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Review Article

CARBAPENEM HYDROLYSING METALLO- ß-LACTAMASES: A REVIEW

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ABSTRACT

The introduction of carbapenems into clinical practice nearly three decades ago, marked a great advance in the treatment of resistant bacterial infections. However, during the last few years, there has been an alarming increase in the reports of spread of carbapenem resistant *Enterobacteriaceae* along with the emergence of highly nosocomial clones. Acquired carbapenem resistance is largely attributed to the production of carbapenem-hydrolysing enzymes called carbapenemases which include metallo-beta-lactamases (MBL). The rapid dissemination of these MBL producers is worrisome, since they exhibit an unrivaled resistance spectrum to carbapenems as well as nearly all beta-lactam drugs. This review focuses on the types of carbapenem hydrolyzing metallo-beta-lactamases produced by important pathogens which have been reported in various parts of the world. The review discusses the Imipenemase (IMP) type, the Veronese Imipenemase (VIM) type and the New Delhi Metallo (NDM) type of metallo-beta-lactamases, their importance, detection and surveillance. Since the genes associated with MBL production are transferable, a significant threat can be expected in the future due to the emergence of novel enzyme types and Variants.

Keywords: Carbapenems, Imipenemase, Veronese imipenemase, New Delhi Metallo beta lactamase, Resistance

INTRODUCTION

The rapid spread of antibiotic resistance among pathogens is an emerging threat and a matter of particular concern worldwide. Today, carbapenems are often used as a last resort in the treatment of multi drug resistant Gram negative infections in view of their resistance to hydrolysis by most beta- lactamases[1].

Carbapenems are prescribed for the treatment of intra-abdominal infections (complicated appendicitis and peritonitis), bacterial meningitis and skin infections. They are the mainstay of therapy in patients with serious hospital-acquired infections and are even known to be effective against methicilllin resistant *S.aureus*. Carbapenems used commonly in India include Imipenem and Meropenem. The latter is highly potent against *Enterobacteriacae, Pseudomonas* species, *Acinetobacter*species, *H. influenza* and anaerobic bacteria, with a fairly good potency against Gram positive cocci[2]. Carbapenems have a high degree of toxicity and their mode of action is by inhibition of cell wall synthesis in bacteria. In addition, they are also known to cause an increase in the permeability of the outer membrane of cells and affect their efflux system. They have a high range of activity and are considered as the drug of choice for infections resistant to penicillins and cephalosporins[3]. Carbapenems consist of a tetrahydropyrolidine ring connected to a hydrophilic side chain by a reaction catalysed by an inorganic metal[4]. The structure of carbapenems is similar to penicillin, except that the nitrogen atom in penicillin at position one is replaced with a carbon atom[5] (Fig. 1).

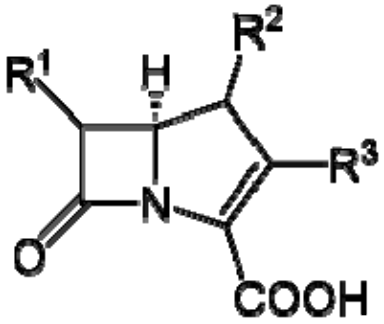

Fig. 1: Structure of Carbapenem Backbone

Though the introduction of carbapenems into clinical practice has been thought to represent a great advance in the treatment of serious bacterial infections, recently carbapenem resistance has been frequently observed in non-fermenting Gram-negative bacteria *Pseudomonas aeruginosa* and *Acinetobacter baumanii*[6]. In a recent report, the Infectious Diseases Society of America specifically addressed three categories of emerging multiple drug resistant Gram-negative bacilli, which included carbapenem-resistant organisms like *Acinetobacter* species, *Klebsiella* and *Pseudomonas*[7].

Carbapenem resistance has been attributed to various causes such as reduced expression of outer membrane proteins, increased efflux systems and production of carbapenemases-beta-lactamases which can inactivate carbapenems by causing their hydrolysis[8]. Based on molecular studies, the Ambler classification separates carbapenem hydrolyzing enzymes into three groups: A, B and D (Table 1)[9].The carbapenemases which are derivatives of class A or class D enzymes usually mediate carbapenem resistance in *Enterobacteriaceae* or *Acinetobacter* species and include NmcA, Sme1-3, IMI-1, KPC1-3, and GES-2[10]. Despite the avidity of these enzymes for carbapenems, they do not always mediate high-level resistance and not all are inhibited by clavulanic acid[9].In contrast, oxacillinases hydrolyze carbapenems poorly but are able to confer resistance and are only partially inhibited by clavulanic acid.

