Review Article

Emergence and Spread of Carbapenem Antimicrobial Resistance: A Review on Mechanism of Drug Resistance and Laboratory Detection

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Abstract

Antimicrobial resistance (AMR) is a growing global concern, exacerbated by the misuse of antimicrobial agents in humans and animals. Carbapenems, a category of β-lactam antibiotics, play a vital role in the management of severe infections induced by resistant bacterial strains. However, the rise of carbapenem-resistant *Enterobacteriaceae* (CRE) has emerged due to overuse and the development of carbapenemases, enzymes that hydrolyze carbapenems. CRE infections are associated with high mortality rates and limited treatment options, necessitating combination therapy approaches. Effective detection methods for carbapenemase production include phenotypic tests like the Modified Hodge Test and Carba NP test, alongside molecular methods. Overall, a multifaceted approach is essential to combat the threat posed by CRE and Carbapenemase-producing Enterobacterales, ensuring effective management and treatment of resistant infections.

Keywords: Antimicrobial resistance, carbapenem-resistant *Enterobacteriaceae*, extended-spectrum β-lactamase, Penicillin-binding proteins, β-lactamase genes

Résumé

La résistance aux antimicrobiens (RAM) est une préoccupation mondiale croissante, exacerbée par l'utilisation abusive des agents antimicrobiens chez l'homme et l'animal. Les carbapénèmes, une catégorie d'antibiotiques β-lactamines, jouent un rôle essentiel dans la prise en charge des infections graves induites par des souches bactériennes résistantes. Cependant, l'augmentation du nombre d'entérobactéries résistantes aux carbapénèmes (ERC) est due à leur surutilisation et au développement des carbapénémases, des enzymes qui hydrolysent les carbapénèmes. Les infections à ERC sont associées à des taux de mortalité élevés et à des options thérapeutiques limitées, nécessitant des approches thérapeutiques combinées. Les méthodes efficaces de détection de la production de carbapénémases comprennent des tests phénotypiques comme le test de Hodge modifié et le test Carba NP, ainsi que des méthodes moléculaires. Globalement, une approche multidimensionnelle est essentielle pour lutter contre la menace posée par les ERC et les EPC, afin de garantir une prise en charge et un traitement efficaces des infections résistantes.

Mots-clés: Résistance aux antimicrobiens, Entérobactéries résistantes aux carbapénèmes, Protéines de liaison à la pénicilline, β-lactamase à spectre étendu, Gènes de β-lactamase

Introduction

Antimicrobial resistance (AMR) risk is rapidly increasing worldwide.[1] Governments worldwide are addressing this significant threat to modern medicine. The formation of AMR in microbes is natural, but abuse of antimicrobial agents in people and animals exacerbates it.[2] Antimicrobial usage is a

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primary cause of AMR.[3,4] The limited availability of novel antimicrobials to substitute ineffective ones underscores the necessity to preserve the efficacy of current medicines. Certain bacteria exhibit innate resistance to multiple classes of antimicrobial drugs.[5] Acquired resistance cases are particularly alarming; bacteria previously susceptible to an antimicrobial agent develop resistance to it due to the selective pressure exerted by its use. Vertical evolution is the term used to describe resistance that results from chromosomal mutation. In contrast, horizontal evolution is the term used to describe resistance that is formed through the assimilation of genetic material from other resistant organisms.[6] A primary factor contributing to the swift dissemination of AMR throughout bacterial populations is the presence of resistance-conferring genes on plasmids or other highly mobile genetic components, which are autonomously duplicated and exchanged between bacterial cells and species.[7] Following the approval of a newly identified antimicrobial drug for therapeutic application, clinically relevant resistance frequently emerges within months to years.[8]

The Discovery of Carbapenems

Carbapenems are derivatives of thienamycin, an antibiotic synthesised by the soil bacterium *Streptomyces cattleya*.[9] Carbapenems are β-lactam antimicrobials with proven efficacy in severe infections caused by extended-spectrum β-lactamase (ESBL) producing *Enterobacteriaceae*. β-Lactam antibiotics are antibacterial agents with a β-lactam ring in their molecular structure. Beta-lactam antibiotics are broadly grouped into penicillins, cephalosporins, monobactams, and carbapenems. Carbapenems differ from penicillins by a carbon atom replacing the sulfur at position 1 and a double bond between C2 and C3 in the five-membered thiazolidine ring, also a trans-1α-hydroxyethyl side chain in the trans configuration at C6 confers the excellent β-lactamase stability, which is associated with the broad spectrum of activity of carbapenems.[10] Carbapenems currently in clinical use are imipenem, meropenem, doripenem, ertapenem, and panipenem. They are bactericidal in activity and structurally differ from one another based on the side chain. They have broad-spectrum activity and act against Gram-positive and Gram-negative bacteria, including anaerobes.[11]

