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Review Article

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Carbapenem Resistance Mechanisms, Carbapenemase Genes Dissemination, and Laboratory Detection Methods: A Review

Rawan Samy Abou-assy[1], Magda Mohammed Aly[1,2*], Reda Hasan Amasha[1], Samyah Jastaniah[1], Fawaz Alammari[3], Mohammed Shamrani[3]

[1]Department of Biology, College of Science, King Abdulaziz University, Jeddah, Saudi Arabia.
[2]Department of Botany and Microbiology, Faculty of Science, Kafrelsheikh University, Kafrelsheikh Govvernorate, Egypt.
[3]Department of Microbiology, King Faisal Medical Complex, Taif, Makkah, Saudi Arabia.

****Email:*** *mmmohammad@kau.edu.sa*

ABSTRACT

In recent years, carbapenem-resistant Enterobacteriaceae (CRE) has expanded quickly throughout the world, posing a serious threat to public health. They are also very difficult to treat and are associated with a high fatality rate. The class of antimicrobials known as carbapenems is regarded as the most trustworthy and last line of defense. It is possible to distinguish between CRE resistance mechanisms that produce carbapenemase enzymes and those that do not. The three classes of β-lactamases, class A, class B, and class D, which have the highest clinical significance among nosocomial pathogens, class D, contain a variety of carbapenemase types. Carbapenem resistance genes have been disseminated by the vertically intrinsic inheritance method and the horizontally acquired inheritance method. The primary factor contributing to the rise in CRE prevalence is the plasmid-mediated horizontal transfer of carbapenemase genes. For the clinical prevention and treatment of these infections, CRE, particularly carbapenemase-producing Enterobacteriaceae (CPE), must be accurately and promptly detected. For application in clinical microbiology laboratories, numerous CRE phenotypes and genotypes fast detection techniques have been created. In this article, we summarized the various mechanisms of carbapenem resistance and the classification of carbapenemases enzymes, and we compared the advantages and limitations of the carbapenem resistance detection methods.

Key words: *Carbapenemase, CRE, Carbapenem resistance, Enterobacteriaceae, β-lactamases*

INTRODUCTION

Infections caused by carbapenem-resistant bacteria is a worldwide urgent public health problem and is extremely hard to treat and have been related to a high death rate [1, 2] and turning into an inexorably troublesome issue in health care settings and hospitals [3, 4].
Due to their wide range of activity and stability, cephalosporins and carbapenems have been the mainstay of treatment for serious infections brought on by Enterobacterales bacteria that are resistant to -lactams. However, due to the high demand for carbapenems in clinical settings, the widespread acquisition of resistance genes to these essential drugs has compromised their efficacy. Since there are frequently few effective antibacterial choices, most treatments rely on medications that carry a risk of toxicity or other safety issues [5]. Severe infections brought on by carbapenem resistance have been linked to high fatality rates, frequently surpassing 40%. When compared to bacteremia caused by carbapenem-sensitive isolates, research on the Enterobacteriaceae (CRE) has repeatedly shown that bacteremia generated by carbapenem-resistant isolates is linked with

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unacceptable high mortality [6]. The rest of the world is still lagging behind, whereas Europe and the USA have implemented monitoring programs for the tracking of antibiotic resistance, particularly carbapenem. The Global Antimicrobial Resistance Surveillance System (GLASS) was launched in 2015 by the World Health Organization (WHO) to fill this gap and bridge available data, more countries enrolled in the program of map carbapenem resistance Enterobacteriaceae [7, 8].

Gram-negative organisms, including Klebsiella pneumoniae, Pseudomonas aeruginosa, and Acinetobacter baumannii, frequently exhibit carbapenem resistance, which may be inherent or caused by genes that encode for transferable carbapenemase [1]. In terms of carbapenem hydrolysis and geographic distribution, the most effective carbapenemases are the bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and $bla_{OXA\text{-}48}$ kinds [9]. Particularly in oncological patients and those with impaired immune systems, carbapenem-resistant *K. pneumoniae* is a common source of nosocomial infections and is linked to high fatality rates of up to 50%. To prevent the spread of antimicrobial resistance from becoming endemic in healthcare settings, it is necessary to conduct ongoing surveillance, molecular characterization, and identification of the source of the mechanisms behind carbapenem resistance [10]. Magiorakos and colleagues proposed consensus definitions for MDR (bacteria resistant to more than three antibiotic classes), extensively drug-resistant (XDR), and pan-drug-resistant (PDR) bacteria in 2012. These definitions were developed by experts from the Centers for Disease Control and Prevention (CDC) [11]. These criteria would classify the majority of the carbapenemase-producing enterobacteriaceae (CPE) that are currently encountered as MDR and a sizable portion of CPE like XDR. The concurrent use of the terms carbapenem-resistant Enterobacteriaceae/Enterobacterales (CRE), CPE, and carbapenemase-producing carbapenem-resistant Enterobacteriaceae has resulted in further terminology problems (CP-CRE). The phrases carbapenemase-producing organisms (CPO) and carbapenem-resistant organisms (CRO) are also occasionally used. The Enterobacteriaceae that the CDC initially classified as CRE were those that were resistant to both third-generation cephalosporins and carbapenem [12]. CRE are currently defined as any Enterobacteriaceae or Enterobacterales that are documented to produce a carbapenemase or are resistant to any carbapenem antibiotic, including intermediate resistance [12]. Additionally, resistance to a non-imipenem carbapenem is necessary for those Enterobacteriaceae, such as Proteus mirabilis, that may have an innately lower susceptibility to imipenem [13]. By doing molecular tests for the presence of carbapenemases, the CDC Toolkit advises that carbapenemase production be verified [13, 14].

