ISSN 2515-8260 Volume 10, Issue 01, 2023

A Review on Molecular Description of Carbapenem Resistant Gram-Negative *Bacilli*

B.S.Saravanan[1], S.Swarupa Gnana Sudha Meriam[2], D. J. Mukesh Kumar[3*], C. Venkataramaniah[4]**

[1] Department of Microbiology, RVS Dental College & Hospital, Sulur, Coimbatore, Tamilnadu, India

[2] Department of Microbiology, Government Erode Medical College & Hospital Perundurai, Tamilnadu, India

[3] Centre for Materials Engineering and Regenerative Medicine, Bharath Institute of Science and Technology, BIHER, Selaiyur, Chennai, Tamilnadu, India

[4] Department of Anatomy, Bharath Medical College & Hospital, Selaiyur, Chennai, Tamilnadu, India

***Corresponding Author:**

Email: fio7rio@yahoo.co.in
Email: itsmemukesh@gmail.com
Mobile: +91-9941178552
Mobile: +91-9884553310

ABSTRACT

The rise in multi-drug resistant Gram-negative bacteria during the past few years has posed a serious danger to public health care. Our understanding of how antibiotic resistance mechanisms emerge and spread across bacterial strains has been enhanced by recent advanced molecular techniques. The resistant pathogens use a variety of resistance mechanisms, including cell membrane porosity changes, antibacterial target modification, and destruction of antibacterial agents. This section will discuss and explain the molecular characterisation of carbapenem resistance as well as their detection techniques. Understanding these mechanisms can be crucial for the development of novel antimicrobial drugs as well as for specific treatment considerations about the usage of antibiotics other than carbapenem and beta-lactams. Although the majority may be carried out quickly with a molecular device, molecular approaches are presently used for identifying carbapenem-resistant species. Whole-genome sequencing delivers clear idea on the comprehensive evaluation of whole genome and has the potential to develop into a very potent tool in regular clinical settings. The cost of genome sequencing is still high, and an automated system for data processing is needed. It might take a while before this method is routinely used in medical laboratories, especially in underdeveloped nations. To create innovative antibacterial medicines that can enhance pathogen prognosis, it can be crucial to comprehend these processes and the importance of the formation of carbapenem resistance strains.

Keywords: Antimicrobial resistance, Molecular methods, Gram-negative bacteria, Carbapenem resistant.

ISSN 2515-8260 Volume 10, Issue 01, 2023

Introduction

Gram-negative bacilli (GNB) nosocomial outbreaks of multi-drug resistance (MDR) bacteria, notably carbapenem-resistant *Klebsiella pneumoniae* (CRKP), have been viewed as a possible danger globally over the past ten years. The infections caused by these microorganisms that are resistant to antibiotics that relate to healthcare are now extremely dangerous and challenging to treat. The expense of treating these infections therefore can increase for the medical system. The CRKP isolates are among the most significant extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae that can destroy carbapenems, the last line of defence compared to the common MDR GNB infections. The synthesis of class A, B and C carbapenemase enzymes are primarily accountable for the pathway of CR [1]. Numerous reports on these newly-emerging CRKP classes may be found in *K. pneumoniae* linked to severe illnesses [1, 2]. GNB ability to produce transmissible plasmid-encoded carbapenemase enzymes, also known as *K. pneumoniae* carbapenemase, is now a growing worldwide health concern [3]. These antimicrobials are presently utilized as the first line of treatment for severe infections brought on by *K. pneumoniae* that produces ESBLs because carbapenems are relatively stable and can resist hydroxylation produced by beta-lactamase hydrolysis. lung infection [4, 5]. However, the rise of CRKP and MDR pathogens is a result of the abuse of carbapenems. As the treatment regimen becomes more difficult to implement, these infections have the capacity to develop carbapenemase enzymes that are resistant to numerous antimicrobials [2, 6, 7].

