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REVIEW ARTICLE

EMERGING CARBAPENEMASES: BRINGING A STEP CLOSER TO EXTREMELY DRUG RESISTANT BACTERIA

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ABSTRACT

Carbapenemases are β- lactamases that confer resistance to the carbapenems (e.g., imipenem, meropenem, ertapenem, and doripenem). Most often these enzymes confer resistance to the other b-lactam agents as well, including extended-spectrum cephalosporins. The enzymes are usually found in bacterial isolates that are already resistant to nearly all other antimicrobial agents, and treatment options for infections caused by them are significantly limited. This important mechanism of resistance is emerging in the USA include *Klebsiella pneumonia* carbapenemases (KPC), while in India it is New Delhi metalo betalactamase (NDM) and in spain it is Verona imipenem hydrolyzing enzyme (VIM). Prevention and control of this emerging resistance is complicated by the occurrence of low-level carbapenem resistance isolates that are hard to detect in the clinical microbiology laboratory. This article will review the epidemiology, diagnostic methods and therapeutic options of emerging carbapenemases.

INTRODUCTION

Carbapenem and carbapenemases

In 1950s and 60s broad-spectrum antibiotics became available for the treatment of Gram-negative infections, however bacteria acquired a range of mechanisms to evade these agents. In particular, beta-lactam antibiotics such as penicillins became vulnerable to hydrolysis by enzymes called beta-lactamases. In the mid 1970s new beta-lactamase stable cephalosporin compounds, were introduced but unfortunately novel extended-spectrum beta-lactamases (ESBL) soon emerged thus compromising these new compounds. The first hospital outbreaks caused by ESBL producers occurred in France in the mid-1980s, soon followed by large outbreaks in the United States. ESBL producers, are now widespread worldwide, and often are multidrug-resistant (MDR) also to fluoroquinolones and aminoglycosides[1]. A further class of beta-lactam antibiotics, the carbapenems, came into clinical use in 1985. These drugs combine exceptional intrinsic antibacterial activity with stability to most of the prevalent beta-lactamases, including ESBLs and have become the treatment of choice for infections due to the ESBL-producing strains, which are increasingly diagnosed in hospital settings. Four carbapenems currently have FDA approval for clinical use: imipenem, meropenem, ertapenem, and doripenem. Regrettably, it has become clear that bacteria also can acquire carbapenem- hydrolysing β -lactamase (carbapenemases). Such enzymes have emerged in various parts of the world, including Europe, the Indian subcontinent and the US. Carbapenemases represent the most versatile family of lactamases, with a breadth of spectrum unrivaled by other β lactam-hydrolyzing enzymes. Although known "carbapenemases," many of these enzymes recognize almost all hydrolysable β lactams, and most are resilient against inhibition by all commercially available β - lactamase inhibitors.[2, 3]

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Classification of carbapenemases

Classification by Ambler, based on amino acid homology has resulted in four major classes, which correlate well with the functional scheme. Molecular classes A, C, and D include the β-lactamases with serine at their active site, whereas molecular class B β lactamases are all metalloenzymes with an active-site zinc. The epidemiologically most relevant carbapenemases fall into classes A, B, and D.[1]

Class A: It included
KPC **(**K. pneumonia carbapenemases);
SME **(**Serratia marcescensenzyme),
NMC-A/IMI (Not metalloenzyme carbapenemase/imipenem-hydrolysing beta-lactamase)
GES (Guiana extended spectrum)

Class B*:* Includes the metallo-beta-lactamases (MBLs)
IMP (imipenemase),
VIM (Verona integron-encoded metallo-beta-lactamase)
NDM – 1 (New Delhi metallo-beta-lactamase)

Class D: Includes the oxacillin hydrolyzing (OXA) type carbapenemases which are mostly found in Acinetobacter spp., although OXA-48 occurs in Enterobacteriaceae**.**

Epidemiology International and National

The first transferable carbapenemase identified in Gram-negative bacteria was an IMP-like MBL in the Far East, followed by VIM types in Europe.[1] The current epidemiology of carbapenemases generally follows patterns of increasing occurrences that are country specific. Presumably this is due to multiple factors, including antibiotic usage, dosing regimens, and local hospital practices concerning isolation of patients with multi resistant pathogens.

NEW DELHI METALLO-BETA-LACTAMASE (NDM):

It is a newly-described metallo-beta-lactamase (MBL), first identified in 2008 in single isolates *of Klebsiella pneumoniae* and Escherichia coli**,** both recovered from a patient in Sweden.[4] Like other acquired MBLs, NDM-1 hydrolyses all beta-lactam antibiotics except for aztreonam, which is usually inactivated by co-produced extended-spectrum or AmpC beta-lactamases. Most isolates with NDM type, also contain ESBL and acquired amp**C** genes, which makes them resistant to all antibiotics except polymyxins, tigecycline and, occasionally, certain aminoglycosides.[4] Cases have also been reported in from Australia, Canada, Singapore, US and, according to recent media reports, from China, Israel, Japan, Kenya, Malaysia, Oman and Taiwan. Recently NDM has been reported from Bombay and Varanasi in India.[5,6,7,8,9,10] In most of the studies the majority of cases described, had a history of recent travel and hospital admission on the Indian subcontinent, but there was also a smaller proportion of cases who had received hospital care in Balkan countries. In a recent study published in lancet infectious disease molecular study and strain typing of the NDM strains isolated from patients in UK did not showed any clonal relatedness to Indian strain and till date there is no scientific evidence that NDM originated from Indian subcontinent.