However, Metallo Beta-Lactamases (MBL) belonging to class B exhibit potent hydrolyzing activity not only against carbapenems but also to other beta-lactam antibiotics. They the possess the characteristic hallmark of being universally inhibited by ethylene diamine tetra acetic acid[3] (EDTA) as well as other chelating agents of divalent cations, a quintessential feature of MBLs that correlates with their mechanistic function. The Imipenemase (IMP) and the Veronese Imipenemase (VIM) genes responsible for MBL production are plasmid borne and can be rapidly transferred from one cell to another. This phenomenon has lead to the horizontal transfer of the MBL genes to other species. Today, these enzymes are not restricted to a particular species in a particular region but are prevalent nearly all over the world in various species. Recent studies in 2008[11] have described an additional, novel acquired carbapenemase called New Delhi Metallo-1 (NDM-1), from New Delhi, India, also belonging to the class B of beta lactamases. MBL producing strains have been reported to be an important cause of nosocomial infections and their spread poses a significant threat to human health as it leaves no option for treatment of serious infections. Since MBLs hydrolyze virtually all classes of beta lactams and we are several years away from the implementation of their therapeutic inhibitor, their continued spread could lead to a clinical catastrophe. The present review focuses on the types of MBLs and in particular examples of transferable MBLs from *Pseudomonas* species, *Acinetobacter* species, and Enterobacteriaceae, including their epidemiology, methods for detection and the need for their surveillance.

Table 1: Ambler classification of carbapenemases

Ambler Classification	Type of enzymes	Hydrolysis spectrum				Inhibited by Clavu-lanic acid	EDTA
		Amino-penicill-ins	Extended spectrum Cephalos-porins	Aztreonam	Carbap-enem		
A	NmcA; Sme-1 to Sme-3; IMI-1	○	ΔΔ	E	○	+/-	-
A	KPC-1	○	E	○	○	+	-
A	GES-2	○	○	○	E	+	-
B	IMP-1 to IMP-9	○	○	ΔE	○	-	+
B	VIM-1 to VIM-3	○	○	ΔE	○	-	+
D	OXA-23 to OXA-2	○	○	ΔE	E	+/-	-

Key: - (○) High level; (E) low level; (Δ) no hydrolysis.

Prevalence of resistance to Carbapenems

Metallo-beta-lactamase (MBL) producing *Pseudomonas aeruginosa* was first reported in Japan[12] in 1988 and since then has been found in various parts of the world including

East Asia[13], Europe[14], Australia [15] and South America[16]. Acquired carbapenemases are a large group of beta lactamases of high structural diversity that, in most instances, hydrolyse not only carbapenems, but also oxyimino-cephalosporins and cephamycins[17]. Various types of these carbapenemases producing *Enterobacteriaceae* have slowly spread to Greece, United States, Latin America and China giving rise to country wide epidemics. Local outbreaks have been reported in Poland and Italy. The strains isolated have been shown to possess plasmids and transposons bearing MBL genes which spread when patients are transferred between hospitals. Such introductions into healthcare systems across country borders have led to international epidemics by secondary local or regional transmission[11, 17, 18].

According to the 2009 data from the European Antimicrobial Resistance Surveillance Network (EARS-Net, formerly EARSS) the rates of carbapenem-resistance among invasive *K. pneumoniea* infections were: 43.5% in Greece, 17.0% in Cyprus, 1.3% in Italy, 1.2% in Belgium and below 1% in the other 23 reporting countries[19]. After the initial report of NDM-1 from Sweden in 2008[11], the Health Protection Agency (HPA) in the United Kingdom (UK), concerned over the rapid increase in the number of cases of human colonisation and infection with NDM-1 and other carbapenemase-producing *Enterobacteriaceae* in hospitals across the country, issued a national alert in July 2009[20]. Similarly to the first case of NDM-1 reported by Yong *et al.*, the majority of the patients with NDM-1-positive bacteria in the UK had a history of travel to India or Pakistan, where many of them had been hospitalised with various indications, including elective surgery and renal dialysis[20, 21].

Phenotypic detection of carbapenemases

Carbapenemase production may confer certain phenotypic characters to the organisms depending on its species, type of enzyme produced, presence of additional resistance mechanisms and the expression level [18, 22]. The Modified Hodge Test (MHT) is the most commonly used method to detect organisms producing MBLs and it is the only method so far recommended by the Clinical and Laboratory Standards Institute (CLSI). Alternatively the E-test may also be used but because of its high cost its use is found to be limited.

The Modified Hodge Test (MHT)

Carbapenemase production is detected by the MHT when the test isolate produces the enzyme and allows growth of a carbapenem susceptible strain (*E.coli* ATCC 25922) towards a carbapenem disk. The result is a characteristic clover leaf-like indentation[23, 24] (Fig. 2). This assay is sensitive only to carbapenemase production and does not give any information on the type of enzyme produced by the isolate.