While higher-level fight to carbapenems detected in strains that experience the lack of outer membrane porins due to the existence of *Klebsiella pneumoniae* carbapenemase (KPC-2) and KPC-3, some pathogenic bacterial strains might show mild carbapenem resistance, particularly those that harbor KPC-1 [6, 7-9]. One specific K that can produce KPC-2 or KPC-3. The *K. pneumoniae* clone, also known as clonal complex (CC) 258, is now found all over the world. The KPC gene and numerous other acquired antibacterial resistance-granting genes are typically found in this clone [8, 9]. Within K, further carbapenemases were discovered. *K. pneumoniae* strains that have extended spectrum oxacillinases OXA-48 and metallo-betalactamases (MBLs) like IMP, VIM, and metallo-beta-lactamase-1. India was the country where the New Delhi metallo-beta-lactamase 1 (NDM-1) in *K. pneumoniae* strains were first discovered. The majority of Africa, Asia, and North America are now all home to *K. pneumoniae* isolates that produce NDM-1 [9]. Turkey was the first country to report oxacillinase-48-type (OXA-48) producing *K. pneumoniae*; it has now expanded to other countries in Europe [10]. KPC-producing strains are related to the spread of nosocomial contagions among the three main carbapenemases found in *K. pneumoniae*. The NDM and OXA-48 strains, on the other hand, have a long history of being connected to population and hospital transmitted infection [11].

Previous research revealed that the OXA-48 enzyme-producing gene is known to be much more likely than the KPC and NDM genes to disperse throughout various enterobacterial species [12]. For the identification of carbapenemases in bacteria, various techniques are used. These techniques range in sensitivity, specificity, benefits, and drawbacks and can be either phenotypic-based or molecular-based detection methods [12, 13]. Multiplex real-time Polymerase chain reaction (PCR) is one of the more modern molecular techniques for detecting carbapenemases genes and is well known for being highly specific, efficient, and prominent for the detection of bacteria that produce carbapenemase [14]. This technique has made it possible to track the global spread of *K. pneumoniae*, which produces carbapenemase and has been identified in numerous nations.

In addition to the remarkable rise in antibiotic resistance over the past few years, GNB are becoming more common in many nations everywhere. Carbapenems were one of the latter

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options for giving MDR-GNB, particularly *Enterobacteriaceae* that produce ESBLs. In non-fermentative GNB and in *Enterobacteriaceae*, specifically in *K. pneumoniae* as opposed to *Escherichia coli*, CR has been documented [15].

The most prevalent bacteria causing a variety of nosocomial and community-acquired diseases, including pneumonia, peritonitis, urinary tract followed by meningitis contagions, are these GNB, which are significant nosocomial pathogens [16]. Since resistant to carbapenems can occur in conjunction with fighting to another kinds of antibiotics, like aminoglycosides and fluoroquinolones, developed carbapenemase-encoding genes represent a genuine medical problem for antimicrobic administration. Carbapenem-hydrolyzing -lactamases, such as class A beta-lactamases, class B metallo—lactamases and class D-lactamases, can cause carbapenem resistance in GNB. First discovered in *P. aeruginosa*, OXA- lactamases have found in other GNB, like *A. baumannii* and *Enterobacteriaceae*. The carbapenemase that was most regularly seen in *Enterobacteriaceae* is the OXA-48 enzyme. Neither clavulanic acid nor ethylene diamine tetra-acetic acid block this carbapenemase. It exhibits a wide range of penicillin-specific activity, but only moderate action towards third-generation cephalosporins, aztreonam, and carbapenems. Particularly in *P. aeruginosa* strains, the reduced permeable brought on by the deletion or modification of oprD porin which is situated in outerior membrane has mostly related to CR. Additionally, *P. aeruginosa* carbapenemase development may be linked to imipenem resistant mechanisms [17].

