

CARBAPENEMASE PRODUCING GRAM NEGATIVE BACTERIA IN TERTIARY HEALTH CARE SETTING: THERAPEUTIC CHALLENGES

Sanghamitra Datta, Chand Wattal

Department of Clinical Microbiology, Sir Ganga Ram Hospital
Rajinder Nagar, New Delhi, India

Abstract: The emergence and the spread of multi-drug resistant gram negative pathogens is a major public health concern particularly in the hospitals and health care settings. The gradual increase in incidence of these organisms reflects both, their de-novo selection due to antibiotic usage and their spread between patients. Carbapenemases represent the most versatile family of the β -lactamases having a broad spectrum hydrolysing activity to most of the potent β -lactams. The options of treating these infections are limited and the lack of development of broad spectrum new antimicrobial agents at par with carbapenems has prompted us to re-evaluate the use of colistin. Awareness as well as detection of these organisms in a hospital set up, coupled with stringent hospital infection control practices and judicious use of antibiotics will help us fight this deadly menace.

INTRODUCTION

The emergence of antibiotic resistant organisms is a major public health concern, particularly in hospitals and other health care settings. They seem to be biologically fit and are capable of causing serious life threatening infections that are difficult to manage as treatment options are limited. Probably antibiotic resistance in bacteria is a demonstration of the survival of the fittest with a serious outcome as treatment failure. There is a concern that in the not too distant future we may be faced with growing number of untreatable infections.

It was less than a decade after the introduction of carbapenems and or cephalosporins in the clinical practice in 1980's that gram negative bacteria resistant to these agents emerged. Genes coding for beta-lactamase enzymes have mutated continuously in response to heavy pressure of antibiotic use leading to the development of newer broad spectrum β -lactamases. Carbapenemases represent the most versatile family of β -lactamases that hydrolyze not only carbapenems but other beta-lactams as well, with the occasional exception of monobactams. Most of them resist inhibition by all commercially viable beta-lactamase inhibitors.

CLASSIFICATION

Carbapenemases belong to two major molecular families, distinguished by the hydrolytic mechanism at the active site. The first carbapenemases described were the metalloenzymes as naturally occurring in several gram positive bacilli and gram negative species like *Bacillus cereus*, *Stenotrophomonas maltophilia*, *Flavobacterium* and *Chryseobacterium* spp. These enzymes used zinc as their active site and were inhibited by EDTA thereby establishing them as metalloenzymes or molecular Class B carbapenemases¹. Subsequently more carbapenem-hydrolysing enzymes emerged not inhibited by EDTA. These enzymes utilised serine as their active sites and were grouped as Class A and Class D as per the Ambler's classification². Until the early 1990's, all carbapenemases were described as species specific chromosomally encoded beta-lactamases each with well defined characteristics. However the identification of plasmid encoded IMP-1 (active on imipenem) a metallo- β -lactamase in *P.aeruginosa*³, OXA-23, a class D carbapenemase in *Acinetobacter baumannii*⁴ and KPC-1 a class A carbapenemase in *K.pneumoniae* has changed the pattern of carbapenemase dissemination. What was

once considered a problem of clonal spread has now become a global problem of inter-species dispersion. Due to the proliferation of new members of established carbapenemase families, it is important to understand the properties of these enzymes with all their strength and limitations.

Class A carbapenemases

The Ambler Class A carbapenemases belong to functional group 2f as defined by Bush et al⁵. These β -lactamases have been detected in *Enterobacter cloacae*, *Serratia marcescens*, and *Klebsiella* spp. Bacteria expressing these enzymes are characterized by reduced susceptibility to imipenem, and their MICs can range from mildly elevated (e.g., imipenem MICs of ≤ 4 μ g/ml) to fully resistant. Their hydrolytic mechanism requires an active-site serine at position 70 in the Ambler numbering system for Class A β -lactamases⁶. Their hydrolytic profile is reflected in Table 1. A fourth member of this class, the GES β -lactamases, was originally identified as an ESBL family, but over time, variants were discovered that had low, but measurable, imipenem hydrolysis. This subgroup of GES enzymes is also classified as functional group 2f carbapenemases.

