Carbapenemases: A Review

Shalley Dahiya[1], Pooja Singla[2], Uma Chaudhary[3], Bijender Singh[4]

[1]Medical Officer, Department of Microbiology, BPS Government Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India, [2]Assistant Professor, Department of Microbiology, SHKM Government Medical College, Mewat, Haryana, India, [3]Senior Professor and Head, Department of Microbiology, Pandit Bhagwat Dayal Sharma Post Graduate Institute of Medical Sciences, Rohtak, Haryana, India, [4]Medical Officer, HCMS, Medical Officer, Bahadurgarh, Haryana

ABSTRACT

Carbapenems are widely regarded as the drugs of choice for the treatment of severe infections caused by extended-spectrum beta-lactamases producing *Enterobacteriaceae*. The emergence of carbapenem-resistant organisms is worrisome since antimicrobial treatment options are very restricted. Resistance of organisms against carbapenem, imparted by the presence of carbapenemase, is an emerging global health problem with high morbidity and mortality. Carbapenemases are a large and diverse family of microbial enzymes that hydrolyze not only carbapenems but also other beta-lactam antibiotics. Detection of carbapenemase-producing organisms in the clinical microbiology laboratory is a matter of major importance for the choice of appropriate therapeutic schemes and the implementation of infection control measures. The detection of carbapenemase producers, however, poses a number of difficulties, as it cannot be based simply on the resistance profile, and as the relevant methodology based on specific tests has not yet been well standardized.

Keywords: Antibiotics, Carbapenemases, Carbapenems, *Enterobacteriaceae*, Metallo-beta-lactamases, Modified Hodge test

Corresponding Author: Dr. Shalley Dahiya, Department of Microbiology, BPS Government Medical College for Women, Khanpur Kalan, Sonepat, Haryana, India. Phone: +91-9812532499, E-mail: docshalley@gmail.com

INTRODUCTION

Enterobacteriaceae are members of the intestinal flora. They are the most common cause of infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis, and catheter-associated infections. *Enterobacteriaceae* are the cause of community- and hospital-acquired infections. They have the tendency to spread easily among humans (hand carriage, contaminated food, and water). They acquire genetic material through horizontal gene transfer, mediated mostly by plasmids and transposons.[1] *Escherichia coli* and *Klebsiella pneumoniae* possessing extended-spectrum beta-lactamases (ESBLs) enzymes continue to beat our best clinical efforts. They are capable of hydrolyzing penicillin's (e.g., ampicillin and piperacillin), cephalosporins of the first, second, third, and fourth generations, and the monobactam aztreonam (but not the cephamycins or carbapenems).[2] It is, therefore, mandatory to maintain the clinical efficacy of carbapenems, which have become antimicrobial drugs of last resort.[1] With the introduction of penicillin, beta-lactam antibiotics have always been the antimicrobial agents of choice. Unfortunately, bacterial beta-lactamases presents a significant threat to the efficacy of these life-saving antibiotics.[3]

Bacterial resistance to beta-lactam antibiotics is achieved by any one of the following mechanisms: The first, by the production of beta-lactam hydrolyzing beta-lactamase enzymes, second, by the utilization of beta-lactam insensitive cell wall transpeptidases, third, by the active expulsion of beta-lactam molecules from Gram-negative cells by way of efflux pumps.[4] A wide variety of bacteria synthesize beta-lactamases, including aerobic Gram-positive, Gram-negative, and anaerobic species. In the Gram-positive bacteria, beta-lactamases are secreted to the outside membrane environment as exoenzymes. In the Gram-negative bacteria, they remain in the periplasmic space, where they attack the antibiotic before it can reach its receptor site.[5] Beta-lactamases are encoded by genes on chromosomes or plasmids.[6]