GNB that manufacture broad spectra beta-lactamases has the capacity to withstand number of antimicrobial treatments, including third generations cephalosporins and monobactams, making them a serious issue in medical settings all over the world. These bacteria include *P. aeruginosa*, *A. baumannii*, and other groups of *Enterobacteriaceae* group. Because of its capability to resist many beta-lactamase enzymes, carbapenems were among the latter line of defence against thoughtful infections caused by many GNB that produce ESBLs [15]. However, excessive use of carbapenems have resulted in the development of carbapenem resistant *Enterobacteriaceae* (CRE) and carbapenem resistant *A. baumannii* (CRAB) and *P. aeruginosa* that is resilient to carbapenem. CRE, CRP, and CRAB each have unique mechanisms for carbapenem resistance. CRP and CRAB battle carbapenems by using the mixture of carbapenemase enzymes (CE), mutations various carbapenemases that had been found and classified into classes, and CRE isolates, for instance, are proficient of deactivating carbapenem through the creation of CE. OXA-51-like, OXA-23, and so on enzymes are related with CRAB separates which has linked to major nosocomial contaminations in several nations. These categories are now frequently detected in CRE [16, 17].

The OXA-48 enzyme, which was first discovered in Turkey in *K. pneumoniae*, has lately been found in Europe and the Middle East. While the KPC enzyme was initially found in *K. pneumoniae* separates in the United States, it has since spread to 38 American states. There have lately been reports of KPC in Canada, Europe, Australia, and India. The international health community is now deeply concerned about the development of CRE due to the restricted antibiotic alternatives accessible to treat illnesses instigated by CR-GNB. Therefore, it was believed that the best ways to control CR-GNB infections include early recognition of the condition, good hand hygiene habits, and sensible antibiotic administration. Many clinical laboratories face difficulties in accurately detecting carbapenem-resistant organisms (CROs) in both clinical and screening cultures. The identification of carbapenem resistance using simply routine antibiotic susceptibility tests (ASTs) is confounded by variations in CR activity among carbapenemases, even though ASTs were still necessary to choose the best dealing option. For instance, Ambler class A (KPC) hydrolyses almost all beta-lactams, but several beta-lactamase inhibitors, like boric acid, and tazobactam, can decrease their hydrolytic activity.

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Although OXA-48 types have low action towards cephalosporins and carbapenems, Ambler class B have a wide variety of action compared to these antibiotics. Additionally, there are currently no beta-lactamase inhibitors that can suppress the activities of Ambler class D. Several phenotypic and genotypic recognition techniques can recognize CRB and distinguish among different carbapenemase groups. They differ, nonetheless, in terms of sensitivity, performance standards, and price. This review's objective is to give a comprehensive overview of all currently used techniques for CR-GNB molecular characterisation. ways for detection. Numerous ways to detect CR-GNB have emerged over the past ten years. These techniques comprise phenotypic-based techniques for measuring the action of CE, like growth-based tests, quick colorimetric techniques, immunochromatographic (IC) followed by molecular-based techniques, such as whole-genome sequencing (WGS) and PCR-based techniques based on phenotypes [18].

Antibiotic susceptibility test

Ertapenem have been cited as the highest subtle gauge for the activation of carbapenemases, and disc diffusion susceptibility patterns is thought as first-line approach for initial identification of carbapenemases. The E-test, broth micro- and agar-dilutions followed by automatic antibacterial susceptibility testing (AST) structures are just a few of the platforms that can be utilized to conduct this test employing minimum inhibitory concentration (MIC) breakpoints as a confirmatory screening method. Various testing techniques have shown variation in their capacity to identify carbapenem resistance. Certain CP-GNB isolates continue to be susceptible to or intermediary to various carbapenems, despite the European Committee on AST or EUCAST recommendation that carbapenem MIC divisions be determined. For instance, some Enterobacteriaceae isolates possess the blaKPC gene yet are susceptible to the antibiotics ertapenem, meropenem, and imipenem [19]. Using the MIC divisions of 2 mg/L/zone diameters 22 mm for all the carbapenems, the Clinical and Laboratory Standards Institute (CLSI) released rules in 2009 for the detection of CROs. The modified Hodge test should be used to validate any *Enterobacteriaceae* isolates that have carbapenem MIC divisions of 2 or 4 g/ml and thus are battle to all third-generation cephalosporins. This recommendation was made through the CLSI one year later (MHT) [20]. This method has strong KPC detectability but poor NDM and IMP detection sensitivity. Another issue with the CLSI guidelines is that few CRE-releasing OXA-48-type enzymes might be vulnerable to third-generation cephalosporins [20]. In 2016, the CLSI recommended, when Enterobacteriaceae separates are linked to a misgiving of carbepenemase construction depends on up-to-date carbapenem breakpoints, confirmatory tests or a molecular method must be carried out [21]. The analysis of tedious ASTs to identify CROs depends on carbapenem divisions has been improved by both CLSI and EUCAST, however these methods still do not successfully identify all or the majority of CROs. Routine ASTs are, in fact, beneficial and required for treatment-related objectives, but not for screening or epidemiological ones testing of integrated disk synergy and multiple-disk mechanisms. Some phenotypic methods for differentiating between different forms of carbapenemase include multi-disk migration testing. These techniques rely on the interaction between carbapenems and beta-lactamase suppressors. These suppressors comprise boronic acids for KPC recognition, dipicolinic acid for MBL, clavulanate for ESBL, cloxacillin for AmpC [22]. Due to their ease of use and affordability, multi-disk tests are frequently utilized. The sensitivity of these tests varies, ranging from 90 to 100 percent. Numerous commercialization phenotype multi-disk assays were established to recognize and distinguish many carbapenemase varieties.