Mechanism of Action of Carbapenems

Bacterial cell walls are complex structures composed of a peptidoglycan polymer. The last transpeptidation step in the synthesis of peptidoglycan is enabled by transpeptidase enzymes, which are penicillin-binding proteins (PBPs). Carbapenems and other β-lactams share a structure with acylated D-alanyl-D-alanine, the terminal amino acid residues of the peptidoglycan. This structural similarity allows carbapenems to bind irreversibly to the active site of PBPs, inhibiting transpeptidation of the peptidoglycan layer through crosslinking and disrupting cell wall formation. Finally, autolysins, bacterial surface enzymes, cause bacterial cell death. Autolysins may form cell wall nicks that attach new peptidoglycan units. Inhibition of cell wall production by β-lactam drugs, along with autolysis, leads to weak areas in the cell wall, allowing membrane extrusion.[12,13] Carbapenems inhibit cell wall synthesis by binding to most high molecular weight PBPs. They traverse the outer membrane of Gram-negative bacteria through specific outer membrane proteins to reach the periplasmic space and bind with PBPs. Penicillin-binding proteins vary among different bacterial species. PBP-1 is responsible for the formation of the cell wall, PBP-2 is responsible for maintaining the rod-like shape, and PBP-3 is responsible for bacterial septum formation. Carbapenems preferentially bind to PBPs 1a, 1b, 2, and 4, and to a lesser extent PBP3, which is the primary target of aminopenicillins and cephalosporins. The affinity of carbapenems to multiple PBPs of various bacteria contributes to the broad spectrum of activity of these agents[14-16] [Table 1].

The Emergence of Carbapenem Resistance

The number of bacterial species with ESBL genes has grown since 2000, and isolates of *Escherichia coli* from the community that can form ESBLs that hydrolyse nearly all β-lactam agents, aside from carbapenems, have been reported globally. This has led to a rise in the clinical application of carbapenems. As a result, there were more clinical bacterial isolates that produced carbapenemases, which are β-lactamases that hydrolyse carbapenems. Therefore, carbapenem resistance – the capacity of bacteria to proliferate and endure in the presence of clinically significant carbapenem concentrations – has emerged as a result of the abuse of carbapenems.[17-19]

Mechanism of Carbapenem Resistance: Carbapenem Resistance in Negative Bacteria

Resistance to carbapenem is mediated by four mechanisms[20]

1. Overproduction of beta-lactamase enzyme
2. Diminished permeability (alteration in porin channel)
3. Efflux mechanism
4. Production of altered and low-affinity PBPs.

Overproduction of beta-lactamases enzyme

An enzyme called beta-lactamase hydrolyses the beta-lactam ring, hence deactivating medications. Chromosomal genes or transferable genes on plasmids and transposons encode β-lactamases. Moreover, integrons usually have several resistance factors, so β-lactamase genes (bla) generally live there. While integrons can help spread multidrug resistance throughout several bacterial species, transposable elements move it. The effectiveness of the beta-lactamase in hydrolysing beta-lactam antibiotics relies on: (1) its hydrolysis rate, (2) The bacterial cell's production of β-lactamase, (3) the target protein's (PBP) susceptibility to the antibiotic. and (4) the speed at which the antibiotic diffuses into the periplasm of the cell.

Table 1: List of common carbapenem drugs with mechanism of action and spectrum of activity

Drugs	Mechanism of action	Spectrum of activity
Imipenem*	It inhibits the third and final stage of bacterial cell wall synthesis by preferentially binding to specific PBPs. Imipenem binds to all PBP subtypes but has the highest affinity for PBP-2 and PBP-1 B	It is a broad-spectrum antibiotic active against aerobic and anaerobic gram-positive and gram-negative bacteria. It is particularly active against *P. aeruginosa* and *enterococci*[14]
Meropenem	Meropenem has a higher affinity for PBP-2, PBP-3, and PBP-4 of E. coli and P. aeruginosa and PBP-1, PBP-2, and PBP-4 of *S. aureus*	It is very similar to imipenem. meropenem is more active against *Enterobacteriaceae*, Haemophilus influenzae, gonococcus, and *P. aeruginosa*[17]
Doripenem	Doripenem inhibits cell wall formation and facilitates bacterial cell lysis	Doripenem has good activity against both Gram-positive and Gram-negative bacteria. It appears to have superior *in vitro* activity against *P. aeruginosa* compared to imipenem and meropenem[18]
Ertapenem	It preferentially binds to PBP-2, then PBP-3, but also has a strong affinity for PBP-1a and PBP-1b	It has no activity against *P. aeruginosa and A. Baumannii*[18]