Classification of beta-lactamases can be defined according to two properties, functional and molecular. The functional classification by Bush *et al.* proposes to classify the known beta-lactamases into four major functional groups (Groups 1-4) according to the group-

specific substrate or inhibitor profiles. Group 2 is differentiated into multiple subgroups. The updated system comprises Group 1 (Class C) cephalosporinases; Group 2 (Classes A and D) broad-spectrum, inhibitor-resistant, and ESBLs and serine carbapenemases; and Group 3 metallo-beta-lactamases (MBLs). In this functional classification scheme, carbapenemases are found primarily in Groups 2f and 3.[7] The second, a less commonly encountered group of beta-lactamases, or Class B beta-lactamases. These use a divalent transition metal ion, most often zinc, linked to a histidine, or cysteine residue. The divalent transition metal ion reacts with the carbonyl group of the amide bond of most penicillins, cephalosporins, and carbapenems but not monobactams.[5]

CARBAPENEMASES

The carbapenems are a class of beta-lactam antibiotics that differ from the penicillins. There is the substitution of a carbon atom for a sulfur atom. There is addition of a double bond to the five-membered ring of the penicillin nucleus (Figure 1).

They bind the bacterial penicillin-binding proteins, which are important for elongation and cross-link the peptidoglycan of the bacterial cell wall. This binding impairs the construction of the cell wall, inhibits cell growth, and results in cell lysis and death. Carbapenems were first introduced in 1980. They are usually used as the last choice in treating serious infections caused by multidrug-resistant strains of Gram-negative bacilli. These are stable to beta-lactamases including the ESBLs and AmpC produced by Gram-negative bacilli.[8-10]
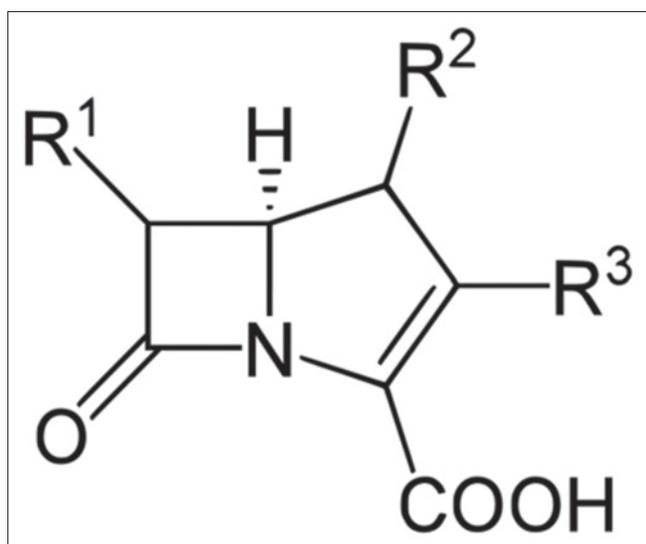

Figure 1: Structure of a carbapenem

Based on molecular studies, carbapenem-hydrolyzing enzymes can be classified into two groups. Serine enzymes possess a serine moiety at the active site. MBLs require divalent cations, usually zinc, as metal cofactors for enzyme activity. The serine carbapenemases belong to Class A or Class D enzymes. They usually result in carbapenem resistance in *Enterobacteriaceae* or *Acinetobacter* spp.[11]

Class A Serine Carbapenemases

Class A serine carbapenemases are members of functional Group 2f. They can hydrolyze a broad variety of beta-lactams, including carbapenems, cephalosporins, penicillins, and aztreonam. However, they all are inhibited by clavulanate and tazobactam.[11] The enzymes characterized from *Enterobacteriaceae* include not metalloenzyme carbapenemase-A (NMC-A), Serratia marcescens enzyme (SME) 1-3, "imipenem-hydrolysing beta-lactamase" (IMI-1), *K. pneumoniae* carbapenemases 1-3 (KPC), and Guiana extended-spectrum (GES-2).[7,11]

