ACQUIRED INFECTIONS [36]	
KPC, IMP, VIM	Hospital-acquired infections
OXA-48, NDM	Hospital- and community-acquired infections

Carbapenem-resistant enterobacterial isolates which are not carbapenemase producers have been identified as a source of hospital-acquired infections (mostly *K. pneumoniae* and *Enterobacter* sp.) [36].

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, urinary tract infections**.

No specific virulence factors seem to be associated with carbapenemase producers.

MAIN TYPES OF INFECTION [15, 49]		
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 also 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 *Enterobacteriaceae* [13, 14, 15]. **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 [14, 49]. An increased attributable mortality has been shown for infections due to carbapenemase producers compared to that due to susceptible strains [15].

The **choice of the optimal antibiotic therapy** is largely based on the detailed analysis of the **antibiotic susceptibility testing results**. In many cases, the antibiotic choice remains limited to **colistin,** parenteral **fosfomycin, gentamicin, amikacin and tigecycline** [14, 27, 45, 55]. The **infection site** and the **diffusion of the antibiotics** at the infected site are also factors to consider for optimal antibiotic choice. **Antibiotics should not be used in monotherapy** to treat carbapenemase producers in order to **prevent further selection of antibiotic resistance** and, theoretically, **improve clinical efficacy**.

→ Treating infections due to carbapenemase producers in *Enterobacteriaceae*

It has recently been proposed that carbapenems, provided they exhibit low MIC values, may be administered for treating carbapenemase producers at a high dosage and prolonged infusion regimen and preferably in association with an aminoglycoside or colistin [14, 27].

However, most of the current recommendations are based on studies performed with KPC and VIM producers and not with OXA-48 and NDM producers. Furthermore, around 20% of OXA-48 producers do not produce an ESBL and may remain susceptible to extended-spectrum cephalosporins [9].

→ Treating infections due to imipenem-resistant *P. aeruginosa* isolates with OprD2 modification

Treatment alternatives may include **broad-spectrum cephalosporins**, **aminoglycosides** and **fluoroquinolones** - antibiotics to which many strains remain susceptible. A **combination of antibiotics** should be preferred to monotherapy, although recently debated [28, 52]. No study has yet reported on the evaluation of treatments of infections due to carbapenemase-producing *P. aeruginosa*. The choice of the best antibiotic combination should be based on analysis of the antibiotic susceptibility testing results. **Meropenem**, **colistin** and parenteral **fosfomycin**, or parenteral **rifampicin** may be included in the antibiotic combination, provided that *P. aeruginosa* is naturally resistant to tigecycline [48, 52].

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

Tigecycline and **colistin** have been proposed, but the optimal antibiotic treatment for these infections remained unknown [2, 44, 48].

POSSIBLE ASSOCIATION OF ANTIBIOTICS, DEPENDING ON SUSCEPTIBILITY TEST RESULTS AND MIC DETERMINATION	
Enterobacteriaceae	• Colistin, parenteral fosfomycin, gentamicin, and tigecycline in bi- or tri-therapy • Carbapenem (if low MIC), at high dosage and prolonged infusion + aminoglycoside or colistin
P. aeruginosa	• **Impermeability**: broad-spectrum cephalosporins, aminoglycosides or fluoroquinolones • **Carbapenemase**: meropenem, colistin, parenteral fosfomycin or rifampicin
A. baumannii	• Tigecycline and colistin

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 [24].

Detection of carbapenemase-producing isolates in clinical specimens is first based on a careful analysis of susceptibility testing results. Recently, both the **CLSI (US) and EUCAST (Europe) breakpoints** for carbapenems have been **lowered significantly** to allow **better detection of carbapenem-resistant isolates** (www.clsi.org; www.eucast.org).

Screening cut-off values for carbapenemase producers are advocated by the EUCAST **(Figure 5)** and **meropenem** has been proposed as the indicator antibiotic with the best sensitivity/specificity ratio [26].

Figure 5: Breakpoints, MIC values and screening cut-off values of carbapenems for *Enterobacteriaceae* and *A. baumannii*, as updated in 2014.

	BREAKPOINTS (mg/L)				SCREENING CUT-OFF
	EUCAST		CLSI®		EUCAST
	R >	S ≤	R ≥	S ≤	
Imipenem	8	2	4	1	> 1
Meropenem	8	2	4	1	> 0.12
Ertapenem	1	0.5	2	0.5	> 0.12
Doripenem	2	1	4	1	

P. Nordmann, personal communication, adapted from Wayne, PA. M100-S24, CLSI, 2014 / www.eucast.org

Why search for carbapenemase activity rather than carbapenem resistance?

