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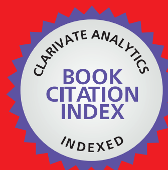

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Chapter

Carbapenemases

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Abstract

Carbapenems are usually regarded as the last treatment option for serious infections caused by Gram-negative and Gram-positive microorganisms. Although they are stable to hydrolysis by most β-lactamases, their usage as the last resort antbiotics was seriously compromised by the appearance of carbapenem-hydrolyzing enzymes called carbapenemases. These enzymes are produced mostly by *Enterobacteriaceae* and Gram-negative nonfermentative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. True carbapenemases belonging to Ambler molecular classes A, B, and D are often encoded by genes embedded in mobile genetic elements like plasmids, integrons, and transposons, which often harbor multiple resistance determinants limiting further the treatment options. At present, large number of nosocomial and community-acquired infections caused by worldwide spread of carbapenem-resistant Gram-negative bacteria have become a major public health problem. Although polymyxins remain active, in vitro reports of benefits of combination schemes favor this strategy against carbapenemase-producing Gram-negative bacilli.

Keywords: carbapenems, carbapenemases, *Enterobacteriaceae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*

1. Introduction

Carbapenems are bactericidal antibiotics often used as a last treatment option for severe infections caused by isolates producing extended-spectrum and/or AmpC β-lactamases. However, their stability to β-lactamases of Gram-negative bacteria is not absolute. From the first report of carbapenemases in the 1980s, the last decade is marked with the rapid spread of carbapenemases-producing organisms, becoming a public health concern worldwide.

2. Carbapenem antibiotics

Carbapenems are β-lactam antibiotics with broad antimicrobial spectrum, which encompasses Gram-positive and Gram-negative aerobic organisms and anaerobic bacteria. They often remain as the last treatment option for infections caused by multiresistant Gram-negative isolates. Their in vitro efficacy is due to good penetration through the outer membrane of Gram-negative bacteria, high affinity for penicillin-binding protein (PBP) molecules and excellent stability against β-lactamases [1]. The carbapenems bind to PBP-1 and PBP-2 and induce spheroplast formation and cell lysis without filament formation. They comprise imipenem, meropenem, ertapenem, and doripenem. In general, imipenem, and

doripenem are potent also against Gram-positive bacteria, while meropenem, ertapenem, and doripenem are slightly more effective against Gram-negative organisms. Ertapenem is not active against *P. aeruginosa* or *A. baumannii*, while doripenem has lower minimum inhibitory concentrations (MICs) than imipenem and meropenem versus *P. aeruginosa* and *A. baumannii* [1] Meropenem has an advantage over imipenem because it is not susceptible to renal dihydropeptidase and can be administered without cilastatin which is neurotoxic and contraindicated in neurological patients. Carbapenems are stable against wide range of serine-based β-lactamases including extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases or against strains with inducible and derepressed expression of chromosomal AmpC β-lactamases. Although the stability against these common enzymes is not complete, the rate of hydrolysis is slow and only becomes significant when coupled with reduced outer membrane permeablity. Carbapenems are strong inducers of chromosomal β-lactamases, but β-lactamase induction does not affect their activity [2]. However, induction of chromosomal AmpC beta-lactamase can cause antagonism with penicillins and cephalosporins, and thus carbapenems should not be administed in combination with other β-lactams.

3. Classification, historical background, and properties of carbapenemases

Some β-lactamases, named carbapenemases, can efficiently hydrolyze carbapenems [3]. These include chromosomal enyzmes, which are ubiquitious in some unusual pathogens and mostly environmental isolates (e.g., *Stenotrophomonas maltophilia*, *Aeromonas* spp., *Flavobacterium* spp., *Bacteroides fragilis*) and acquired carbapenemases of classes A, B, and D [3]. Although several class C carbapenemases have been described (ACT-1, CMY-2, CMY-10, and ADC-68), their producers usually exhibit reduced susceptibility to carbapenems as a consequence of low enzyme's catalytic efficiency and a permeability defect. Therefore, they are not considered as true carbapenemases [4].

Based on the structure of their active site, carbapenemases are devided into two groups: class A-penicillinase and class D-oxacillinases; they contain serine at their active site and are inhibited by β-lactam inhibitors including clavulanic acid and tazobactam. The second group is metallo-β-lactamases belonging to class B which contain zinc atom at the active site and is inhibited by metal chelators such as ethylenediaminetetraacetic acid (EDTA) [5].

