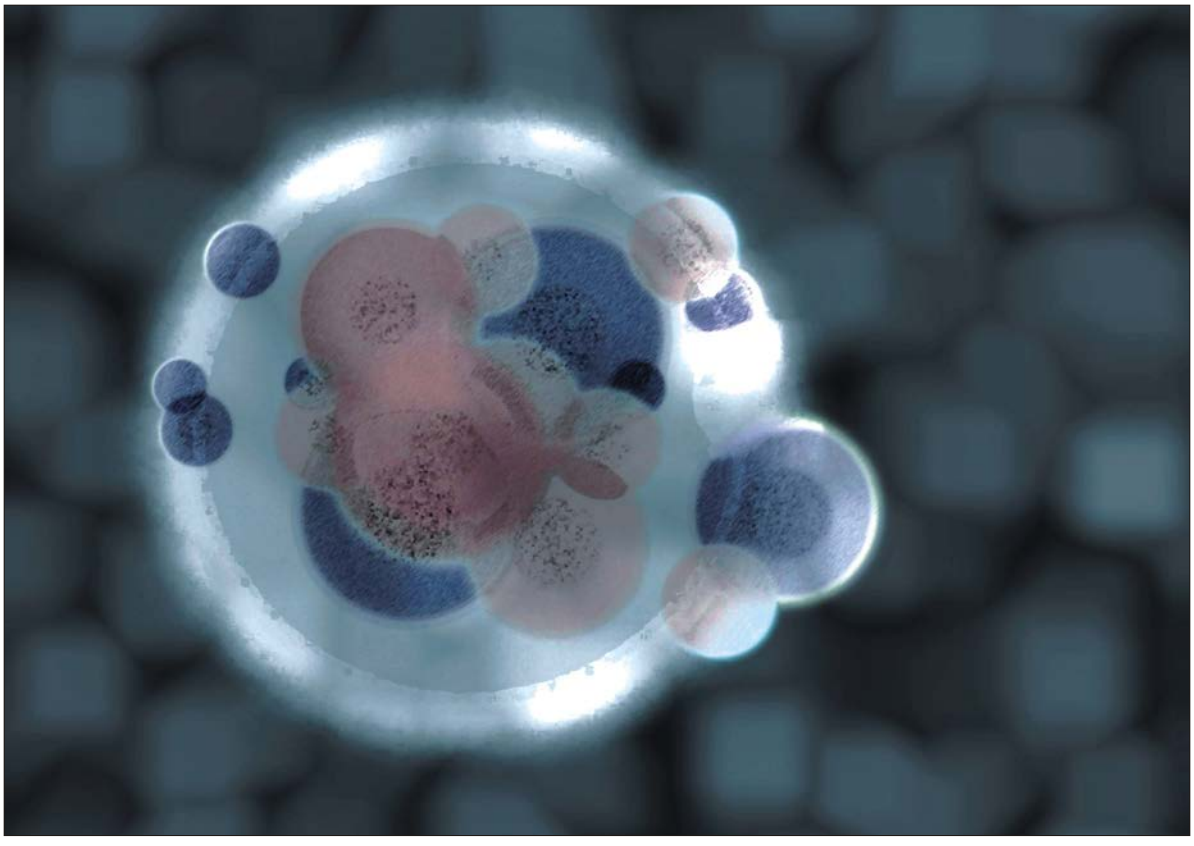

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Global Emergence of Nosocomial Gram-negative Pathogens Possessing Carbapenem-hydrolyzing β-lactamases

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Abstract: Carbapenem antibiotics are typically reserved for serious nosocomial infections. Several classes of β-lactamases have emerged that possess carbapenem-hydrolyzing activity. Class A KPC-type β-lactamases have recently emerged in *Klebsiella pneumoniae* isolates from the northeastern United States. Class D OXA-type β-lactamases are typically found in *Acinetobacter baumannii* and *Pseudomonas aeruginosa* isolates from southern Europe. Class B metallo-β-lactamases are also typically found in *A. baumannii* and *P. aeruginosa*; regions in the Far East and Europe have been especially affected by bacteria carrying these enzymes. Most nosocomial pathogens that possess a carbapenem-hydrolyzing β-lactamases are frequently resistant to other classes of antibiotics, including aminoglycosides and fluoroquinolones. For infections caused by these highly resistant Gram-negative pathogens, polymyxin antibiotics are often administered, although concerns remain regarding their efficacy and toxicity.