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

- As they are mostly plasmid-located, particularly in *Enterobacteriaceae*, they are **more easily spread** [8].
- All three main types of carbapenemase genes, namely the ***bla*KPC**, the ***bla*NDM** and ***bla*OXA-48** genes have the ability to **spread within enterobacterial species**.
- The ***bla*KPC** and the ***bla*NDM** genes have been identified in *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*, showing their ability to **cross the species barrier**.
- **Carbapenemase producers** are also associated with **other** structurally-unrelated **resistance traits**.

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

In contrast, **resistance due to impermeability** 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 impermeability could **revert to susceptibility when antibiotic selection pressure stops**, while this is not the case for carbapenemases.

How to detect carbapenemase producers as infectious agents?

Any suspicion of carbapenemase activity should be based on the analysis of the **antibiotic susceptibility results** [33]. In a clinical laboratory, detection 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 [19, 33]. This technique requires the development and the validation of a specific protocol, a period of incubation time (3 to 5 h), additional centrifugation steps, a MALDI-TOF instrument and trained personnel [19].

➔ Rapid colorimetric detection of a pH change (0.5 to 1.5 hours)

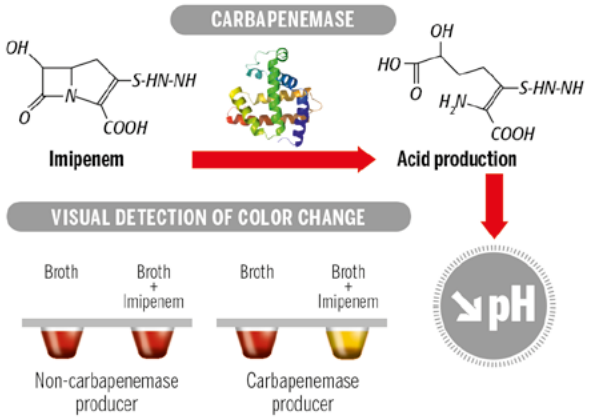
(RAPIDEC® CARBA NP or "lab-developed" Carba NP test)

This test is based on detection of hydrolysis of the ß-lactam ring of a carbapenem molecule (imipenem). Hydrolysis 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 strip. **(Figure 6)**.

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 [10, 34]. These techniques detect **carbapenemase activity in *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*** [10, 34].

The detection of *in vivo* production of a carbapenemase using the Modified-Hodge test has been used for years [49]. This method should now be abandoned since it is both time-consuming (results obtained within 72 h) and lacks specificity and sensitivity.

Figure 6: The principle of colorimetric detection of carbapenemase activity



How to identify the carbapenemase type?

Determination of the exact carbapenemase type (gene identification) is currently required in two clinical situations.

- **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

➔ 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) [19, 26]. **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. 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 [19, 26, 53]. The sensitivity of MBL detection has been improved by supplementing the culture media with zinc. Phenotypic detection of MBLs is reliable when dealing with *Enterobacteriaceae* 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 *Enterobacteriaceae* or in *A. baumannii* since the enzymatic activity of OXA-type carbapenemase is not inhibited by clavulanic acid, tazobactam, sulbactam or zinc chelators.

High level resistance to temocillin and piperacillin-tazobactam in *Enterobacteriaceae* exhibiting resistance or reduced susceptibility to a carbapenem may be predictive of the production of OXA-48 carbapenemases.

Preliminary identification of carbapenemase production (Ambler class A, B, D) can be made rapidly by using the RAPIDEC® CARBA NP test or the "lab-developed" Carba NP test [11].

DIAGNOSIS

Molecular characterization of carbapenemase genes [26, 27, 33]

Molecular techniques are mainly based on **PCR technology** and may be followed by sequencing of the entire coding region **(Figure 7)**. PCR-based methods include simplex, multiplex and real-time assays. Hybridization and microarrays may also be used.

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 expression in clinically-relevant bacterial species has not yet been assessed.

The main disadvantages of molecular techniques as screening techniques are their cost, expensive equipment, and for some techniques, the need for trained microbiologists [33].