The genes for SME, IMI, and NMC-A beta-lactamases are chromosomally located. They are rare on account of no association with the mobile element. SME-1 was first detected in England from two *S. marcescens* isolates that were collected in 1982. The NMC-A and IMI were isolated from rare clinical isolates of *Enterobacter cloacae* in the United States, France, and Argentina. NMC-A and IMI-1 have 97% amino acid identity and resemble SME-1, with approximately 70% amino acid identity. These chromosomal beta-lactamases are induced in response to imipenem and cefoxitin.[7]

KPC and GES family of carbapenemases are plasmid encoded. KPC carbapenemases have the greatest potential for spread due to its location on plasmids. The KPC carbapenemases differ from the other functional Group 2f enzymes by two important characteristics. First is the presence of the KPC enzymes on transferable plasmids. The second, their substrate hydrolysis spectrum includes the aminothiazole oxime cephalosporins, such as cefotaxime. Although, the KPC beta-lactamases are predominantly found in *K. pneumoniae*, there have been reports of these enzymes in *Enterobacter* species and in *Salmonella* spp.[7]

The intensive care antimicrobial resistance epidemiology surveillance project discovered the first member of the KPC family in a *Klebsiella* clinical isolate from North Carolina in 1996. This isolate was resistant to all beta-lactams tested, but carbapenem minimum inhibitory concentration (MICs) decreased in the presence of clavulanic acid.[12]

After the discovery of KPC-1, a single-amino-acid variant, KPC-2 was reported along the east coast of the

United States. KPC-2 was first identified in 2003 as the result of a point mutation in KPC-1. It was identified in four isolates with imipenem MICs of 2-8 μg/ml from Baltimore, from 1998 to 1999. The KPC-2 producing gene resides on a transferable plasmid. All isolates exhibited reduced susceptibility to imipenem, but none were technically resistant according to approved Clinical and Laboratory Standards Institute (CLSI) breakpoints.[7]

A single-amino-acid variant of KPC-2, KPC-3, was reported from a 2000 to 2001 *K. pneumoniae* outbreak in New York. KPC-3 was also detected in *Enterobacter* spp., where MICs for imipenem were also not in the resistant range.[7,13]

KPC carbapenemases hydrolyse beta-lactams of all classes, with the most efficient hydrolysis observed for nitrocefin, cephalothin, cephaloridine, benzylpenicillin, ampicillin, and piperacillin. Imipenem, meropenem, cefotaxime, and aztreonam, are hydrolyzed 10-fold-less efficiently than the penicillins and early cephalosporins. Weak but measurable hydrolysis is observed for cefoxitin and ceftazidime, giving the KPC family a broad hydrolysis spectrum that includes most beta-lactam antibiotics. The KPC family can spread easily due to its location on plasmids. It is the most often present in *K. pneumoniae*, an organism known for its ability to accumulate and transfer resistance determinants. The treatment of infections caused by these organisms is extremely difficult because of their multidrug resistance and hence results in high mortality rates.

The GES/integron-borne cephalosporinase (IBC) family of beta-lactamases was first described in 2000. The IBC-1 was isolated from an *E. cloacae* isolate in Greece and GES-1 in a *K. pneumoniae* isolate from French Guiana.[7]

Class B MBLs

This class of beta-lactamases can hydrolyze carbapenems and known by its resistance to the commercially available beta-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 can hydrolyze cephalosporins and penicillins. However, they lack the ability to hydrolyze aztreonam. This hydrolysis is based on the interaction of the beta-lactams with zinc ions in the active site of the enzyme. This results in the distinctive trait of their inhibition by ethylenediaminetetraacetic acid (EDTA), a chelator of Zn^{2+} and other divalent cations. A considerable amount of sequencing work has depicted high variability in primary sequences and molecular structures of MBLs. The first MBLs for which an amino acid sequence was determined was the metallo-beta-lactamase from

Bacillus cereus, the prototypical metallo-beta-lactamase for many years.[7]