Table 1.: Substrate profile of carbapenemases

Molecular class	Functiona l group	Enzyme	Hydrolytic Profile					Inhibition profile	
			Penicilli ns	Early cephalosporins	Extended cephalosporins	Aztreon am	Carbapenem	EDT A	Clavul anate
A	2f	NMC	+	+	+	+	+	-	+
		IMI	+	+	+	+	+	-	+
		SME	+	+	±	+	+	-	+
		KPC	+	+	+	+	+	-	+
		GES	+	+	+	-	±	-	+
B	3	IMP	+	+	+	-	+	+	-
		VIM	+	+	+	-	+	+	-
		GIM	+	+	+	-	+	+	-
		SPM	+	+	+	-	+	+	-
		SIM	+	+	+	-	+	+	-
		NDM	+	+	+	-	+	+	-
D	2d	OXA	+	+	±	-	±	+	±

+ Strong hydrolysis, ± Weak hydrolysis, - No Hydrolysis

Correspondence: Dr. Sanghamitra Datta, Consultant, Department of Clinical Microbiology, Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi, India, Phone- +911- 42251047, e-mail: sanghamitra_micro@yahoo.co.in

Chromosomal Class A carbapenemases

SME- (“*Serratia marcescens* enzyme”), IMI- 1 (“imepenem hydrolysing²-lactamases”) and NMC- A (“not metalloenzyme carbapenemase”). The genes for these three enzymes are chromosomally located, a fact which may have contributed to their rarity. Subsequent chronological discovery of Class A carbapenemases is shown in Table 2.

Table 2.: Chronological discovery of Ambler’s Class A Carbapenemases

Enzyme	Clinical strains	Origin (year)	Encodement site
SME-1	<i>S.marcescens</i> S6 & S8	UK 1982	Chromosome
SME-2	<i>S.marcescens</i> 4126	USA 1992	Chromosome
SME-3	<i>S.marcescens</i>	USA 2004	Chromosome
IMI-1	<i>E.cloacae</i> 1413B	USA 1984	chromosome
NMC-A	<i>E.cloacae</i> NOR-1	France 1990	chromosome
KPC-1	<i>K.pneumoniae</i> 1534	USA 1997	non-conjugative plasmid
KPC-2	<i>K.oxytoca</i> 3127	USA 1998	Conjugative plasmid
KPC-3	<i>K.pneumoniae</i> CL5761	USA 2001	Conjugative plasmid
KPC-4	<i>Enterobacter</i> spp.E624	UK 2004	Conjugative plasmid
GES-2	<i>P.aeruginosa</i> GW-1	South Africa 2000	Integron in Plasmid
GES-4	<i>K.pneumoniae</i> KG502	Japan 2002	Integron in Plasmid
GES-5	<i>E.coli</i> 365.02	Greece 2003	Integron in Plasmid
GES-6	<i>K.pneumoniae</i> 78.01	Greece 2003	Integron in Plasmid

Table 3.: Phenotypic and Genotypic tests to detect MBL

Technique	Use	Substrate and/or reagents	Advantages	Disadvantages
Clinical microbiology	Disk approximation	Ceftazidime and Imipenem-meropenem and	Easy to use	Disk and absence of disk placement not standardized and are always easy to interpret
	Disk diffusion	Imipenem and EDTA	Easy to use and relatively easy to interpret	Disk not standardized; MBL-producing bacteria can be imipenem sensitive
	Microdilution test	Imipenem and EDTA and L-lysine/phenothiazine	Based on reduction in MICs, easy to interpret	Specialized and labor intensive; MBL-producing bacteria can be imipenem sensitive
	E-test	Imipenem and EDTA	Easy to use and relatively easy to interpret	MBL-producing bacteria can be imipenem sensitive and therefore cases may be missed
	Carbapenem hydrolysis	Meropenem and EDTA	Very sensitive and devoted to be the gold standard	Highly specialized, labor intensive, and interpretation not straightforward
Molecular detection	PCR for genes for IMP, VIM, etc.		Easy to perform, specific for gene family	Requires table-steady DNA primers, cannot differentiate between variants, may not detect new variants
	DMG probes		Specialized, labor intensive	Probe required for each gene family, cannot differentiate between variants
	Cloning and sequencing		Molecular gold standard	Labor intensive, interpretation of data requires experience

SME-1 was the first Class A carbapenemase identified from two *Serratia marcescens* isolates collected in London in 1982 prior to any carbapenem marketing⁷.

IMI-1 was the second type of chromosomally encoded carbapenemase of Ambler group Class A that was identified from two *E. cloacae* strains isolated in Southern California in 1984 (7). IMI-1 shares 95% amino acid identity with NMC-A, has a very similar hydrolysis profile and is inducible like NMC-A.

NMC-A was the third type of first Class A carbapenemase identified from *E.cloacae* NOR-1 clinical isolates in 1990. First isolated from a patient in Paris.