K. pneumoniae carbapenemase **(KPC)**

It was first detected in 1996 in North Carolina, then spread along the east coast of the US, and finally across the whole country, posing a significant threat with 70% or higher mortality in bacteraemic patients.[12] Outside the US, *K. pneumoniae* with KPC have spread widely in Israel and Greece, with outbreaks or isolated cases in hospitals in other European countries. Spread is also occurring in China and Latin America, India. Many *K. pneumoniae* isolates with KPC enzymes belong to a single clonal complex, CC11, and predominantly to a single sequence type, ST 258, containing different variants of the bla_{KPC} gene. Apart from *K. pneumoniae,* KPC enzymes have been found in other species of Enterobacteriaceae (e.g. K. oxytoca**,** Entero bacter spp., E.coli) and, more recently, also in *Pseudomonas spp.* and *Acinetobacter baumanni.* As with the MBL producers, few treatment options remain, although some isolates remain susceptible to few aminoglycosides (gentamicin, isepamicin) as well as to polymyxins (such as colistin) or tigecycline.[13,14]

OXA-type carbapenemases

OXA-48 was first described in Turkey during an outbreak of *K. pneumoniae* in Istanbul but has since attained international distribution not only among *K. pneumoniae* but also *E. coli* .[15] By 2009, strains with OXA-48 enzyme were being reported from the Middle East, India, Europe and North Africa.[1]

IMP, VIM

The most common MBLs are the IMP- and VIM-type enzymes. The IMP-type enzymes have become widespread among *Pseudomonas* spp., *Acinetobacter* spp., and members of the *Enterobacteriaceae* in Japan. The geographical distribution of these genes was expanded with the report of *bla*IMP-2 in an *Acinetobacter baumannii* isolate in Italy in 1997. The VIM family of β-lactamases is currently comprised of 14 members, found mainly in *P. aeruginosa* but increasingly being detected in members of the *Enterobacteriaceae*. VIM-2 is the most widely disseminated globally.[1]

Other Class A Carbapenemases

It includes chromosomally encoded SME, IMI, and NMC-A enzyme families and members of the plasmid-borne GES β-lactamases. These are rarely reported and display a unique susceptibility profile in that they are resistant to carbapenems, penicillins, and aztreonam but susceptible to extended-spectrum cephalosporins.[1]

Carbapenemases reported from India

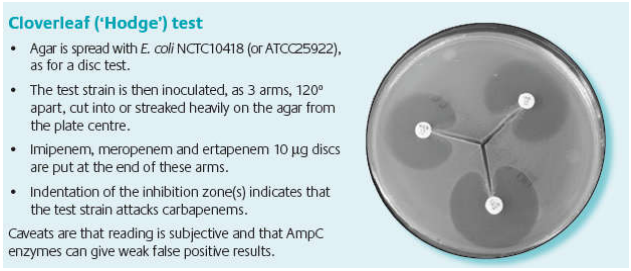
A study was conducted at CMC vellore in 2006 on MBL producing pseudomonas aeruginosa, Among 57 MBL producing *Pseudomonas* spp. five strains had *bla*VIM genes, including a newly characterized *bla*VIM 18 gene.[16] In 2005-06 a large multicentric study was conducted by surveillance of antimicrobial resistace group (SARI), in which 164 *pseudomonas spp* were collected from all over India. Of these 20 *pseudomonas spp* were MBL positive by E test and PCR for detecting IMP/VIM was done on 17 strains. 15 were positive for VIM, none for IMP and 2 strains remained unidentified.[17] In 2009 investigators from SGPGI, Lucknow while working on Ventilator associated pneumonia reported that 12 of the 64 isolates were MBL producers and *bla*IMP was documented as most common gene followed by *bla*VIM.[18] In 2010, a study in Mumbai revealed 24 carbapenem-resistant Enterobacteriaceae, 22 of which were NDM-1 producers. Of these 22 organisms, 10 were klebsiella species, 9 were *Escherichia coli*, 2 were enterobacter species, and 1 was *Morganella morganii* — illustrating the ability of the plasmid to spread rapidly among strains of Enterobacteriaceae.[9] A more extensive recent study has shown widespread distribution of NDM-1– producing *Enterobacteriaceae* in Bangladesh, India, Pakistan, and