3.1 Class A carbapenemases

Serin carbapenemases of molecular (Ambler) group A are rare, detected mainly in *Enterobacteriaceae*. They belong to the functional subgroup 2f according to the classification scheme proposed by Bush et al. [6, 7]. The genes encoding them are located on either chromosome or plasmid. This group comprises SME-1 (*Serratia marcescens)* [8], SME-2 (*S. marcescens)* [9], SME-3 (*S. marcescens)* [10], IMI-1 (*Enterobacter cloacae)* [11], NMC-A (*E. cloacae)* [12], KPC-1, KPC-2, and KPC-3 (*Klebsiella pneumoniae*) [13–15] and GES-2 (*P. aeruginosa)* [16]. They confer resistance to aminopenicillins, ureidopenicillins, first- and second-generation cephalosporins, aztreonam, and imipenem. Imipenem is hydrolyzed very slowly without causing a clinically relevant resistance [17]. SME β-lactamase was reported for the first time in *S. marcescens* in the UK in 1982 [8]. Infections caused by SME-positive *S. marcescens* are usually sporadic without potential of causing

outbreaks. IMI and NMC β-lactamases have been sporadically detected in isolates of *E. cloacae* in the USA, France, and Argentina [9, 10]. These β-lactamase are encoded chromosomally and have never been described on transferable genetic elements explaining their sporadic reports wordwide [17]. KPC-1 is a novel class A enzyme isolated in 1996 from *K. pneumoniae* in the USA [13]. The discovery of KPC-1 was quickly followed by two new allelic variants: KPC-2 and KPC-3 [14]. Subsequent revision of the $bla_{\mathrm{KPC-1}}$ sequence demonstrated that KPC-1 and KPC-2 are identical enzymes [13–15]. KPC-3 differs from KPC-2 (the former KPC-1) by one amino acid change (H272Y) [14]. Although, so far, more than 20 different KPC variants have been described, KPC-2 and KPC-3 remain the most commonly identified. Unlike other class A carbapenemases, they hydrolyze expanded-spectrum cephalosporins and all carbapenems. KPC β-lactamases are weakly inhibited by clavulanic acid and tazobactam and are often coharbored with OXA-1 conferring resistance to β-lactam inhibitor combinations. They are not inducible and are encoded on transferable plasmids. A 10-kb Tn3-type transposon Tn*4401* with two insertion sequences IS*Kpn6* and IS*Kpn7* is repeatedly reported as the main genetic structure enhancing the spread of bla_{KPC}-type genes [5]. Recently, a chromosal integration of the bla_{KPC} gene was shown in *K. pneumoniae*, thus enabling easier maintenance of the gene in bacterial population [18]. Today, KPC enzymes are highly prevalent, not only found in *K. pneumoniae* but also in an increasing number of *Enterobacteriaceae, P. aeruginosa*, and *A. baumannii*. They are not only found all over the USA but also in Central and South America, while in European countries, KPC-producing isolates are mostly prevalent in Mediterranean countries, especially in Italy and Greece, countries reported as having endemic situation [19, 20].

They are also still present in Middle East where in 2007 a nationwide strategy was implemented to contain the outbreak faced by Israeli hospitals [21].

KPC-producing isolates are also reported in Asia, especially China where a hypervirulent *Klebsiella pneumoniae* strain K1-producing KPC is concerning [22, 23].

The main transporter in the worldwide dissemination of KPC-producing enzymes is succesfull clone *Klebsiella pneumoniae* ST258 [24]. In contrast to the majority of other class A carbapenemases, KPCs are typically plasmid mediated and usually carried on IncF plasmid. Plasmid location of bla_{KPC} genes is responsible for the rapid dissemination of those important resistance determinants [23, 24].

GES-2 β-lactamase was derived by point mutation from GES-1 extended-spectrum β-lactamase (with amino acid change G170 N) which was described in *K. pneumoniae* isolate from French Guinea [16, 25]. GES-2 β-lactamase was first reported from *Pseudomonas aeruginosa* from South Africa, was plasmid mediated, and conferred resistance or reduced susceptibility to carbapenems [26]. Because the enzymes have broad hydrolysis spectrum that includes penicillins and expanded-spectrum cephalosporins, they were initially classified as ESBLs. Their hydrolysis spectrum was expanded in 2001 to include imipenem. GES β-lactamases are related to integron-borne-cephalosporinase—IBC) ESBLs, but confusing IBC names have been converted to GES [2, 3, 17]. Genes encoding GES family of β-lactamases are located on integrons embedded in plasmids with a possibility of conjugal transfer. However, they appear rarely in contrast to KPC β-lactamases and are not frequently associated with nosocomial outbreaks [3, 17, 26]. At present, the number of GES variants is increasing (with more then 20 described), but only several of them (mainly GES-2, -6, -14, -20) display carbapenemase activity (others are mostly ESBL). GES-type carbapenemases were identified in *Pseudomonas aeruginosa*, in *Acinetobacter baumannii*, and *Enterobacteriaceae* as well (mostly *Klebsiella pneumoniae*, *Serratia marcescens*, and *Enterobacter* spp. isolates) and have worldwide

distribution [17, 23]. In spite of the fact that they are plasmid mediated, they do not have a capacity for successful dissemination like KPC [23].