The presence of chromosomal MBLs is directly correlated with the prevalence of the producing species. However, there is a dramatic increase in the detection and spread of the acquired or transferable families of these metalloenzymes. The most common MBLs families include the Verona integron-encoded MBLs (VIM), "active on imipenem" (IMP), "German imipenemase" (GIM), and "Seoul imipenemase" (SIM) enzymes. These are incorporated as gene cassettes present within a variety of integron structures. The transfer between bacteria is readily facilitated when these integrons become associated with plasmids or transposons.[7]

Transferable imipenem resistance was first detected in 1990 in Japan, in a *Pseudomonas aeruginosa* isolate. It was followed by a second report of a transferable carbapenemase in *Bacteroides fragilis* IMP-1. It was located on a conjugative plasmid in the *P. aeruginosa* clinical isolate. It was also present on an integron in *S. marcescens* and other *Enterobacteriaceae* in Japan. This enzyme hydrolyzed imipenem, penicillins, and extended-spectrum cephalosporins but not aztreonam. EDTA inhibited the hydrolytic activity. The addition of zinc ions restored the hydrolytic activity. Another prevalent family of integron-associated MBLs is composed of the VIM enzymes. VIM-1 was first isolated in Verona, Italy, in 1997. VIM-2 was identified in France in 1996.[14,15]

Identification of the "Sao Paulo MBLs" (SPM-1) MBLs defined a new family with 35.5% amino acid identity to IMP-1. SPM-1 was first isolated in a *P. aeruginosa* strain in Sao Paulo, Brazil. GIM-1 was isolated in Germany in 2002. GIM resembles 30% in homology to VIM, 43% in homology to IMPs, and 29% in homology to SPM. GIM-1 resembled to the other acquired MBLs as it was found in five clonal *P. aeruginosa* isolates within a Class 1 integron on a plasmid. At present, it is not reported elsewhere in the world. The latest family of acquired MBLs was described in Korea. The enzyme SIM-1 has the closest amino acid identity to the IMP family (64-69%).

Class D Serine Carbapenemases

These beta-lactamases can hydrolyze cloxacillin or oxacillin at a rate of >50% than for benzylpenicillin and hence are known as OXA enzymes. They readily hydrolyse carbenicillin. OXA related enzymes now comprise the second largest family of beta-lactamases.[10] OXA enzymes are difficult to purify due to low yield and difficult to characterize biochemically owing to low hydrolysis rates and biphasic kinetics for some substrates. The OXA carbapenemases characterized biochemically have measurable hydrolytic activity

Table 1: Substrate and inhibition profiles of the carbapenemases[7]

Molecular classes	Functional group	Enzyme	Hydrolysis profile[a]				Inhibitor profile[b]	
			Penicillins	Cephalosporins	Aztreonam	Carbapenems	EDTA	Clavulanic acid
A	2f	NMC	+	+	+	+	−	+
		IMI	+	+	+	+	−	+
		SME	+	+	+	+	−	+
		KPC	+	+	+	+	−	+
		GES	+	+	−	±	−	+
B	3	IMP	+	+	−	+	+	−
		VIM	+	+	−	+	+	−
		GIM	+	+	−	+	+	−
		SPM	+	+	−	+	+	−
D	2d	OXA	+	+	−	±	−	±

[a]+: Strong hydrolysis (generally, k_{cat} of >2/s), ±: Weak hydrolysis (generally, k_{cat} of 0.5-2/s), −: No measurable hydrolysis reported (generally, k_{cat} of <0.5 s^{-1}),
[b]+: Reported inhibition, ±: Variable inhibition among beta-lactamase family members, −: No inhibition reported, EDTA: Ethylenediaminetetraacetic acid, NMC: Not metalloenzyme carbapenemase, SME: *Serratia marcescens* enzyme, KPC: *K. pneumoniae* carbapenemases, GES: Guiana extended-spectrum, VIM: Verona integron-encoded metallo-beta-lactamases, GIM: German imipenemase, SPM: Sao Paulo metallo-beta-lactamases

against the penicillins, some cephalosporins, and imipenem (Table 1).[16,17]

DETECTION OF CARBAPENEMASES

Detection of carbapenemase activity in a clinical isolate is a difficult and challenging task for a clinical microbiology laboratory. An elevated carbapenem MIC raises suspicion that a carbapenemase is involved in a clinical infection.