Carbapenem actions and resistance mechanisms

The extremely distinctive structure of carbapenem agents—typically characterized as carbapenem attached to a B-lactam ring—provides protection from the majority of b-lactamases as well as metallo-b-lactamases (MBL), leading to their extended antibacterial activity [10]. The five-membered ring of carbapenems is similar to that of penicillin, but a double bond is added between C-2 and C-3, and the sulfur atom at position C-1 is changed to a carbon atom **(Figure 1)** [15].

The most dependable and last-resort class of antimicrobials, carbapenems are bactericidal β-lactam antibiotics that have been shown to be effective in treating severe infections brought on by bacteria that produce extended spectrum β-lactamases (ESBL) [16]. Due to their concentration-independent ability to kill pathogenic bacteria, carbapenems are frequently favored over other antimicrobials in the treatment of life-threatening illnesses [17]. Due to the Enterobacteriaceae family's growing sensitivity to cephalosporin antimicrobials, only a few agents of the carbapenem class are currently in use worldwide. These include imipenem, meropenem, doripenem, ertapenem, panipenem, and biapenem. The cyclic amine types of carbapenems, such as imipenem, meropenem, and doripenem, are particularly effective against a wide range of gram-positive, gram-negative, and anaerobic bacteria. However, carbapenems are ineffective against Enterococcus faecalis, methicillin-resistant Staphylococcus aureus, and Stenotrophomonas maltophilia [18].

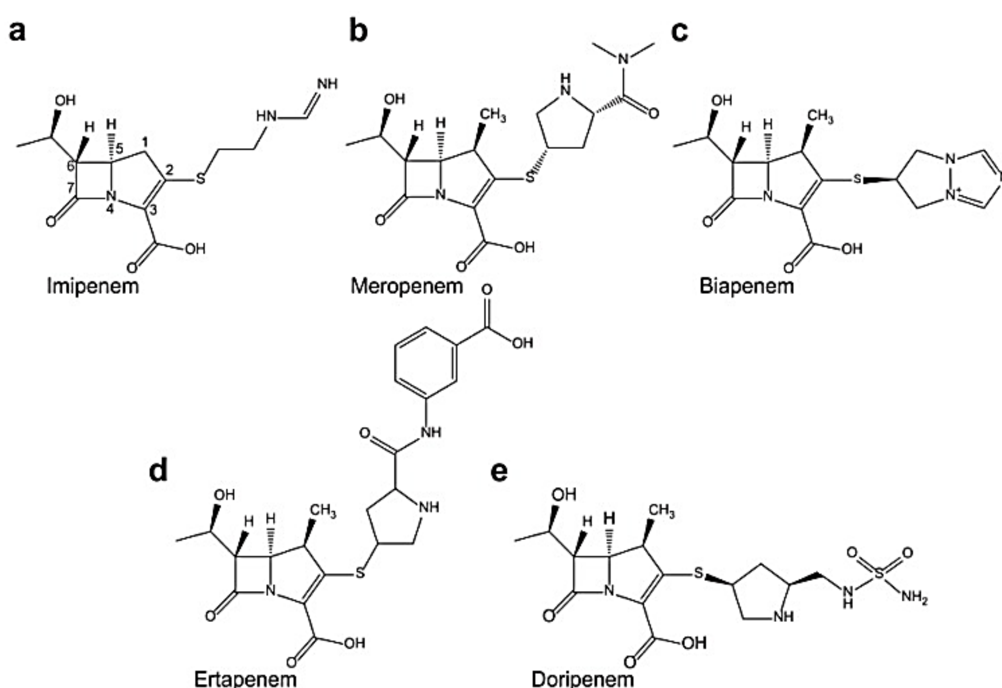


Figure 1. Chemical structures of a) imipenem; b) meropenem; c) biapenem; d) ertapenem; and e) doripenem. The β-lactam nucleus is numbered.

Particularly, panipenem, which belongs to the cyclic amine group, is only marginally effective against strains of Gram-negative bacteria. In comparison, doripenem has lower minimum inhibitory concentrations (MICs) and is very stable against hydrolysis of the majority of -lactamases [19]. In contrast to imipenem, which is used to treat *A. baumannii* and *P. aeruginosa*, doripenem is less susceptible to and hydrolyzes carbapenemase much more slowly than imipenem (by a factor of 2 to 150) [20].

Currently, doripenem, meropenem, and imipenem are three different forms of carbapenems that are used in clinical practice as antipseudomonal medicines. Ertapenem and meropenem imipenem must be administered intravenously in order to be effective because they are poorly absorbed when taken orally. Although this is a rare medical problem for these drugs, carbapenems have minor effects on hepatic metabolism that cause hepatotoxicity and jaundice [2]. Ertapenem has an in vivo half-life of around 4 h, making it acceptable for once-daily treatment. Meropenem, imipenem, and doripenem have half-lives of about 1 h each. Compared to other carbapenems, imipenem is known for its dose-dependent gastrointestinal adverse effects [20]. Dehydropeptidase-1 (DHP-1) is an enzyme found in the renal tubules that is responsible for most carbapenems' breakdown, hence co-administration of a DHP-1 inhibitor such as cilastatin is necessary. Because each carbapenem agent has a distinct role and has little to no allergic cross-reactions in hospitals, choosing a carbapenem agent for a serious infection requires careful consideration [21].