3.2 Class B carbapenemases

Metallo-β-lactamases (MBLs) are clinically the most relevant carbapenemases [23, 27]. This group of enzymes catalyzes the β-lactam hydrolysis by active site containing one or more bounded zinc ions that promote formation of nucleophilic hydoxide further attacking the β-lactam ring. The first descriptions of metallo-β-lactamases included studies on environmental and opportunistic bacteria such as *Stenotrophomonas maltophilia*, *Bacillus cereus*, *Aeromonas* spp., *Legionella gormanii*, *Pseudomonas stutzeri*, *Shewanella* spp., *Myroides odoratus*, and so on, which carry MBLs on their chromosome but could present potential reservoir of mobile carbapenemase determinants [27, 28].

The metallo-β-lactamases or class B carbapenemases are further divided into subclasses (B1, B2 and B3), but the largest number of clinically relevant MBLs belong to B1 subclass, including the most frequently described Verona integron-encoded MBL (VIM), imipenemase (IMP), and New Delhi MBL (NDM). Those MBLs are usually located within different integron structures, and these integrons are associated with mobile plasmids or transposons facilitating the transfer of resistance genes between bacteria [3–5]. Acquired MBLs are reported from all over the world but mostly from Europe and Far East [27]. Transferable imipenem resistance was first detected in 1990 in Japan in *P. aeruginosa* isolate [29]. The β-lactamase-conferring imipenem resistance was designated IMP-1 and was located on a conjugative plasmid [30]. The same enzyme was later found on an integron in *S. marcescens* and other *Enterobacteriaceae* also in Japan. The first member of IMP family reported in Europe was IMP-1 found in *A. baumannii* in Italy [31]. The gene encoding this enzyme was located on class 1 integron [32]. Since that time, the IMP family was found throughout the world, but most reports originate from Far East [4, 22, 27, 33]. IMP-2 β-lactamase was found in an *Acinetobacter baumanni* isolate from Italy in 1997 and shares 85% of amino acid identity with IMP-1 with similar kinetic parameters, while IMP-3 was reported in *Shigella flexneri* from Japan. That was the first appearance of MBL in typical community-acquired pathogen [34]. IMP-3 enzyme was previously known as MET-1, but the substrate specificity, that is, preferential hydrolysis of cephalosporins (and not penicilins and carbapenems), was a direct consequence of a single-base mutation (although the genes differ in two bases and two amino acid changes, that is, S262G and E126G). The gene and the enzyme were then renamed to $bla_{IMP\text{-}3}$ or IMP-3 β-lactamase. Since IMP-1 shows broader substrate profile, it seemed that the gene blaIMP-3 was an ancestor of bla_{IMP}-1 [27]. IMP-4 MBL was first reported from *A. baumannii* associated with nosocomial outbreak in Hong Kong in 2001 [35]. IMP-5 was identified in urinary isolate of *A. baumannii* from Portugal in 2002, highly resistant to penicillins, broad-spectrum cephalosporins, including ceftazidime, ceftriaxone, cefepime, cefpirome, and to aztreonam, but remained susceptible to ampicillin/sulbactam, aminoglycosides, and quinolones [36]. IMP-5 showed greater homology with IMP-1, IMP-3, and IMP-4 than with IMP-2. $Bla_{\mathrm{IMP\text{-}5}}$ was the only gene cassette inserted into a class 1 integron, named In76. This was the first IMP-β-lactamase in Portugal and the second in Europe [36]. There are more than 50 allelic IMP variants reported so far [37]. They are usually devided into IMP-1 and IMP-2 cluster, suggesting their different phylogenetic origin. IMP-12, a very different variant outside the IMP-1 cluster (it has 85% similarity to IMP-1), has a unique structural feature and shows reduced catalytic effieciency toward penicillins [38]. IMP-51 was recently identified in a *P. aeruginosa* clinical isolate in Vietnam and showed increased doripenem- and meropenem-hydrolyzing activities

[39]. IMP-type enzymes have been reported all over the world in *Enterobacteriaceae, P. aeruginosa*, and *A. baumannii* but still represent the dominant carbapenemases in Japan and in Southeast Asia [33, 38, 40]. Analysis of the genetic platform of bla_{IMP} genes has shown that most of them are embedded as gene cassettes in class I integron, harboring also other resistance genes, such as aac (mediating resistance to aminoglycosides), bla_{OXA} (different serine oxacillinases), resistance to antiseptics, or chloramphenicol [38, 40, 41].