Plasmid encoded Class A carbapenemases

KPC (“*Klebsiella pneumoniae* carbapenemase”), The first member of the KPC family was discovered through the ICARE surveillance project in a *Klebsiella pneumoniae* clinical isolate from north Carolina in 1996 that was resistant to carbapenems, extended-spectrum cephalosporins and aztreonam⁸. Although the KPC β -lactamases are predominantly found in *K. pneumoniae*, there have been reports of pressure of these enzymes in *Enterobacter* spp. and in *Salmonella* spp. also. KPC-2 was first identified in 1998-1999 as the result of a point mutation in KPC-1. Concurrent to the increasing reports of KPC-2 a single amino acid variant of KPC-2, KPC-3 was reported from a 2000-2001 in *K.pneumoniae*⁹. KPC family has a broad hydrolysis spectrum that includes most²-lactam antibiotics. The KPC family has the greatest potential for spread due to its location on plasmids, especially since it is most frequently found in *K.pneumoniae*, an organism notorious for its ability to accumulate and transfer resistance determinants. In addition, the clonal spread seen in several epidemics points to difficulties with infection control for this organism. Treatment of infections caused by these organisms is extremely difficult because of their multidrug resistance, which results in high mortality rates¹⁰.

GES-2 (“Guiana extended spectrum-2”) The GES/IBC family of β -lactamases is an infrequently encountered family that was first described in 2000 with reports of IBC-1 (for “integron-borne cephalosporinase”) from an *E.cloacae* isolate in Greece and GES-1 in a *K. pneumoniae* isolate from French Guiana (2). The most recently reported Class A enzyme with carbapenemase activity is GES-2, which is a point mutant derivative of the extended-spectrum betalactamase GES-1 and was identified from a *Pseudomonas aeruginosa* isolate in South Africa (Table2). Like bla_{KPC-1}, bla_{GES-2} was located on a large-size plasmid and on a class 1 integron. Other members are reflected in Table 2

Class B Metallo-betalactamases

This class of β -lactamases is characterized by the ability to hydrolyze carbapenems and by its resistance to the commercially available β -lactamase inhibitors but susceptibility to inhibition by metal ion chelators. The substrate spectrum is quite broad; in addition to the carbapenems, most of these enzymes hydrolyze cephalosporins and penicillins but lack the ability to hydrolyze aztreonam. The mechanism of hydrolysis is dependent on interaction of the β -lactams with zinc ions in the active site of the enzyme, resulting in the distinctive trait of their inhibition by EDTA, a chelator of Zn²⁺ and other divalent cations. Major distinctive properties included the requirement of Zn²⁺ for the efficient hydrolysis of β -lactams and a lack of inhibition by clavulanic acid and tazobactam.

Chromosomal Class B Metallobetalactamases (MBL)

The first metallo- β -lactamases detected and studied were chromosomal enzymes present in environmental and opportunistic pathogenic bacteria such as *Bacillus cereus*, *Aeromonas* spp. and

Stenotrophomonas maltophilia. Fortunately, with the exception of *S. maltophilia*, these bacteria have not been frequently associated with serious nosocomial infections, as they are generally opportunistic pathogens, and the chromosomal metallo- β -lactamase genes are not easily transferred.

Plasmid encoded Class B MBL

There has been a dramatic increase in the detection and spread of the acquired or transferable families of these metalloenzymes. The most common metallo- β -lactamase families include the VIM, SPM, IMP, GIM, and SIM enzymes, which are located within a variety of integron structures, where they have been incorporated as gene cassettes. When these integrons become associated with plasmids or transposons, transfer between bacteria is readily facilitated. Since their initial discoveries, SPM, GIM and SIM MBLs have not spread beyond their countries of origin. However VIM and IMP continue to be detected world-wide with an overall trend of these two MBLs moving beyond *P.aeruginosa* and into the *Enterobacteriaceae*.

IMP-1 (for "active on imipenem") was the first carbapenemase identified as a source of acquired resistance to carbapenems in a *S. marcescens* isolate in 1991 in Japan. The substrate and its inhibition profile is reflected in Table 1.

Currently the IMP family members have increased their count to 18 and are found throughout the world¹¹.

VIM ("Verona integron-encoded metallo- β -lactamase") was first isolated in Verona, Italy from a *P.aeruginosa* isolate in 1997 with the identification of VIM-2 in France in 1996 which was subsequently reported¹¹. The VIM family consists of 14 members with occurrences mostly in *P.aeruginosa* within multiple integron-cassettes structures. As found for bla_{IMP} genes, the bla_{VIM-1} gene was integrated to a gene cassette into a class 1 integrons¹¹.