For instance, Mast Diagnostics has created a brand-new technique called Mastdiscs ID suppressors grouping recognition disks, which relies on a straightforward computation of

suppressing zone of shared disks that incorporate enzyme suppressors to identify carbapenemases. This approach shown decent inequitable power in KPC and MBL identification amongst other investigation. We looked at another carbapenemase identification test, the Rosco Diagnostica Neo-Sensitabs (RDS). These researchers found that both phenotype assays dearth specificity for finding OXA-48-like enzymes. However, they was capable to differentiate among genes of the OXA-48 type and various MBL genes, including NDM, VIM, and IMP.

In order to distinguish CRE that produce OXA-48 from those that produce other carbapenemases, a novel phenotypic test known as the OXA-48 disk study was recently been developed. Multi-disc tests were generally easy to carry out and understand, and they seem to be helpful for medical research laboratory that do not have entree to or cannot pay for molecular methods. This method was demonstrated to have outstanding performance for noticing OXA-48 medium with a chromogenic base for screening. All higher-risk patients, like the elderly, those in intensive care units (ICUs), those who are immunocompromised, those who are traveling from locations where CRE is prevalent, should be checked for Carbapenem-resistant Enterobacteriaceae carriers. Rectal/perirectal swabs or fecal samples are advised for the detection of CRE gastrointestinal carriers [23]. One of the best screening techniques for quickly detecting CRE is chromogenic based medium. They are selective for a particular resistance trait because they were depends on a chromogenic enzyme substrate, particular antibiotics. Several commercial chromogenic agars, including Colorex KPC, and Brilliance CRE, are presently utilized for the recognition of CPB. Most of these media have been tested in numerous prior studies for their capacity to identify carbapenemase classes; these media showed a variety of sensitivities that ranged from 13 to 100% [23]. The ability to identify OXA-48 manufacturers has a poor sensitivity. The Supercarba medium, created a new screening medium which demonstrated a higher sensitivity of 96.5 percent for all carbapenemase producers like OXA-48 producers. Though, this medium seems to only identify carbapenemases in lactose-fermenting microbes. Its short shelf period is 7–10 days, once organized which was a drawback in medical microbiology. The sensitivity and selectivity of this intermediate were both 100% for the recognition of CPE isolates that produced KPC, MBL, and OXA-48 types. Additionally, the enriching stage, which may takes up to 24 hours, can be skipped when using chromogenic-based medium as a screening tool. This can speed up the process of finding carbapenemase producers. Additionally, the mSuperCarba medium's shelf lifetime has been extended to up to one month. Identifying carbapenemase proteins in samples of Enterobacteriaceae is simple with the MHT. This method is based on the capability of a strain that produces carbapenemase to reduce the amount of carbapenem and encourage the development of a sample of *E. coli* that is sensitive to carbapenem [23]. Due to the MHT's capacity to identify KPC production and by CLSI's recommendation of it as a confirming test for carbapenemases from 2009 to 2017, it is widely used in medical microbiology labs. The MHT is distinguished by reduced susceptibility to NDM, MBL, various OXA types, and SME, among other carbapenemases. The MHT also lack selectivity and have the possible to yield false-positive outcomes. Additionally, it can accurately detect various carbapenemases, like VIM, IMP, or OXA-48 [22, 23].