PBPs=Penicillin-binding proteins, *P. aeruginosa=Pseudomonas aeruginosa, E. coli=Escherichia coli, S. aureus=Staphylococcus aureus*, H. influenza=Hemophilus influenzae

Diminished permeability (due to impaired expression of certain outer membrane proteins)

The outer membrane of Gram-negative bacteria is an important barrier to drug penetration and drug resistance. Diminished permeability is primarily due to the absence of OprD porin channel, usually in conjunction with the production of beta-lactamases. Beta-lactamases are located in the periplasmic space between the inner cytoplasmic membrane and outer lipopolysaccharide membrane. Concentrating beta-lactamases protect the PBPs from exposure to active beta-lactam antibiotics.

Overproduction of efflux pumps

Since affinity is determined by physicochemical characteristics (such as electric charge, aromatic, or hydrophobic qualities) rather than chemical structures, efflux pumps can typically recognise a wide variety of substrates. This explains why numerous structurally unrelated antimicrobials can be expelled by MDR efflux pumps. The efflux-mediated β-lactam resistance of Gram-negative bacteria, including Acinetobacter species and *Pseudomonas aeruginosa*, is widely recognized. Carbapenem resistance may result from the overexpression of efflux pumps that are active against carbapenems.[21]

Production of altered penicillin-binding proteins

This may be due to the mutation in the PBP gene that lowers the binding affinity to beta-lactam antibiotics.

Classification of Beta-lactamases

There are many types of beta-lactamases, and many classification schemes have been proposed. The Ambler molecular classification (based on molecular structure) and the Bush-Jacoby-Medeiros classification (based on functional similarities) are the two most widely used classification systems.[22]

Ambler classification

Ambler classification divides β-lactamases into four major classes A, B, C, and D. Enzymes of classes A, C, and D require serine for deactivation of antibiotics, while enzymes from class B, also called Metallo-β-lactamase (MBL), require one or two zinc ions in their mechanism of action.[23]

Bush-Jacoby-Medeiros classification

This classification system classifies the enzymes according to their substrate profile and susceptibility to β-lactamase inhibitors, such as clavulanic acid, into several functional groups, and each group is further divided into several subgroups. There are three major groups: (a) Group 1 - cephalosporins (class C). (b) Group 2 - serine beta-lactamases (class A and D). (c) Group 3-Metallo beta-lactamases (class B).[24]

Class C - β-lactamases preferentially hydrolyze cephalosporins and are not inhibited by clavulanic acid. These β-lactamases are usually encoded on the chromosome and inducible, although they may also be plasmid-encoded and produced constitutively. Class D - They are also referred to as oxacillinase (OXA)-type enzymes because of their preferential ability to hydrolyze oxacillin (rather than penicillin). Enzymes in this group are variably affected by the beta-lactamase inhibitors clavulanate, sulbactam, or tazobactam. Among the heterogeneous OXA group (which includes more than 100 enzymes), six subgroups have been identified with varying degrees of carbapenem-hydrolyzing activity: OXA-23, OXA-24/OXA40, OXA-48, OXA-58, OXA-143, and OXA-51. OXA-163 is an exception, hydrolysing broad-spectrum cephalosporins but carbapenems at a very low level, and being susceptible to β-lactamase inhibitors. The first five groups are carried on transmissible plasmids, while the last group, OXA-51, is chromosomally encoded.[25] (Classification of β-Lactamases is presented in Table 2)

History and Epidemiology of the Most Clinically Encountered Carbapenemases

Class A carbapenemases

An enzyme that typically hydrolyzes penicillins preferentially, but some also have cephalosporinase or carbapenemase activity. They are inhibited by β-lactamase inhibitors such as clavulanic acid. Point mutations can render the enzyme resistant to inhibitors or extend the spectrum of activity to include