- **MHT Positive** test has a clover leaf-like indentation of the *E.coli* 25922 growing along the test organism growth streak within the disk diffusion zone.
- **MHT Negative** test has no growth of the *E.coli* 25922 along the test organism growth streak within the disk diffusion zone.

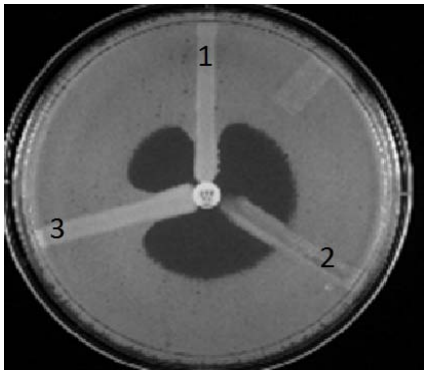

Fig. 2: The MHT performed on a 100 mm MHA plate

(1) *K. pneumoniae* ATCC BAA 1705 showing positive result; (2) *K. pneumoniae* ATCC BAA 1706 showing negative result; (3) A clinical isolate showing positive result

The Epsilometer test (E-test) MBL strip method

For ease of application for most microbiology laboratories, the E test MBL strip is recommended[25], where one half of the strip is impregnated with an imipenem gradient across seven dilutions and the other half with another imipenem gradient overlaid with constant concentration of EDTA (Fig. 3). This division in the strip

Fig. 3: E-test MBL Strip

Acinetobacter sp. expressing a VIM-2 MBL. The intersection of the ellipses at the strip is read from two halves, i.e., at the section with imipenem alone (IP) and imipenem plus EDTA (IPI).A reduction in

the MIC of imipenem of more than or equal to 3 dilutions in the presence of EDTA is interpreted as a positive test.

aids in deciphering whether the type of carbapenemase produced is affected by EDTA or not. For most MBLs the half which contains the drug along with EDTA shows a greater zone of clearance as compared to the drug alone.

Double disk synergy test

There are also few inhibitor tests which help to identify MBL producers. These make use of synergism between an inhibitor and the the carbapenem. The inhibitor used is mostly EDTA which chelates the zinc ions and thus there is loss of carbapenemase activity. Other tests like the double disk synergy test and combined disk test exhibits high level of sensitivity even in low levels of carbapenem resistance. A double-disk

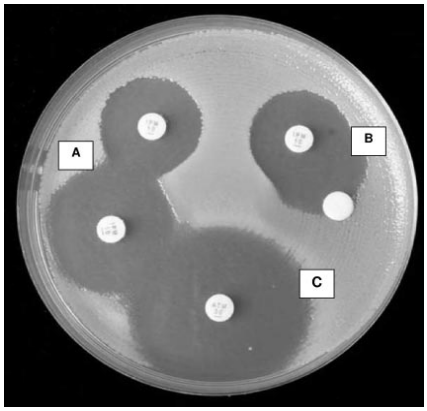

Fig. 4: **The phenotypic appearance of MBL-producing *Serratia marcescens* isolate carrying the *bla*IMP-4 gene**

(A) Combined-disk test, using two imipenem (10 microgram) disks, one with 292 microgram EDTA, showing an increase in zone inhibition of >4 mm around the disk with EDTA. (B) Double-disk synergy test, using an IPM (10 microgram) disk placed 20 mm (center to center) from a blank filter disk containing 292 microgram EDTA.(C) Aztreonam (30 microgram) disk with a >30 mm zone of inhibition.

synergy test (Fig. 4) using imipenem (IPM) and 0.5 M EDTA [26,27] and a combined-disk test using either two IPM disks or two meropenem disks, one containing 930 microgram [28] or 750 microgram[29] of EDTA, have both been reported as reliable methods for the detection of MBLs in carbapenem-resistant *Pseudomonas* and *Acinetobacter* strains.

Spectrophotometric methods

These are the reference methods that should be used to detect carbapenemases. They measure the amount of hydrolysis of carbapenem in the presence or absence of inhibitors using crude cell extracts or enzyme preparations by spectrophotometric methods. They may provide additional information about the enzyme but are however laborious, technically demanding and expensive. Thus, they are carried out in reference laboratories[30].

Genotypic detection methods

Genotypic methods for detecting MBLs exploit the use of DNA recombination techniques in conjunction with molecular techniques. The plasmid bearing the bla gene is first purified by plasmid extraction procedures and the bla gene is then isolated using restriction digestion. The gene is then transformed into a host cell (most ideally used host is *E.coli* DH10B) by electroporation[31]. The transformants are selected by plating on LB plates containing suitable antibiotic and used for Polymerase Chain Reaction (PCR). The transformants may also be subjected to Minimum Inhibitory Concentration (MIC) testing in order to find out if the ability to hydrolyse carbapenems is retained in the vector organism or not. Moreover, the PCR derivatives can be subjected to Pulse field gel electrophoresis or Southern blotting to analyze the relatedness between other bla genes found in other organisms or within the same species. Also Basic Local Alignment Search Tool (BLAST) analyses can be performed to compare the similarities with the already identified MBL sequences.

