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MINI-REVIEW

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The Intriguing Carbapenemases of *Pseudomonas aeruginosa*: Current Status, Genetic Profile, and Global Epidemiology

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Worldwide, *Pseudomonas aeruginosa* remains a leading nosocomial pathogen that is difficult to treat and constitutes a challenging menace to healthcare systems. *P. aeruginosa* shows increased and alarming resistance to carbapenems, long acknowledged as last-resort antibiotics for treatment of resistant infections. Varied and recalcitrant pathways of resistance to carbapenems can simultaneously occur in *P. aeruginosa*, including the production of carbapenemases, broadest spectrum types of β-lactamases that hydrolyze virtually almost all β-lactams, including carbapenems. The organism can produce chromosomal, plasmid-encoded, and integron- or transposon-mediated carbapenemases from different molecular classes. These include Ambler class A (KPC and some types of GES enzymes), class B (different metallo-β-lactamases such as IMP, VIM, and NDM), and class D (oxacillinases with carbapenem-hydrolyzing capacity like OXA-198) enzymes. Additionally, derepression of chromosomal AmpC cephalosporinases in *P. aeruginosa* contributes to carbapenem resistance in the presence of other concomitant mechanisms such as impermeability or efflux overexpression. Epidemiologic and molecular evidence of carbapenemases in *P. aeruginosa* has been long accumulating, and reports of their existence in different geographical areas of the world currently exist. Such reports are continuously being updated and reveal emerging varieties of carbapenemases and/or new genetic environments. This review summarizes carbapenemases of importance in *P. aeruginosa*, highlights their genetic profile, and presents current knowledge about their global epidemiology.

INTRODUCTION TO *PSEUDOMONAS AERUGINOSA* AND SIGNIFICANCE OF CARBAPENEM RESISTANCE

A versatile, opportunistic, and multidrug resistant pathogen, *Pseudomonas aeruginosa* remains a significant cause of infections with high morbidity and mortality [1], including hospital-acquired and ventilator-associated pneumonias, urinary tract, surgical site, burn, and bloodstream infections [2,3]. It shows high propensity to infect

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Abbreviations: ST, Sequence type; PDC, *Pseudomonas*-derived cephalosporinases; RND, Resistance-nodulation-division; KPC, *Klebsiella pneumoniae* carbapenemases.

Keywords: *Pseudomonas aeruginosa*, carbapenemases, resistance, epidemiology

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immunocompromised hosts, patients in intensive care units, and those with structural lung diseases like cystic fibrosis [4,5]. The tenacious nature of *P. aeruginosa*, its dynamic array of antimicrobial resistance pathways, and its substantial burden on healthcare, have impelled its ranking as a critical priority pathogen by the World Health Organization, which is the highest in its three-tier list of pathogens indicating global urgency to develop new therapeutics against this challenging organism [6].

The formidable pathogenic profile of *P. aeruginosa* is greatly linked to its assortment of virulence factors [7], and to numerous and variable resistance determinants, known to confer resistance to multiple antibiotics including β-lactams, aminoglycosides, fluoroquinolones, colistin, and tigecycline, leading to multidrug or even pandrug resistance [8,9]. Most antibiotics are not effective against *P. aeruginosa* due to its disconcerting levels of intrinsic and acquired resistance [5], in addition to recently characterized adaptive resistance, whereby gene expression changes with growth or environmental conditions, including exposure to stress, and is responsible for recalcitrance and relapse of infections [10]. Specifically, the emergence of pseudomonal resistance to carbapenems has become a global health concern. Pioneered by imipenem, and including the approved compounds meropenem and doripenem, these broadest spectrum and greatest potency β-lactam antibiotics represent last-resort options for *P. aeruginosa* infections [11,12]. Nevertheless, resistance to these compounds in *P. aeruginosa* is on the rise, caused by an arsenal of mechanisms. Most commonly, intrinsic loss or decrease in the porin protein OprD, involved in carbapenem uptake, was demonstrated as the most frequent mechanism [13-15]. Moreover, overexpression of efflux pumps of the resistance-nodulation-division (RND) family, mainly the MexAB-OprM efflux pump, plays a major role in carbapenem resistance in *P. aeruginosa* by expelling these compounds to the extracellular environment [16,17]. The role of porin inactivation is known to account for imipenem resistance, while efflux pump overexpression is mainly associated with meropenem resistance [18], but has lower impact on doripenem [19]. In addition, the overproduction of chromosomally encoded, inducible AmpC cephalosporinases, in isolates with accompanying resistance mechanisms, could positively contribute to carbapenem resistance, with ertapenem being completely excluded from these pathways as it has more limited spectrum and is naturally ineffective against *P. aeruginosa* [20,21].