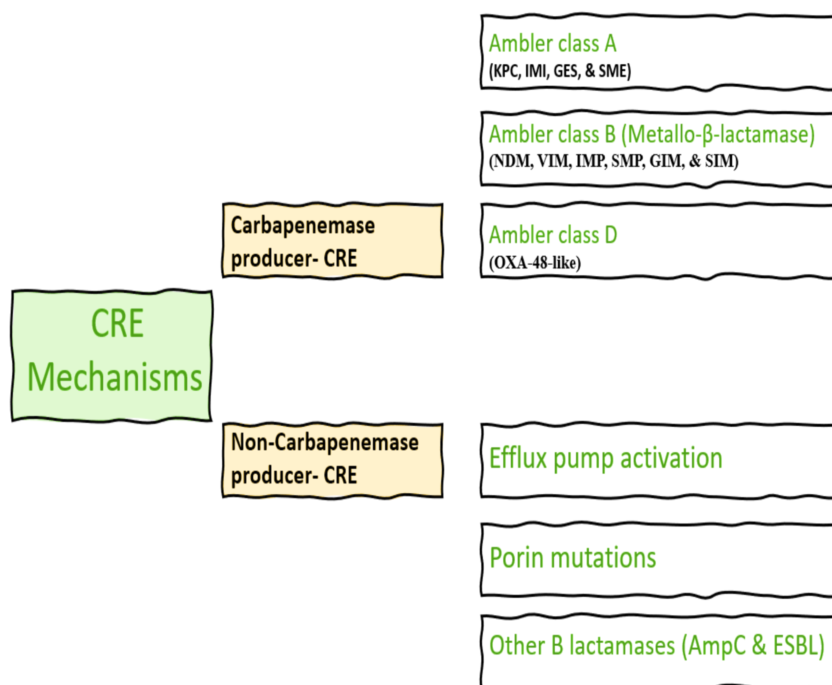
Action of carbapenems

Carbapenems are potent members of the β-lactam family of antimicrobials that are structurally related to penicillin, mode of action of carbapenems is initiated by penetrating the cell wall of bacteria, binding with penicillin-binding proteins (PBPs), and the result in inactivation of intracellular autolytic inhibitor enzymes, ultimately killing the bacterial cell as a result of bacterial cell wall synthesis inhibition, PBPs which are that play an important role in the formation and maintenance of the cell wall structure [22].

According to current knowledge, transpeptidase inhibition is the primary enzyme target of carbapenems during the synthesis of bacterial cell walls, which effectively prevents their peptide cross-linking activities during peptidoglycan biosynthesis. In Gram-negative bacteria, this results in the glycan backbone weakening due to autolysis, and eventually the cell is destroyed by osmotic pressure [23]. According to Codjoe and Donkor (2018), the main inhibitory series of PBPs are 1a, 1b, 2, and 3, and their lethal effect is the inactivation of an inhibitor of autolytic enzymes within the cell wall, which results in the destruction of the bacteria [1]. However, binding to PBPs 4, 5, and 6 is not lethal. Additionally, the PBPs of gram-positive and gram-negative bacteria differ, as do the affinities of certain PBPs for particular carbapenem drugs. These variations can alter the range of activity [24, 25].

Mechanism of carbapenem resistance

According to Beatriz and Perez-Gracia, there are two main groups of CRE resistance mechanisms **(Figure 2)**. Producing carbapenemase enzymes (CP-CRE) was included in the first group, while Non-CP-CRE was included in the second. Based on Ambler's classification, CP-CRE might be further classified into three classes. A class Ambler (KPC, IMI, GES, & SME). Ambler class B, also referred to as metallo-β-lactamase (MBL), is the second class. Instances of this class include (NDM, VIM, IMP, SMP, GIM, & SIM). The OXA-48-like family, which includes many oxacillinase variations, is collectively referred to as Ambler class D, which is the third class (OXA). Despite not producing carbapenemases, members of the second category (Non-CP-CRE) nonetheless produce other -lactamases, such as Type C ampicillinase (AmpC) or ESBLs, which are accompanied by changes to porins or efflux pumps [26].



Klebsiella pneumoniae *carbapenemase* (KPC), Imipenemase/non-metallo-β-carbapenemase (IMI), Guiana extended-spectrum β- lactamase (GES) and Serratia marcescens enzyme (SME). metallo-β-lactamase (MBL), New-Delhi metallo-β-lactamase (NDM), Verona integron-borne metallo-β-lactamase (VIM), Imipenemase/ metallo-β-carbapenemase (IMP), Sao Paulo metallo-β-lactamase (SMP), German imipenemase (GIM) and Seoul imipenemase (SIM), Oxacillinase (OXA), ampicillinase (AmpC), Extended-spectrum β-lactamases (ESBLs).

Figure 2. Different mechanisms of carbapenem resistance in Enterobacterales.

Porin-mediated resistance

Bacteria can restrict the amount of carbapenems that can enter the periplasmic area where PBPs are found. This method entails modifications to the porin expression gene or changes to the porin-encoding gene that result in deficiencies or the complete loss of the associated porin [27]. For instance, the main mechanism of carbapenem resistance in P. aeruginosa isolates is the downregulation of the orp D porin gene [10]. Additionally, it was shown that *K. pneumoniae* had a significant level of ertapenem resistance due to the altered expression of omp K35 and omp K36 [28].

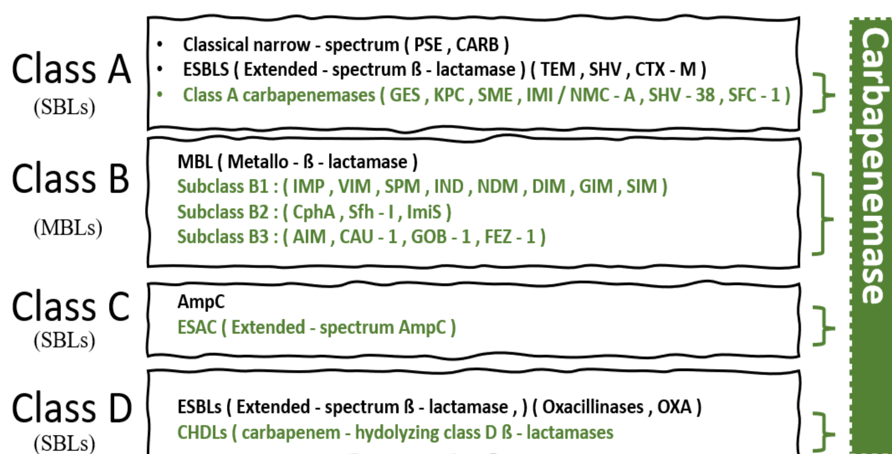
Overproduction of efflux pumps

The efflux pump's main job is to prevent harmful compounds like antibiotics from entering the bacterial cell by expelling them. It also contributes to pathogenicity, mostly by promoting colonization to shield bacteria from naturally occurring antimicrobial substances on mucosal surfaces [29]. Since affinity for substrates is based on physiochemical properties (such as electric charge, aromaticity, or hydrophobicity) rather than chemical structures, efflux pumps are typically able to recognize a wide range of substrates. However, overexpression of