Types of metallo-beta-lactamases acting against Carbapenems

The Imipenemase (IMP) type

The IMP type of MBL is a transferable type of beta- lactamase which confers the property of hydrolyzing imipenems as well as some extended spectrum cephalosporins. They are insensitive to most inhibitory agents but are however susceptible to aztreonam. The first indication of the IMP type was found in the Japanese region in a *Ps. aeruginosa* strain GN 17203 in 1988[12]. The IMP genes are located on a transferable conjugated plasmid of about 120kD which could be readily mobilised to other Pseudomonas strains. This same gene was then transferred to four Serratia marcescens collected32 in seven general hospitals in Japan in 1993. So far a number of such IMP variants have been identified which brings to light that the problem of metallo- beta lactamases is not only prevalent in Japan but also in Europe[33, 34], Canada[35] and Hong Kong[36]. IMP-1 was first detected in *K. pneumoniae* DB96 in 1999 when a clinical isolate was analyzed from a blood sample of a leukemic patient in Singapore[37]. This isolate showed imipenemase activity which was confirmed by E-tests showing matt growth upto 3 microgram/ml but individual colonies were seen growing even upto 32 microgram/ml of the strip. The highly resistant colony was designated as DB96M, which was tested repeatedly and it showed the same degree of resistance. Another variant, DB96R, obtained after repeated subcultures was however inhibited at imipenem concentrations of 3 microgram/ml. MICs of DB96 and DB96M were identical to all beta-lactamases including carbapenems but that of DB96R was less resistant only to carbapenems. The carbapenemase produced by all three variants was a protein of pI 9.0 detected by isoelectric focusing. The activities of all 3 proteins were similar and did not vary significantly.

Further, two amplicons were generated from DB96 using primers based on published sequences for *P. aeruginosa*101/1477[38] and subjected to sequencing using blaIMP-1 primers. The resultant sequence was submitted to BLAST 2.0 and found to be identical to blaIMP-1 from *S. marcescens* (Genbank accession no. S71932), *P.aeruginosa* (AJ 223604) and *K.pneumoniae* (B 29636). The fact that the patient had not travelled to Japan in the recent past was confirmed. Thus it may be possible that the blaIMP-1 gene may have been transferred independently through plasmids. In this case the outer membrane proteins (OMP) also play an important role in carbapenem resistance. To prove this the OMP of all three variants as well as those from carbapenem susceptible organisms was extracted and subjected to SDS-PAGE gel electrophoresis (Fig. 5).

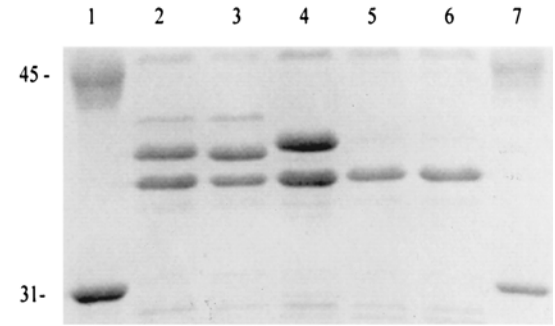

Fig. 5: Outer membrane profiles of K. pneumoniaeisolates in Sodium dodecyl sulphate- Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Lanes 1 and 7, molecular weight markers (in kilodaltons); lane 2, carbapenem-susceptible control isolate 207; lane 3, carbapenem-susceptible control isolate 504; lane 4, DB96R; lane 5, DB96; lane6, DB96M. (Image cropped so all markers not seen)

Both, DB96 and DB96M showed a decreased expression of a 39kD OMP compared to DB96R and susceptible *K.pneumoniae* controls and all three lacked a 41kD OMP present in the controls. Thus the 39kD protein is a major porin and the increased level of resistance was seen when the blaIMP-1 gene was present in conjunction with the absence of the OMP.

Both these mechanisms help in carbapenem resistance in blaIMP-1 positive organisms.

Another study carried out by Hisakazu et al showed a new carbapenem hydrolyzing metallo- beta lactamase, IMP-6. This enzyme was reported to be found on the plasmid pKU501 borne by *S. marcescens* and it encoded an enzyme which caused hydrolysis of meropenem to a very high degree as compared to other carbapenems. The DNA sequence of this IMP-6 showed the presence of a Gly codon at position 196 instead of Ser found in the original IMP-1 gene. This point mutation suggests that the hydroxyl group of Ser-196 plays an important role in the hydrolysis of meropenem. But it also showed loss of activity against penicillin G and piperacillin[39]. This gene is however found exclusively in *S. marcescens* and *A.baumanii* but may be transferred to other organisms in the future.