Parallel to such intrinsic resistance, *P. aeruginosa* can attain carbapenem resistance through acquisition of carbapenemase genes. *P. aeruginosa* genome is among the largest bacterial genomes and maintains a blend of genes acquired through horizontal transfer, and localized within integrons and mobile elements like transposons, insertion sequences, genomic islands, and plasmids [9]. Among these, genes encoding carbapenemases are particularly relevant, due to wide spectrum of antibiotics affected [22]. Since the first description of the plasmid-encoded, transferrable IMP metallo-β-lactamase in *P. aeruginosa* more than 2 decades ago [23], numerous carbapenemases of Ambler classes A, B, and D have been described in this organism [8], whereby they continue to escalate the heterogeneity of carbapenem resistance mechanisms and complicate treatment.

CLASSES OF CARBAPENEMASES IN *P. AERUGINOSA* AND THEIR GENETIC ENVIRONMENT

Carbapenemases are β-lactamases with the most versatile nature and broadest spectrum of activity, capable of hydrolyzing carbapenems, in addition to most other β-lactam antibiotics with few exceptions. At the molecular level, carbapenemases belong to classes A, B, and D of the Ambler classification [24], although infrequent carbapenemases of class C exist, possibly reducing susceptibility to carbapenems via weak catalytic activity coupled to permeability defects [25]. The ability of *P. aeruginosa* to act as a reservoir and a dispersion trajectory for transferable carbapenemases constitutes a threat for antimicrobial therapy [22]. The major properties of *P. aeruginosa* carbapenemases are described below, and their names and abbreviations are shown in Table 1. Also, examples of recent studies reporting *P. aeruginosa* carbapenemases are summarized in Table 2.

Ambler Class A Carbapenemases in P. aeruginosa

Class A carbapenemases hydrolyze penicillins, classical cephalosporins, monobactams, and carbapenems, with hydrolysis dependent on their serine active site [24]. In 2001, Poirel and Colleagues described a self-transferable 100-kb plasmid of *P. aeruginosa* grown from blood cultures of a South African patient with pneumonia. The plasmid harbored a β-lactamase gene, bla_{GES-2}, whose product hydrolyzed expanded-spectrum cephalosporins and imipenem. GES-2 activity was less inhibited by clavulanic acid and tazobactam, common inhibitors of Ambler class A enzymes [26]. In addition to GES-2, GES-5-producing isolates of *P. aeruginosa* were identified in medical settings in Japan since 2014, and its gene was acquired and chromosomally encoded [27,28]. They were also detected in Dubai [29], as well as in Saudi Arabia [30] among sequence type (ST) 235 lineage, the most prevalent global clone associated with multidrug resistance [31]. GES-6 is another *P. aeruginosa* carbapenemase identified on a new type of class 1 integrons named In1076 and is chromosomally located [32]. GES-20 was

Table 1. List of Names and Abbreviations of Carbapenemases Discussed in this Article

Carbapenemase name	Abbreviation
Ambler Class A	
Guiana extended spectrum	GES
Klebsiella pneumoniae carbapenemase	KPC
Ambler Class B	
Central Alberta metallo-β-lactamase	CAM
Dutch imipenemase	DIM
Florence imipenemase	FIM
German imipenemase	GIM
Hamburg metallo-β-lactamase	HMB
Imipenemase metallo-β-lactamase	IMP
New Delhi metallo-β-lactamase	NDM
Seoul imipenemase	SIM
Sao Paulo metallo-β-lactamase	SPM
Verona integron-encoded metallo-β-lactamase	VIM
Ambler Class D	
Oxacillinase	OXA