Another prevalent family of integron-associated metallo-β-lactamases is composed of VIM enzymes. VIM means Verona integron-encoded metallo-β-lactamase because the first enzyme in this family was reported in *Pseudomonas aeruginosa* isolate from Verona in 1997 [42]. $Bla_{\mathrm{VIM\text{-}1}}$ gene was located within class 1 integron repeatedly showing the important role in the dissemination of IMP and VIM genes not only in nonfermentative bacteria but also in *Enterobacteriaceae* [4]. VIM-2 was described in *Pseudomonas aeruginosa* from France that was isolated in 1996 and shared 90% similarity with VIM-1 [43]. VIM-3 was reported in *P. aeruginosa* isolates associated with nosocomial outbreak from Taiwan [44]. The enzyme was chromosomally encoded and differed in two amino acids from VIM-1, namely C178A and A443G. VIM-4 β-lactamases were identified in *K. pneumoniae* and *Enterobacter cloacae* isolates from Italy in a patient previously treated with carbapenems and has one amino acid change in comparison to VIM-1 (S228A), being more active against imipenem than VIM-1 [45]. There are more than 40 allelic variants of VIM enzymes reported so far, and they mainly belong to three sublineages—VIM-1-like, VIM-2-like, and VIM-7-like enzymes, representing phylogenetically the most divergent variants [38, 46]. VIM-2-like enzymes have been associated mostly with *P. aeruginosa*, whereas VIM-1-like enzymes, in particular, VIM-4, have been frequently reported in strains of the *Enterobacteriaceae* [38, 40, 46]. It is interesting to mention that if VIM-1 or VIM-4 enzymes are found in *E. cloacae* and *K. pneumoniae*, mostly of ST147 and 11, they are usually carried on incompatibility group N or A/C plasmids, whereas in *E. coli*, those genes are often associated to IncFI/II plasmids [38, 41]. VIM-7, a more divergent enzyme, with lower efficiency for cephalosporins, was for the first time reported in the USA in 2001, and it was the first MBL in North America [47, 48]. North America was spared from MBLs until early 2000s. VIM enzymes are today widely distributed in all continents and at present the most common MBL found all over the world. In Mediterranean countries, such as Italy and Greece, VIM-positive *P. aeruginosa* strains of clonal complex 235 and 111 have been involved in nosocomial outbreaks [38]. The activity of aztreonam, as the only β-lactam not hydrolyzed by MBLs, is strongly compromised by the copresence of ESBLs, plasmid-mediated AmpC, or other serine β-lactamase together with MBLs [49, 50]. Bla_{VIM} genes are typically embedded in class 1 integrons, which can be incorporated in either plasmids or chromosome. Plasmids carrying bla_{VIM} genes in *Enterobacterriaceae* belong most frequently to IncA/C or IncN group [23, 38, 41].

NDM is a new family of metallo-β-lactamases unrelated to other MBLs. The first NDM-1 positive isolate from Europe was reported in Sweden from a patient who contracted a *K. pneumoniae* infection in India in late 2007 [51]. At the beginning, NDM enzymes spread in the Indian subcontinent, and they are still endemic in India, Pakistan, and Bangladesh. They were found not only in clinical isolates but also in public tap water and sewage water [52].

The spread soon involved the UK, historically related to India, but until now, it was described as present in all continents. However, it should be noted that Balkan region, Arabian Peninsula, and North African countries are considered as possible additional reservoirs of NDM-producing strains [38, 53, 54].

So far, the NDM enzymes have been found mostly in *Enterobacteriaceae* (with the importance of epidemic *Klebsiella pneumoniae* ST147, 11 and 14 and *E. coli*

ST131 and 405*),* *P. aeruginosa*, and *A. baumannii*. *Enterobacteriaceae*-producing NDM β-lactamases pose a serious public health concern because huge conjugative plasmids carrying the bla_{NDM} genes can have up to 14 other antibiotic resistance determinants and can trasfer this resistance to other bacteria, resulting in multidrug resistance or pan-drug resistance phenotypes [55].

The genetic platform of bla_{NDM} gene mostly involves transposon Tn*125* with two flanking IS*Aba125* elements, often truncated in *Enterobacteriaceae* and part of broad-host-range plasmid including IncA/C (predominant plasmid type), F, R, H, N, L/M, and X types [38, 41, 53, 54]. NDM producers can acquire additional β-lactamases, even carbapenemases, and are recognized as the source of community-acquired infections [54, 55]. The highest distribution of NDM variants (more than 15 so far) is detected in *K. pneumoniae* and *E. coli* species from Asian continent with China and India still serving as the major reservoir of NDM producers [38, 54].