Further resistance to imipenem and meropenem are results of changes in expression of porins and efflux proteins. A surveillance report carried out in 2006 on P. aeruginosa40 showed that loss of a porinOprD in the absence of hydrolyzing enzymes increases resistance to imipenem. Moreover, overexpression of the efflux pump mexAB-oprM resulted in reduced susceptibility to meropenem coupled with loss of OprD. Little information is available regarding the metallo-beta-lactamase, IMP-18. The blaIMP-18-positive single isolates of *P. aeruginosa* were identified in hospitalized patients from Mexico and US[41, 42]. The spread of IMP-18 into multiple unrelated isolates from Puerto Rico was also reported and this indicates the movement of blaIMP-18 by mobile genetic elements. In the absence of acquired carbapenemases, carbapenem MICs were less than or equal to 16 microgram/ml. The presence of IMP-18 in OprD-deficient isolates also corresponded with increased carbapenem MICs (32 to >64 microgram/ml).

In addition, these isolates overexpressed the mexAB-oprMpump, which may have further elevated the meropenem MICs. The absence of OprD coupled with IMP-18 appeared to have a synergistic effect on the carbapenem MICs. Since all IMP-18-positive isolates were also OprD deficient, the effect of these enzymes alone on carbapenem susceptibilities has yet to be determined. Continued exploration into DNA analysis of carbapenem and meropenem resistance genes led to the discovery of about almost 18 such IMP genes which are responsible for creating havoc in the field of effective antibiotic therapy. The types of IMP genes found in some Gram negative organisms are listed in Table 2.

Table 2: Types of IMP genes

Organism	IMP Type
E.coli	IMP-1
P.aeruginosa	IMP-1,IMP-2,IMP-4,IMP-7,IMP-9,IMP-10,IMP-11,IMP-13,IMP-16,IMP-18
K.pneumoniae	IMP-1 and IMP-8
A.baumanii	IMP-1,IMP-2,IMP-4,IMP-5,IMP-6,IMP-11

The veronese imipenemase (VIM) Type

After the identification of the IMP genes there was another type of MBL that was found to confer the ability of carbapenem resistance. These types are mainly borne on integrons on plasmids. They are the second most dominant group of transferable MBLs[11]. The VIM enzymes are resistant to a number of beta lactams like piperacillin, ceftazidime, imipenem and aztreonam. These enzymes are dependent on metal ions, which is indicated by loss of activity on addition of EDTA and restoration upon addition of Zn^{+2}. Therefore these observations indicate production of a metalloenzyme. It is closely related to BCII from *B.cereus* sharing 39% amino acid identity [11].

Studies carried out on VIM genes indicate that they are borne within variable regions of class I integrons. Integrons are genetic structures capable of capturing gene cassettes and allowing horizontal gene transfer in bacteria An integron consists of a gene encoded enzyme, integrase which helps in dissemination of the gene cassette along a plasmid or chromosome, it possesses a promoter region to drive its expression and an attC sequence which flanks gene cassette and enables it to be integrated to the attl site. VIM enzymes were first described in Verona, Italy, from a *Pseudomonas aeruginosa* isolate in 1997[44]. Cornaglia *etal* carried out analysis of isolates of patients suffering from nosocomial infections caused by *P.aeruginosa* from the University Hospital of Verona. Tests like Susceptibility tests, Agar dilution method for MIC, beta-Lactamase assays, Isoelectric focusing analysis, Pulsed-field gel electrophoresis (PFGE) (Fig. 6) and Colony blot hybridization were performed[44]. Eight different strains of *P.aeruginosa* were isolated and their susceptibility profiles are listed in the table 3. It is worth noting that these strains were resistant to carbapenems by two mechanisms, production of beta-lactamase as well as loss of the porin protein OprD. In 2002 this same gene was reported in *E.coli* in Athens, Greece[45].

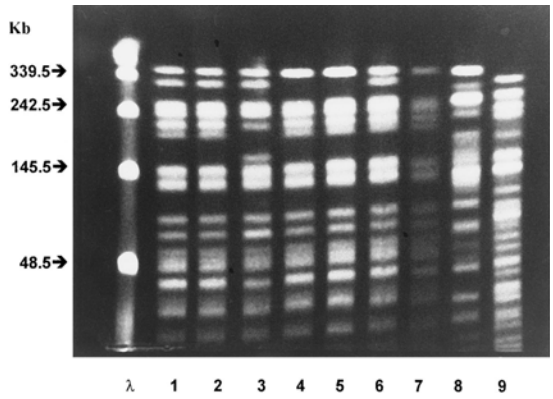

Fig. 6: Pulsed-field gel electrophoresis of XbaI-cleaved genomic DNA of Pseudomonas aeruginosaclinical isolates Lambda-Ladder DNA marker

(DNA sizes indicated). Lane 1, VR-143/97. Lane 2, VR-146/97.Lane 3, VR-155/97.Lane 4, VR-158/97.Lane 5, VR-170/97.Lane 6, VR-174/97.Lane 7, VR-186/98.Lane 8, VR-193/98.Lane 9, VR-148/98 (control strain, not related to the outbreak).