Table 2: Classification of β-lactamase

Ambler molecular class	Major subtypes*	Preferred substrates	Inhibitor†	Main genetic localization	Representative enzyme (S)
A	Gram-positive β-lactamase 2a	Penicillin's	Clavulanic acid	Chromosome or plasmid	PC1
	Gram-negative β-lactamase 2b	Penicillins, early cephalosporins	Clavulanic acid	Plasmid or chromosomal	TEM-1, SHV-1
	Extended-spectrum β-lactamase 2be	Penicillins, extended-spectrum cephalosporins, aztreonam	Clavulanic acid	Plasmid	TEM-24, SHV-12, CTX-M-15
	Inhibitor-resistant TEM β-lactamase 2br	Penicillins	Clavulanic acid‡	Plasmid	TEM-30, SHV-10
	Carbenicillin-hydrolyzing β-lactamase 2c	Carbenicillin	Clavulanic acid‡	Plasmid	PSE-1, CARB-3
	Cephalosporin-hydrolyzing β-lactamase 2e	Extended-spectrum cephalosporins	Clavulanic acid	Chromosome	CepA
	Carbapenem-hydrolyzing β-lactamase 2f	Carbapenems	Clavulanic acid‡	Chromosome or plasmid	KPC-2, SME-1
B	Metallo-β-lactamase 3a	All β-lactams except monobactam	Ethylenediamine tetraacetic acid$, divalent cation chelators	Chromosome or plasmid	IMP-1, VIM-2, NDM-1
C	AmpC-type β-lactamase 1	Cephalosporins	Cloxacillin	Chromosome or plasmid	AmpC, CMY-2
D	Oxacillin-hydrolyzing β-lactamase 2d	Oxacillin	Clavulanic acid‡	Chromosome or plasmid	OXA-1, OXA-10
	Extended-spectrum β-lactamase 2de	Extended-spectrum cephalosporins	Clavulanic acid‡	Plasmid	OXA-11, OXA-15
	Carbapenem-hydrolyzing β-lactamase 2df	Carbapenems	Clavulanic acid‡	Plasmid	OXA-23, OXA-40, OXA-48

*The updated Bush-Jacoby group is indicated. †Tazobactam and sulbactam have activities similar to those of clavulanic acid. ‡Indicates relatively weaker inhibition. Cilastatin is an inhibitor of dehydropeptidase-1 (an enzyme found in the renal tubule border) that metabolizes imipenem, for the reduction of nephrotoxicity of imipenem cilastatin is added clinically. NDM=New Delhi metallo-beta-lactamase, VIM=Verona integron-encoded metallo-β-lactamase, $ EDTA=Ethylenediamine tetra acetic acid$, divalent cation chelators, KPC=*Klebsiella pneumoniae carbapenemaseenzyme*, IMP=Imipenemase metallo-β-lactamase enzyme, OXA=Oxacillinase β-lactamase enzyme

Table 3: Description of the common carbapenemase enzyme

	Microorganisms	Geographic distribution	Molecular epidemiology
NDM (beta lactum)	Widespread in *Enterobacteriaceae* (esp. *K. pneumoniae* and *E. coli*)	Indian sub-continent	Plasmid spread among strains is more important than clonal spread
VIM	Mostly *K. pneumoniae*	Greece	Plasmid spread among strains is more important than clonal spread
IMP	Not defined	Scattered worldwide; no clear associations	Mostly plasmid spread
KPC	*K. pneumoniae*, occasionally other *Enterobacteriaceae*	USA since 1999. Israel and Greece; outbreaks elsewhere in Europe	Some plasmids spread; some clones spread
OXA 48	Widespread *K. pneumoniae*	Turkey, Mid-East and N. Africa	Mixture of plasmid and clone spread

K. pneumonia=Klebsiella pneumonia, E. coli=Escherichia coli, NDM=New Delhi metallo, VIM=Verona integron-encoded MBL, KPC=*K. pneumoniae* carbapenemases, IMP=Imipenemase, OXA=Oxacillinase

third-generation cephalosporins and aztreonam (so-called ESBLs. Class A carbapenemases include *Klebsiella pneumoniae* carbapenemases (KPCs), imipenem-hydrolyzing β-lactamase (IMI), Guiana extended spectrum carbapenemase (GES), Serratia fonticola carbapenemase, Serratia marcescens enzyme, and non-metallo-carbapenemase-A. KPCs hydrolyze all β-lactams, and isolates with the blaKPC gene are multidrug-resistant. IMI isolates are infrequently identified due to their atypical AMR. GES carbapenemases are infrequent but rapidly increasing.[26]

Class B carbapenemases

Class B enzymes are the broadest-spectrum enzymes, are inhibited by chelating agents, and can hydrolyze all β-lactams except aztreonam, resulting in considerably limited therapeutic options. Bacillus cereus MBL, first-class B enzyme BCII, was discovered in 1966. By 1989, only four MBLs were identified, but the discovery of plasmid-encoded imipenem-resistant *Pseudomonas*-type carbapenemases (IMP) in 1991 rekindled clinical interest. MBLs are primarily plasmid-encoded, making them easier for microbial pathogens to spread. New Delhi