Key words: Carbapenemase, antibiotics, β-lactamases

INTRODUCTION

Antimicrobial resistance in hospital-acquired pathogens is associated with adverse clinical outcomes and increased healthcare expenditures. Carbapenem antibiotics are often reserved for the therapy of serious nosocomial infections due to Gram-negative bacilli. In particular, carbapenem antibiotics are often the agents of choice for therapy of infections due to *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacteriaceae* possessing extended-spectrum β-lactamases. Because these nosocomial pathogens are frequently resistant to other classes of antibiotics (including fluoroquinolones and aminoglycosides), effective therapeutic regimens are severely restricted when carbapenem resistance develops.

Although efflux systems, decreased cell wall permeability and altered penicillin-binding proteins can contribute to β-lactam resistance, in Gram-negative pathogens the primary defense are β-lactamases. β-lactamases have been categorized into four Ambler molecular classes. Classes A, C and D β-lactamases all possess serine at the critical site. Class B β-lactamases require the presence of zinc and are referred to as metallo-β-lactamases. In this review, β-lactamases that contribute to carbapenem resistance are examined.

CLASS A β-LACTAMASES

Several class A β-lactamases have been found to possess hydrolytic activity against carbapenems (Table 1). Sme-1 and Sme-2 have been recovered from carbapenem-resistant isolates of *Serratia marcescens*[1,2]. Sme-1 and Sme-2 were recovered from isolates in several cities in the United States and in London., but to date have remained distinctly unusual. Both enzymes were inhibited by clavulanic acid and tazobactam. The β-lactamase IMI-1 was recovered in two clinical isolates of *Enterobacter cloacae* from a hospital in California[3]. The bacteria carrying Sme and IMI β-lactamases remained susceptible to third generation cephalosporins and their identification actually preceded the introduction of carbapenems into clinical practice. The NmcA β-lactamase has been recovered in isolates of *E. cloacae*[4-6]. NmcA β-lactamase was chromosomally-encoded, closely related to IMI-1 and to Sme-1 and preferentially hydrolyzed imipenem. A fifth enzyme, GES-2, was found in an isolate of *Pseudomonas aeruginosa* from South Africa[7].

As noted, the finding of Sme, IMI, NmcA and GES-2 β-lactamases has remained rare. In contrast, bacteria carrying KPC-type β-lactamases appear to be emerging. The first of these enzymes, KPC-1, was found in a carbapenem-resistant strain of *K. pneumoniae*[8]. The finding of KPC-2, which differs from KPC-1 by a single

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Table 1: Susceptibility patterns of bacteria possessing class A β-lactamases with carbapenem-hydrolyzing activity

