Review

Carbapenem resistance in *Enterobacteriaceae*: here is the storm!

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The current worldwide emergence of resistance to the powerful antibiotic carbapenem in *Enterobacteriaceae* constitutes an important growing public health threat. Sporadic outbreaks or endemic situations with enterobacterial isolates not susceptible to carbapenems are now reported not only in hospital settings but also in the community. Acquired class A (KPC), class B (IMP, VIM, NDM), or class D (OXA-48, OXA-181) carbapenemases, are the most important determinants sustaining resistance to carbapenems. The corresponding genes are mostly plasmid-located and associated with various mobile genetic structures (insertion sequences, integrons, transposons), further enhancing their spread. This review summarizes the current knowledge on carbapenem resistance in *Enterobacteriaceae*, including activity, distribution, clinical impact, and possible novel antibiotic pathways.

Carbapenem resistance: a worsening situation

Enterobacteriaceae are rod-shaped, Gram-negative bacteria that are normal inhabitants of the intestinal flora and among the most common human pathogens, causing infections that range from cystitis to pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections. They are the most common source of both community- and hospital-acquired infections, with *Escherichia coli* being by far the most important pathogen for humans. *Enterobacteriaceae* spread easily between humans by hand carriage as well as contaminated food and water and have a propensity to acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons [1–3]. This combination is why emerging multidrug resistance in *Enterobacteriaceae* is of the utmost importance for clinical therapy.

β-Lactams are diverse antibiotic molecules that are classified according to their chemical structures. Carbapenems (imipenem, ertapenem, meropenem, and doripenem) are the latest developed molecules that possess the broadest spectrum of activity (Box 1). Since the 2000s, the spread of community-acquired *E. coli* isolates producing extended-spectrum β-lactamases (ESBLs) capable of hydrolyzing almost all antibiotic β-lactams except carbapenems has been reported worldwide [4]. The consequence of this emerging phenomenon has been an increased consumption of carbapenems. These antibacterial agents are crucial for treating life-threatening nosocomial, or hospital-acquired, infections, such as those linked with transplantations, hospitalizations in intensive care units, and surgery, and the emergence of carbapenem resistance may jeopardize or stop the development of modern techniques in medicine.

In *Enterobacteriaceae*, carbapenem resistance arises from two main mechanisms: (i) acquisition of carbapenemase genes that encode for enzymes capable of degrading carbapenems, or (ii) a decrease in the uptake of antibiotics by a qualitative or/and quantitative deficiency of porin expression in association with overexpression of β-lactamases that possess very weak affinity for carbapenems (Figure 1). Both of these mechanisms are discussed in detail below.

The most important carbapenemases are categorized as three types of enzymes: (i) the KPC type enzymes first described in the US but now found worldwide; (ii) the VIM, IMP, and NDM metallo-β-lactamases; and (iii) the OXA-48 type enzymes circulating among Mediterranean countries and progressively disseminating to other geographical areas.

It is clear that very few novel antibiotics will be launched in the next few years, making the issue of carbapenem-resistant *Enterobacteriaceae* of primary importance worldwide.

Non-carbapenemase mediated carbapenem resistance in *Enterobacteriaceae*

The outer membrane of Gram-negative bacteria forms a hydrophobic barrier that protects the cell against external agents such as heavy metals and detergents. This membrane contains specific proteins, called porins, that form hydrophilic channels to allow the selective uptake of essential nutrients and other compounds, including antibiotics (Figure 1) [5,6]. The primary porins in *Enterobacteriaceae* involved in taking up antibiotics belong to the OmpF or OmpC families [6]. Thus, any changes in the number or activity of bacterial porins might have an effect on antibiotic resistance. Mutation of a gatekeeping loop or central channel in the porin proteins, a loss of porin expression, or a shift in the types of porins found in the outer membrane can decrease sensitivity to antibiotics (Figure 1). Porin synthesis may be regulated in response to antimicrobial compounds or aromatic products via several cascades involving the *mar* and *sox* operons, with a subsequent decrease in the number of porins in the outer membrane [7].

Carbapenem resistance was first observed in enterobacterial isolates, especially among *Enterobacter* spp. (~1% of the strains) overexpressing a chromosomal *ampC*

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Keywords: antibiotic; Gram-negative bacteria; carbapenemase; β-lactamases.

  doi:10.1016/j.molmed.2012.03.003

Box 1. The β-lactam family

Mechanism of action

β-Lactams act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. The final transpeptidation step in the synthesis of the peptidoglycan is facilitated by transpeptidases known as penicillin-binding proteins (PBPs). PBPs vary in their affinity for binding penicillin or other β-lactam antibiotics, and the amount of PBPs varies among bacterial species.

β-Lactam antibiotics have structures similar to D-alanyl-D-alanine, the terminal amino acid residues on the precursor N-acetyl muramique (NAM)/N-acetyl glucosamine (NAG) peptide subunits of the nascent peptidoglycan layer. The structural similarity between β-lactam antibiotics and D-alanyl-D-alanine facilitates their binding to the active site of PBPs. The β-lactam nucleus of the molecule irreversibly binds to the Ser403 residue of the PBP active site. This irreversible inhibition of the PBPs prevents the final crosslinking (transpeptidation) of the nascent peptidoglycan layer, disrupting cell wall synthesis.

Under normal circumstances, peptidoglycan precursors signal a reorganization of the bacterial cell wall and, as a consequence, trigger the activation of autolytic cell wall hydrolases. Inhibition of crosslinkage by β-lactams causes a build-up of peptidoglycan precursors, which triggers the digestion of existing peptidoglycan by autolytic hydrolases without the production of new peptidoglycan. As a result, the bactericidal action of β-lactam antibiotics is further enhanced and eventually the structural integrity of the cell wall decreases to the point where lysis occurs.