efflux pumps active on carbapenems may cause carbapenem resistance, which can expel many structurally unrelated antimicrobials [11]. Gram-negative bacteria with efflux-mediated carbapenem resistance, like *P. aeruginosa* and Acinetobacter species, are well known [30].

Enzyme-mediated resistance

The majority of the time, resistance results from the production of β-lactamases, also known as carbapenemases, which are able to hydrolyze carbapenems and other β-lactam antimicrobials. This resistance mechanism poses the greatest threat because the genes encoding these enzymes are carried on transposons, plasmids, or other mobile genetic elements, which can be horizontally transferred to other bacterial species. Three of the four ambler classes of β-lactamases, classes A, B, and D, which have the highest clinical importance among nosocomial pathogens, have been found to have a wide diversity of carbapenemases in Enterobacteriaceae based on their molecular structures. The most popular technique for categorizing β-lactamases is the Ambler Classification system, which is based on amino acid homology and is thought to be the most straight forward β-lactamase classification scheme **(Figure 3)** [31]. Serine β-lactamases, which refer to Classes A and D, have a serine residue at the active site to aid in ring opening (SBLs). According to the Bush-Jacobi-Medeiros functional classification approach, Class B includes Metallo-β-lactamases (MBLs), which feature active sites that utilize zinc ions to mediate bond hydrolysis.



Metallo-b-lactamases (MBLs) and serine β-lactamases (SBLs)

Figure 3. Molecular structure classification of β-lactamases using the Ambler method [31].

According to the Bush-Jacobi-Medeiros technique **(Figure 4)**, β-lactamases are divided into groups of 1 to 3 based on how quickly they break down β-lactam substrates and how well the inhibitor works [32, 33]. SBLs can be inhibited by β-lactamase inhibitors such clavulanic acid, sulbactam, and/or tazobactam. On the other hand, metal ion chelators, such as dipicolinic acid, ethylenediaminetetraacetic acid (EDTA), or o-phenanthroline, which are all not licensed for clinical usage, inhibit MBLs instead of these inhibitors, which do not impact MBLs [27]. Despite having some extended activity toward carbapenems, the clinical relevance of a fourth class (Class C) remains uncertain [25]. Class D and class A or class C β-lactamases only share about 16% of their amino acid composition overall [34].

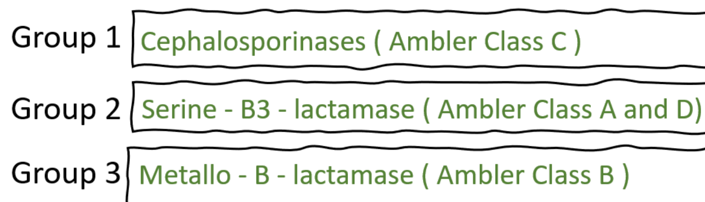


Figure 4. The functional classification of β-lactamases using the Bush-Jacobi-Medeiros method [32, 33].

Classification of carbapenemase enzymes

Class A Carbapenemases

The following enzymes are chromosomally encoded: NmcA (not metalloenzyme carbapenemase A), SME (Serratia marcescens enzyme), IMI-1 (imipenem-hydrolyzing β-lactamase), and SFC-1. These enzymes hydrolyze carbapenems and are somewhat inhibited by clavulanic acid (*Serratia fonticola* carbapenemase-1). The others are plasmid encoded like, KPC (KPC-2 to KPC-13), IMI (IMI-1 to IMI-3) and derivatives (GES-1 to GES-20) of GES (Guiana extended spectrum).

In 1996, the first KPC enzyme (KPC-2) was isolated and characterized in North Carolina, USA, from a *K. pneumoniae* clinical isolate [35]. Since then, the KPCs are the predominant after a few years of their discovery, had spread worldwide and caused outbreaks in many North American, Asian, European and African countries. KPC producers have evolved to be multidrug resistant to β-lactams as a result of that limiting therapeutic options for treating KPC-related infected patients [36], there are currently 12 additional variants of bla_{KPC} existing globally [37], KPC resistant clones are disseminating internationally in various areas that have different multi-locus locations with additional β-lactamase content which differ by size, number and structure of plasmids to compare producer isolates from different countries. However, a single genetic element (transposon Tn4401) has been found in bla_{KPC} genes [38].

An outbreak brought on by K. pneumoniae that produces KPC-2 was documented in a Greek hospital in 2007. 22 hospitalized patients who had no prior history of visiting regions infested with KPC-producers were afflicted by the outbreak. 20 out of 32 (62.5%) of the patients who were infected by a K. pneumoniae outbreak that produced KPC-3 and were examined in Columbia in 2008 died. Another outbreak involving 16 patients in intensive care units and driven by a KPC-3-producing *K. pneumoniae* strain was looked into in Italy in 2009 [3]. According to a study on CRE isolates recovered between 2013 and 2016 in a health system in Northern California, 20.8% of the analyzed isolates carried the bla_{KPC} gene, making up 38.7% of those with carbapenemase genes [39].