Table 2. Examples of Studies Reporting Carbapenemases in *Pseudomonas aeruginosa* since 2020

Ambler class	Carbapenemase	Source of Isolation	Country	Year	Reference
A	KPC-2	Ascitic fluid	Brazil	2021	[63]
	KPC-2	Respiratory, surgical, and urine samples from ICU patients	China	2021	[64]
	KPC-90	Fecal screening sample	China	2022	[65]
	GES-24	Long-term care facilities	Korea	2020	[66]
B	VIM-5	Various clinical isolates	Nigeria	2021	[67]
	VIM	Various clinical isolates	Malaysia	2021	[68]
	VIM-1, VIM-2, VIM-4	Clinical and screening specimens from critical care units	Germany	2022	[69]
	VIM-6	Various clinical samples	Kenya	2022	[70]
	IMP-1, IMP-7, IMP-10, IMP-34, IMP-41	Various clinical samples	Japan	2022	[71]
	IMP-6	Urine	Korea	2022	[72]
	NDM-1	Sputum or endotracheal aspirates of COVID-19 patients	Egypt	2020	[73]
	NDM-1	Clinical and screening specimens from critical care units	Germany	2022	[69]
	NDM-1	Various clinical samples	Kenya	2022	[70]
	NDM-1	Urine	Korea	2022	[72]
D	OXA-913	Skin specimen of a dog with pyoderma	Korea	2021	[74]
	OXA-486	Feces of a red deer sampled in a humanized area	Portugal	2022	[75]

described in prevalence approaching 85% among carbapenem-resistant *P. aeruginosa* collected from Mexican hospitals and was chromosomally encoded on embedded class 1 integron arrays [33].

Currently, the plasmid-borne *Klebsiella pneumoniae* carbapenemases (KPCs) are among the most prevailing and widely distributed carbapenemases. While well acknowledged in *Enterobacteriaceae* family, the first KPC-2 identification in *P. aeruginosa* was in Columbia in 2007 with a suggested chromosomal gene location [34]. However, bla_{KPC-2} genes in this organism are mostly carried by plasmids of different sizes, associated with Tn*4401b* or a part of the Tn*4401* sequence [35]. A recent detailed understanding of the genetic background of KPC-carrying plasmids harbored by *P. aeruginosa* was described, with 29-kb bla_{KPC-2} -carrying plasmid, pR31-KPC, including two accessory modules, the IS*26*- bla_{KPC-2} - IS*26* unit and IS*26*-ΔTn*6376*-IS*26* region, separated by a 5.9-kb backbone region [36]. Almost a decade ago, it was anticipated that emergence of unrelated plasmids, differing in size and incompatibility group, and harboring diverse genetic structures containing bla_{KPC-2} in *P. aeruginosa* would eventually assume a dissemination pattern close to that in *Enterobacteriaceae* [37]. Currently, the spread of successful international clones, the variable existence of bla_{KPC-2} on integrons, transposable elements, and plasmids, accompanied by gene rearrangement events like transposition and recombination, and antimicrobial pressure, may all have driven the observed spread of bla_{KPC-2} in *P. aeruginosa* [38].

Ambler Class B Carbapenemases in P. aeruginosa

These are metallo-β-lactamases that use zinc-dependent hydrolysis to confer resistance to all β-lactams except aztreonam. They are resistant to β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam, but susceptible to inhibition by metal ion chelators like ethylene diamine tetraacetic acid (EDTA) [39]. The most common families of Class B include IMP, VIM, NDM, GIM, and SIM, whose encoding genes are located within various integrons as gene cassettes. When these integrons become associated with plasmids or transposons, horizontal transfer between bacteria is highly probable [40]. Interestingly, the initial discoveries of four families of metallo-β-lactamases including IMP [23], VIM [41], SPM [42], and GIM [43] were from *P. aeruginosa*, indicating this organism as a favorable reservoir for metallo-β-lactamases. At least 32 different variants of IMP and 23 variants of VIM exist in *P. aeruginosa* [8], and some reports indicate that 30% of resistant strains possess a metallo-β-lactamase [44]. According to a recent whole-genome sequencing analysis, internationally disseminated *P. aeruginosa* high-risk clones that are multidrug resistant, such as the most frequently reported ST111, ST175, ST233, ST235, ST277, ST357, ST654, and ST733 are at the origin of the wide dissemination of NDM, VIM, and IMP [45]. In Brazil, the emergence of *rmtD1* gene, encoding aminoglycoside resistance, was recently reported in *P. aeruginosa* isolates carrying KPC-2 and/or VIM-2, featuring a pan-resistant phenotype [46]. The infuriating spread of the metallo-β-lactamase NDM-1, which displays tighter binding to most β-lactams and which has spread among many Gram-negative bacteria was captured by *P. aeruginosa*, adding to its arsenal of resistance weapons. After its initial reporting in Serbia [47], NDM-1-positive *P. aeruginosa* isolates have been recovered throughout the world [48]. In 2021, an emerging clone, ST308, was identified including NDM-1-producing *P. aeruginosa*, in addition to the global high-risk ST235 clone which was predominant and has circulated for the previous 14 years in Singapore [49].