The MHT was excluded from the present edition of CLSI 2018 owing to its problem of false positives and negative consequences. Nevertheless, the MHT is still useful despite its lower sensitivity to various OXA types, NDM, and MBL carbapenemases. Moreover, the MHT limits selectivity and can yield false-positive findings when looking for some samples that express AmpC in conjunction with porin alterations [22]. Reduced susceptibility to certain forms of OXA, NDM and MBL carbapenemases on the other hand, is a characteristic of MHT [23]. Additionally, the MHT lacks selectivity and can yield false-positive outcomes

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when looking for some isolates that produce AmpC in conjunction with porin mutations [24]. The problem of false positives and false-negative results led to the MHT being excluded from the current CLSI 2018 edition. Despite these drawbacks, the MHT's simplicity, affordability, and moderate sensitivity make it helpful for initial screening of many CRE separates in many medical research laboratory [24]. NP Carba. A phenotypic colorimetric assay called the Carba NP is used to find CPE. The RAPIDEC CARBA NP is one of the commercially available Carba NP tests, and it measures imipenem hydrolysis, which alter pH and cause the colorimetric pH indicator to shift (phenol red) [24]. In comparison to other phenotypic testing, this method offers many benefits, including easiness, rapidity, cost-effectiveness, and specificity. The CLSI and EUCAST have suggested the Carba NP as a assenting test for *Enterobacteriaceae* followed by other CR-GNB that produce carbapenemase [21]. The Carba NP's sensitivity for the majority of carbapenemases varied between 73 and 100% in numerous trials [24]. Nevertheless, few class A carbapenemases, like GES-5 and SME-1 and some carbapenemases with lower imipenem hydrolysis action, like OXA-48, demonstrate low sensitivity to the Carba NP. According to a different investigation, MacConkey and Drigalski agar-cultured isolates of the Carba NP produced ambiguous results. According to this study, the Carba NP is negatively impacted by both media.

Carbapenem resistance

The 3 major mechanisms of carbapenem resistance include β-lactamase hydrolysis, efflux pump up-regulation, and porin channel changes. Figure 1 exemplifies the general mode of actions of CR.

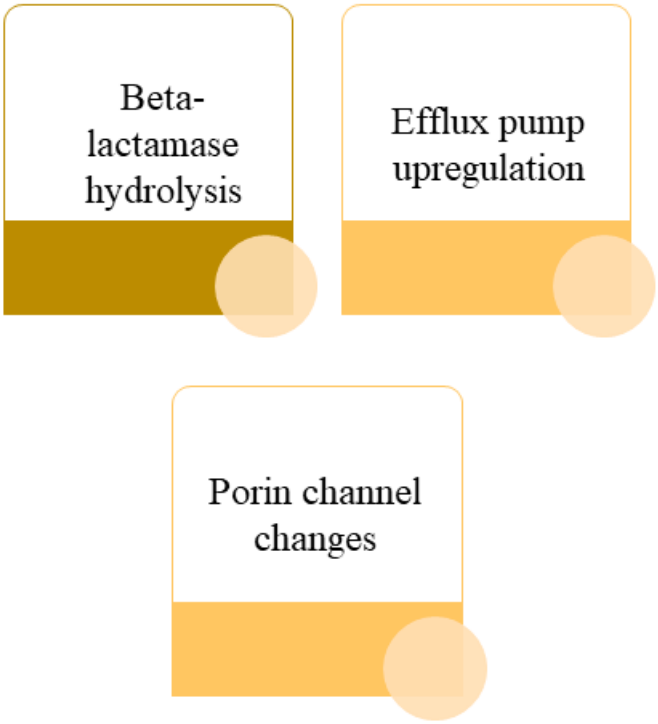

Figure 1. Mode of actions of CR.