A global surveillance study conducted through 2012–2014 enrolled 202 medical centers from 40 countries all over the world and identified 471 MBL-producing isolates of *Pseudomonas aeruginosa* and *Enterobacteriaceae* with 32 different MBL variants. Among them, seven novel allelic MBL variants were identified (VIM-42, -43, -44 and =45, IMP-48 and -49 and NDM-16), differing by one amino acid from previously reported enzymes proving still dynamic genetic background of these worldwide disseminated enzymes [56].

Other MBL-encoding genes are mostly limited to not only a single report or to particular geographic regions, including genes encoding SPM-1, GIM-1 and SIM-1, but also more recently described AIM-1, KHM-1, DIM-1, BIC-1, and TMB-1 [38, 57–60]. GIM–MBL, unlike other class B carbapenemases, has two zinc ions in the active site.

SPM β-lactamases, originally described in *P. aeruginosa* strain from Brazil, are mostly limited to South America and have been associated with outbreaks at many hospitals. There were two imported cases in Europe until now, one in Switzerland, and one in the UK [57, 61, 62]. Unlike the other MBL-encoding genes, bla_{SPM} gene was not part of gene cassette or integron, but it was located on plasmid and flanked with two expanded form is insertion segment common region (IS*CR*) elements probably providing its mobilization capacity [57]. The gene was also described in *A. baumannii* strain from Tehran [63].

3.3 Class D carbapenemases (carbapenem hydrolyzing class D β-lactamases; chdls)

The main characteristic of these enzymes is their hydrolytic activity toward oxacillin. Ambler group D carbapenemases include oxacillinases typical for *Acinetobacter baumannii*. [64]. OXA enzymes (the name came since they hydrolyze isoxazolylpenicillins much faster than penicillins) were recently divided into 12 subgroups: OXA-23-like, OXA-24/40-like OXA-48, OXA-51-like, OXA-58-like, OXA-143-like, OXA-253, OXA-211, OXA-213, OXA-214, OXA-229, and OXA-235 [65]. The amino acid sequence identities between members of the same subgroup are more than 90%, whereas the identities between enzymes that belong to different subgroups are less than 70% [65]. Enzymes belonging to OXA-51 group are naturally occurring β-lactamases of *A. baumannii* (often used as marker of identification) and are normally expressed at low levels but can be overexpressed as a consequence of the IS*Aba1* location upstream of the genes [64]. The clinically significant resistance is usually expressed in combination with other mechanisms like porin loss or upregulated efflux pumps. OXA-23 subgroup has been reported worldwide but most reports are coming from *Acinetobacter baumannii* isolates from Latin America. OXA-23-encoding gene is usually located on transposon Tn2006,

almost always bracketed on both sides by IS*Aba1* insertion sequence [5, 65–67]. It could be located on transferable plasmids, but it was also described integrated in the *A. baumannii* chromosome. OXA-24/40 subgroup was reported less frequently from *A. baumannii*, mostly from Iberian Peninsula and the USA [68]. It confers high level of carbapenem resistance and is usually chromosomally encoded. OXA-58 and OXA-143 are usually plasmid mediated, first reported in France and Germany, respectively [69, 70]. Similar to OXA-23, $bla_{\mathrm{OXA\text{-}58}}$ is located within transposon usually bracketed by two copies of IS*Aba3*. OXA-72 is the most prevalent allelic variant found in Europe, Far East, and South America [65, 71–73]. OXA-72 and OXA-23 variants have not only been identified in long-term care facilities but also in environmental samples such as municipal wastewater stressing the fact that strains resistant to antimicrobials are constantly circulating between the hospitals, community, and the nature [74–76].

Newer oxacillinase subgroups were mostly described as naturally occurring enzymes with low carbapenemase activity in *Acinetobacter* "non-*baumannii*" isolates like OXA-134a and OXA-143 in *A. lwoffi*, OXA-211 in *A. johansonii*, OXA-213 in *A. calcoaceticus*, OXA-214 in *A. haemolyticus*, OXA-229 in *Acinetobacter bereziniae*, and OXA-235 in *Acinetobacter schindleri* [65, 66, 70]. However, it is suggested that those enzymes present a reservoir of enzyme variants that could evolve to a more significant level and could be easily transferred to other bacterial species.

OXA-48 β-lactamase was originally described in a *Klebsiella pneumoniae* isolate from Istanbul, Turkey, in 2001 [77]. It is now widespread not only in *Klebsiella pneumoniae* but also in other *Enterobacteriacea*. Turkey was reported as having the highest epidemiologic level called endemic in 2015. The most important reservoirs of OXA-48 carbapenemases are linked with Turkey, India, Middle East, and North African countries [78]. $bla_{OXA\text{-}48}$ gene is usually located in pOXA-48a plasmid, which belongs to the group of IncL/M plasmids. This plasmid has high conjugation rate, and it is self-conjugative, explaining partly the global dissemination of OXA-48 enzymes [53]. The spread of $bla_{OXA\text{-}48}$ gene is further mediated by I*S1999* insertion sequence located upstream of the Tn*1999* transposon which is usually carried on L/M plasmid. In the majority of European countries, at present, it is the most prevalent type of carbapenemase among *Enterobacteriaceae* [65, 78].