Petropoulou *et al*. identified a set of 19 *Klebsiella* isolates with imipenem MICs in the susceptible range of 1-4 µg/ml. They suggested the production of MBLs by disk testing with imipenem in the presence and absence of EDTA. It was then confirmed by polymerase chain reaction (PCR) as VIM-1. The five related *Klebsiella* strains with the VIM-1 gene showed imipenem MICs ranging from 2 to 64 µg/ml (susceptible to high-level resistance). Decreased permeability due to the absence of the outer membrane porin OmpK36 was a contributing factor in the most resistant isolates, with an imipenem MIC of 64 µg/ml.[7,18]

The KPC serine carbapenemases also have been reported to be difficult to detect. These carbapenemase demonstrate imipenem MICs as low as 2 µg/ml, and a low inoculum results in susceptible MICs by broth microdilution. Tenover *et al*. processed 15 characterized imipenem- and meropenem- non-susceptible KPC-producing isolates for imipenem and meropenem resistance using CLSI broth microdilution, E test, Microscan WalkAway, BD Phoenix Sensititre Autoreaders, VITEK, and VITEK2. The automated systems reported carbapenem susceptibility in this collection of isolates ranging from 6.7% to 87%, depending on the system used. Day-to-day variation was observed. E test results were discordant due to the presence of colonies in the zones of inhibition.[19-21]

Phenotypic Tests for Detection of Carbapenemase Production

The modified Hodge test (MHT) is the only method of carbapenemase detection so far recommended by the CLSI.[22,23] This general phenotypic test is widely used as a method for the carbapenemase detection. The underlying principle of the test is inactivation of a carbapenem by either whole cells or cell extracts of the carbapenemase-producing organisms. This allows a carbapenem-susceptible indicator strain to extend growth toward a carbapenem disc, along the streak of inoculum of the test strain or extract thereof. This test is sensitive for the detection of a carbapenemase-mediated mechanism of resistance to carbapenems. However, it does not provide information regarding the type of carbapenemase involved. Moreover, the reports of false-positive results generated by CTX-M-producing strains with reduced outer membrane permeability are an added drawback. Some investigators have raised the issue of difficult interpretation of the MHT for weak carbapenemase producers, particularly for MBLs in *Enterobacteriaceae*.[24] In the isolates of MBL producers, the addition of zinc sulfate may improve the MHT performance.[25] The accurate determination of levels of susceptibility to meropenem and the MHT are the sensitive methods for detection of KPC-producing isolates in the endemic regions, although with insufficient specificity.[26]

Microbiological Tests with Inhibitors

The inhibitory effect of boronic acids, usually 3-aminophenylboronic acid (APB) forms the basis of specific phenotypic assays for the identification of KPC-producing strains (and of those producing other Class A carbapenemases). However, the mechanism of inhibition by boronic acids is not known. Several modifications of such tests are available which differ in their performance. One of these studies quotes the double disc synergy test approach to work well, but the evaluations done

so far finds combined disc test to be better. Meropenem or imipenem are the preferable compounds among several indicator beta-lactams tested so far. Different cut-off values of zone diameter differences between discs with a carbapenem plus APB and the carbapenem are proposed to indicate the production of KPC (or another Class A carbapenemase) (≥4 to ≥7 mm). There is excellent specificity in diagnosing KPC-producing isolates and differentiating them from plasmid-mediated AmpC-producing isolates with the use of meropenem discs, with or without 400 μg of APB. Besides the disc diffusion approaches, a second method is developed in which MICs of carbapenems are observed both in the absence and in the presence of APB (0.3 g/L) by agar dilution. Reduction of carbapenem MIC in the presence of APB by a three-fold or greater is proposed as the cut-off value for positive isolates.[24,27,28]