Diversity of β-lactams

The β-lactam family of molecules is composed of four groups: penicillins, cephalosporins, monobactam, and carbapenems (Figure I).

β-Lactam resistance in *Enterobacteriaceae*

In *Enterobacteriaceae*, the main mechanism involved in β-lactams resistance is the production of enzymes, named β-lactamases, that possess hydrolytic activity against β-lactamines. Depending on their activity profile, four groups of β-lactamases could be defined:

- Penicillinases inactivate penicillins but do not degrade cephalosporins, aztreonam, or carbapenems.
- Cephalosporinases preferentially inactivate cephalosporins and aminopenicillins, but do not affect other penicillins (carboxy- and ureido-penicillins), aztreonam, and carbapenems.
- Extended-spectrum β-lactamases (ESBL) inactivate all β-lactams except carbapenems. These enzymes are inhibited by clavulanic acid.
- Carbapenemases inactivate at least carbapenems and, depending on the enzyme, other types of β-lactam molecules as well (Table 1).

β-Lactamases inhibitors that derivate from β-lactams

Three clinically used β-lactamases inhibitors derivate from the β-lactams: clavulanic acid, sulbactam, and tazobactam (Figure I). Those molecules have weak intrinsic antimicrobial activity, despite sharing the β-lactam ring that is characteristic of β-lactam antibiotics. However, the similarity in chemical structure allows the molecule to act as a suicide substrate by covalently interacting with the active sites of β-lactamases secreted by the bacteria.

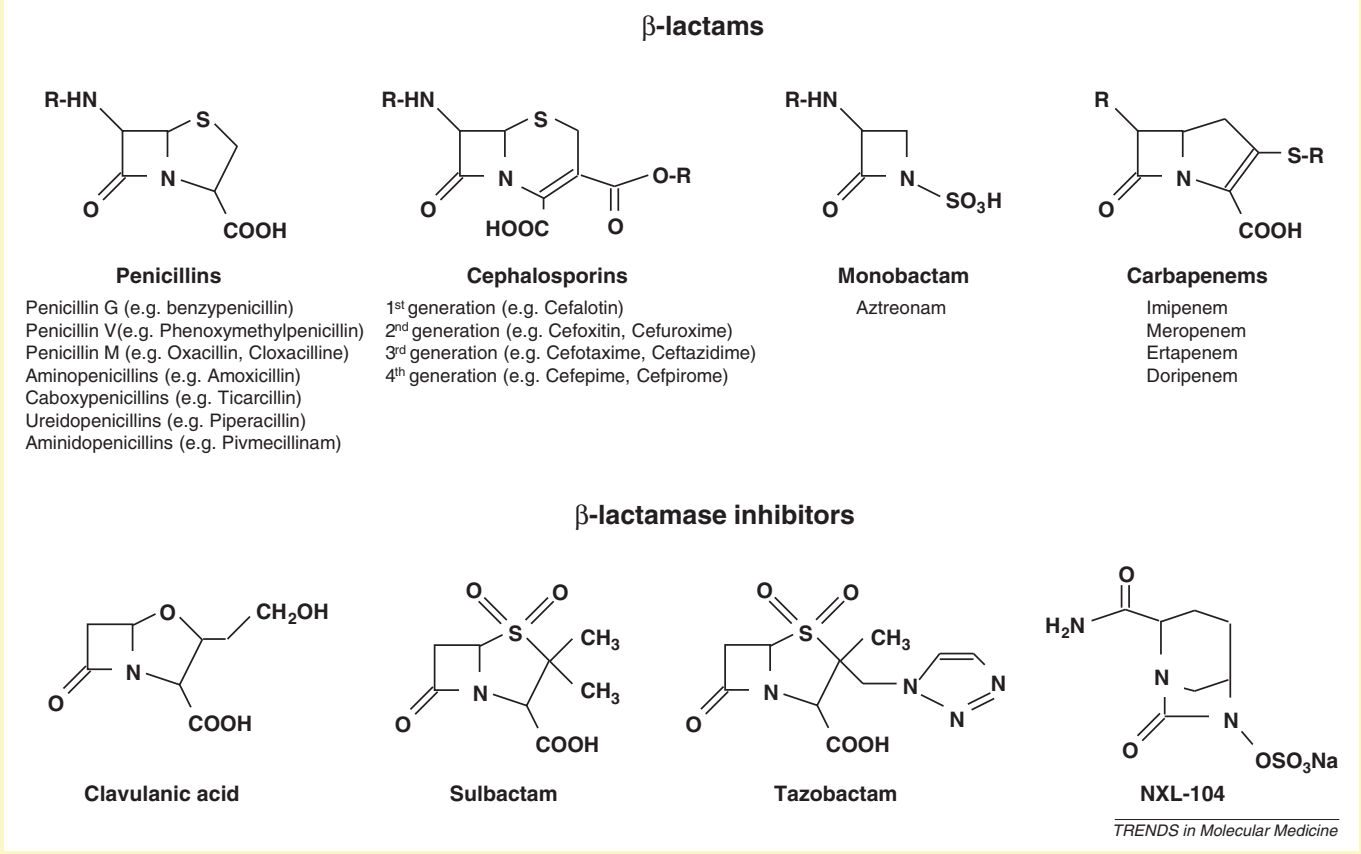

Figure I. Chemical structures of the main β-lactams and β-lactamase inhibitors that are clinically available.

gene encoding an intrinsic cephalosporinase and exhibiting modifications in their OmpC or OmpF porins [8–11]. Similar mechanisms were reported for *Serratia* sp., *Citrobacter freundii*, and *Morganella morganii*. This mechanism of carbapenem resistance has also been observed in other enterobacterial species that do not express an intrinsic cephalosporinase, such as *E. coli*, *Klebsiella pneumoniae* and *Salmonella* spp. In those cases, resistance corresponds