SPM-1 ("Sao Paulo MBL") A new family of MBL with 35.5% amino acid identity to IMP-1 was first isolated in *P.aeruginosa* strain in Sao Paulo, Brazil. Since the initial report single clones of SPM-1 has caused multiple outbreaks with high mortality in Brazil. Genetic analysis revealed that it was not part of an integron but instead was associated with a new type of transposable structure with potential recombinase and promoter sequences¹².

GIM-1 ("German imipenemase") was isolated in Germany in 2002. It has 30% homology to VIM, 43% homology to IMPs and 29% homology to SPM. It is found within a class 1 integron on plasmid.

SIM-1 ("Seoul imipenemase") Latest member of the MBL family was isolated from Korea. It has the closest amino acid identity with the IMP family. It was discovered in a large scale screen of imipenem resistant *Pseudomonas spp.* and *Acinetobacter spp.*

NDM-1 - a new MBL—designated New-Delhi metallo-1 is swiftly emerging¹³. A Swedish patient of Indian origin travelled to New-Delhi and acquired a UTI caused by carbapenem resistant *K.pneumoniae* typing to complex ST-14. The isolate *K.pneumoniae* 05-506 was shown to possess a metallo- β -lactamase but was negative for previously known MBL genes¹³. Gene libraries and amplification of class 1 integrons revealed three resistant genes found on a 180kb region that is easily transferable to the recipient strains conferring resistance to all antibiotics except for fluoroquinolones and colistin¹⁴. NDM-1 shares very little identity with other MBLs; the most similar being VIM-1/VIM-2 with only 32.4% identity¹⁵. It can hydrolyse all β -lactams except for aztreonam. The broad resistance carried on these plasmids is a further worrying development for India that already has high level of antibiotic resistance.

Class D-The Oxa-beta-lactamases

OXA ("oxacillin-hydrolysing") β -lactamases represented one of the most prevalent plasmid encoded β -lactamases in the late 1970's and

early 1980s. Currently there have been 102 unique OXA sequences identified of which 9 are extended spectrum β -lactamases and at least 37 are considered to be carbapenemases. The first OXA β -lactamase with carbapenemase activity was described by Paton et al. in 1993. The enzyme was purified from a multidrug-resistant *A. baumannii* strain that was isolated in 1985 from a patient in Edinburgh, Scotland. The enzyme was designated ARI-1 (for "Acinetobacter resistant to imipenem") and was later demonstrated to reside on a large plasmid. Their activity is inhibited by clavulanic acid except for OXA-23, which is resistant to clavulanic acid. OXA-23 to OXA-27 are carbapenemases in Class D family.

EPIDEMIOLOGY AND CLINICAL IMPACT AND INFECTION CONTROL

Carbapenem antibiotic design was inspired by the natural product thienamycin, produced by the soil organism *Streptomyces cattleya*¹⁶. Because of the prevalence of these molecules in the soil, it is only logical to expect that enzymes capable of degrading these β -lactams would be produced by environmental organisms such as *Bacillus cereus* and *Bacillus anthracis*, bacteria with well-characterized metallo- β -lactamases that would provide a selective advantage for growth of these environmental species². The problem of carbapenemase-mediated resistance intensified once genes for these enzymes became associated with acquired genetic mobile elements such as plasmids and integrons. Environmental organisms may provide genetic material as a source of these enzymes and clinical strains may disperse this information by the help of mobile piece of genetic material (transposon) within the hospital setting and into the surrounding environment. Infection with carbapenemase-producing *Enterobacteriaceae* (CRE) is emerging as an important challenge in health care settings¹⁷. Analysis of 2007 data regarding health-care associated infections reported to CDC indicated that 8% of *K.pneumoniae* isolates were carbapenem resistant compared to 1% in 2000¹⁷. The emergence and spread of carbapenem resistant gram negative organisms is another in a series of worrisome public health development and underscores the immediate need for aggressive detection and control strategies. Patients with unrecognized carbapenem resistant colonization of CRE in the normal flora have served as reservoirs for transmission during health-care-associated outbreaks¹⁸. Colonisation of native flora is not a common mode of transmission in non-fermenters like *P.aeruginosa* and *Acinetobacter spp.* which are ubiquitous in origin and a common inhabitant in hospital milieu. Outbreaks with carbapenemase producing organisms have been due to lack of adherence to infection control measures. Studies claim that the control of outbreak was hindered due to lack of adherence to infection control practices. Only 62% compliance to gown and gloves and 42% to hand washing or alcohol based hand rub have led to these outbreaks¹⁹. Experience from the outbreak suggests that early detection through use of targeted surveillance and introduction of strict infection control measures including reinforcement of hand hygiene and contact precautions can help control the spread of these organisms. For all acute care facilities, CDC and HICPAC (Health Care Infection Control Practices Advisory committee) recommend an aggressive infection control measure. In countries like US routine microbiologic surveillance of persons admitted such as that performed in some facilities to detect MRSA is not recommended for CRE. Countries with higher incidence of these organisms should implement the "Tier 2 infection control strategies outlined in 2006 HICPAC guidelines"²⁰.