Class B Carbapenemases

The mechanism of hydrolysis depends on the interaction of the β-lactam drugs with zinc ions in the active site of the enzyme. The genes encoding these enzymes are frequently found within a variety of integron structures and integrated into the gene cassettes. These enzymes are typically in the class of β-lactamases with the ability to hydrolyze carbapenems but are sensitive to inhibition by EDTA, a chelator divalent cation especially Zn^{2+} [40]. Metallo-lactamase families that are most frequently found include:

1. New Delhi metallo-β-lactamase 1 (NDM-1)

   An Indian-born Swedish patient who had recently visited New Delhi, India, and had been infected with a carbapenem-resistant *K. pneumoniae* strain for a urinary tract infection in 2008 was found to have an NDM-producing *K. pneumoniae* isolate. In the future, the SENTRY Antimicrobial Surveillance Program stated that NDM-producing Enterobacteriaceae (*Enterobacter cloacae, K. pneumoniae*, *and Escherichia coli*) have been detected in Indian hospitals since 2006 and possibly even earlier. Additionally, NDM-producers have been isolated from drinking and seepage water (street pools of water) samples collected in New Delhi, which poses a serious health risk [41].

   By the middle of 2010, travelers from other nations, notably Europe and the United States, may have brought the $bla_{\mathrm{NDM\text{-}1}}$ gene with them on their travels, as the same strains have been discovered in environmental samples from India [41]. In this category, at least 28 variations havent been characterized and recognized.

Although they have also been discovered in conjunction with *A. baumannii and P. aeruginosa* species, bla_{NDM} genes are prominent in K. pneumoniae and E. coli isolates [42].

2. Imipenem-resistant Pseudomonas (IMP)-type carbapenemases
   Japan was where this kind was initially identified in the 1990s. These enzymes were extensively studied in Pseudomonas species, Acinetobacter species, and other members of the Enterobacteriaceae family. In addition to Europe, since the initial reports from Europe in 1997, the bla_{IMP} genes have also been discovered in Canada and Brazil. The U.S. and Australia have since experienced a steady spread of these gene variations from other Far Eastern nations. Currently, 91 different types of IMP-type carbapenemases have been discovered [43].

3. VIM (Verona integron-encoded metallo-β-lactamase)
   In 1997, a new family of carbapenemases was discovered in Verona, Italy. Pseudomonas putida and P. aeruginosa are the bacteria with the highest frequency of bla_{VIM} genes, which are mostly integron-β-associated metallo-lactamases and have more than 71 members in total [44].
   In terms of the plasmids, they are carried on and the fact that they are integron-associated, the VIM and IMP families share some commonalities. Amino acid sequence diversity in the VIM family is up to 10%, in the IMP family it is 15%, and between the two families it is 70%. Both are vulnerable to all β-lactam inhibitors and hydrolyze all β-lactams except monobactams [45].

4. GIM (German imipenemase)
   In 2004, five clonally related *P. aeruginosa* clinical isolates found in North Rhine-Westfalia, Germany, had the carbapenemase GIM-1 (German imipenemase). Later, in 2012, five additional GIM-1-producing P. aeruginosa isolates from the same area were discovered. The bla_{GIM-1} gene was shown to be on plasmids, and the bla_{GIM-1} gene cassette, together with the bla_{OXA-2} and the aminoglycoside resistance genes aac_{A4} and aad_{A1}, was discovered to be embedded in a class 1 integron structure. *P. putida, E. cloacae, S. marcescens, E. coli, K. oxytoca, Citrobacter freundii, Acinetobacter pittii, P. aeruginosa*, and *K. pneumoniae* were shown to carry the bla_{GIM-1} gene [46].
   SIM beta-lactamases are Class B beta-lactamases that are capable of hydrolyzing a wide variety of beta-lactams, including penicillins, narrow- to expanded-spectrum cephalosporins, and carbapenem, the SIM family of beta-lactamases appear to be transferable through integrons [47].

Class D Carbapenemases

These carbapenemases, of the Oxacillinase (OXA) enzyme class, are serine-β-lactamases that are only weakly inhibited by clavulanic acid or EDTA. They also have weak activity against carbapenems [48]. The OXA β-lactamase with carbapenemase activity was initially discovered. The main issue with OXA carbapenemases is their propensity to rapidly evolve and broaden their spectrum of action, making it challenging to determine their true prevalence rates. OXA β-10, OXA -11, and OXA -15 are recognized as extended-spectrum cephalosporinases, whereas OXA -23 and OXA -48 are recognized as carbapenem-hydrolyzing D β-lactamases. There have been 484 unique bla_{OXA} sequences identified over the years, and at least 37 are considered to be carbapenemases (CHDLs) [1, 49].

Currently, the most prevalent CP gene found in K. pneumoniae is bla_{OXA-48}, which is ubiquitous in Turkey, the Middle East, North Africa, and Europe. Environmental Acinetobacter species have been shown to carry the non-nosocomial OXA-24 type, but the OXA-23 type, which is transmitted internationally, is more common in Saudi Arabia, the United States, and Europe. The OXA-58 group has been extensively described in many epidemics around the world [50, 51]. OXA-48 was initially identified in a *K. pneumoniae* isolate from Istanbul, Turkey, in 2001 [52]. A few years later, Istanbul was the first city to report an outbreak of infections brought on by K. pneumoniae that produces OXA-48 [53]. In France, OXA-48-producing K. pneumonia isolates were found to be the source of an outbreak in 2010. Hospital outbreaks have been recorded in Russia and the Netherlands [54]. In, Egypt, Germany, France, and other countries have all reported sporadic occurrences of isolates that produce OXA-48 [55]. According to a recent study conducted in Egypt, samples of K. pneumoniae most frequently included the carbapenemase gene bla_{OXA-48} (74%) [56]. Another study done in Iran using clinical samples of blood, urine, and sputum found that the carbapenemase gene bla_{OXA}-48 was the most common (72%), followed by bla_{NDM} (31%). The majority of the A. baumannii strains' chromosomes contain bla_{OXA} enzymes with carbapenem-hydrolyzing activity; however, bla_{OXA-23} and bla_{OXA-48} have also been discovered on plasmids isolated from enteric bacteria. A

wide range of bacteria, including Acinetobacter, Shewanella, Pseudomonas, and Burkholderi, include these OXA-like enzymes [56, 57].