β-lactamase	Bacteria	No. of isolates (patients)	Country of origin	Imipenem MIC (μg mL^{-1})	Concomitant* resistance	Concomitant susceptibility	References
Sme-1	*S. marcescens*	1	England	16		MEM, CAZ, FEP	[1]
Sme1, 2	*S. marcescens*	25	USA	256-512	MEM, ATM, FOX	CAZ	[2]
IMI-1	*E. cloacae*	2	USA	>32	TZB, FOX,	CAZ, CTX, MEM	[3]
NmcA	*E. cloacae*	1	France	16	FOX	MEM, ATM, CTX	[4,5]
NmcA	*E. cloacae*	1	USA	>32	MEM, FOX	TZB, CAZ, CTX, TMP-SMX, CIP, GM	[6]
GES-2	*P. aeruginosa*	1	S. Africa	16	MEM, CAZ, TZB, ATM, GM, CIP	TOB	[7]
KPC-1	*K. pneumoniae*	1	USA (NC)	16		MEM, CAZ, TZB, ATM, GM	[8]
KPC-2	*S. enterica*	1	USA (MD)	16	MEM, CAZ, TZB, ATM	FEP	[9]
KPC-2	*K. oxytoca*	1	USA (NY)	32	MEM, CAZ, TZB, ATM, TMP-SMX	GM	[10]
KPC-2	*E. cloacae*	4	USA (MA)	8	CAZ, FEP		[11]
KPC-2	*Enterobacter* spp.	3	USA (NY)	24->32	ERT		[12]
KPC-2	*K. pneumoniae*	4	USA (MD)		ERT, ATM	FOX, CIP, FEP, AK, GM	[13]
KPC-2	*K. pneumoniae*	19	USA (NY)	4 ->64	CAZ, TZB, AK, CIP		[14]
KPC-2	*K. pneumoniae*	76	USA (NY)	>32	CAZ, TZB, AK, CIP	PXB, TIG	[15]
KPC-3	*E. coli*	7	USA (NJ)	3-16			[16]
KPC-3	*K. pneumoniae*	24	USA (NY)	>4	CAZ, TZB, AK, GM	PXB, TET	[17]
KPC-2	*K. pneumoniae*	96	USA (NY)	8->32	MEM, ERT, CAZ	PXB, TIG, DOX, GM	[102]

* AK= amikacin; ATM = aztreonam; CAZ= ceftazidime; CIP= ciprofloxacin; CTX= cefotaxime; DOX=doxycycline; ERT= ertapenem; FEP= cefepime; FOX= cefoxitin; GM= gentamicin; MEM= meropenem; PXB= polymyxin B; TET=tetracycline; TIG= tigecycline; TMP-SMX= trimethoprim-sulfamethoxazole; TZB=piperacillin-tazobactam

Table 2: Susceptibility patterns of bacteria possessing class D β-lactamases with carbapenem-hydrolyzing activity

β-lactamase	Bacteria	No. of isolates (patients)	Country of origin	Imipenem MIC (μg mL^{-1})	Concomitant* resistance	Concomitant susceptibility	References
OXA-23	*A. baumannii*	1	Scotland	16	FOX, ATM	CAZ, GM, CIP	[26,27]
OXA-23	*A. baumannii*	8	Brazil	>32	MEM, CAZ, TZB, SULB, AK, GM, CIP	PXB	[28]
OXA-23	*P. mirabilis*	10	France	0.25-0.5			[29]
OXA-24	*A. baumannii*	29	Spain	128	MEM, CAZ, ATM, CIP	TOB	[31]
OXA-25	*A. baumannii*	1	Spain	64	MEM, CAZ, PIP, ATM, FEP		[30]
OXA-26	*A. baumannii*	1	Belgium	64	MEM, CAZ, PIP, ATM	FEP	[30]
OXA-27	*A. baumannii*	1	Singapore	16	MEM, CAZ, PIP, ATM		[30]
OXA-40	*A. baumannii*	1	France	256	MEM, CAZ, TZB, ATM, GM, CIP	AK, COL	[32]
OXA-40	*A. baumannii*	82	Spain	>16	TZB, CAZ, ATM, GM, AK, CIP		[33]
OXA-48	*K. pneumoniae*	1	Turkey	64	MEM, CAZ, TZB, FOX, FEP		[34]
OXA-58	*A. baumannii*	7	France	32	MEM, CAZ, FEP, TZB, ATM	COL	[36]

*AK= amikacin; ATM = aztreonam; CAZ= ceftazidime; CIP= ciprofloxacin; COL= colistin; DOX= doxycycline; FEP= cefepime; GM= gentamicin; MEM= meropenem; PXB= polymyxin B; SULB= sulbactam; TOB= tobramycin; TZB=piperacillin-tazobactam

amino acid change, has been reported in sporadic isolates of *Salmonella enterica*[9], *K. oxytoca*[10] and *Enterobacter* sp.[11,12]. Isolates of *K. pneumoniae* with KPC-2 have also been identified[13] and appear to be rapidly emerging in the New York City region, resulting in hospital outbreaks[14,15].