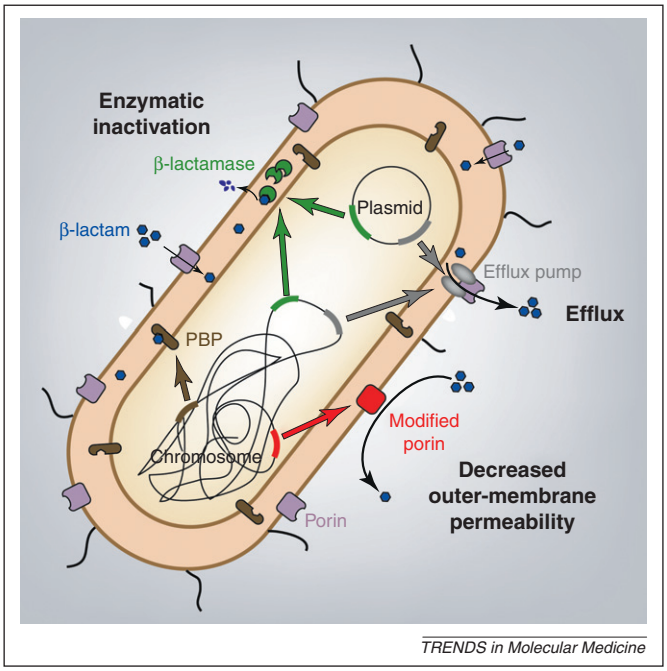

Figure 1. Primary mechanisms of β-lactam resistance in *Enterobacteriaceae*. Hydrophilic channels formed by porins allow the uptake of β-lactams through the outer membrane of the bacterium. In the periplasmic interspace, β-lactam molecules bind irreversibly to the penicillin-binding proteins (PBPs), leading to the inhibition of the peptidoglycan synthesis. Primary mechanisms of β-lactam resistance in *Enterobacteriaceae* include the following: (i) enzymatic inactivation of the antibiotic by chromosome- and/or plasmid-encoded enzymes possessing hydrolytic activity against β-lactam molecules; (ii) decreased outer membrane permeability through production of modified porins, loss of porin expression, or a shift in the types of porins found in the outer membrane; and (iii) efflux of the antibiotic to the outside of the bacterium through production of an efflux pump.

to a combination of plasmid-encoded AmpC expression together with decreased cell membrane permeability owing to modifications in OmpK35/36 for *K. pneumoniae*, in OmpF and OmpC for *E. coli*, and in OmpF for *Salmonella typhimurium* [12–14]. In those cases, strains are nosocomial- or community-acquired (human and animals isolates) and the plasmid-encoded AmpC enzymes are diverse (e.g. CMY-2, DHA-1, ACC-1) [8,10,15–17]. Plasmid-borne AmpC type β-lactamase genes originate from diverse Gram-negative bacilli such as *Hafnia alvei*, *Morganella morganii*, and *Aeromonas* spp. [18]. Plasmid-mediated cephalosporinase genes are often associated with coresistance to other antibiotics (e.g. aminoglycosides, tetracycline, sulfonamides) as a consequence of the colocalization of antibiotic resistance genes on the same plasmids [10]. The selection of isolates with carbapenem resistance or decreased carbapenem susceptibility has been sometimes observed during a carbapenem-based treatment [10,17,19].

Similarly, production of an ESBL in combination with outer membrane permeability defects can be responsible for carbapenem resistance in *Enterobacteriaceae* [8,19,20]. In that case, ESBL-encoding genes (e.g. bla_{TEM}, bla_{SHV}, and $bla_{\mathrm{CTX\text{-}M}}$) are plasmid-located. This mechanism was reported for almost all enterobacterial species including *K. pneumoniae*, *E. coli*, *Salmonella* spp., and *Enterobacter* spp. Ertapenem-resistant and ESBL-producing *K. pneumoniae* have been recently described in Italy and were found to be widely disseminated [20]. Those isolates carried a novel OmpK36 porin variant (OmpK36 V) that contributed to ertapenem resistance associated with reduced susceptibility to meropenem and susceptibility to imipenem [20]. The identification of ertapenem-resistant isolates is an emerging phenomenon as a consequence of the worldwide dissemination of community-acquired CTX-M-producing isolates [4]. Similarly, overexpression of AcrA, an efflux pump component, was described as responsible for imipenem resistance in *Enterobacter aerogenes* [21]. Nevertheless, several studies suggest that carbapenem resistance in isolates with porin defects is unstable [8]. This could be the consequence of lower fitness and decreased growth ability of strains with an outer membrane permeability defect.

In addition, strains that do not produce carbapenemase but which are carbapenem resistant are usually less resistant to antibiotics of other families. Their carbapenem resistance trait is not transferable, as opposed to most of the strains harboring carbapenemase genes. For this reason, carbapenem-resistant isolates that do not produce carbapenemases are considered of less clinical concern than carbapenemase-producing strains.

Carbapenemase-mediated carbapenem resistance in *Enterobacteriaceae*

The first carbapenemases identified in *Enterobacteriaceae* were SME-1 in London in 1982 [22] and IMI-1 in the USA in 1984 [23]. The first carbapenemase reported as a serine carbapenemase in the primary literature was the chromosomally-encoded NmcA from an *Enterobacter cloacae* clinical isolate [24]. Since then, carbapenem-resistant *Enterobacteriaceae* have been reported worldwide, primarily as a consequence of widespread acquisition of carbapenemase genes [25]. A large variety of carbapenemases belonging to three molecular classes of β-lactamases has been identified in *Enterobacteriaceae*: the Ambler class A, class B and class D β-lactamases (Table 1). In addition, rare chromosome-encoded cephalosporinases (Ambler class C or AmpC) produced by *Enterobacteriaceae* may possess slight extended activity toward carbapenems, but their clinical significance remains debatable [25].