Poirel L.*etal* described in 1999 in context with VIM-1 and VIM-2 that the latter shared 90% amino acid identity with VIM-1[46]. The MIC values for VIM-1 positive organisms are tabulated in Table 3. The amino acids that may be involved in the catalytic site of these enzymes were identical[47,48,49]. VIM-2 has a broad hydrolysis profile which includes most of the beta-lactams, except aztreonam, cefsulodin and cefpirome. Its activity was higher against imipenem than against meropenem. It was reported that 50 μM EDTA inhibited its activity and 2mM $ZnCl_2$ restored its activity[46].

Variants of the VIM genes were also reported in Bulgaria and Germany in 2006. These were VIM-15 and VIM-16. Two *Ps. aeruginosa* strains, 166301 and 9551, were isolated in 2005 and 2006[50] which showed carbapenem resistance. MICs, determined by the agar dilution technique, are shown in Table 4. The results of a Hodge test and a double-disk synergy test with EDTA[51], carried out, demonstrated the production of carbapenemase enzyme which is sensitive to EDTA inhibition, thereby confirming the presence of MBLs. PCR with specific bla_{vim}genes was also carried out to sequence the whole gene and its environment. The results showed that the gene shares close homology to VIM-2 genes except for one nucleotide substitution in each of the variants causing an amino acid to differ from that found in the VIM-2 gene. The 9551 gene had an A584T substitution leading to tyrosine 218 phenyalanine substitution. All other VIM genes have a tyrosine at that position except VIM-7. And the 9551 gene showed a nucleotide substitution causing a serine 54 leucine substitution. All other VIM genes carry a

serine. To analyse if the change in amino acid caused any difference in resistance the VIM genes of strains 166301, 9551 and VIM-2 genes were cloned in *E.coli* DH5alpha. The transformants showed a significant increase in carbapenemase activity with the expression of the newly introduced VIM genes. The MICs are tabulated in Table 4

Detection of VIM-19 in *Klebsiella pneumoniae*[52] conferred the property of complete or intermediate resistance to all beta-lactams including carbapenems like imipenem, meropenem and ertapenem and colistin. This strain showed susceptibility to only tigecycline. PCR for its genes showed positive for bla_{TEM}, bla_{CTX-M},bla_{CMY}, bla_{VIM}, bla_{KPC} genes. Mapping results showed that bla_{KPC} gene was located in a transposon. It is postulated that VIM -19 is a result of carbapenem pressure in hospitals where VIM related producers are commonly present. Some of the organisms showing VIM positive activity are shown in Table 5.

Table 3: MIC values of various antibiotics for *P.aeruginosa* carrying bla_{VIM-1} genes

Isolate	MIC of Anti-microbial agent (mg/mL)											
	Imi	Mero	Ctri	Czid	CEP	Atm	Pip/Taz	Tic/Clv	Gm	Amik	Tm	Cpfx
VR-143/97	>128	128	>128	>128	>128	16	>128	>128	64	32	32	>128
VR-146/97	>128	>128	>128	>128	>128	8	>128	>128	>128	>128	>128	>128
VR-155/97	>128	>128	>128	>128	>128	32	>128	>128	>128	>128	>128	>128
VR-158/97	>128	128	>128	>128	>128	8	>128	>128	32	32	32	>128
VR-170/97	>128	128	>128	>128	>128	16	>128	>128	>128	64	64	>128
VR-174/97	>128	>128	>128	>128	>128	8	>128	>128	>128	64	64	>128
VR-186/98	>128	>128	>128	>128	>128	4	>128	>128	>128	64	64	>128
VR-193/98	>128	>128	>128	>128	>128	4	>128	>128	128	32	32	>128

Table 4: Antibiotic susceptibilities of wild-type and transformant strains

Antibiotics	MIC (microgram/ml)					
	P. aeruginosa		*E. coli* DH5alpha			
	9551 (VIM-15)	166301(VIM-16)	pBC-VIM-15	pBC-VIM-16	pBC-VIM-2	Host strain
Amoxicillin	512	>512	256	256	256	4
Piperacillin-tazobactam	128	128	8	8	4	1
Ceftazidime	64	64	0.5	0.5	1	0.13
Cefotaxime	>256	>256	4	2	1	0.03
Cefepime	32	32	0.03	0.03	0.03	0.016
Aztreonam	16	32	0.016	0.016	0.016	0.016
Meropenem	128	>128	0.03	0.03	0.03	0.016
Imipenem	>128	>128	0.5	0.25	0.25	0.25
Gentamicin	0.5	>128	0.13	0.13	0.13	0.13
Tobramycin(4microgm/ml)	8	>128	0.25	0.25	0.25	0.25