A third enzyme KPC-3, which has an additional amino acid substitution, has been found in *E. cloacae*[12], *E. coli*[16] and *K. pneumoniae*[17]. A hospital outbreak, also in New York City, involved KPC-3 possessing *K. pneumoniae*[17]. Unlike the chromosomal Sme, NmcA and IMI-1 β-lactamases, KPC enzymes have been found to reside on transmissible plasmids[9,17], likely accounting for the variety genera carrying these enzymes. Most of the hospital outbreaks of *K. pneumoniae* have involved a small number of strains, however[14,15,17]. The *K. pneumoniae* possessing KPC enzymes isolates tend to be resistant to all β-lactam antibiotics and other classes of antibiotics, including aminoglycosides and fluoroquinolones. Therefore, therapy of infections due to these pathogens is extremely difficult. Occasional isolates retain susceptibility to gentamicin and amikacin. Most KPC-carrying *K. pneumoniae* are susceptible to the glycylcycline tigecycline and to polymyxin B, although resistance to the latter agent has been documented[15].

Class C: Chromosomal AmpC β-lactamases can slowly hydrolyze imipenem. When expression of an AmpC enzyme is coupled with an additional mechanism of resistance, frank carbapenem resistance can result. The combination of porin loss and class C β-lactamase expression is an important cause of imipenem resistance in *P. aeruginosa*[18,19] and *Acinetobacter baumannii*[20]. Occasional isolates of *E. aerogenes*[21,22] and *K. pneumoniae*[23-25] can also develop resistance to imipenem secondary to the combination of porin loss and AmpC expression.

Class D: Several groups of class D oxacillin-hydrolyzing enzymes possess carbapenem-hydrolyzing activity (Table 2). OXA β-lactamases are typically inhibited by NaCl but variably inhibited by clavulanic acid. The first group, consisting of OXA-23[26-29] and OXA-27[30] have 99% homology with one another. OXA-23 (also known as ARI-1) is not inhibited by clavulanic acid and was first recovered in isolate of *A. baumannii* from Scotland[26,27]. A strain of *A. baumannii* carrying OXA-23 has been found in hospitals in Brazil[28]. OXA-23 has also been found in a strain of *Proteus mirabilis* from France, although this strain remained susceptible to imipenem[29]. One isolate of *A. baumannii* with OXA-27 from Singapore has been reported; this enzyme was only weakly inhibited by clavulamic acid[30]. The second cluster of OXA enzymes possessing carbapenem-hydrolyzing activity include OXA -24[31] -25[30] -26[30] and -40[32] An outbreak of *A. baumannii* carrying OXA-24 has been reported in Spain; tobramycin was the only reported antibiotic displaying *in vitro* susceptibility[31]. Single isolates of *A. baumannii* carrying OXA-25[30] OXA-26[30] and OXA-40[32] have been documented; all were highly resistant to β-lactams. An outbreak of two major strains of *A. baumannii* possessing OXA-40 has also been documented in Spain[33]. A third unrelated enzyme, OXA-48, has been recovered in a carbapenem-resistant strain of *K. pneumoniae* from Turkey[34]. This isolate also carried multiple β-lactamases and had a porin defect, likely contributing to high-level β-lactam resistance. The fourth unrelated enzyme is OXA-58, recovered in carbapenem-resistant isolates of *A. baumannii* in France[35,36]. Six patients from a burn unit were found to harbor the same strain of OXA-58 possessing *A. baumannii*. This enzyme was found residing on a plasmid and was detected in an unrelated environmental isolate of *A. baumannii*[36]. Several isolates of *A. baumannii* from Argentina possessing unidentified OXA-type enzymes have also been reported[37,38]. Many OXA β-lactamases are not easily transmissible and possess only weak carbapenemase activity[26,29-31,35]. When OXA-24 was inserted into a susceptible *E. coli*, the MIC for imipenem rose from 0.12 to 1 μg mL^{-1}[31]. It appears that concomitant porin defects are necessary in order to reach a significant level carbapenem resistance in OXA-24 possessing *A. baumannii*[39].