DETECTION OF CARBAPENEMASES

A difficulty in detecting CRE (Carbapenemase producing

Enterobacteriaceae) is due to the fact that some strains that harbor *bla_{KPC}* have minimal inhibitory concentrations (MICs) that are elevated but still within the susceptible range for carbapenems. Because these strains are susceptible to carbapenems, they are not identified as potential clinical or infection control risks using current susceptibility testing guidelines. To address this challenge, in January 2009, CLSI published a recommendation that carbapenem-susceptible *Enterobacteriaceae* with elevated MICs or reduced disk diffusion zone sizes be tested for the presence of carbapenemases using the modified Hodge test (MHT)²¹. The MHT is a phenotypic test used to detect carbapenemases in isolates demonstrating elevated but susceptible carbapenem MICs and has demonstrated sensitivity and specificity exceeding 90% in identifying carbapenemase-producing *Enterobacteriaceae*. If the MHT reveals the presence of a carbapenemase, CLSI recommends that a remark of carbapenemase producer be added to the microbiology report to inform clinicians and infection control staff. As treatment option on MHT-positive, carbapenem-susceptible isolates is limited, CLSI guidelines do not recommend any changes regarding the reporting of susceptibility results and they should be reported as such. Strains of *Enterobacteriaceae* that test intermediate or resistant to carbapenems should be reported as such and do not need to be subjected to the MHT.

Unfortunately, there are no standardized phenotypic testing criteria available to detect MBLs or OXA-D carbapenemases. Non-fermenters or *Enterobacteriaceae* carrying MBLs are often carbapenem intermediate or susceptible and can be missed when using imipenem or meropenem in the detection method. The different phenotypic and genotypic tests used to detect MBL is listed in Table 3¹¹. Though E-test has a high sensitivity of 94% and specificity of 95% false-negative results have been reported. It has also been observed that EDTA alone has inhibitory action against some bacteria due to permeabilization of the outer membrane and can lead to false positive results. E-test metallo- β -lactamase detection tests have also yielded false-positive results with OXA-23-producing *A. baumannii*¹¹. *P. aeruginosa* isolates with an imipenem MIC of 16 μ g/ml and *Acinetobacter* spp. isolates with an imipenem MIC of 8 μ g/ml, whereas for *Enterobacteriaceae*, an MIC of 2 μ g/ml may be appropriate candidates for screening for MBL¹¹s.

THERAPEUTIC AGENTS

Polymyxins and tigecycline are the antimicrobials most often used for treatment of infections caused by carbapenem-resistant *Enterobacteriaceae*²². Polymyxin B and Polymyxin E have been used in clinical practice. Discovered more than 50 years ago, has been reintroduced as a valuable therapeutic agent with efficacy against multidrug-resistant gram-negative bacteria due to a shortage of new antimicrobials with activities against these organisms. The emergence of multi-drug resistant gram-negative bacteria and the lack of development of promising new anti-microbial agents have prompted the medical community to re-evaluate the use of Colistin (polymyxin E). Colistin resistance is emerging in clinical isolates of carbapenem-resistant *K. pneumoniae*. Although colistin and tigecycline do not show a synergistic effect on carbapenem resistant *K. pneumoniae* in vitro, they are not antagonistic and may have an additive effect when used together and prevent the emergence of resistance to these antibiotics²³. The combination or monotherapy can be used in multi-drug resistant non-fermenters as well, though tigecycline is not active against *P.aeruginosa* due to efflux by MesXY-OprM²⁴. The limitation of tigecycline as treatment option in urinary tract infection and blood stream infections is well known.

CONCLUSION

Awareness regarding the resistant isolate prevalent in hospital environment followed by infection control practices is the first step that a clinical microbiologist can take to address this problem. Expertise in detection of these organisms in the laboratory is important as high carbapenem MICs are not always evident. Hospitals and other acute care facilities should adopt aggressive infection control strategies to battle these pathogens. Evaluation of effective antibiotic options and its judicious use shall help in the fight against carbapenemase-producing organisms besides the infection control measures.

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