There are four categories of AMR mechanisms: (1) limited drug absorption; (2) changing a druggable target; (3) inactivated drug; and (4) active-based drug efflux. Instances of intrinsic resistance tactics comprise lowering drug uptake, -inactivation, and -efflux, whereas instances of acquired fight tactics comprise pharmaceutical target change, drug-inactivation, and -efflux. Gram +ve and -ve microbes use different kinds of processes, which can be attributed to structural variations among other factors. Gram positive bacteria are less likely to use efflux processes and fewer likely to use inhibiting drug uptake because they lacked an LPS

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outer membrane. All four fundamental mechanisms are used by gram-negative microbes. The basic pathways of AR are shown in Figure 2.

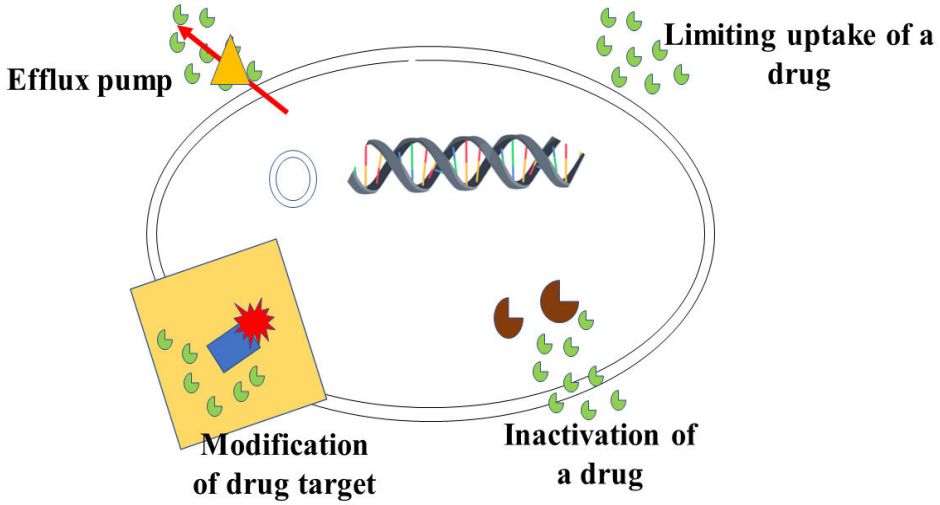

Figure 2. Mechanisms of AMR.

β-Lactamases formation using gram negative bacteria

Depending on the level of the enzymes' activity, gram-negative bacteria create different -lactam antibiotic degrading enzymes that break down penicillin's, certain suppressor-based -lactam antibiotics, carbapenem, and other antibiotics. Table 1 depicts the few examples of carbapenemase identified in GNB from various source whereas table 2 depicts the anti-bacterial tested towards CRB.

Table 1. Examples of carbapenemases detected in Gram-negative bacteria from animal and environmental samples.

S. No.	Source of animal or environment		Microbial species	Identified carbapenemase
1	Water	Coastal water	A. towneri	OXA-58
		Wastewater	Citrobacter freundii	OXA-204
		Hospital wastewater	Raoltella ornitholitica	KPC-2
2	Animals	Cats	Salmonella enterica	IMP-4
		Domestic parrot	A. baumannii	OXA-72
		Cattle	A. baumannii	OXA-24
		Birds	Numerous Enterobacteriaceae species	OXA-48
		Companion animals	Acinetobacter baumannii	OXA-23 & 48
3	Samples from	Soft coral	Pseudobacteriovorax	PAN-1

	environment		antillogorgiicola	
		Marine sediments	Zhongshania aliphaticivorans	ZHO-1

Note:

PAN denotes Pseudobacteriovorax antillogorgiicola carbapenemase

ZHO means Zhongshania aliphaticivorans carbapenemase.

IMP represents imipenemase

OXA specifies oxacillinase followed by

KPC denotes Klebsiella pneumoniae carbapenemase

Table 2. Anti-bacterial normally tested towards carbapenem resistant bacteria.