Class B: Metallo-β-lactamases belong to the molecular class B of Ambler and are distinguished by the presence of zinc ion at their active site. They are inhibited by metal chelators such as Ethylene Diamine Tetraacetic Acid (EDTA) and thiol-based compounds and are not affected by the suicide β-lactamase inhibitors clavulanate, sulbactam and tazobactam. Metallo-β-lactamases efficiently hydrolyze virtually all broad-spectrum β-lactam antibiotics (but not monobactams), conferring resistance to penicillins, expanded-spectrum cephalosporins and carbapenems.

Metallo-β-lactamases are characteristically found as chromosomally-encoded enzymes in several bacterial species, including *Stenotrophomonas maltophilia, Chryseobacterium (Flavobacterium) meningosepticum, Aeromonas hydrophila, Bacillus cereus* and *B. fragilis*[40,41]. *Stenotrophomonas maltophilia* has emerged as an important nosocomial pathogen[42]. Identification of a plasmid-like element containing the intrinsic *S. maltophilia* metalloenzyme is disconcerting, since it could be spread to other Gram-negative species[43].

Over the past decade, acquired metallo-β-lactamases have been increasingly isolated from a variety of Gram-negative bacteria including *Pseudomonas* sp., *Acinetobacter* sp. and *Enterobacteriaceae*. The spread of metallo-β-lactamase-carrying bacteria in hospitals poses a serious threat to effective antimicrobial therapy of nosocomial infections. Four major groups of mobile metallo-β-lactamases have been recognized: The IMP, VIM, SPM and GIM groups. Their genetic determinants are often carried on mobile gene cassettes inserted into plasmid or chromosomal-borne integrons[44-46]. The IMP- and VIM-type enzymes are the prominent representatives, with 17 IMP-type and 11 VIM-type variants reported to date (www. lahey.org/studies/) and their presence is now recognized worldwide.

IMP-1 was first detected in 1991 in an isolate of *S. marcescens* from Japan[47] and soon after was found in several other Gram-negative species[48-52]. In a Japanese survey conducted in 1996-1997, 1.3% of *P. aeruginosa* and 4.4% of *S. marcescens* isolates produced IMP-1. This enzyme was also detected in other Gram-negative bacilli, including *K. pneumoniae, Citrobacter freundii, Enterobacter* sp., *Proteus vulgaris, Providencia rettgeri, Pseudomonas putida* and *fluorescens, Burkholderia cepacia, Alcaligenes xylosoxidans* and *Acinetobacter* sp.[49]. More recently, a longitudinal sample of Japanese isolates from the SENTRY Antimicrobial Surveillance Program, collected from 1998 to 2002, revealed that metallo-β-lactamase-producing strains of several species have persisted in Japan[53]. The IMP β-lactamases have spread to other regions in Asia (including Korea, Taiwan and Hong Kong) and have been reported in bacteria from Europe and Canada[54-59].

The second major family, VIM-type metallo-β-lactamases, were initially described in isolates from European countries. VIM-1 was identified in a