The Ambler class A carbapenemases

Three major types of class A carbapenemases are known, corresponding to the NmcA/IMI, SME, and KPC enzymes [26]. All three enzyme types hydrolyze a broad variety of β-lactams including penicillins, cephalosporins, carbapenems, and aztreonam (Table 1) by a hydrolytic mechanism involving an active site serine at position 70 (Ambler numbering of class A β-lactamases) [27]. Their hydrolytic activity is inhibited *in vitro* by clavulanic acid and tazobactam (Table 1). A fourth type of enzyme corresponding to the GES type β-lactamases was originally considered a 'classical' ESBL family because GES-1 does not possess carbapenemase activity, but variants have subsequently been identified that possess weak but significant carbapenemase activity.

The SME enzymes have been only identified in *Serratia marcescens*. This family includes three variants (SME-1, -2, and -3), all chromosomally-encoded [28], that are recovered sporadically throughout the USA [29,30]. The

Table 1. Principle carbapenemases in *Enterobacteriaceae*

Ambler class	Name of the enzyme	Plasmid/ chromosome	Hydrolysis spectrum						Inhibitor
			Penicillins	First generation cephalosporins	Second generation cephalosporins	Third generation cephalosporins	Aztreonam	Carbapenems	
A	SME-1 to -3	Chromosome	++	++	–	+	+	+	Clavulanate, tazobactam, sulbactam, NXL-104
	NMC-A	Chromosome	++	++	–	+	–	++	
	IMI-2	Plasmid	++	++	–	+	–	++	
	GES-4, -5, -6	Plasmid	++	++	+	+	–	+	
	KPC-2 to -12	Plasmid	++	++	–	++	+	++	Clavulanate, tazobactam, boronic acid, sulbactam
B	IMP-1 to -33	Plasmid	++	++	++	++	–	++	EDTA
	VIM-1 to -33	Plasmid	++	++	++	++	–	++	
	NDM-1 to -6	Plasmid	++	++	++	++	–	+	
	KHM-1	Plasmid	++	++	++	++	–	++	
D	OXA-48	Plasmid	++	++	+/–	+/–	–	+	NaCl
	OXA-181	Plasmid	++	++	+/–	+/–	–	+	

The (+) or (++) annotations refer to the sole hydrolysis rate and do not take into account the level of carbapenem resistance observed in the corresponding bacterial host. Italicized inhibitors cannot be used in clinical practice. An annotation of (–) indicates, no detectable hydrolysis.

chromosome-encoded IMI and NmcA enzymes have been detected in rare isolates of *Enterobacter* spp. in the UK, France, and Argentina [23,28,31]. The gene encoding the IMI-2 variant has been identified as plasmid-located in environmental *Enterobacter asburiae* strains recovered from several US rivers [32], as well as in a single *E. cloacae* strain isolated in China [33].

The first representative of the GES type family enzymes was reported in 2000 and that family now includes 20 variants [34,35]. All the GES variants possess the ability to hydrolyze broad-spectrum cephalosporins (Table 1), but some variants possess amino acid substitutions within their active sites (positions 104 and 170 according to the Ambler classification) that enlarge their spectrum of activity against carbapenems [36]. This is the case for GES-2, -4, -5, -6, -11, and -14. GES-4, -5, and -6 have been reported in *Enterobacteriaceae*, whereas GES-2, -11, and -14 are still restricted to *Pseudomonaceae* or *Acinetobacter*. Although still rare, according to scattered reports GES enzymes have been identified worldwide [37,38].

KPC enzymes are currently the most clinically significant enzymes among class A β-lactamases. The first KPC-producing strain (KPC-2 in *K. pneumoniae*) was identified in 1996 in the Eastern part of the USA [39]. Since then, 11 KPC variants have been identified (KPC-2 to KPC-12) (http://www.lahey.org). Within a few years, KPC-producing strains disseminated widely and have been identified over the entire US territory (although still mainly among hospitals in the East coast states), as well as Puerto Rico, Columbia, Italy, Greece, Israel, and China [40,41]. Outbreaks of KPC-producing strains have also been reported in many European countries and in South America [40,41]. Although community-acquired KPC-producing strains have been reported in the USA and Israel a few years ago they remain rare [41]. Indeed, KPC enzymes have been reported mostly from nosocomial *K. pneumoniae* isolates and to a much lesser extent from other enterobacterial species [41]. Attributed mortality to infections due to KPC-producing strains is high (50% or more) [42–44], partly due to the fact that KPC-producing strains are usually multidrug resistant leading to failure in first line therapy and to very limited therapeutic options [41].

Of note, one specific *K. pneumoniae* clone (ST258) expressing the gene bla_{KPC-2} has been extensively identified worldwide [45], indicating that it may have contributed significantly to the spread of the bla_{KPC} genes. However, within the same geographical area several KPC clones may disseminate, differing by MLST type, β-lactamase content, and plasmid sizes. However, there is striking evidence that the bla_{KPC} genes are always associated with a given genetic element (transposon Tn*4401*) (Figure 2). Tn*4401* is a Tn*3*-like transposon that has been identified in isolates from different geographical origins and of different sequence types in *Enterobacteriaceae* and *Pseudomonas aeruginosa* [45]. Tn*4401* is found inserted at different loci and on plasmids varying in size and incompatibility group [46]. In addition, Tn*4401* is capable of transposition creating a 5-bp target site duplication but without any target site specificity [46].

The class B metallo β-lactamases (MBL)

Class B β-lactamases exhibit a broad spectrum of hydrolytic activity including all penicillins, cephalosporins, and carbapenems, with the exception of monobactam aztreonam (Table 1). Their activity is not inhibited by commercially available β-lactamase inhibitors (clavulanic acid, tazobactam, or sulbactam). Hydrolysis is dependent on the interaction of the β-lactam with Zn^{2+} ion(s) in the active site, explaining the inhibition of their activity by EDTA, a chelator of divalent cations (Table 1).