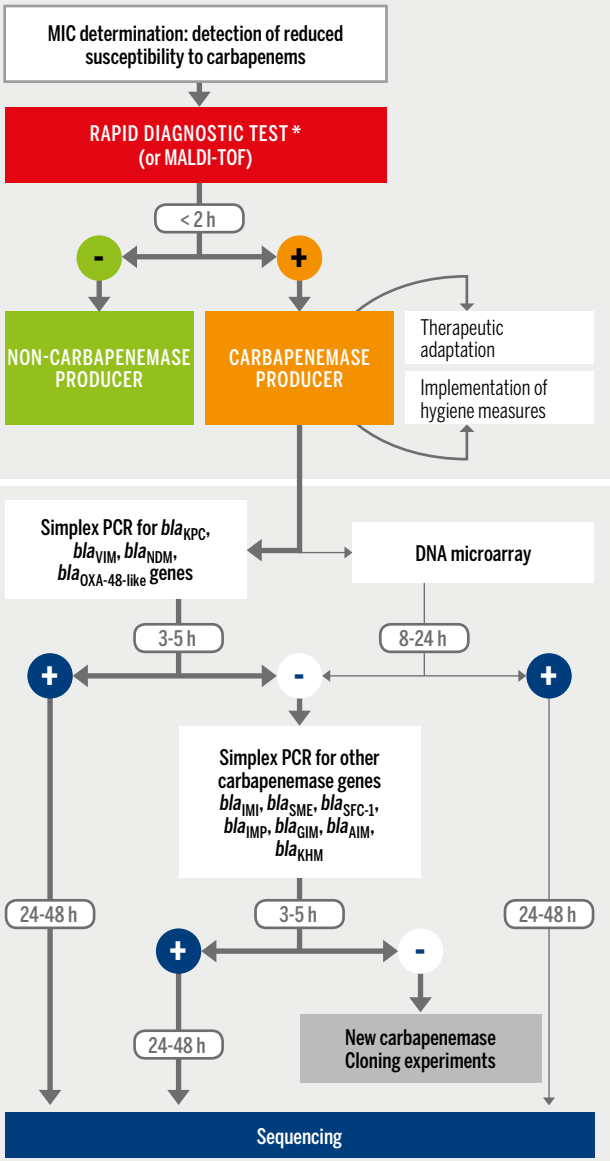
In addition, sequencing of the entire gene may be needed for several carbapenemase genes, such as the OXA-48 derivatives, in order to differentiate for example OXA-163 - which is a true ESBL without significant carbapenemase activity - from OXA-48, which is a true carbapenemase [43].

Finally, totally novel emerging carbapenemase genes may remain undetected by commercially-available molecular based techniques which only screen known genes.

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)**.

Figure 7: Strategy for detecting and identifying carbapenemase producers from cultured *Enterobacteriaceae*



Adapted from Dortet L, et al. Antimicrob Agents Chemother. 2014;58(4):2441-5.

* 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 *Enterobacteriaceae* [1, 33, 47]**:**

- **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 any foreign country 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 ICUs, in transplant units and immuno-compromised patients may be recommended [1, 33, 47].

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** (P. Nordmann, L. Poirel, personal communication).

Screening of non-carbapenemase related carbapenem-resistant Gram-negative bacilli: no specific recommendations are known, 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 (*Enterobacteriaceae, 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 ***Enterobacteriaceae*, rectal swabs and stools** are the most suitable clinical samples for performing screening of carbapenemase producers and carbapenem-resistant isolates **(Figure 8)**. In the case of ***P. aeruginosa*, environmental screening** may be also useful since water-borne sources of outbreak are often identified. In the case of ***A. baumannii***, additional **skin or nasal swabs samples** may be useful for detection of carbapenem-resistant isolates [49].

Direct identification of carbapenemases from clinical specimens

→ Molecular methods

Direct identification of several carbapenemase genes using molecular-based techniques is possible (see page 19). Currently, molecular techniques are most recommended in an outbreak situation due to their cost **(Figure 9)**. 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 **(Figures 8, 9)**.

→ 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.

→ 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.

This **enrichment step** is particularly recommended **during an outbreak situation (Figure 9)** [1, 49]. 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 *Enterobacteriaceae*** [1].

Its disadvantage is the additional time (12h - 24h) needed to detect carbapenemase production. The efficiency of this enrichment step has not been evaluated for NDM and OXA-48 type producers in *Enterobacteriaceae*, nor for carbapenem-resistant *P. aeruginosa* and *A. baumannii* isolates.