S. No.	**Antibacterial**	**MIC breakpoint Showing resistance**	**Occurrence of susceptible isolates**	**Remarks**
1	Trimethoprim-sulphamethoxazole	Greater than 4	Less than 5x	Sparse information available
2	Amikacin	Greater than 16	50 x	Cross resilient not the rule based on actual subcategory of altering enzyme expressed
3	Piperacillin-tazobactam	Greater than 16	Less than 5x	Resistance generally linked with higher-level expression of broad spectrum beta-lactamases
4	Colistin	Greater than 2	80 x	Broth microdilution suggested to evade major errors
5	Imipenem	Greater than 8	Less than 5x	Variable degrees of resistance might happen as per the stage of carbapenemase expression and concurrent existence of other mode of actions of carbapenem resistance

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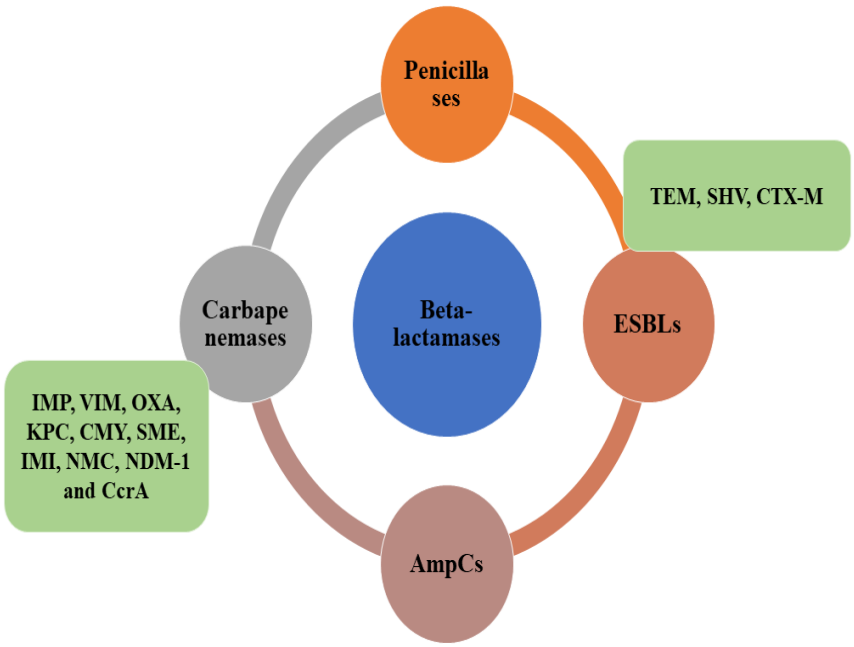

Figure 3. Classification of β-Lactamases.

As a result, the beta-lactamases are classified as shown in Figure 3. The below beta-lactamases are among those posing a hazard from antibiotic resistance:

a penicillase is an enzyme that attacks the beta-lactam compound of penicillin antibiotics, although not their extended-spectrum counterparts, such as cephalosporins.

b. ESBLs: The broad-spectrum cephalosporins cefotaxime, and ceftazidime, and also the oxyimino-monobactam aztreonam, are hydrolyzed by ESBLs. They can sometimes result from alterations that change the amino acid conformation in the active site of genes that code for the phenotype development of beta-lactamases including TEM-1, -2, or SHV-1. According to reports, more and more particular ESBLs are being detected. These ESBLs are encoded by the plasmid and render Cephem medications useless. The therapeutic options for a variety of contagions produced by GNB are restricted by ESBLs. It would be challenging to treat a water-borne illness case in toddlers or neonates caused by an ESBL producer, particularly if the aetiology was CR [25, 26]. ESBLs alone do not render carbapenems inactive, but they can deliberate resistance to them because of later chromosomal porin changes that prevent -lactam drugs from penetrating the bacteria [27].