MBL (NDM) and Verona integron-encoded MBL (VIM) have been identified, with aztreonam being hydrolyzed by bacteria co-expressing SBLs and MBLs.[27]

The New Delhi metallo-β-lactamase enzymes

Although NDM carbapenemases have also been discovered from *Acinetobacter* spp. and *P. æruginosa,* the distribution of NDM enzymes is mostly linked to the transmission of conjugative plasmids among *Enterobacteriaceae*. In 2008, the NDM-1 enzyme was discovered in a *Klebsiella pneumoniæ* isolate from a patient who had previously been hospitalised in New Delhi, India, and had a urinary tract infection. Additionally, the *E. coli* strain found in the patient's faeces carried the blaNDM-1 gene. Following the initial isolation of isolates that produced NDM-1 and had a clear epidemiological connection to the Indian subcontinent, NDM-1-positive isolates from clinical samples were found in various parts of Bangladesh, India, Pakistan, and the United Kingdom.[28]

Class C carbapenemases

Class C β-lactamases preferentially hydrolyze cephalosporins and are not inhibited by clavulanic acid. These β-lactamases are usually encoded on the chromosome and inducible, although they may also be plasmid-encoded and produced constitutively AmpC β-lactamases are significant cephalosporinases encoded on the chromosomes of numerous *Enterobacteriaceae* and select other organisms. The inaugural bacterial enzyme identified to degrade penicillin was the AmpC β-lactamase from *E. coli*. In 1965, Swedish researchers initiated a comprehensive investigation into the genetics of penicillin resistance in *E. coli*. Mutations exhibiting progressively increased resistance were designated as ampA and ampB. A mutation in an ampA strain that led to diminished resistance was subsequently classified as ampC. AmpC β-lactamases provide resistance to cephalothin, cefazolin, cefoxitin, the majority of penicillins, and β-lactamase inhibitor-β-lactam combos. Although much of the amplifier terminology has evolved, the designation ampC has remained constant. The ampC gene sequence from *E. coli* was reported in 1981. It varied from the sequence of penicillinase-type β-lactamases, such as TEM-1, yet, akin to them, it possessed serine at its active site. AmpC enzymes are categorized as class C in the Ambler structural classification of β-lactamases; however, in the functional classification by Bush *et al.*, they are designated as group 1.[25]

Class D carbapenemases

These include the enzymes known as OXA, which derive their name from their ability to hydrolyze oxacillin effectively. Enzymes in this group are variably affected by the beta-lactamase inhibitors clavulanate, sulbactam, or tazobactam. Among the heterogeneous OXA group (which includes more than 100 enzymes), six subgroups have been identified with varying degrees of carbapenem-hydrolyzing activity: OXA-23, OXA-24/OXA40, OXA-48, OXA-58, OXA-143, and OXA-51. OXA-163 is an exception, hydrolysing broad-spectrum cephalosporins but carbapenems at a very low level, and being susceptible to β-lactamase inhibitors. The first five groups are carried on transmissible plasmids, while the last group, OXA-51, is chromosomally encoded.[25] The OXA-48 enzyme, which hydrolyses carbapenems, exhibits poor hydrolysis activity toward carbapenems and strong hydrolysis activity toward penicillins. In addition, β-lactamase inhibitors have little effect on it, which is why this enzyme has recently drawn interest. Although they are commonly detected in Acinetobacter species, other OXAβ-lactamases, such as OXA-23, OXA-24/40, and OXA-58, have comparatively low carbapenemase activity. The absence of inhibitors for this class of enzymes is one of their biggest risks.[29,30]

Oxacillinase-48 enzyme

The enzymes hydrolyze penicillins at a high level and carbapenems at a low level, sparing broad-spectrum cephalosporins, and are not susceptible to β-lactamase inhibitors. When combining permeability defects, OXA-48-like producers may exhibit a high level of resistance to carbapenems. The blaOXA-48-type genes are consistently located on plasmids. The present distribution of the blaOXA1 gene is mostly associated with the propagation of a singular 62 kb IncL/M-type self-transferable plasmid that lacks any supplementary resistance genes. OXA-48 type carbapenemases have primarily been identified in North African nations, the Middle East, Turkey, and India, which serve as significant reservoirs. However, the presence of OXA-48 producers in European countries is now well-documented, with several reported hospital outbreaks. The identification and detection of numerous OXA-48-like producers can be difficult, as they often do not display resistance to broad-spectrum cephalosporins or simply show diminished susceptibility to carbapenems. Consequently, effective screening and detection technologies are necessary to prevent and manage their spread.[31] Description of common carbapenemases enzyme is presented in Table 3.