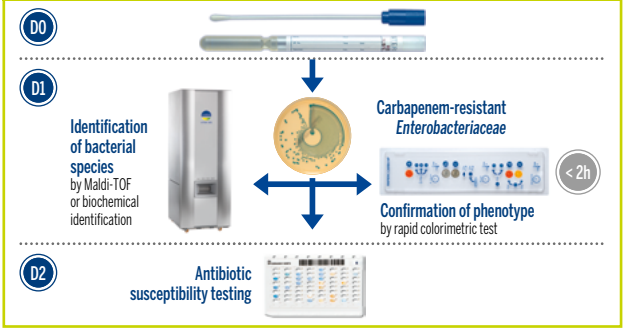
Specimens should be plated on **selective media, ideally chromogenic media** for ease of use and better specificity [16, 17, 19, 26, 33].

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 [16, 17, 19, 26, 33].

Consequently, using chromogenic culture media for the screening of carbapenemases, followed by phenotypic confirmation (colorimetric test) is currently the best screening strategy for *Enterobacteriaceae*.

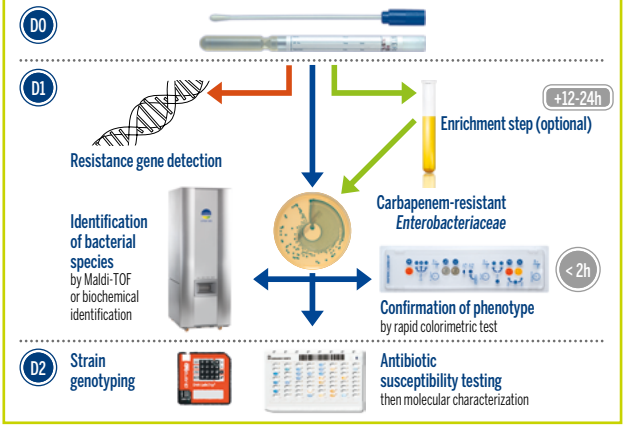
To date, none of the screening media have been evaluated comparatively for detection of carbapenemase producers or carbapenem-resistant *P. aeruginosa* and *A. baumannii* isolates.

Figure 8: Strategy for detecting carriers of carbapenemase producers in *Enterobacteriaceae* OUTSIDE an outbreak situation



P. Nordmann, personal communication

Figure 9: Strategy for detecting carriers of carbapenemase producers in *Enterobacteriaceae* DURING an outbreak situation



P. Nordmann, personal communication

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**. 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 *Enterobacteriaceae* (www.cdc.gov).

These recommendations may also apply for the prevention of the spread of NDM or OXA-48 producers in *Enterobacteriaceae*, 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 [47].

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 [47].

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 *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii* [49].

The role of chlorhexidine bathing to interrupt transmission of carba-penemase producers is not established. Similarly, decontamination of the gut flora for carbapenemase producers remains highly debatable.

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 [14].

SIX CORE MEASURES FOR PREVENTION OF CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* 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 2012 CRE Toolkit: http://www.cdc.gov/hai/organisms/cre/cre-toolkit/

CONCLUSION

Although rarely reported a decade ago, carbapenem-resistant Gram-negative bacilli are increasingly identified worldwide. 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 [14]. 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 [33].

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

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, carbapenems**, and the only option while waiting for novel marketed broad-spectrum antibiotics.

LIST OF ABBREVIATIONS

CDC	Center for Diseases Control and Prevention
CLSI	Clinical Laboratory Standards Institute
EDTA	Ethylene Diamine Tetraacetic Acid
ESBL	Extended Spectrum Beta-Lactamases
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IMP	Imipenemase
KPC	*Klebsiella Pneumoniae* Carbapenemase
MALDI-TOF	Matrix Assisted Laser Desorption Ionization Time-of-Flight
MBL	Metallo-Beta-Lactamase
MIC	Minimum Inhibitory Concentration
NDM	New Delhi Metallo-ß-lactamase
OXA-48	Oxacillinase of type OXA-48
PCR	Polymerase Chain Reaction
VIM	Verona Integron-encoded Metallo-ß-lactamase
WHO	World Health Organisation

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bioMérieux S.A. • 69280 Marcy l'Étoile • France
Tel.: + 33 (0)4 78 87 20 00 • Fax: +33 (0)4 78 87 20 90
www.biomerieux.com