OXA-48 hydrolyzes carbapenems at a low level, shows very weak activity against expanded-spectrum cephalosporins, and does not significantly hydrolyze ceftazidime and cefepime but in combination with impermeability can lead to high-level resistance to carbapenems [79]. OXA-48 significantly hydrolyze only penicillins and narrow-spectrum cephalosporins and like other class D enyzmes is not inhibited by clavulanic acid, sulbactam, or tazobactam leading to elevated MICs of amoxycillin/clavulanate and piperacillin/tazobactam, as shown in **Table 1**. Resistance to expanded-spectrum cephalosporins occurs only in strains positive for addtional ESBL or AmpC. Some OXA-48 variants, including OXA-181 (differing by four amino acids from OXA-48) and OXA-162 (with one amino acid substitution T213A) were also found in *Enterobacteriaceae* [78]. OXA-162 variant is also able to hydrolyze ceftazidime, whereas OXA-48 is not. Interestingly, OXA-181 is often associated with other carbapenemase genes, and many reports from Indian subcontinent describe isolates coproducing OXA-181, NDM-1, and VIM enzymes [80].

In a recent report, environmental species, *Shewanella xiamenensis* was identified as the progenitor of the $bla_{\mathrm{OXA\text{-}181}}$ gene [81]. It is therefore hypothesized that a mobilization of a chromosomal gene to plasmid happened, and then the gene disseminated further in clinically relevant species. This work emphasizes the constant interplay between microorganisms in the environment, community, and clinics. Gram-negative bacteria are ubiquitous in nature and are widely distributed in soil and water where they can survive for a long time posing a possible reservoir of

Type of carbapenemase	Substrate profile						Inhibiton by	Country of origin	Host species	Reference
	Penicillins	**Narrow-spectrum cephalosporins**	**Expanded-spectrum cephalosporins**	**Aztreonam**	**Imipenem**	**Meropenem**				
Class A										
IMI	+	+	—	—	+	—	Clavulanic acid	France	*E. cloacae*	[11]
NMC	+	+	—	—	+	—	Clavulanic acid	France	*E. cloacae*	[12]
SME-1	+	+	—	—	+	—	Clavulanic acid	United States	*S. marcescens*	[8]
SME-2	+	+	—	—	+	—	Clavulanic acid	United States	*S. marcescens*	[9]
SME-3	+	+	—	—	+	—	Clavulanic acid	United States	*S. marcescens*	[10]
KPC-1	+	+	+	+	+	+	PBA	United States	*K. pneumoniae*	[13]
KPC-2	+	+	+	+	+	+	PBA	United States	*K. pneumoniae*	[13, 15]
KPC-3	+	+	+	+	+	+	PBA	United States	*K. pneumoniae*	[14]
GES-2	+	+	—	—	+	—	Clavulanic acid	French Guinea	*P. aeruginosa*	[16]
Class B										
IMP-1	+	+	+	—	+	+	EDTA	Japan	*P. aeruginosa*	[29]

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Type of carbapenemase	Substrate profile						Inhibiton by	Country of origin	Host species	Reference
	Penicillins	**Narrow-spectrum cephalosporins**	**Expanded-spectrum cephalosporins**	**Aztreonam**	**Imipenem**	**Meropenem**				
IMP-2	+	+	+	—	+	+	EDTA	Italy	***A. baumannii***	[32]
IMP-4	+	+	+	—	+	+	EDTA	Japan	***A. baumannii***	[35]
IMP-5	+	+	+	—	+	+	EDTA	Portugal	***A. baumannii***	[36]
VIM-1	+	+	+	—	+	+	EDTA	Italy	*P. aeruginosa*	[42]
VIM-2	+	+	+	—	+	+	EDTA	France	*P. aeruginosa*	[43]
VIM-3	+	+	+	—	+	+	EDTA	Taiwan	*P. aeruginosa*	[44]
VIM-4	+	+	+	—	+	+	EDTA	France	*E. cloacae, K. pneumoniae*	[45]
VIM-7	+	+	—	—	+	+	EDTA	USA	*P. aeruginosa*	[47]
NDM-1	+	+	+	—	+	+	EDTA	Sweden	*K. pneumoniae*	[51]
GIM-1	+	+	+	—	+	+	EDTA	Germany	*P. aeruginosa*	[58]
SPM-1	+	+	+	—	+	+	EDTA	Brasil	*P. aeruginosa*	[57]