The first MBLs were detected from environmental and opportunistic bacteria such as *Bacillus cereus* [47,48], *Aeromonas* spp. [49,50], and *Stenotrophomonas maltophilia* [51]. Those bacteria are mostly opportunistic and soil-dwelling pathogens, and their MBL genes are intrinsic and chromosome-borne. Since the 1990s, a dramatic increase in acquired or transferable MBL genes has been described in *Pseudomonas* spp. and *Enterobacteriaceae*.

The most common types of acquired MBLs identified in *Enterobacteriaceae* include the IMP and VIM groups, together with the emerging NDM group, whereas the group KHM-1 is rare [52]. Generally, the level of carbapenem resistance observed for MBL-producing strains may vary, and attributed mortality associated with MBL production ranges from 18% to 67% [53].

IMP type β-lactamases were among the first acquired MBLs identified and were detected in *Pseudomonas* spp., *Acinetobater* spp., and *Enterobacteriaceae* [54,55]. In *Enterobacteriaceae*, IMP-1 was first reported from a *S. marcescens* isolate in Japan in 1991 [56]. Since then, 33 IMP variants have been assigned and IMP type carbapenemase producers have spread worldwide. The endemic spread of IMP type enzymes has been reported from Japan, Taiwan, East of China, and more recently from Greece, although outbreaks and single reports have been reported in many other countries [55]. It has been suggested that selection of IMP type genes occurred first in Japan as a consequence of heavy carbapenem usage. Analysis of the bla_{IMP} genetic environments most often revealed features of class 1 integrons (Figure 2) [55]. Class 1 integrons are DNA-based structures that harbor antibiotic resistance genes, named gene cassettes, that are coexpressed from a single promoter. Resistance genes encode decreased susceptibility to unrelated antibiotic molecules (e.g. β-lactams, aminoglycosides, sulfonamides, and chloramphenicol). The genes identified among the bla_{IMP}-containing integrons often encode resistance to aminoglycosides (*aacA4*, *aadA1*, and *aadB*), class D β-lactamases (bla_{OXA}), and chloramphenicol (*catB*) (Figure 2) [55]. Class 1 integrons may integrate unrelated resistance genes but are not mobile by themselves. However, they are usually identified inside transposon structures, thus allowing their spread.

Another family of MBLs includes the VIM (for Verona integron-encoded metallo-β-lactamase) enzymes. VIM-1 was first identified in Italy in 1997 [57], and shortly after the VIM-2 variant was reported in France from a *P. aeruginosa* isolate [58]. The VIM group of enzymes contains 33 variants, mainly identified from *P. aeruginosa* and to a lesser extent from enterobacterial isolates. The genes also correspond to gene cassettes located inside class 1 integrons (Figure 2) [59]. VIM-2 is the MBL most often reported worldwide, with an endemic spread in at least Southern Europe (Greece, Spain, Italy) and Southeast Asia (South Korea, Taiwan) [59].

The other acquired MBLs include SPM-1, GIM-1, SIM-1, DIM-1, and AIM-1; however, these enzymes have so far not been identified in enterobacterial strains [60]. Recently, the β-lactamase KHM-1 was identified in Japan from a single *Citrobacter freundii* clinical isolate that had been recovered in 1997 [61].

Discovered in 2008 in Sweden from an Indian patient previously hospitalized in New Delhi, India [62], NDM-1-positive *Enterobacteriaceae* are now the focus of worldwide attention. NDM-1-producing bacteria have been identified mainly in the UK, India, and Pakistan [63], but numerous studies during the past year reported NDM-1-producing strains worldwide [64]. Most (but not all) of the patients infected or colonized by NDM-1 producers had traveled to the Indian subcontinent or have some relationship with India, Pakistan, or Bangladesh. Those data indicate that the Indian subcontinent is a reservoir of bla_{NDM-1} genes. In addition, several NDM-1-producing enterobacterial isolates have been reported in patients having a relationship with the Balkan states or the Middle East, suggesting that those areas might constitute a secondary reservoir of NDM-1-producing strains [65,66]. The spread of NDM-1 in the Middle East may be explained by a close relationship with the Indian subcontinent (population exchange and trade). The bla_{NDM-1} gene is not associated with a single clone, a single species, or to a specific plasmid backbone but has been identified from unrelated Gram negatives, including mostly enterobacterial species, harbored by different plasmid types [67]. Plasmids carrying the bla_{NDM-1} gene are diverse in size, incompatibility group, and associated resistance genes. Noticeably, all NDM-1-producing strains may express many other unrelated resistance genes, such as those that encode other carbapenemases (OXA-48 type, VIM type), AmpC cephalosporinases, ESBLs, and resistance to aminoglycosides (16S RNA methylases), macrolides (esterases), rifampicin (rifampicin-modifying enzymes), and sulfamethoxazole. The association of such a high number of plasmid- and chromosome-encoded resistance genes (including resistance to fluoroquinolones) in single isolates had been rarely observed and explains the multidrug resistance patterns observed. Many NDM-1 producers remain susceptible only to tigecycline (a tetracycline analog), colistin, and to a lesser extent to fosfomycin [68]. Although bla_{NDM-1}-positive isolates are predominantly nosocomial *K. pneumoniae* isolates, the bla_{NDM-1} gene has been identified in community-acquired *E. coli* [69]. Accordingly, the spread of NDM-1 producers might be wider than expected, considering that the colonization rate of NDM-1 producers in one Pakistani city has been estimated at more than 18% [70]. In addition, NDM-1-producing isolates have been found in tap and environmental water in New Delhi among many unrelated Gram-negative species, including environmental bacterial species such as *Vibrio cholera*, indicating it is widespread in the environment, at least in India [71]. Currently, six NDM variants have been identified. Although most NDM-producing strains described are enterobacterial species, some NDM-2 variant has been identified in *Acinetobacter baumannii* [72].