Table 5: Types of VIM genes found in various organisms

Organism	VIM type
E.coli	VIM-1
P.aeruginosa	VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, VIM-7, VIM-8, VIM-9, VIM-10, VIM-11a, VIM-11b, VIM-15, VIM-16
K.pneumoniae	VIM-1, VIM-4, VIM-5
A.baumanii	VIM-2

The New Delhi Metallo (NDM) type

Acquired carbapenemases confer extensive antibiotic resistance to *Enterobacteriaceae* and represent a public health threat. A novel acquired carbapenemase, New Delhi metallo-beta-lactamase 1 (NDM-1), has recently been described in the United Kingdom and Sweden in 2008 in single isolate of *K.pneumoniae* and *E.coli* in patients treated in New Delhi, India[11]. Due to lack of epidemiological data within India, the exact prevalence of NDM-1 enzyme is not known. NDM-1 is a 180kb long region present on the plasmid of *K.pneumoniae* 05-506. Its molecular mass is 128kD, it is monomeric and hydrolyses all beta-lactam antibiotics except for aztreonam, but organisms producing NDM-1 are susceptible to only colistin and less consistently tigecycline[11, 21]. Cases reported by Yong *et al*.[11] show that most of the NDM-1 positive patients in UK had previously travelled to India or Pakistan. Results based on these reports indicate that many of these bacteria possess bla_{NDM-1} on conjugative plasmids and *K.pneumoniae* & *E.coli* are the most frequent hosts[14].

Sequencing of the NDM-1 gene was carried out and it showed type 14 complex, the closest type of genes being the VIM-1/VIM-2 types sharing 32.4% similarity. The gene product was an MBL but when tested was negative for all known MBL genes. Using information from gene libraries and amplification of class I integrons, the bla_{NDM-1} gene carried three resistance conferring regions; the first was bla_{cmy-4} flanked by ISEcP1 on one side and blc on the other. The class I integron contained a gene arr-2, a novel erythromycin esterase gene, ereC, and A1 &cml A7. The new MBL gene was flanked by IS26 and *K.pneumoniae* genomic DNA[11].

Apart from having unique residues at the active site which gives it additional properties, it also has new inserts between positions 162 and 166 which is unlike that in other MBLs. Kinetics of this enzyme revealed that it binds more tightly to most cephalosporins and also to penicillin. It does not bind to carbapenem as tight as the IMP-1 or VIM-2 enzymes but the conversion of carbapenems to the inactive state happen at a rate similar to VIM-2. NDM-1 was produced by both, a *K .pneumoniae* isolate from urine of the patient. *E.coli* isolated from a fecal sample from the same patient also possessed the same gene suggesting in vivo transfer[14]. This is an indication of horizontal gene transfer among unrelated bacteria which may give rise to a plethora of NDM positive genera in the future causing a major threat to treatment of nosocomial infections.

To confirm that this enzyme belonged to the MBLs, MHT was also performed and the clover leaf type indentation was observed in four of the samples analyzed[53]. Molecular analysis by PCR was carried out using these four samples. First, the DNA was extracted by the heat boil method and this DNA was subjected to single target PCR. Agarose gel electrophoresis was performed using the amplified product and it was visualized under UV light (Fig. 7).

Studies carried out with this same gene at Mumbai showed an increasing number of NDM-1 positive isolates from ICU blood cultures, oral swabs, urine, sputum and bronchoalveolar lavage. Among them *P.aeruginosa* and *K.pneumoniae* have increased significantly in the past 5 years and are at a much higher frequency as compared to *E.coli, A.baumanii* and *Enterobacter spps*. (Fig. 8). NDM-1 has been able to accumulate at such a fast pace possibly due to efficient plasmid transfer. Hence, screening methods should be made necessary in tertiary care centers where there is a risk of further dissemination into the community to curb the spread of such resistant organisms. Sometimes carbapenemases may not be detected soon enough because their MIC values are below the current break points which may result in failure of the ongoing antibiotic treatment. Hence, direct PCR detection of NDM-1 genes is a faster means of avoiding ineffective treatment.