Type of carbapenemase	Substrate profile						Inhibiton by	Country of origin	Host species	Reference
	Penicillins	**Narrow-spectrum cephalosporins**	**Expanded-spectrum cephalosporins**	**Aztreonam**	**Imipenem**	**Meropenem**				
SIM-1	+	+	+	—	+	+	EDTA	South Korea	*P. aeruginosa*	[59]
DIM-1	+	+	+	—	+	+	EDTA	The Netherlands	*P. aeruginosa*	[60]
Class D										
OXA-48-like	+	+	—	—	+	—		Turkey	*K. pneumoniae*	[77]
OXA-23 (ARI-1)	+	+	+	+	+	+		UK	***A. baumannii***	[67]
OXA-24/40-like	+	+	+	+	+	+	NaCl	Spain	***A. baumannii***	[68]
OXA-58-like	+	+	+	+	+	+		France	***A. baumannii***	[69]
OXA-143-like	+	+	+	+	+	+		Germany	***A. baumannii***	[70]
OXA-253	+	+	+	+	+	+		Germany	***A. baumannii***	[65]

The most prevalent allelic variants are shown. Abbreviations: PBA-phenylboronic acid; EDTA-ethylenediaminetetraacetic acid.

Table 1.
Classification, substrate profile, susceptibility to inhibitors, host species, and county of origin of carbapenemases.

resistance determinants. Main characteristics of the most prevalent allelic variants of the Ambler classes A, B, and D carbapenemases are shown in **Table 1**.

4. Clinical significance of carbapenemases

Carbapenemase-producing Gram-negative bacteria can cause a wide spectrum of infections including bacteremia, nosocomial pneumonia, wound infections, endocarditis, and urinary tract infections. Risk factors for carbapenem-resistant isolates usually include procedures involving manipulations of the gastrointestinal tract, trauma, orthopedic procedure, or life-threatening conditions like septicemia and septic shock. Those infections are often associated with high hospitalization cost, treatment failures, longer hospital stay, and high mortality rates [82–84].

5. Laboratory detection

Appropriate and timely laboratory detection of carbapenemase-producing microorganisms is of crucial importance to implement adequate antimicrobial therapy. Screening for carbapenemase production is usually based on reduced inhibition zones around carbapenem disks in routine disk-diffusion testing or elevated MICs for carbapenems. However, the level of resistance depends on the type of carbapenemase, allelic variant, gene expression, and/or the presence of additional resistance mechanisms such as porin loss or upregulation of efflux pumps.

Recommendations for carbapenem susceptibility breakpoints have been established by both Clinical Laboratory Standards Institute (CLSI) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST), but no global consensus exists with EUCAST breakpoints being generally lower [85, 86]. However, some strains with low-level resistance are still being missed. EUCAST has also established epidemiologic breakpoints to distiguish between wildtype isolates and those with carbapenem-resistant determinants [86].

Ideally, methods for identifying carbapenemase should have a short turnaround time to ensure timely implementation of control measures. This could be challenged by difficulties in detecting carbapenemase producers, since MICs to carbapenems could be elevated but within susceptible range or even low, as described in *Enterobacteriacea*e and *A. baumannii*. Particularly in OXA-48-producing organisms, the MICs of carbapenemas can be very low. The screening usually relies on the reduction of the inhibition zone around ertapenem disk [87, 88]. However, relevant methodology with specific laboratory test has not yet been standardized. Modified Hodge test is the only test recommended by CLSI for the phenotypic detection of carbapenemase producers but often lacking sensitivity and specificity. There are also several inhibitor-based tests using different inhibitors (EDTA and phenantroline as inhibitors of MBLs, phenylboronic acid as inhibitor of KPC) in combination with carbapenem (e.g., meropenem) or cephalosporin (e.g., ceftazidime) in different formats—disk-diffusion or broth dilution or E-test [88]. There is no specific inhibitor that could be used in detection of class D carbapenemases, but there are reports on using temocillin disk (or combined with avibactam) for this purpose [88]. Carba NP (derived from the names Nordmann-Poirel) test is a simple biochemical test based on hydrolysis of imipenem detectable by a change of color of the indicator due to decrease of pH. It is applicable in most microbiological laboratories, although the reference standard in detection of carbapenemase production is spectrophotometric measurement of carbapenem hydrolysis in the presence or absence of inhibitor, but it is still reserved for reference laboratories [89]. Recently, a new method for the detection of carbapenemases was described [90].