Carbapenem-resistant *Enterobacteriaceae*

According to CLSI M100, 28th ed. Carbapenem-resistant *Enterobacteriaceae* (CRE) can be defined as *Enterobacteriaceae* that are resistant to one or all of the following carbapenems: imipenem, meropenem, ertapenem, or doripenem (Ertapenem nonsusceptibility is the most sensitive indicator for carbapenemase production) and resistant to one or more agents in third-generation cephalosporins: Ceftriaxone, cefotaxime, and ceftazidime.

Laboratory Method of Testing for Carbapenem-resistant *Enterobacteriaceae*

Susceptibility test findings from automated systems or disc diffusion are the primary basis for identifying carbapenemase producers in clinical laboratories across the globe. There are two categories for the Laboratory Method used to characterize CRE.[32]

Phenotypic methods for carbapenemase production

Many phenotypic tests are available for the detection of carbapenemases. Modified Hodge Test (MHT), Carba NP

test, Carbapenem Inactivation Method (CIM), Modified CIM (mCIM), EDTA-Modified Carbapenem Inactivation Method (eCIM).[33]

Phenotypic test

Different Phenotypic assays are based on the following principle:

a. Growth-based assays which evaluate resistance based on growth in the presence of an antibiotic (e.g. MHT) and mCIM
b. Hydrolysis techniques, such as matrix-assisted laser desorption–ionization-time of flight mass spectrometry and Carba NP, that identify the hydrolysis product catalyzed by carbapenemase enzymes. Several studies highlight various phenotypic test options, which are accurate, user-friendly, and practical. No single test can meet all the requirements of the "perfect" test.

Modified hodge test

The most well-known method for detecting carbapenemase is the MHT. A clinical isolate is streaked away from a disc impregnated with either imipenem or meropenem that was previously put on an agar plate that had been infected with a lawn of an *E. coli* strain that is susceptible to carbapenem. In order for the carbapenem-susceptible *E. coli* isolate to grow unhindered around the streak line close to the carbapenem disc, giving it a cloverleaf appearance, the MHT depends on the ability of carbapenemase manufacturers to lower the local concentration of carbapenem antibiotics. The assay exhibits limited sensitivity for MBLs but acceptable sensitivity for the majority of carbapenemases, especially KPC enzymes. Yet, given the quick spread of *Enterobacteriaceae* that produce NDM, failure to identify this epidemiologically significant resistance mechanism could have serious repercussions.[34]

- Advantage of the MHT: It is inexpensive, relatively easy to perform, and uses reagents available in most clinical laboratories. It is less costly than the Carba NP test
- Limitations of MHT: The presence of isolates that produce ESBLs or AmpC cephalosporinases, along with porin mutations, frequently leads to false-positive results in the MHT, which has a reported specificity of approximately 91%. A study by Pasteran *et al*. found that MHT may yield false-positive results, primarily due to CTX-M-producing strains exhibiting reduced outer membrane permeability and high-level AmpC production. A study found that MHT is less reliable for detecting NDMs, VIMs, and IMPs producing bacteria; however, it may be useful for detecting KPC and OXA-48 producers. However, several studies reported that the sensitivity and specificity for MHT were 61% and 93% respectively. A study indicated that MHT was effective for detecting carbapenemases; however, it exhibited low sensitivity and specificity for metallo-enzymes. They reported that phenotypic methods were growth-dependent and time-consuming, requiring 18–24 h for completion. These methods were not clinically useful, and the results were subjective.[34]

Carba NP test and variants

The Carbapenemase Nordmann-Poirel test identifies carbapenemases through the *in vitro* hydrolysis of imipenem in bacterial extracts, resulting in color changes within approximately 2 h. Hydrolysis of imipenem yields a carboxylic derivative that lowers the pH, causing a phenol red indicator to shift in colour from red to yellow. The sensitivity for detecting most carbapenemases ranges from 73% to 100%. The sensitivity for OXA-48-like carbapenemases is notably lower, with one study reporting a rate of only 6%.