The intrinsic and acquired carbapenemase genes dissemination in gram-negative bacilli

Due to differences in cell walls, externally decreased membrane permeability, broad-specificity drug-efflux pumps, and the presence of various broad-spectrum-β-lactamases, Gram-negative bacteria are typically more resistant to a variety of antimicrobials and several chemotherapeutic agents than Gram-positive bacteria. After the discovery and introduction of β-lactam antibiotics into clinical practice, the evolution and advent of inactivating enzymes were well recognized. Penicillinases were the first β-lactam-hydrolyzing enzymes, followed by cephalosporinases, ESBLs, and, more recently, MBLs and carbapenemases, to broaden their range of activity. The MBLs have significantly reduced the effectiveness of carbapenems, which are frequently used as last-resort medications [58, 59].

Historically, *Acinetobacter* spp. has been the primary source of class D carbapenemases. The OXA-48 group, however, is primarily found in the order Enterobacterales [36]. The tailored mechanisms, known as extrinsic resistance, are typically carried in transferable elements like plasmids, in contrast to the general intrinsic mechanisms, which are primarily attributed to chromosomal genes. This second type of resistance makes it difficult to control infections because it can spread both vertically and horizontally. When genes are transferred horizontally, bacteria may acquire genetic material from unrelated bacteria or their environment, whereas when genes are transferred vertically, bacteria inherit their genetic makeup from parental cells in the previous generation. The three horizontal gene transfer mechanisms are transformation, transduction, and conjugation [60]. The source of genetic material that bacteria take in varies amongst them. For the other two procedures, DNA [61] will be taken from a donor, either a phage or a plasmid via transduction or conjugation, respectively. In transformation, bacteria take in foreign DNA from the environment. Resistance genes are distributed both within and across species through conjugation. However, transduction has a larger role in the dissemination of virulence genes, and it is still unknown how much bacteriophages contribute to the spread of resistance genes. It's possible for resistance to carbapenems to develop due to both intrinsic and acquired resistance mechanisms [22].

Intrinsic Resistance of Gram-Negative Bacilli

The term "intrinsic resistance" refers to the tendency of a large number of bacteria, including pathogens and commensals, to be resistant to specific classes of antimicrobial agents. This intrinsic resistance restricts drug choices for treatment and can raise the risk of developing acquired resistance [1].

For instance, opportunistic and environmental pathogenic bacteria, such as Stenotrophomonas maltophilia, Bacillus cereus, and Aeromonas species, are the main sources of MBLs' early chromosomal detection in nature. Stenotrophomonas maltophilia is the only one of these opportunistically harmful bacteria that is frequently linked to hospital-acquired illnesses. Others typically carry chromosomal Metallo-β-lactamase enzymes, which are not transferrable, from the groups that had a serine-based hydrolytic mechanism of action, but in the middle of the 1990s, chromosomal MBLs were found in the majority of carbapenem-resistant P. aeruginosa and then Acinetobacter spp. in clinical specimens. This occurrence was explained by the fact that the MBL genetic materials are mobile [55, 61, 62].

Acquired resistance of gram-negative bacilli

There are various methods for spreading acquired carbapenemases among bacterial isolates, and many of the ones reported in Enterobacteriaceae are plasmid-mediated. Additionally, other significant pathways that confer carbapenem resistance have been identified recently [63].

The former is supported by the former's widespread distribution of sequence type (ST) 258 KPC-producing K. pneumoniae [64]. Additionally, certain clades within ST258 have been linked to the presence of particular KPC genes: ST258A, which corresponds to clade I, has been found to be strongly linked to KPC-2, while ST258B, which corresponds to clade II, tends to carry KPC-3 [65]. This suggests that KPC-2 and KPC-3 have developed associations with particular clones of *K. pneumoniae* through recombination events and transfer of mobile genetic elements, such as transposons and plasmids, and that these associations have persisted as bacteria have spread from one person to another. The genomic region in charge of capsular polysaccharide production varies amongst these subtypes as well. But there is also evidence of outbreaks predominantly brought on by horizontal gene transfer, according to reports [66]. A hybrid plasmid co-containing $bla_{\text{IMP-4}}$ and $bla_{\text{NDM-1}}$ was recently discovered in a ST20-K28 carbapenem-resistant K. pneumoniae strain, according to research. The development of novel

hybrid plasmids should be continuously monitored since it may endanger efforts to prevent antibiotic resistance [67].

The KPC gene was discovered in 66 different strains of Enterobacteriaceae, consisting of 13 different species, including Klebsiella, Enterobacter, and Citrobacter, in a five-year single-center CPE outbreak investigation. They discovered evidence for the spread of KPC-carrying bacteria from person to person as well as for the transfer of plasmids between diverse bacteria and the transfer of KPC-containing transposons across plasmids [68]. Additionally, an epidemic analysis from Norway discovered that KPC-2-containing plasmid-mediated indigenous spread was to blame for the transfer of carbapenem resistance from K. pneumoniae to Enterobacter asburiae [69]. Bacterial clones and mobile genetic components carrying resistance genes interact continuously. OXA-48 is more closely related to IncLM-type plasmids regardless of ST type, as opposed to the close association between ST258 and KPC [65]. In conclusion, clonal multiplication and transmission, or horizontal transmission mediated by plasmids, is probably a factor in the current global CPE epidemic. The difficulty of detecting plasmid-mediated transmission, however, may have led to an underestimation of findings to date [68].