Boronic acid-based methods seem to exhibit high sensitivity in the detection of KPC producers. However, further evaluation of their specificity is required. There is a tendency for false-positive results because of high-level expression of AmpC-type cephalosporinases and porin alterations by organisms with reduced susceptibility to carbapenems. It is not surprising that boronic acids exhibit significant inhibition of AmpCs. It is noteworthy that the APB-based assays failed to detect the KPC-producing *Klebsiella* isolates in the case of co-production of VIM enzyme.[24,29,30]

Tsakris *et al*. tested 57 KPC-producing isolates by using discs containing 400 μg of phenyl boronic acid as an inhibitor and several beta-lactams as the antibiotic substrates. In comparison to the beta-lactam discs alone, they found significantly increased (>5 mm) inhibition zone diameters with combination of BA with cefepime and all carbapenems.[28]

Doi *et al*. found that 10 KPC-producing isolates produced increased zone diameter ≥5 mm by addition of APB to ertapenem or meropenem (but not imipenem) discs in comparison to the carbapenem disc alone.[27]

A group of experts have investigated the utility of APB for detection of other Class A carbapenemases. They found 100% sensitivity and specificity of BA-based MIC tests utilizing imipenem-APB to differentiate Class A carbapenemase-producing bacteria from non-carbapenemase producing bacteria. These tests use a cut-off of ≥3-fold reduction in MIC compared with imipenem alone.[24]

There are several inhibitor-based tests for the specific detection of MBL producers. These are based on the synergy between MBL inhibitors and a carbapenem and/or an oxyimino-cephalosporin (ceftazidime) as

indicator beta-lactam compounds. The MBL inhibitors are EDTA, EDTA plus 1, 10-phenanthroline, thiol compounds, and dipicolinic acid these tests take advantage of the metalloenzyme dependence on zinc ions and use the chelating agents to inhibit beta-lactam hydrolysis. Various formats (disc diffusion or broth dilution) of EDTA-based synergy tests have been the most common used and evaluated.

The E test MBL is not appropriate for the detection of MBL-producing *Enterobacteriaceae* with low imipenem MICs (≤4 mg/L). The new E test strips show better preliminary results for detection of MBLs in *Enterobacteriacea*. However, it is important to note that MBL inhibitors act non-specifically and affect other structures and processes (e.g., outer membrane permeabilization in *P. aeruginosa*).[31]

A group of experts from the European antimicrobial resistance surveillance system and EUCAST recommends the following procedures for the detection of Class A and B enzymes in *Enterobacteriaceae*. Suspect the production of Class A enzymes (KPC or other enzymes), when there is a difference of ≥4 mm in the zone diameter between meropenem (10 μg) and meropenem plus boronic acid (600 μg). The boronic acid can also inhibit Class C enzymes. Compare between the zone diameters of meropenem and meropenem plus cloxacillin (750 μg) discs. The increase in diameter by ≥5 mm suggests the presence of a strain hyper producing the chromosomal AmpC or producing a plasmid-encoded AmpC.

To detect the Class B enzymes use disc combination test using meropenem and meropenem plus EDTA (0.25 M). An increase in the zone diameter of ≥5 mm is taken as positive. This procedure is, overall, similar to that proposed by different investigators who recommend the use of different discs containing a carbapenem disc with EDTA and boronic acid but with different concentrations. However, these protocols cannot discriminate Class A carbapenemases from the combination of Class C beta-lactamases or ESBLs and porin loss.