Table 3: Susceptibility patterns of bacteria possessing metallo-β-lactamases

β-lactamase	Bacteria	No. of isolates (patients)	Country of origin	Imipenem MIC (μg mL^{-1})	ATM MIC (μg mL^{-1})	Concomitant susceptibility	References
IMP	*P. aeruginosa*	15	Japan	2->128	4->128	MEM, CAZ, GM, TOB, AK, CIP	[52]
IMP	*P. aeruginosa*	53	Japan	2->128	1->128	CAZ, GM, CIP	[51]
VIM	*P. aeruginosa*	7	Greece	≥>128	32-64	CAZ, FEP, CIP, GM, AK, TZB	[61]
VIM-1	*P. aeruginosa*	8	Italy	>128	4-32	MEM, FEP, TZB, GM, AK, CIP	[69]
VIM	*P. aeruginosa*	45	Korea	8->128		CAZ	[66]
VIM	*P. putida*	7	Korea	64->128		CAZ	[66]
IMP	*A. baumannii*	9	Japan	8-16	4-32	CAZ, CTX, AK, GM	[50]
IMP-4	*Acinetobacter* sp.	23	Hong Kong	0.25-16	2-64	TZB, CTX	[56]
IMP	*Acinetobacter* sp.	17	Korea	8-32		CAZ	[66]
VIM	*Acinetobacter* sp.	16	Korea	4-32		CAZ	[66]
GIM	*P. aeruginosa*	5	Germany	>8	8-16	TZB, CAZ, CIP, GM, TOB	[46]
IMP-8	*K. pneumoniae*	17	Taiwan	0.25->256	0.06->256	CAZ, GM, TOB, CIP	[55]
IMP-8	*E. cloacae*	20	Taiwan	0.25-8	0.002->256	GM, TOB, CAZ	[70]
IMP	*S. marcescens*	4	Japan	8->128	2-8	CAZ, CIP	[48]

* AK= amikacin; ATM = aztreonam; CAZ= ceftazidime; CIP= ciprofloxacin; CTX= cefotaxime; FEP= cefepime; GM= gentamicin; MEM= meropenem; TOB= tobramycin; TZB=piperacillin-tazobactam

P. aeruginosa isolate collected in Verona, Italy in 1996[60] VIM-type variants have since been recovered in clinical isolates of *P. aeruginosa*, *Acinetobacter* sp. and enteric Gram-negative bacteria (including *Escherichia coli*, *K. pneumoniae, S. marcescens, E. cloacae*) from Europe, the Far East and the United States[61-64].

SPM-1 and GIM-1 appear thus far to be restricted to *P. aeruginosa* isolates in Brazil and Germany, respectively[45,46].

Initially, only sporadic isolates were found possessing metallo-β-lactamases. However, reports of nosocomial outbreaks soon followed[52,55,59,61,65-67]. In some regions, metalloenzyme-producing Gram-negative pathogens have become highly endemic. In one medical center in Italy, a significant increase in the prevalence of imipenem-resistant *P. aeruginosa* was noted; bla_{VIM} was found in 64 of 89 resistant isolates[65]. In this outbreak, molecular typing revealed that the bla_{VIM}-positive isolates generally belonged to two clusters. Isolates of VIM-possessing *P. aeruginosa* were detected from inpatients, residents of long-term care facilities and outpatients[65]. A recent report from SENTRY Antimicrobial Surveillance Programme suggests that the emergence of metallo-β-lactamases has become a national problem in Italy[67]. The original integron harboring the bla_{VIM-1} gene cassette, In70, was found in different strains throughout Italy. In addition, other novel integrons were identified and a considerable degree of variability in metallo-β-lactamases and integrons existed between geographically distinct regions.

Another study on the prevalence of metallo-β-lactamase -producing isolates came from 28 KONSAR hospitals in Korea[66]. In this study, 11.1% of resistant *Pseudomonas* sp. and 15.1% of *Acinetobacter* sp. carried metallo-β-lactamases[66]. Molecular typing showed that many of the bla_{VIM}-positive *P. aeruginosa* isolates belonged to one large cluster, suggesting intra- and inter-hospital spread of this strain.

Although considered efficient carbapenemases, a close analysis of susceptibility patterns of various metallo-β-lactamases -producing bacteria reveals an imperfect correlation between the presence of various enzymes and carbapenem susceptibility. Carbapenem MICs in the susceptible- or intermediate range have been frequently encountered, particularly among *Enterobacteriaceae* (Table 3), suggesting other mechanisms are needed for high-level carbapenem resistance. Variations in the degree of metallo-β-lactamase expression, decreased outer membrane permeability[40,68, 69] and/or expression of active efflux systems may also contribute to carbapenem resistance. Isolates with carbapenem MICs below the breakpoint for susceptibility will not be suspected of carrying one of these β-lactamases and therefore go unrecognized. The accurate detection of metallo-β-lactamase-producing isolates is important for the timely institution of infection control measures and prevention of further dissemination.