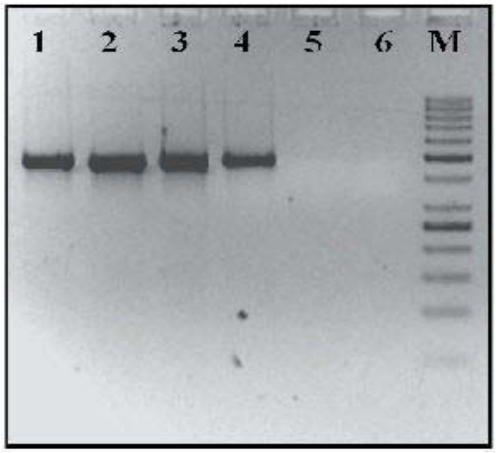

Fig. 7: Gel picture of PCR results of NDM-1

1, 2, 3 and 4 represent NDM positives; 5 and 6 represent NDM negatives; and M-50 basepair DNA ladder. (Image is cropped to so all markers are not seen)

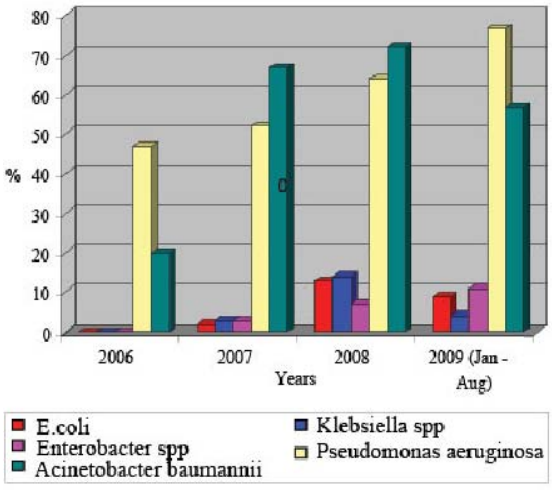

Fig. 8: Percentage of ICU blood cultures from 2006- 2009

Statistics on NDM-1

Till June 2009 the Antibiotic Resistance Monitoring and Reference Laboratory (ARMRL) had received 17 further producers[53]. Of the total 21 UK producers comprised *K. pneumoniae*[54], *E. coli*[55], *Enterobacter spp.*,[56] and *Citrobacter freundii*[9], from 18 patients and 16 hospitals scattered across England, with one in Scotland. NDM-1 has become the most frequent carbapenemase in isolates referred to ARMRL, and the most widely scattered. Cases from Europe included two cases from Austria[57], two from Belgium[58], one from Denmark[59], one from France[60], two from The Netherlands[61, 62], one from Sweden[11] and twenty nine from UK[21]. Cases were also reported from Australia[15], Canada[63], Singapore[64], US[65], India[11, 65] and Germany[66]. However, the ability of this genetic element to spread rapidly among *Enterobacteriaceae* means that there will almost certainly be numerous secondary cases throughout the world that are unrelated to travel to the Indian subcontinent.

CUNCLUSION

Ever since the first detection of an MBL in Japan in 1988 in *P.aeruginosa,* reports of MBL production have been obtained from *Bacterioides fragilis*[12], *K .pneumoniae, E.coli, Enterobacter aerogenes, E.cloacae, Citrobacter freundii, Proteus vulgaris, Acinetobacter spps.* and *Alcaligenes xylosoxidans*[28]. But the most frequent among these are *P.aeruginosa* and *Acinetobacter* species. MBL producers are being reported with an increasing frequency all over the world for causing prolonged nosocomial outbreaks accompanied by serious infections[67]. The occurance of an MBL–positive isolate in a hospital setting poses a therapeutic problem as well as a serious concern for infection control and management[68]. The data reviewed in this article may vary with the updates of newer cases of carbapenemases. But the overall idea behind it is to bring to light the seriousness of this situation. Cases once detected should not be neglected but should be treated as soon as possible. It means that newer antibiotics will have to be discovered which combat infections caused by these organisms, which should be stronger than the existing carbapenems. They should have a wide range of activity as well as be stable to inactivating enzymes produced by these pathogens. The most effective antibiotics till date are colistin, aztreonam and tigecycline[53]. The emergence of resistant entities reflects the indiscriminate use of antibiotics because such strains are doubtlessly an outcome of antibiotic pressure. Thus antibiotics should be chosen judiciously and less severe infections should be treated with non carbapenem drugs to preserve the efficacy of carbapenems. Since most organisms which are carbapenemase-positive are enteric bacteria, the obvious route of infection would be the faeco-oral route by contaminated food or water and hence proper sanitation and hygiene must be provided[14]. In hospitals, patients with infections of an MBL-positive pathogen must be kept isolated and necessary precautions must be taken for the safety of all the other patients in that hospital. Ideally all patients must first be screened for colonization of multi-drug resistant organisms in endemic countries[14] and surveillance must be strengthened to monitor and control the emerging carbapenemase- producing resistant pathogens.

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