It is possible to accurately differentiate KPC producers from KPC-negative *Klebsiella* by using meropenem discs with 400 μg of boronate. The authors claim that these disc tests can easily detect carbapenemases the first isolation day. There is no need of estimation of E test carbapenem MICs and clover leaf test.[27,28]

Spectrophotometric Assays to Detect Carbapenemase

The spectrophotometric measurement of carbapenem hydrolysis is the method of choice for the detection of carbapenemase production in a suspected

carbapenemase-producing isolate. The details regarding the type of enzyme are obtained by performing hydrolysis of carbapenems with crude cell extracts or partially purified enzymes. The hydrolysis is done in the presence or absence of inhibitors (i.e., EDTA for MBLs, tazobactam or clavulanic acid for KPCs). The spectrophotometric assays demand labor and technical expertise, hence are performed in reference laboratories.[29]

Molecular Methods to Detect Carbapenemase Genes

Simplex and multiplex PCRs, real-time PCR, DNA hybrid technique, and sequencing are usually used for the detection of carbapenemase genes (bla_{KPC}) in research laboratories and reference centers. Nowadays, some clinical laboratories routinely perform these molecular methods to avoid the problems of the phenotypic detection of carbapenemase-producing organisms.[31]

Akter *et al.* conducted a study to detect the molecular causes of multidrug resistance in *Klebsiella*. They selected the 10 multidrug resistant phenotypes that showed mucoid phenotype on MacConkey media, for plasmid DNA extraction procedure and agarose gel electrophoresis. The analysis revealed that all seven out of 10 isolates contained plasmid forming a unique banding pattern. It may be epidemiology significant to have strong association between plasmid profiles and drug resistance patterns.[32]

The spectrophotometry assay to detect hydrolysis of a carbapenem, followed by PCR to identify bla_{KPC} gene is the gold standard to confirm the presence of a KPC. However, the limitations of this genotypic method are that it is a time-consuming procedure for a clinical microbiology laboratory and usually requires verification of isolates in a reference laboratory.[33]

CONCLUSION

Emerging antibiotic resistance has become a critical issue, due to a shortage of new antibiotic options. Clinicians must prescribe antibiotics according to the status of the patient in a manner that does not lead to further drug resistance. There is a need to select an appropriate initial antibiotic regimen for empiric therapy followed by rotation of different antibiotic classes. To avoid unnecessary use of broad-spectrum antibiotics, one should detect the resistant bacteria. Failure to detect carbapenemases has contributed to their uncontrolled spread and therapeutic failures. The carbapenemases should be detected routinely in clinical laboratories by using appropriate methods and reported to clinicians to implement the judicious use of antibiotics.

REFERENCES

1. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase - Producing *Enterobacteriaceae*. Emerg Infect Dis 2011;17:1791-8.
2. Pitout JD, Laupland KB. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: An emerging public-health concern. Lancet Infect Dis 2008;8:159-66.
3. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev 2010;23:160-201.
4. Wilke MS, Lovering AL, Strynadka NC. Beta-lactam antibiotic resistance: A current structural perspective. Curr Opin Microbiol 2005;8:525-33.
5. Samaha-Kfoury JN, Araj GF. Recent developments in beta lactamases and extended spectrum beta lactamases. BMJ 2003;327:1209-13.
6. Rice L. Evolution and clinical importance of extended-spectrum beta-lactamases. Chest 2001;119:391S-6.
7. Queenan AM, Bush K. Carbapenemases: The versatile beta-lactamases. Clin Microbiol Rev 2007;20:440-58.
8. Rodloff AC, Goldstein EJ, Torres A. Two decades of imipenem therapy. J Antimicrob Chemother 2006;58:916-29.
9. Matsumoto T, Kumazawa J, Nagayama A. Sensitivities to four carbapenems of bacteria isolated from patients with refractory complicated urinary tract infections and the detection of carbapenemase-producing *Pseudomonas aeruginosa*. J Antimicrob Chemother 1996;38:322-4.
10. Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, *et al.* Comparative review of the carbapenems. Drugs 2007;67:1027-52.
11. Walsh TR, Toleman MA, Poirel L, Nordmann P. Metallo-beta-lactamases: The quiet before the storm? Clin Microbiol Rev 2005;18:306-25.
12. Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD, *et al.* Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2001;45:1151-61.
13. Woodford N, Tierno PM Jr, Young K, Tysall L, Palepou MF, Ward E, *et al.* Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3, in a New York Medical Center. Antimicrob Agents Chemother 2004;48:4793-9.
14. Watanabe M, Iyobe S, Inoue M, Mitsuhashi S. Transferable imipenem resistance in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 1991;35:147-51.
15. Poirel L, Naas T, Nicolas D, Collet L, Bellais S, Cavallo JD, *et al*. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-beta-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob Agents Chemother 2000;44:891-7.
16. Bush K, Jacoby GA. Updated functional classification of beta-lactamases. Antimicrob Agents Chemother 2010;54:969-76.
17. Walther-Rasmussen J, Høiby N. OXA-type carbapenemases. J Antimicrob Chemother 2006;57:373-83.
18. Petropoulou D, Tzanetou K, Syriopoulou VP, Daikos GL, Ganteris G, Malamou-Lada E. Evaluation of imipenem/imipenem EDTA disk method for detection of metallo-beta-lactamase-producing *Klebsiella pneumoniae* isolated from blood cultures. Microb Drug Resist 2006;12:39-43.
19. Smith Moland E, Hanson ND, Herrera VL, Black JA, Lockhart TJ, Hossain A, *et al.* Plasmid-mediated, carbapenem-hydrolysing beta-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J Antimicrob Chemother 2003;51:711-4.
20. Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, *et al.* Rapid spread of carbapenem-resistant *Klebsiella pneumoniae*