A recent study focused on the genetic environment of bla_{NDM-1} revealed a strong association between the genes bla_{NDM-1} and ble_{MBL}, the latter gene encoding a functional bleomycin resistance protein (Figures 2 and 3) [73]. It was shown that the bla_{NDM-1} and ble_{MBL} genes are part of the same operon and coexpressed under control of the same promoter, the latter being partially formed by the 3′ extremity of the insertion sequence IS*Aba125* (Figures 2 and 3) [73]. Production of the bleomycin resistance protein decreases the spontaneous RecA-dependent mutation rate, contributing to the stabilization of bla_{NDM-1} positive isolates. This suggests that the emerging carbapenemase resistance NDM-1 might be also selected by bleomycin-like molecules that are produced, at the very least, by environmental species such as *Streptomyces* spp. Strains that produce bleomycin resistance protein (and consequently NDM producers) might be more prone to persisting

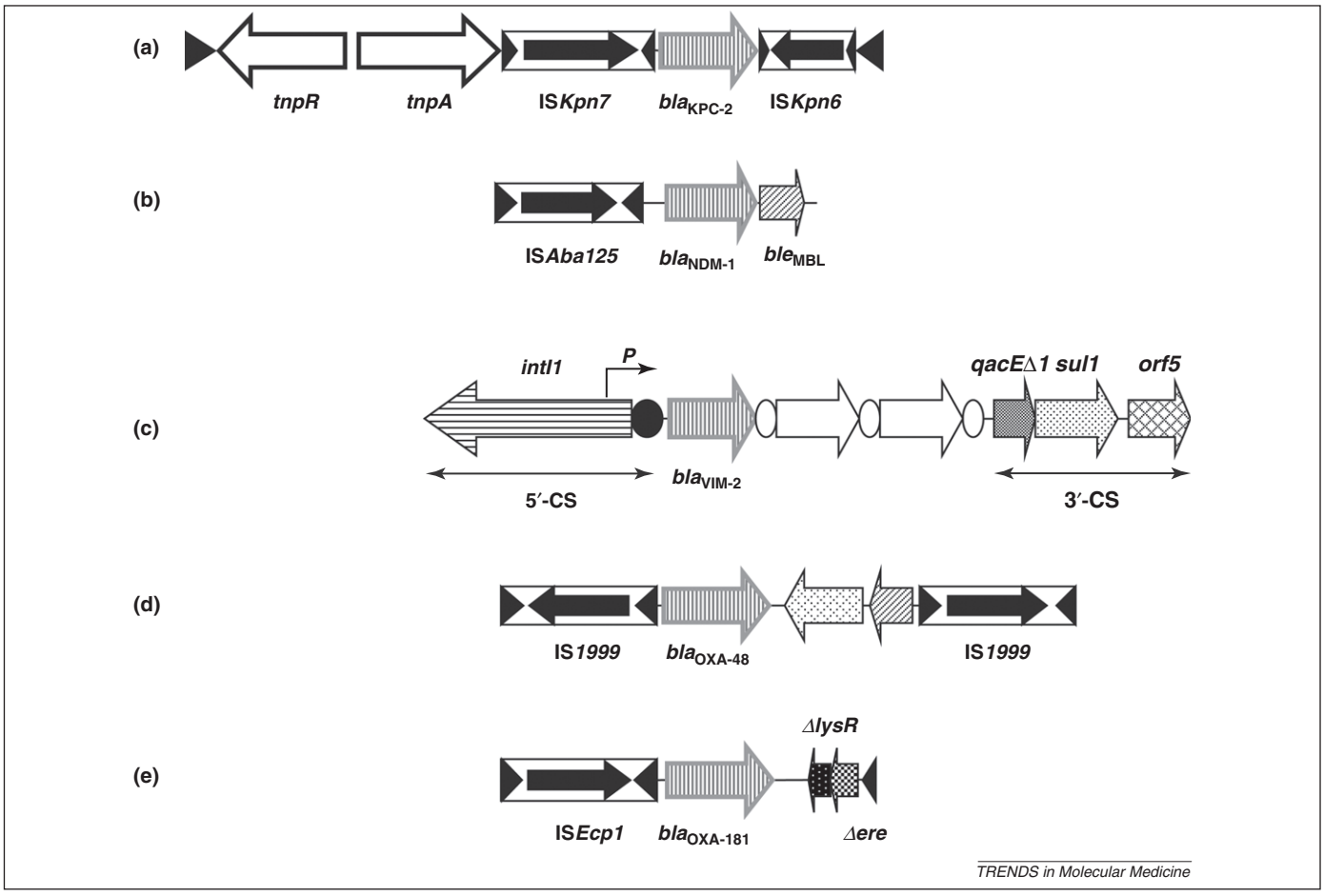

Figure 2. Schematic representation of the main genetic structures involved in carbapenemase genes acquisition in *Enterobacteriaceae*. **(a)** The Tn*4401* type transposon carrying the bla_{KPC-2} gene, comprising the *tnpA* transposase gene, the *tnpR* resolvase gene, and the two insertion sequences IS*Kpn6* and IS*Kpn7*. **(b)** The bla_{NDM-1} gene is bracketed upstream by insertion sequence IS*Aba125* and downstream by the ble_{MBL} gene-encoding resistance to bleomycin. **(c)** A class 1 integron (with its 5′CS and 3′CS conserved regions) harboring the bla_{VIM-2} gene cassette (*intI1* is for the integrase gene, the *attI* recombination site is represented by a black circle, the gene cassettes by white arrows and their respective 59 bp by white circles, and the promoter sequences [*P*] provided by the 5′CS are indicated by a thin arrow). **(d)** The Tn*1999* composite transposon made of two copies of insertion sequence IS*1999* bracketing a fragment containing the bla_{OXA-48} gene. **(e)** The Tn*2013* transposon made of IS*Ecp1* associated with the $bla_{OXA-181}$ gene. Inverted repeats (left and right) are indicated by black triangles.

in the environment due to selection by naturally produced bleomycin-related molecules [73].