The laboratory detection of carbapenem resistance organisms

Both nosocomial infections and community-acquired infections are now being treated unsuccessfully due to CRE. Therefore, there is a critical need for the quick and precise detection of carbapenem resistance and carbapenemase-producing isolates. In clinical laboratories, CRE can be found using a number of different techniques, including automated systems or disc diffusion, MICs, modified Hodge tests, selective agar, spectrometric, synergy tests, whole genome sequencing, and molecular methods. Automated systems or disc diffusion are used in the initial baseline test that predicts CPO; however, detecting the enzymes is challenging due to the numerous mechanisms involved and faulty methodologies used in some clinical laboratories [37, 70].

Phenotype based method

Disc diffusion

On an agar plate containing a test bacterium, impregnated discs carrying a standard dose of an antibiotic drug are inserted to perform disc diffusion. The antibiotic drug diffuses into the Muller-Hinton agar medium over the course of an overnight incubation to promote microbial growth. The sensitivity of the test organism is proportional to the antibiotic's inhibitory zone. For common bacterial infections, bacterial species identification is necessary in addition to this sensitivity testing, and while it is extremely reliable, there are inherent issues with differentiating between acquired and intrinsic resistance [71, 72]. The CLSI disc diffusion assay was used to measure routine antibiotic susceptibilities, and CLSI breakpoints. For the IPM, ETP, and MEM disc zones of inhibition, values below 23 mm indicated sensitivity, values above 20 to 22 mm indicated intermediate resistance, and values above 19 mm indicated resistance. The IPM disc test performs poorly as a screening test for carbapenemases, according to the CLSI criteria for the phenotypic detection of KPC-producing organisms, which are based on lower sensitivity to ETP or MEM [73, 74].

Automated systems

With a standardized inoculum for the test strain, diluted in a specific broth, and a drop of an antimicrobial susceptibility testing indicator added, instruments are used to assess antibiotic susceptibility. The inoculated panel is placed into an automated system instrument after the inoculum (0.5 McFarland Standard) has been poured into it and sealed in a safe location. The panel is then automatically scanned by the instrument, and the data produced is examined using preliminary algorithms and contrasted, as necessary, with the controlled results [64, 75].

The ability of three commercial systems to infer carbapenem resistance mechanisms: Phoenix, Vitek 2, and (MicroScan NM36 & MicroScan NBC39) were compared. Intermediate susceptibility or resistance to at least one carbapenem was detected in 100% (Phoenix), 95% (Vitek 2 and MicroScan NM36), and 91% (MicroScan NBC39) of the 55 test isolates: Vitek 2 failed to detect nonsusceptibility for one *K. pneumoniae* isolate and two *Enterobacter* isolates with ESBL/Amp C in combination with porin loss; the NM36 panel failed for two *K. pneumoniae* isolates with OXA-48 or an IMP enzyme and an *E. cloacae* isolate with Amp C/porin loss; and the NBC39 panel failed for three *K. pneumoniae* isolates with OXA-48 and one isolate each of *K. pneumoniae* and *E. cloacae* with ESBL/Amp C in combination with porin loss [76]. All systems but the Phoenix consistently missed one of these isolates, an *Enterobacter* sp. with SHV-12 ESBL, Amp C activity, and decreased permeability. The CLSI recommendations state that Enterobacteriaceae isolates are suspected of being CPO when the MICs of meropenem, imipenem, or ertapenem are 2-4 µg/ml or 2 g/ml, respectively [77].

The modified Hodge test

On a Mueller-Hinton plate, a susceptible isolate of *E. coli* DH10B is cultivated to produce a confluent growth lawn. Each test isolate suspected of producing a carbapenemase is streaked from an imipenem disk that has been placed in the middle of the plate. Growth of *E. coli* around the imipenem disk or along the streak provides evidence of carbapenemase activity **(Figure 5)**. Despite the low cost and ease of use of this test, it frequently yields false-positive results when used with isolates that produce ESBLs linked to porin loss or changes. NDM-1 carbapenemases were also used to find false-negative results. These factors led to the removal of this test from the CLSI guidelines in 2018 [78, 79].

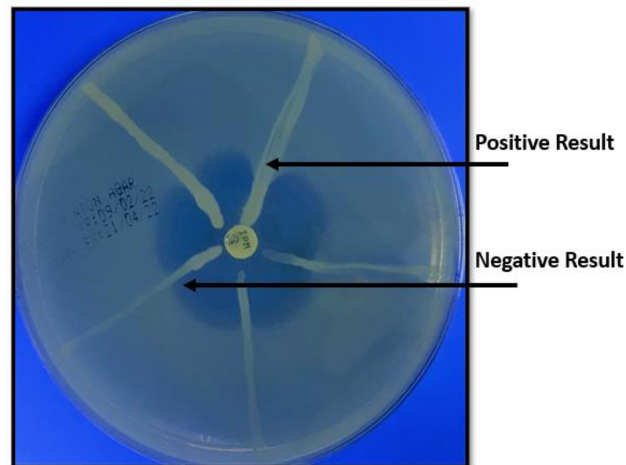


Figure 5. Modified Hodge test for screening for carbapenemase-producing bacteria

Carba NP test

The Carba NP test is a colorimetric microtube assay used to identify the presence of carbapenemase in P. aeruginosa and Enterobacteriaceae. For the detection of enzyme-type carbapenemases of the KPC, NDM, VIM, IMP, and S. marcescens, this test offers good sensitivity and specificity (>90% each), but low sensitivity (11%) for the detection of OXA-48 carbapenemases. It has been reported that the Carba NP test can detect carbapenemase synthesis in CPE that is imipenem susceptible [80]. Recently, a ready-to-use variant (RAPIDECR Carba NP test) for regular use in labs became commercially accessible [81].