Britain, all of which appears to have occurred since the original isolate was discovered in 2008.[19] *Enterobacteriaceae* isolates from five continents obtained as part of the Study for Monitoring Antimicrobial Resistance Trends (SMART) using molecular methods for rapid and accurate detection of b-lactamase genes recently documented NDM-1 positive Indian strains.[20] In another interesting study from BHU, Varanasi the investigators worked on MBL detection in *Enterobacteriaceae*, among 740 strains collected; 64 were positive for MBL and PCR for blaNDM was done which revealed that 54/64 strains were NDM positive.[10]

Laboratory diagnosis of carbapenemases

Minimum inhibitory concentration (MIC) break points for the isolates which are resistant or borderline sensitive for carbapenem by disc diffusion testing is performed and organisms with MICs ≤ 1 mg/L are considered to be susceptible, whereas those with MICs ≥4 mg/L are characterized as resistant for meropenem. The phenotypic confirmatory test for MBL detection includes:[21]

Modified Hodge Test: The test to determine the presence of carbapenemases per the recent guidelines of hospital protection agency, UK.



Test for Metallo beta lactamase

MBL E- strip test

MBL Etest is performed according to the recommendations of the manufacturer (AB Biodisk). A reduction in the MIC of imipenem of three or more 2-fold dilutions in the presence of EDTA is to be interpreted as a positive test indicative of MBL production.[1]

Double disc synergy testing (DDST):

Test strains are adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and used to inoculate Mueller–Hinton agar plates. Depending on the test, a 10 mg imipenem disc or a 30 mg ceftazidime disc is placed on the plate, and an EDTA disc (Rosco, Denmark) is placed at a distance of 10 (edge to edge). After overnight incubation, the presence of any synergistic inhibition zone is interpreted as positive.[1]

Combined disc test (CDT)

Test strains are adjusted to a turbidity equivalent to that of a 0.5 McFarland standard and are used to inoculate Mueller–Hinton agar plates, 10 mg meropenem discs and disc containing Meropenem-EDTA and Meropenem-dipiclonic acid (Rosco, Denmark) are placed on a plate inoculated with the test organism. Zone of inhibition around meropenem and combined disc is to be calculated after 18 h of incubation in air at 35^{o}C and results interpreted positive when zone enhancement is more than 7 mm.[1]

Test for Group A carbapenemases (KPC) Detection.

Combined disc test with meropenem and meropenem + boronic acid (Rosco, Denmark) will be performed and results interpreted as zone enhancement more than 7 mm.[1]

Molecular test for genotypic detection of carbapenemases

PCR analysis is the gold standard method for the detection of MBL producers, but it is not suitable for daily testing in clinical laboratories due to the cost and inconvenience.[1] By PCR we can easily do genotypic detection of NDM, IMP, VIM, KPC, OXA-48, SPM, GIM , GES< NMC and IMI genes. Various format of PCR are available like singleplex PCR, multiplex PCR and real time PCR. In 2007 a multiplex PCR war documented which could simultaneously detect VIM, IMP, GIM, SIM, SPM [23] and recently a multiplex PCR has been reported by which 11 genes blaIMP, blaVIM, blaNDM, blaSPM, blaAIM, blaDIM, blaGIM, blaSIM blaKPC, blaBIC, and blaOXA-48 can be detected simultaneously.[24]

Treatment options for carbapenemase producing bacteria

Carbapenem-resistant Enterobacteriaceae present an increasing and diverse problem, including strains of multiple species with metallo-β-lactamases (IMP, NDM or VIM) and non-metallo (KPC and OXA-48) enzymes as well as those combining an extended-spectrum β-lactamase (ESBL) or AmpC enzyme with porin loss. Most strains, except those with OXA-48 alone, are broadly resistant to β-lactams and have multiple aminoglycoside-modifying enzymes; those with NDM-1 carbapenemase typically also have 16S rRNA methylases, conferring complete aminoglycoside resistance.[1] In a recent study from UK 37 NDM strains were isolated all strains were resistant to imipenem and meropenem, amikacin, tobramycin. One isolate was suseptable to ciprofloxacin, 4 to aztreonam, 33 to colistin and 26 strains were sensitive to tigecycline[19]. Investigators from BHU varansi reported that 85.% of the NDM-1-producing isolates were susceptible to polymyxin B and 46.% were susceptible to tigecycline. For other antimicrobials, 51% showed susceptibility to piperacillin/tazobactam and 22%, 5% and 1% showed susceptibility to amikacin, gentamicin and tobramycin, respectively. Among 23 urinary tract isolates, 8 showed susceptibility to nitrofurantoin.[10] Thus it is required that we perform a large multi centric study to test these bacterial isolates for broad range of antibiotics like chloramphenicol, ciprofloxacin, colistin, fosfomycin, minocycline, nitrofurantoin, temocillin and tigecycline and based on which guidelines can be formulated for starting the empirical therapy in high infection units like ICU.

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