By investigating a worldwide collection of NDM-1-producing enterobacterial isolates, it was shown that the current spread of the bla_{NDM-1} gene was not related to the spread of specific clones, to the spread of specific plasmids, or to the dissemination of one given genetic structure [67]. It was shown that the bla_{NDM-1} gene was always associated at its 5′-end with a remnant of insertion sequence IS*Aba125* that is an IS previously found in *A. baumannii* [74]. Because the $bla_{NDM-1/-2}$ genes have been recently identified as part of a composite transposon (Tn*125*) made of two copies of IS*Aba125*, a working hypothesis is that the bla_{NDM-1} gene had been integrated first into the chromosome of *A. baumannii* from an unknown environmental species and then transferred onto plasmids replicating in *Enterobacteriaceae* (Figure 3). The high G+C content of the bla_{NDM} gene argue for the environmental species, either a Gram positive *Streptomyces*-like species or a Gram negative *Stenotrophomonas*-like species, being the progenitor of this carbapenem resistance trait (Figure 3). NDM-1-producing *E. coli* of the ST type 131 was recently found to be responsible for a community-acquired infection [75]. This ST type corresponds to those of CTX-M-15-producing *E. coli* clone disseminated worldwide [76], further emphasizing the dissemination potency of the NDM-1 resistance trait.

The class D carbapenemases of the OXA-48 type

Class D β-lactamases, also named OXAs for 'oxacillinases', include 232 enzymes, with a few variants possessing some carbapenemase activity. With the exception of one variant (OXA-163) that has a very weak carbapenemase activity, carbapenem-hydrolyzing class D β-lactamases (CHDLs) do not hydrolyze expanded-spectrum cephalosporins (Table 1). Overall, the carbapenemase activity of CHDLs is weak and is not inhibited by either clavulanic acid or by EDTA, but is inhibited by NaCl (Table 1).

Although most of the CHDLs variants have been identified in *Acinetobacter* spp., OXA-48 has been found only in *Enterobacteriaceae*. The first OXA-48 producer was identified from a *K. pneumoniae* isolate recovered in Turkey in 2003 [77]. Since then, OXA-48 producing strains have been extensively reported as sources of nosocomial outbreaks in Turkey [78], and identified in countries of Southern Europe and Africa [79]. Sporadic descriptions or hospital outbreaks