The genetic environment of IPM- and VIM-encoding genes is mainly a class 1 integron, characterized by an integrase *intlI* gene associated with a transposase *tnpA*. This integron belongs to mobile integron elements that are commonly plasmid-mediated; however, some large integrons, classified as superintegrons that harbor hundreds of gene cassettes and homogeneous sites, have been detected in the pseudomonal chromosome [40]. Recently, five types of bla_{VIM-2}-containing integrons were described in Russia, including In56, In559, In59-like, In59, and In249 [50]. Regarding NDM-1, its encoding gene in *P. aeruginosa* was reported as part of the variable region of a complex class 1 integron bearing IS common region 1 (IS*CR1*), and surrounded by IS*Aba125* and a truncated bleomycin resistance gene [51].

Overexpression of Class C Cephalosporinases Associated with Other Mechanisms, or Peculiar Carbapenem-Hydrolyzing AmpC Enzymes

In addition to carbapenemases, another β-lactam resistance mechanism in *P. aeruginosa* is production of chromosomal AmpC enzymes, also called *Pseudomonas*-derived cephalosporinases (PDCs), induced or derepressed to cause penicillin and cephalosporin resistance. Inducible AmpC can be upregulated by subinhibitory concentrations of some β-lactams. Moreover, mutations in regulatory AmpC components can lead to stable expression resulting in resistance [52]. The current knowledge regarding the role of AmpC in carbapenem resistance in *P. aeruginosa* suggests that their mere overexpression does not significantly affect carbapenems, but certainly could contribute to resistance if escorted by additional mechanisms like efflux pump overproduction, poor OprD, and/or carbapenemases [20]. For example, in an investigation from Korea, co-expression of PDC-2 with IMP or VIM resulted in high level carbapenem resistance, unlike either mechanism alone [52]. Also, PDC

Figure 1. **Heatmaps showing the worldwide distribution of *Pseudomonas aeruginosa* carbapenemases**, with colored areas corresponding to the geographical regions with predominance of the specific carbapenemase group. (**a**): KPC; (**b**): GES carbapenemases; (**c**): IMP; (**d**): VIM; (**e**): NDM. The heatmaps were extracted using data from the Antimicrobial Testing, Leadership, and Surveillance (ATLAS). Accessible through: https://atlas-surveillance.com.

genes were detected together with OprD and efflux pump mutations to contribute to high-level imipenem resistance in *P. aeruginosa* isolates from companion animals in Japan [53]. Furthermore, some mutational variants of PDC like PDC-2, PDC-3, PDC-4, or PDC-5 show reduced susceptibility for all β-lactams, including ceftazidime, cefepime, cefpirome, aztreonam, imipenem, and meropenem, compared to PDC-1 [54], putting forward a novel resistance mechanism.