in New York City: A new threat to our antibiotic armamentarium. Arch Intern Med 2005;165:1430-5.

21. Tenover FC, Kalsi RK, Williams PP, Carey RB, Stocker S, Lonsway D, *et al*. Carbapenem resistance in *Klebsiella pneumoniae* not detected by automated susceptibility testing. Emerg Infect Dis 2006;12:1209-13.

22. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing: Twenty-First Informational Supplement. CLSI Document. M100-S21. Wayne, PA, USA: CLSI; 2011.

23. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. Clin Microbiol Infect 2001;7:88-91.

24. Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive screening tests for suspected class A carbapenemase production in species of *Enterobacteriaceae*. J Clin Microbiol 2009;47:1631-9.

25. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-beta-lactamase-producing isolates of *Pseudomonas* spp. and *Acinetobacter* spp. J Clin Microbiol 2003;41:4623-9.

26. Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, *et al.* Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. J Clin Microbiol 2007;45:2723-5.

27. Doi Y, Potoski BA, Adams-Haduch JM, Sidjabat HE, Pasculle AW, Paterson DL. Simple disk-based method for detection of *Klebsiella pneumoniae* carbapenemase-type beta-lactamase by use of a boronic acid compound. J Clin Microbiol 2008;46:4083-6.

28. Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D, *et al.* Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. J Clin Microbiol 2009;47:362-7.

29. Beesley T, Gascoyne N, Knott-Hunziker V, Petursson S, Waley SG, Jaurin B, *et al.* The inhibition of class C beta-lactamases by boronic acids. Biochem J 1983;209:229-33.

30. Giakkoupi P, Pappa O, Polemis M, Vatopoulos AC, Miriagou V, Zioga A, *et al.* Emerging *Klebsiella pneumoniae* isolates coproducing KPC-2 and VIM-1 carbapenemases. Antimicrob Agents Chemother 2009;53:4048-50.

31. Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, *et al.* Acquired carbapenemases in Gram-negative bacterial pathogens: Detection and surveillance issues. Clin Microbiol Infect 2010;16:112-22.

32. Akter J, Chowdhury AM, Mohammad AF. Molecular study on plasmid profiling of clinical isolates of antibiotic resistant *Klebsiella* from Chittagong city. Int J Chem Life Sci 2013;02:1163-5.

33. Hirsch EB, Tam VH. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): An emerging cause of multidrug-resistant infection. J Antimicrob Chemother 2010;65:1119-25.