- Limitation of Carba NP test: The Carba NP test shows variability in sensitivity and specificity for detecting OXA enzymes, particularly with mucoid isolates, which are prone to false negatives. Several studies previously demonstrated that the sensitivity for OXA-48-like detection increased from 56% to 71% when comparing the original Carba NP test to the updated version. In contrast, research by Nordmann, Poirel, and Dortet[32-34] reported 100% sensitivity and specificity for *Enterobacteriaceae* and 94.4% sensitivity for *Pseudomonas* species. However, few studies found that the Carba NP test had only 80% specificity for OXA-48-like *P. aeruginosa* and Enterobacteriaceae, highlighting its limitations.[35]

Carbapenem inactivation method

The CIM test, introduced in 2015, detects carbapenemase-producing bacteria using a 10-µg meropenem disk. If the disk is hydrolyzed after incubation with a carbapenemase-producing isolate, it indicates the presence of the enzyme. The disk is then tested on *E. coli*, with the absence of an inhibition zone confirming carbapenemase production. The CIM boasts high sensitivity (91%–94%) and specificity (99%–100%), and is cost-effective, utilizing readily available materials. However, it has limitations, including difficulties in detecting OXA-type carbapenemases, MBLs that need divalent cations, and low-level carbapenemase expression.[36]

Modified carbapenem inactivation method

A study by the CLSI working group found that the mCIM approach can accurately identify carbapenemases in *Enterobacteriaceae*. The mCIM has a mean sensitivity and specificity of 97% and 99%, respectively, across nine testing sites. It has excellent reproducibility across laboratories for representative carbapenemases from Ambler classes A, B, and D. Zone diameters between 6 and 15 mm are considered positive, while zone diameters between 16 and 18 mm require additional testing. The mCIM was added to the CLSI M100 supplement in 2017. Advantage of mCIM: It can accurately identify the presence of carbapenemases. Limitation of mCIM: Like the MHT, the mCIM has a significant disadvantage in that it needs to be incubated overnight, meaning that, unlike the Carba NP test, data will not be accessible in a single work shift. it cannot distinguish between serine and MBLs.[37]

EDTA-modified carbapenem inactivation method

The mCIM assay effectively detects carbapenemases but cannot differentiate between serine and MBLs. To enhance

its accuracy, a modification involving the addition of EDTA has been suggested. This modified assay operates on the principle that if the test isolate produces a MBL, the presence of Ethylenediaminetetraacetic Acid (EDTA).

Method of test: The method involves adding EDTA to a TSB tube, processing mCIM and eCIM tubes in parallel, and inoculating meropenem disks with *E. coli* ATCC 25922 strain. eCIM results should only be interpreted if mCIM indicates carbapenemase presence, with a ≥5-mm increase in zone diameter indicating MBL-producing strain. The eCIM was added in 2017 to distinguish MBL carbapenemases from serine carbapenemases. Advantage of phenotypic test: Compared to molecular tests, these phenotypic tests are cheaper. Since phenotypic testing requires an additional overnight incubation step, molecular approaches for diagnosis were found to be comparably faster. One potential drawback of eCIM is the possibility of false-negative results when an isolate produces both serine and metallo-carbapenemases. It has been observed that OXA-48-like and NDM enzymes can co-produce; therefore, this is significant.[36,37]