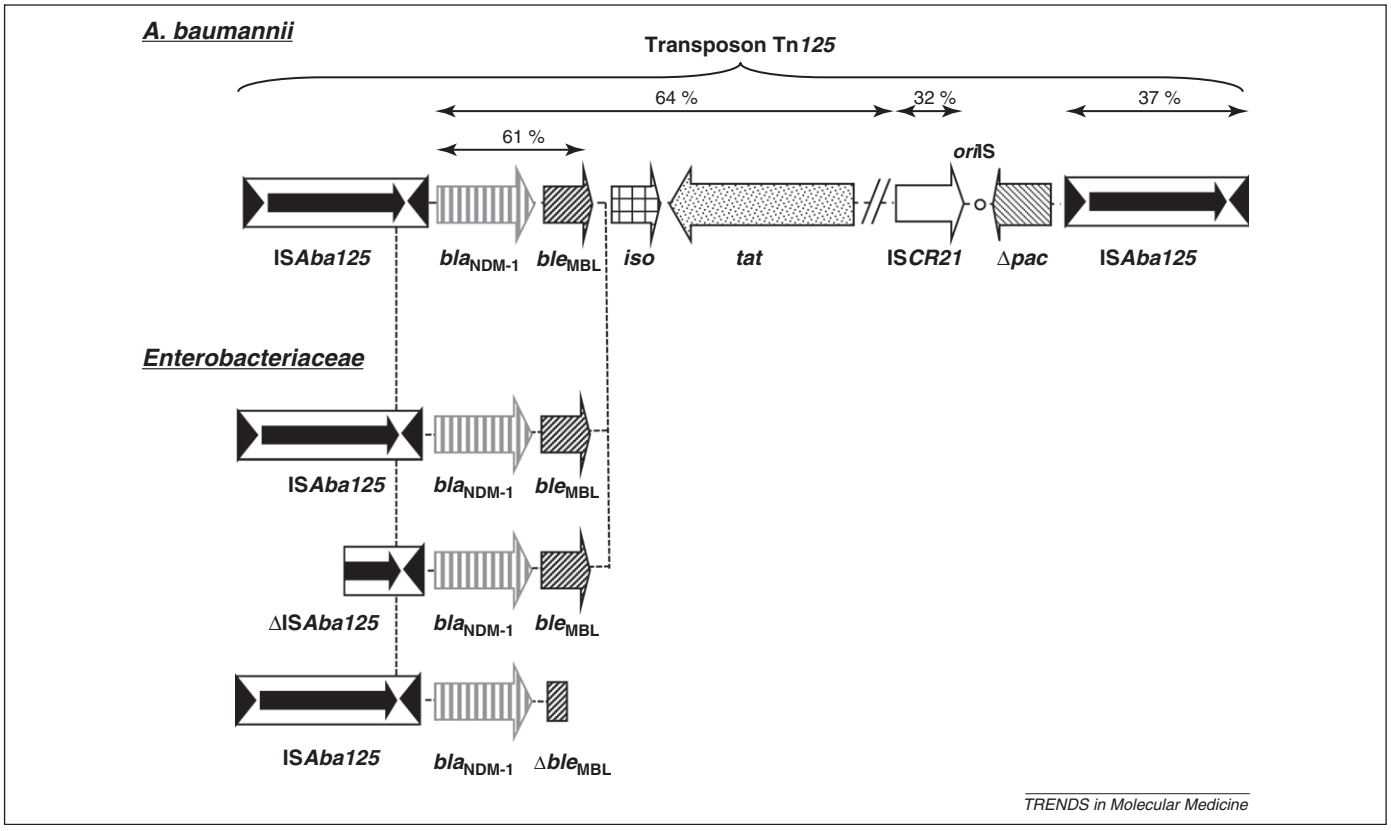

Figure 3. Genetic environment of the bla_{NDM-1} gene in *Acinetobacter baumannii* and *Enterobacteriaceae*. In *A. baumannii*, the bla_{NDM-1} gene has been identified as part of the composite transposon Tn*125* made of two copies of IS*Aba125*. Only part of this transposon was identified in *Enterobacteriaceae*. The guanosine + cytosine nucleotide content (in %) is indicated for several DNA fragments.

involving OXA-48-producing *K. pneumoniae*, *E. coli*, or *E. cloacae* introduced by the transfer of hospitalized patients from endemic areas have been reported in France, Germany, Spain, Netherlands, and the UK. The current spread of OXA-48 producers is a source of great concern.

A single plasmid of approximately 62 kb is the main source of the bla_{OXA-48} gene disseminated in a variety of enterobacterial species [80]. That self-conjugative plasmid possesses an IncL/M type backbone with no other antibiotic resistance gene [81]. The bla_{OXA-48} gene is bracketed by two IS*1999* elements to form a functional composite transposon (Figure 2). Recently, a point-mutant derivative of OXA-48 sharing identical hydrolytic properties (Table 1), namely OXA-181, has been identified in enterobacterial isolates from India or from patients with a link to the Indian subcontinent [82–84]. The $bla_{OXA-181}$ gene was identified in a genetic context totally different from that of bla_{OXA-48}, being located on a non-self-conjugative ColE2 type plasmid approximately 7 kb in size in association with the insertion sequence IS*Ecp1* responsible for its acquisition by a one-ended transposition mechanism [85].

A species close to *Shewanella oneidensis* is the progenitor of the bla_{OXA-48} gene [86], and *Shewanella xiamenensis*, another environmental bacteria, is the natural reservoir of the $bla_{OXA-181}$ gene [87]. Spread of both *Shewanella* spp. and *Enterobacteriaceae* in the same water-borne environment might explain this spread.

Isolates producing the carbapenemases OXA-48 and OXA-181 usually do not exhibit high resistance levels to carbapenems (Table 1). Thus, the detection of OXA-48-like enzymes can be difficult unless those isolates possess associated mechanisms of resistance including ESBL production and/or permeability defects. Therefore, it is probable that the prevalence of OXA-48 or OXA-181 producers is currently underestimated.

Concluding remarks

As highlighted in this review, resistance to carbapenems in *Enterobacteriaceae* may result either from expression of carbapenemases or by combined effects of β-lactamases with no (or very weak) intrinsic carbapenemase activity (i.e. ESBLs or cephalosporinases) and decreased outer membrane permeability.

The real prevalence of carbapenemase-producing bacterial strains remains unknown because many countries worldwide do not report rates of antibiotic susceptibility. Therefore, the worldwide spread of *Enterobacteriaceae* expressing carbapenemases now represents a significant threat for the public health and requires efforts toward detection and infection control strategies. The early identification of carbapenemase-producing strains both in clinical infections and/or at the carriage state is mandatory to prevent the development of difficult or impossible-to-treat infections. Whereas many reports of carbapenemase producers are still related to nosocomial isolates, one of the most important concerns is the spread of carbapenemase-encoding genes in the community, particularly in *E. coli*. This spread will be very difficult to control, whatever the antibiotic and detection policies enacted. At the hospital level, detection of infected and/or colonized

patients should be implemented to prevent outbreaks involving carbapenemase-producing strains, a procedure that can be performed easily considering the availability of microbiological and molecular tools developed for that purpose.

We fear a serious drought of novel molecules in the pipeline of newly developed antibiotics. This is the result of a lack of investment by pharmaceutical companies in antibacterial research and development in favor of the far more lucrative investment in molecules designed to treat chronic diseases.

A few analogs of the major antibacterial classes (cephalosporins, oxazolinidones, quinolones, macrolides, ketolides, streptogramines, and cyclines) are in clinical development, mostly for treating infections due to methicillin-resistant *Staphylococcus aureus*. The situation is worse for developing compounds to treat Gram negative bacteria due to several reasons, including the ease with which antibiotic resistance genes are exchanged and the necessity for antibiotics to cross the outer membrane to be active.

We do not believe that biology-based antibiotics such as phage therapy, antisense RNA, or even replacement of antibiotic-resistant flora by antibiotic-susceptible flora can be productive sources of novel antibiotics. We believe that the genetic plasticity of bacteria will limit any development in that field.

We believe that traditional chemical-based therapy may open new pathways for antibiotherapy. Those novel molecules may result from broad-spectrum screening, identification of new targets in the bacterial metabolism, or analysis of molecules that may interact with the active site of carbapenemases. As an example, NXL104 may inhibit the activity of several class A carbapenemases such as KPC [88]. Several inhibitors of metallo-β-lactamases (VIM, IMP, NDM) may have a bright future, considering their *in vitro* antibacterial activity. The challenge of the next few years will be the race between the creation of effective novel molecules and the spread of carbapenemases worldwide.

Acknowledgments

This work was funded by grants from the UMRS 914 (INSERM and Université Paris XI).

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