The test was called carbapenem inactivation method (CIM), and it was based on degradation of meropenem by carbapenemase. The water suspension of carbapenem-resistant microorganism is incubated with a 10-ug meropenem disk for 2 h. After incubation, the disk is removed from the suspension and placed onto a Mueller-Hinton agar plate previously inoculated with the carbapenem-sensitive microorganism (usually *Escherichia coli*). If the carbapenem-resistant microorganism produces carbapenemase, consequently, meropenem will be hydrolyzed and the indicator (carbapenem susceptible) microorganism will grow close to the disk [90]. CIM method has high sensitivity, comparable to that of Carba NP test, as reported recently [91]. Modified CIM test includes preparation of the bacterial suspension in tryptic soy broth and extending the time of incubation to 4 hours improving the detection of some carbapenemases in *Enterobacteriaceae* [92]. Recently, the use of mass spectrometry (matrix-assisted laser desorption/ionization-time of flight—MALDI-TOFF) based on analysis of degradation of carbapenem-molecule-enabled rapid detection of KPC carbapenemase (in 45 min) or MBL (150 min) [93]. Finally, simplex or multiplex PCR, real-time PCR, or hybridization tests could significantly improve detection of carbapenemase genes in clinical laboratory bypassing the sensitivity and specificity problems with phenotypic tests [89]. However, molecular methods require expensive equipment and trained laboratory stuff.

6. Therapeutic options

Colistin is usually the last resort antibiotic for the treatment of infections associated with carbapenemase-producing Gram-negative bacteria. However, nephrotoxicity and neurotoxicity are of clinical concern [94]. Colistin-resistant isolates of *A. baumannii, P. aeruginosa*, and *K. pneumoniae* have emerged recently leading to pan-drug resistant phenotypes with modified outer membrane lipopolysaccharide [95–97]. The optimal treatment approach for infection caused by carbapenemase-producing Gram-negative bacilli remains a controversy. Still, a combination therapy is strongly recommended and includes two or more antibiotics active in vitro such as colistin, tigecycline, amikacin, aztreonam, or carbapenem in different combination schemes [98, 99]. Colistin is not recommended as monotherapy because of the development of heteroresistance. The combinations of colistin with rifampicin, vancomycin, and meropenem were shown to be synergistic in *A. baumannii* in vitro by chequerboard, 2-well and time-kill metod, but the results of the randomized, controlled clinical trials are controversial [99–101]. If the pathogen is suspected to be MBL producer, aztreonam may present the core drug (if the isolate does not possess other extended-spectrum or ampC β-lactamase), but if the patient is critically ill, the combination therapy with three drugs is preferred. If aminoglycosides or fosfomycin retain in vitro activity, for any serious infection, then they should not be using monotherapy. However, oral fosfomycin should not be used for the management of any infection outside the urinary tract, and in critically ill patients, combination therapy should be considered. Tigecycline is recommended for skin and soft-tissue infection with carbapenemase-producing *A. baumannii* and *Enterobacteriacea*e. If MICs to meropenem are low or moderately high (up to 16 μg/ml), high-dose meropenem can be administered by prolonged infusion [99, 100]. Dual carbapenem-based regimen for carbapenem-resistant *Enterobacteriaceae* usually includes high-dose meropenem or high-dose doripenem with ertapenem, and it is also most effective if the third drug is added [99]. In bloodstream and respiratory tract infections colistin could be added, while in gastrointestinal and biliary tract infections colistin and tigecycline should be included. Newer β-lactam-β-lactamase inhibitor combinations such as ceftolozan/tazobactam seem promising

in treatment of carbapenem-resistant *Pseudomonas aeruginosa* infections [102]. However, it is not effective against MBL-producing organisms. Continued reports should be acknowledged in efforts of optimizing therapy for infections caused by carbapenem-resistant Gram-negative bacili. Furthermore, there is an urgent need to develop new antimicrobials. Although antibiotics still represent the mainstream direction for the treatment, the alternatives to them should be taken into account, including vaccines, bacteriocins, and probiotics. The revival of old antibiotics should be supported by expanding the knowledge of their pharmacokinetics and pharmacodynamics in order to avoid side effects and resistance developments.

7. Conclusions

In the last decade, an epidemic spread of carbapenamases among *Enterobacteriaceae* and Gram-negative nonfermentative bacilli was observed worldwide. The type of the most prevalent carbapenemase in a geographic region differs and might be associated with historical or cultural connections and exchange of people between countries where certain types of carbapenemase are endemic including the transfer of patients or staff across the borders, medical tourism, and migration of refugees. Particularly alarming is the fact that carbapenemases are not restricted only to hospital isolates any more. They have been reported and are continuously circulating between hospitals, long-term care facilities, community, and the environment. In conclusion, facing the global dissemination of carbapenemase-producing Gram-negative bacteria, and awaiting for the new antimicrobials to appear, a reasonable approach including hand hygiene, contact precautions, rational antibiotic usage, and active surveillance should be part of an intensive strategy aimed to reduce the incidence of colonization and infections with carbapenem-resistant microorganisms and to stop the spread of these bacteria into the community or the environment and vice versa.

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