The Carba NP test is carried out in wells, and changes in color from red to orange or yellow show that the tested strains are manufacturing carbapenemases. By employing phenol red as an indicator, the Carba NP test quickly and accurately pinpoints carbapenemase producers by changes in pH values. For example, coupled mechanisms of resistance or isolates that are carbapenem sensitive but express a broad-spectrum β-lactamase without carbapenemase activity might cause non-carbapenemase-mediated imipenem resistance, which can be detected by color within 2 hours [82].

Modified carbapenem inactivation method

This technique has been added to CLSI 2017 for the identification of Enterobacterales that produce carbapenemase [83]. As a result, EDTA is added to the mCIM to enhance it (eCIM). When using this procedure, a 2-mL tube is filled with 20 µL of 0.5 M EDTA in tandem with the mCIM, and the eCIM should be used to interpret the results of the mCIM [84]. To find out whether *P. aeruginosa* and Enterobacteriaceae are producing carbapenemase, employ the modified carbapenem inactivation technique (mCIM) test. The mCIM test makes use of easily accessible chemicals and media, in contrast to the Carba NP test, which necessitates the use of unique reagents that are not frequently used in clinical laboratories. Its process is straightforward, and it's simple to interpret the outcomes. Furthermore, the distinction between serine carbapenemases and MBLs in Enterobacteriaceae can be made using an EDTA-mCIM in addition to the mCIM [77].

Bioluminescence-based carbapenem susceptibility detection assay

Vincent van Almsick and colleagues recently discovered this technique. With a sensitivity and specificity of 99 and 98%, it enables the detection of carbapenem as producing A. baumannii, carbapenemase-producing-CRE, and non carbapenemase-producing-CRE in just 2.5 hours from culture media [85].

Immunochromatographic assays

In order to identify VIM, NDM, KPC, and OXA-48 carbapenemases from grown bacterial colonies in 5 minutes, a number of immunochromatographic methods have been devised. Based on monoclonal antibodies produced through the immunization of mice, these tests [86].

Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry (MALDI-TOF)

This technique involves combining freshly generated bacterial cultures with carbapenem solutions and incubating the mixture for 2–4 hours at 35–37 °C. The combination is then centrifuged, and the supernatant is measured using the mass spectrometry method. The breakdown products and sodium salt of the carbapenem molecule can be seen in the spectra when carbapenemase hydrolyzes it [87].

Spectrophotometric assay

This procedure involves first creating a bacterial crude extract—typically by sonication—and adding it to an imipenem solution that has been buffered. Measurements of the hydrolysis of the β-lactam ring are made using UV spectroscopy [88].

Chromogenic agar preparation

Chromogenic agar preparations, such as CHROM agar KPC, have become popular for the detection of MDR pathogens from surveillance cultures (CHROM agar, Paris, France). By including chromogenic substances and substrates that inhibit the development of additional yeast isolates, Gram-positive bacteria, and Gram-negative bacteria, culture mediums can be made selective. A. baumannii that was resistant to carbapenems may have been identified using a novel formulation that included (K. pneumoniae carbapenemase supplement) [89]. Recent changes to CHROM agar for Acinetobacter have increased organisms' ability to develop selectively for those that are resistant to carbapenems [90]. When compared to Xpert Carba-R, KPC type is detected on CHROM agar with 75.4% sensitivity and 99.8% specificity, according to Moubareck *et al*. While the CHROM Agar KPC screening procedure is less expensive, Xpert Carba-R may be more accurate and quicker [35].

Double-disc synergy testing

Double disc diffusion was carried out using this technique, which was created by Jarlier and colleagues for the identification of Enterobacterales that produce ESBLs, by applying a carbapenem disc and a carbapenem with inhibitor disc on the bacterial culture plate. When a carbapenem disc's inhibition zone size is larger on the side that is closest to a carbapenem with an inhibitor disc than it is on the other side, a favorable outcome is observed. The inhibition zone should resemble a champagne cork or keyhole in shape [91].

A double-sided E-test, imipenem vs. imipenem with EDTA, is one of the variations of double-disc synergy testing that is used as a screening test for MBL manufacturers. Other versions include carbapenem with -clavulanate, -cloxacillin -EDTA, or -2-mercaptoproionic acid. This method is effective for detecting MBL carbapenemases with high resistance to imipenem but may be ineffective with low resistance. They also found that there is no inhibition test that has been verified for OXA-48/OXA-181 producers and that EDTA by itself has inhibitory action against some bacteria and can cause false-positive results [36]. However, extended-spectrum β-lactamase inhibitors, such as clavulanic acid, tazobactam, and sulbactam, are similarly unreliable for phenotypically detecting carbapenemase synthesis in bacterial isolates when used in conjunction with a carbapenem agent [1].

Genotype based methods

There are already a number of molecular methods for detecting carbapenemase genes, and these assays can reveal both the precise nature of the carbapenemase and the presence or absence of the enzymes [74]. To prevent random spread into healthcare facilities and community settings, some genotyping approaches could enhance the detection of unidentified dominant resistance genes and their variants [36, 92].

These assays include:

Traditional simplex and multiplex PCR assays, which are performed on colonies and use the proper primers for each gene, can provide results in 4 to 6 hours with excellent sensitivity and specificity, potentially reducing the risk of infectious disease outbreaks in hospitals. However, the limitation of these contemporary tools is a lack of sequence similarity to genes [93]. The hyplex SuperBug ID test system (bioTRADING, Mijdrecht, Netherlands) is one of the available PCR assays [27, 94].

CONCLUSION

The mortality rates among patients are high as a result of the lengthy detection procedures and constrained treatment regimens that CRE have spread to in a number of healthcare settings around the world in recent decades. In order to effectively treat patients and prevent infections, it is crucial to identify CRE as soon as possible, especially among Enterobacteriaceae that produce carbapenemases. These enzymes were first described on chromosomal genes, and then different carbapenemase genes that are easily transmitted on mobile elements between species began to arise. It is anticipated that clinical microbiology laboratories will frequently use a number of phenotypic and gene-based approaches for the quick detection of carbapenemases.

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