Molecular methods for resistance mechanism

Polymerase chain reaction (PCR)-based techniques are among the most effective and commonly employed fast molecular tools for quantifying and profiling genes that confer resistance at both species and genus levels. This technique enhances the target nucleic acid sequence by the use of particular primers that bind to single-stranded DNA following the denaturation of the target DNA at elevated temperatures.[38,39] Innovations in PCR provide a more expedited and resilient iteration of this method, including conventional PCR, real-time or quantitative PCR (qPCR), reverse transcriptase PCR, digital PCR, multiplex PCR (mPCR), and automated PCR. For example, mPCR facilitates the concurrent identification of several resistance genes by employing multiple primer sets.[39-42] Conventional PCR: The basic idea behind conventional PCR is to amplify specific DNA sequences that correspond to known carbapenemase genes. Application: Identifying well-known genes such as bla KPC, bla_NDM, bla_VIM, bla_IMP, and bla_OXA-48, among others, is frequently employed as a follow-up test after phenotypic screening. Advantage: Excellent specificity, ease of use, and reasonable cost. Limitations: Unknown or novel resistance genes cannot be detected. It requires prior knowledge of the target genes. Real-time PCR, or qPCR, facilitates the swift, concurrent detection and quantification of amplified PCR products by fluorescent dyes, obviating the need for gel electrophoresis.[44,45] Commercially available automated PCR or qPCR techniques filter samples, condense DNA, and amplify and detect significant bacterial genes, hence demonstrating antibiotic resistance in under 2 h.[39,46] For 25 years, PCR-based methods have served as the benchmark for identifying β-lactam resistance genes in *Enterobacteriaceae*. mPCR was devised to identify 11 acquired genes that encode carbapenemase (blaIMP, blaVIM, blaNDM, blaSPM, blaAIM, blaDIM, blaGIM, blaSIM, blaKPC, blaBIC, and blaOXA-48) utilizing three distinct multiplex reaction combinations.[41,47,48] Numerous automated systems were developed to identify the target genes.[41,47,48] Real-time mPCR or qPCR devices provide simultaneous amplification and detection in a single step, hence minimizing contamination risks. GeneXpert is an automated real-time PCR technology utilizing the Carba-R assay to detect and quantify various bacterial species and multiple carbapenemase genes from rectal samples.[42,49] The Check-Direct assay comprises a panel of several multiplex real-time PCR kits utilizing numerous probes, encompassing both narrow and broad-spectrum β-lactamase genes.[41,48,50] A diverse array of mPCR panels was created to enhance analytical performance without necessitating specialized personnel.[43] Nevertheless, these PCR-based assays are costly, constraining their application in resource-limited facilities. Additional molecular techniques, including Fluorescence *in situ* hybridization (FISH), microarray, whole genome sequencing (WGS), and Loop-mediated isothermal amplification (LAMP), have been employed to identify carbapenem resistance.[39,40,51] FISH is a method for identifying specific RNA or DNA sequences using dye-labeled oligonucleotide probes, which are observed through fluorescence microscopy. Microarray-based techniques employ numerous locations on a solid support chip for various oligonucleotides associated with resistance genes to identify labelled DNA fragments in a single assay.[52] The WGS technology screens an entire bacterial sequence for antibiotic-resistant genes. It compares them with known genes in publicly accessible databases, facilitating the prediction of both present and emerging phenotypic and genotypic resistance.[53] Finally, the LAMP assay is a straightforward amplification method that eliminates PCR temperature cycling by utilizing a single temperature for target gene amplification. This technique generates a substantial quantity of DNA replicas in a brief timeframe.[55,56] LAMP has been utilized as a substitute for PCR because of its simplicity and cost-efficiency, particularly in resource-limited laboratory environments. Nonetheless, the approach necessitates a sophisticated primer design.[39,51,54]

Risk Factors for the Acquisition of Carbapenem-resistant *Enterobacteriaceae* Infection

According to a landmark comprehensive study that examines the emergence of CRE across the globe, "CRE Epidemiology and Prevention," the most prominent risk factors for CRE infections are extensive time in medical facilities, exposure to medical instruments, such as indwelling devices, and previous exposure to antibiotics. CRE infections are typically seen in patients with prior healthcare exposure, and medical devices are a common risk factor for CRE acquisition.

Prevention of Carbapenem-resistant *Enterobacteriaceae* Infections

for the prevention of CRE infections following measures should be taken: Active surveillance, strict compliance with

hand hygiene, contact precautions, screening patients without links to known CRE patients in high-risk areas, and rectal swabs sent to the laboratory for identification of CRE may significantly reduce the spread of CRE infection outbreaks. Because different carbapenemases are unique to different bacterial species and to regions where those species are endemic, population movement is seen as a major component of both globalisation and the spread of antibiotic-resistant bacteria. Hence, the preventive measures at the national and international levels are effective for the spread of CRE globally.[57-60]

Perspective on the Future

Epidemiological data gathering is essential for implementing effective and reasonably priced strategies to combat CRE and Carbapenemase-producing Enterobacterales. All healthcare facilities should create and follow guidelines that facilitate the collection of epidemiological data and the prompt reporting of any outbreaks so that the necessary actions may be taken immediately. Antibiotic stewardship programs should be implemented to prescribe and use antibiotics and to manage and monitor infections brought on by clinically significant organisms in medical institutions. Before starting antimicrobial therapy, cultures, sensitivity tests, and MIC determinations for carbapenem-resistant bacteria should be carried out to establish the right dosage and course of treatment. This will help to prevent overprescription and overuse of carbapenems.

To find the best course of action for treating severe CRE infections, more research is desperately needed. Other methods of treating these infections, including phage treatment or quorum sensing quenching, should be considered. To ascertain their effectiveness *in vivo*, antibiotic combinations that have encouraging *in vitro* effects ought to be examined in clinical studies. Guidelines for premarketing research on possible resistance mechanisms should be established at an early stage of the development of novel antimicrobials. Finally, to prevent the abuse or overuse of antimicrobials, stringent restrictions must be implemented to prohibit their administration without a prescription.[61-64]

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Conflicts of interest

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