Ambler Class D Carbapenemases in P. aeruginosa

The class D serine β-lactamases are enzymes capable of hydrolyzing oxacillin and cloxacillin, hence the name oxacillinases and are not inhibited by Ambler classes A or B inhibitors. Originally, these enzymes were identified in *Enterobacteriaceae* and *P. aeruginosa*, and were plasmid-encoded [24]. In 1993, Hall and Colleagues described the first extended-spectrum OXA enzyme, OXA-11, in *P. aeruginosa* recovered from blood cultures of a Turkish burn patient and showed that the enzyme exhibited considerable hydrolysis rates of ceftazidime [55]. Additional extended-spectrum variants were later described, like OXA-13, OXA-14, 15, OXA-18, OXA-28, and OXA-45, with none exhibiting carbapenem hydrolysis. These enzymes were seldom identified in other species, and despite their importance to resistance profile of *P. aeruginosa*, they did not spread, and their epidemiological impact on resistance remains inconclusive [24,56]. Low rates of OXA-23, OXA-40, and OXA-58 families, that mainly exist in *Acinetobacter baumannii*, were detected in *P. aeruginosa* [57] even though their associated genes have both chromosomal and plasmid locations [56]. A specific carbapenemase, OXA-198, was identified in one *P. aeruginosa* strain and its gene was harbored by a class 1 integron carried on a 46-kb nontypeable plasmid [58]. Later, this carbapenemase was identified in a hospital-associated cluster, and its gene belonged to a novel IncP-11 plasmid, whose transfer operon was partly deleted, perhaps limiting horizontal spread [59]. In India, the carbapenem-hydrolyzing OXA-48, predominant in *Enterobacteriaceae*, was detected in *Escherichia coli* and *P. aeruginosa* co-infection. The $bla_{\text{OXA-48}}$ gene was identified on a 60-Kb plasmid previously associated with spread of this resistance trait [60], probably emphasizing genome plasticity of *P. aeruginosa*.

WORLDWIDE EPIDEMIOLOGY OF CARBAPENEMASES IN *P. AERUGINOSA*

The rates of carbapenem resistance in *P. aeruginosa* vary worldwide, with a prevalence of 10-50% in most countries. For example, Canada and the Dominican Republic represent the lowest rates (3.3% and 8% respectively), while Australia, North America, and some

countries in Europe represent rates of 10-30%. By contrast, rates in Brazil, Peru, Costa Rica, Russia, Greece, Poland, Iran, and Saudi Arabia are above 50%, representing predominant areas in which resistance rates are high enough to be concerning for public health [48]. Regarding carbapenamases, a report by the Centers of Disease Control and Prevention in 2018 indicated that 1.9% of carbapenem-resistant *P. aeruginosa* isolates were carbapenemase producers [61]. Worldwide, the dissemination of carbapenemases is greatly variable as shown in Figure 1, with metallo-β-lactamases remaining most predominant, especially the VIM group, followed by IMP and NDM. Others have maintained regional spread like SPM in Brazil, Switzerland, the United Kingdom, China, and India, DIM in Poland, GIM in Germany, SIM in China, HMB in Germany and the United States, CAM in Canada, AIM in Australia, and FIM in Italy. KPC- and GES-producing *P. aeruginosa* clones have been identified in Europe and Asia, while the OXA-type remain the least identified and have been reported in Spain, India, the United Kingdom, and Belgium [62].

CONCLUSION AND OUTLOOK

The data presented in this mini-review constitute a snapshot of the current status of carbapenemases in *P. aeruginosa* which continues to be a major infectious disease concern. This organism represents an exemplary phenomenon of resistance endemicity, acquisition, and dissemination. The literature on carbapenemases originating in *P. aeruginosa* like metallo-β-lactamases, those acquired through its extreme versatility like class A and D enzymes, and the subordinate mechanisms that originate from PDCs, is so extensive and reports from different geographic areas are ongoing. Also, additional variants and original genetic backgrounds are being revealed, and spread of resistant strains will continue, driven by the clinical use of carbapenems. The wealth of such information should prompt novel methodologies like next-generation sequencing and novel detection methods to uncover additional knowledge about carbapenemases in this organism and make thorough assessment of its genome. Although such techniques afford an effective means to track *P. aeruginosa* carbapenemases, their application in molecular epidemiological surveys of this organism remains limited and should be improved. Despite efforts to halt further expansion of carbapenem-resistant *P. aeruginosa*, a permanent cure is still a long way off. Indeed, understanding the properties, epidemiology, and molecular characteristics of carbapenemases remains an essential part of a comprehensive scheme needed for proper containment and the prevention of a possible global health crisis instigated by this organism.

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