Several phenotypic screening methods have been described for identification of metallo-β-lactamases. The inhibition of metallo-β-lactamases by chelating agents, such as EDTA and 2-mercaptopropionic acid (2-MPA), served as the basis for screening methods described by several investigators. Two proposed screening methods involve the double-disk synergy test, using ceftazidime (CAZ) and 2-MPA disks[70] and the imipenem-EDTA disk diffusion method[71]. However, metallo-β-lactamase-producing bacteria may possess additional antibiotic-hydrolyzing enzymes[72] which may decrease the sensitivity of these screening tests. Metallo-β-lactamase-possessing Gram-negative bacteria

Table 4: Summary of clinical studies involving colistin therapy for multidrug resistant pathogens

Bacteria	No. of cases	Site of infections	Therapy	Favorable clinical outcome (%)	Microbiological Cure (%)	Renal toxicity (%)	Comments	References
A. baumannii	21	Respiratory	IV colistin alone	57	66	24	Outcome similar to imipenem group	[92]
A. baumannii and *P. aeruginosa*	71	Respiratory	Inhaled colistin		92	0		[100]
A. baumannii and	20	Respiratory	IV colistin	*25*	37			[86]
P. aeruginosa	12	Urine	IV colistin	83				
	9	Bacteremia	IV colistin	78				
A. baumannii,	29	Respiratory	IV and/or inhaled	76	41	10	65% also with imipenem,	[91]
P. aeruginosa, *A. xylosoxidans*		polymyxin B					38 with aminoglycoside	
P. aeruginosa	18	Respiratory	IV colistin	56	33			[89]
	6	Abdominal		83	67			
A. baumannii and *P. aeruginosa*	60	Mixed	IV Polymyxin B	88	14			[90]

Table 5: *In vitro* studies involving polymyxins against carbapenem-resistant bacteria

Bacteria	No. of isolates tested	Combinations with enhanced activity (% of isolates)	Comments	References
A. baumannii	13	Colistin+rifampin (85%)		[93]
A. baumannii	8	Polymyxin B + imipenem and/or rifampin (87-100%)	Triple combination bactericidal 8/8	[95]
P. aeruginosa	13	Polymyxin B + azithromycin		[97]
P. aeruginosa	10	Polymyxin B + imipenem and/or rifampin (80-100%)	Polymyxin B resistant isolates	[98]
K. pneumoniae	16	Polymyxin B + rifampin	KPC-2	[102]

with carbapenem MICs in the susceptible range can also be missed using these methods. Yan *et al.*[73] developed a modified four-disk diffusion method involving chelating agents, cephalosporins and clavulanic acid. Although higher detection rates were noted for several Gram-negative species, lower specificity was observed for *A. baumanii* and *P. aeruginosa* isolates. A commercially available Etest (AB BIODISK, Solna, Sweden) method has been reported to be sensitive in detection of metallo-β-lactamases in *Acinetobacter* sp., *P. aeruginosa, Serratia* sp., *S. maltophilia* and *B. fragilis*[74]. The Etest method however, will likely miss metallo-β-lactamase -producing *Enterobacteriaceae* with imipenem MICs below 4 μg mL^{-1}[73].

THERAPEUTIC OPTIONS

Because most of the pathogens carrying carbapenem-hydrolyzing enzymes are frequently resistant to all β-lactams and other classes of antimicrobials (aminoglycosides, fluoroquinolones), therapy of serious infections can be exceedingly difficult. For *Acinetobacter* sp., sulbactam frequently demonstrates *in vitro* activity[75-77] and was effective in an animal model of pneumonia[78,79]. Successful clinical outcomes have also been reported with sulbactam therapy[76,80,81]. However, sulbactam resistance hampers *in vivo* effectiveness[78,79] and sulbactam-resistant isolates have been frequently encountered in some reports[82-84]. Tigecycline has also demonstrated *in vitro* effectiveness against carbapenem-resistant *A. baumannii*[85], however clinical experience with this antibiotic is unknown.

The emergence of multidrug resistant *A. baumannii* and *P. aeruginosa* has led to a renewed interest in polymyxin E (colistin) and polymyxin B. Most of these isolates remain susceptible to the polymyxins, which act by displacing cations from the outer bacterial cell membrane. Successful outcomes following polymyxin therapy have been noted in patients with serious infections, including bacteremias, urinary tract infections and neurosurgical-related meningitis (Table 4)[82,86-88]. However, poorer clinical and microbiological response rates (typically ~25-60%) have been noted for therapy of nosocomial pneumonia due to *A. baumannii* and *P. aeruginosa*[86,89,90]. A poor response rate to parenteral colistin therapy has also been documented in an animal model of *A. baumannii* pneumonia[78]. Nephrotoxicity rates of 10-37% have been observed with parenteral polymyxin therapy[86,90,91]. However, in one report involving patients with ventilator-associated pneumonia due to *A. baumannii*, the outcomes and toxicities of colistin therapy were similar to imipenem therapy of the carbapenem-susceptible isolates[92].

The poor response rates involving parenteral polymyxin therapy have prompted searches for antibiotic combinations to improve outcomes (Table 5). Several novel combinations have demonstrated improved activity against multidrug resistant *A. baumannii*. Enhanced *in vitro* activity has been observed with combinations involving sulbactam and azithromycin[77] colistin and rifampin[93] polymyxin with azithromycin[94] and polymyxin B with rifampin and/or imipenem[95]. In an experimental model of pneumonia involving a highly imipenem resistant strain of *A. baumannii* carrying OXA-24, improved outcome was observed with various combinations involving rifampin, sulbactam, tobramycin and imipenem[96]. Similarly, for *P. aeruginosa*, increased *in vitro* killing of highly-resistant strains has been noted

with polymyxin B and azithromycin, imipenem and/or rifampin[97,98]. Whether any of these combinations results in superior clinical or microbiological outcome remains to be determined. Aerosolized colistin or polymyxin B has been used to treat hospital-acquired pneumonia due to these pathogens, with high rates of microbiological clearance and apparently little risk of nephrotoxicity[91,99,100]. Aerosolized colistin therapy has been suggested also as an infection control measure to eradicate respiratory colonization[101]. Fortunately, polymyxin resistance has been unusual to date, occurring in ~0-5% of isolates[77,89,90,94,97-100].

Therapy of infections due to carbapenem-resistant *K. pneumoniae* is equally difficult. For isolates possessing KPC β-lactamases, resistance to all β-lactams, fluoroquinolones and most aminoglycosides is common[14,15,17,101]. Combining imipenem with the β-lactamase inhibitor clavulanic acid only lowers the imipenem MIC by about 2-4 dilutions and is not likely to be an effective regimen[17,101]. Although many of these isolates appear susceptible to tetracycline, the MICs hover around the breakpoint for this antibiotic and time kill studies do not suggest this will be an effective therapy[17,102]. KPC-possessing *K. pneumoniae* are susceptible to the glycylcycline antibiotic tigecycline[15,102], but clinical experience is lacking. About 60% of isolates retain susceptibility to gentamicin and 90% to polymyxin B[102]. Polymyxin B possesses concentration-dependent killing and the combination of polymyxin B and rifampin has demonstrated synergy in time kill studies[101]. Again, the clinical utility of these regimens is unknown.

Many of the outbreaks involving carbapenemase-carrying *A. baumannii*, *P. aeruginosa* and *K. pneumoniae* tend to involve only a small number of strains. Given the toxicity and poor response rates to polymyxins for many of these infections, preventing transmission of these pathogens is crucial. Compliance to rigorous infection control strategies will be essential in reducing the spread of these nosocomial pathogens.

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