Circulation and related hazard of carbapenemases

Clinical studies of the progressive rise of total drug resistance, microbes that manufacture carbapenemases, also known as carbapenemresistant organisms, are resistant to every type of antibiotic currently in use. As a result, carbapenemases are now recognised as a new hazard that can inactivate the last lines of defence for antibiotics. The prevalence of various metallo-lactamase genes worldwide has been documented subsequent the discovery of the classical carbapenemases in the 1990s [28, 29]. It was initially discovered in North Carolina in 2003 and has since spread over the world. KPC are primarily found in Klebsiella species. They operate as the exclusive repository for OXA-48 and their derivatives for Enterobacteriaceae. The metallo-beta-lactamases, which were once less common in the USA were the common cause of CR and are contributing to "development of total drug resistance (TDR)". The KPC gene, blaKPC, is extensively distributed among different species and regions of the world. It is contained within Tn4401, a transposon of the Tn3 type that has the capacity to insert into a number of GNB plasmids. Plasmids with the blaKPC gene are frequently associated with antibiotic resistance genes. Fabrication of carbapenemase is crucial for the evolution of

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resistance, particularly in carbapenem-resistant Enterobacteriaceae (CRE). The expression serin beta-lactamases may be implied by the existence of carbapenemase-producing Enterobacteriaceae (CPE) in a specimen. *E. coli*, *K. pneumoniae*, and *A. baumannii* is frequently originate to produce carbapenemase. Additionally, there is a higher chance of finding carbapenemases in Central Europe. In certain nations including Italy, KPC is endemic [30]. In Portugal, wastewater and river water samples were found to contain Enterobacteriaceae that produce KPC, and in Austria, bacterial isolates from people were found to contain NDM-1, VIM, as well as OXA-48. Consequently, there was an increase in the recognition of *Enterobacteriacea* that produce KPC. No matter the cause of infection—community-acquired bacteria (CRA) due to CRB has been described with a dismal prognosis [31]. Recovery from carbapenem resistance has been controversial due to incidents of co-resistance to other medications that resulted in fatalities. According to Zhang study, although carbapenem resistance negatively affected the mortality of P. aeruginosa bacteremia, the relationship between the two is still debatable. Seven study showed that 26-44 percent of deaths were attributed to CR, indicating that the sequence may alter slightly when bacteraemia is present. In four trials, 8-18.4% of deaths within 30 days were attributed to carbapenemm resistance [32]. Buehrle et al. predicted a number of characteristics that would have made early active therapy beneficial in preventing mortality from carbapenem-resistant Pseudomonas aeruginosa (CRPA) bacteraemia [32]. This demonstrates how a variety of factors can influence whether a carbapenem-resistant bacterial infection recovers or dies, making the infection challenging to treat. The US woman's death from carbapenem-resistant Enterobacteriaceae, which was previously mentioned in this article, and the ensuing catastrophe of 26 diverse antibiotics, remain to emphasize the threat that CR-GNB pose in both medical settings as well as the environment. "CRPA had considerably higher fatality than those contaminated with carbapenem-susceptible *P. aeruginosa* (CSPA),". These reports assisted in placing these CRB on the serious precedence list that calls for the development of novel R and D antibiotics. Keeping your hands clean and using several barriers Hand hygiene is crucial for reducing the CRE, usually, the CRB adhesion to communicated exteriors and preventing the dissemination of the similar to other animate/inanimate exteriors. To decrease CRE population on the hands, it has been suggested to apply antibacterial hand gel. Additionally, this complies with guidelines.

General hygiene practices, preventative measures against food or water source contamination, and hand hygiene must all be practised in tandem. Every time a person encounters sewage, preserved sewage's receiver water bodies, crude recreational waters, etc. that could be dirtied with ARB, they should wash their hands. By doing this, the spread of AMR from the water matrix will be reduced. This must do by the multiple-barrier strategy. The multiple barrier method reduces or prevents the high-risk pathogens, such as CRE, from contaminating drinking water. While some chemical disinfectants might break bacterial cells to proclamation their genome-enhancing genetic transfer to infections, deteriorating the prediction of the contagion they origin, and form lethal complexes by remaining antibiotics in water, some progressive oxidative procedures combined with UV-irradiation and chemical antiseptics have the potential to eradicate ARB and ARGs [33]. Up taking and internalization of CRGNB, ARGs, CPB to eatable fruits (Figure 4).

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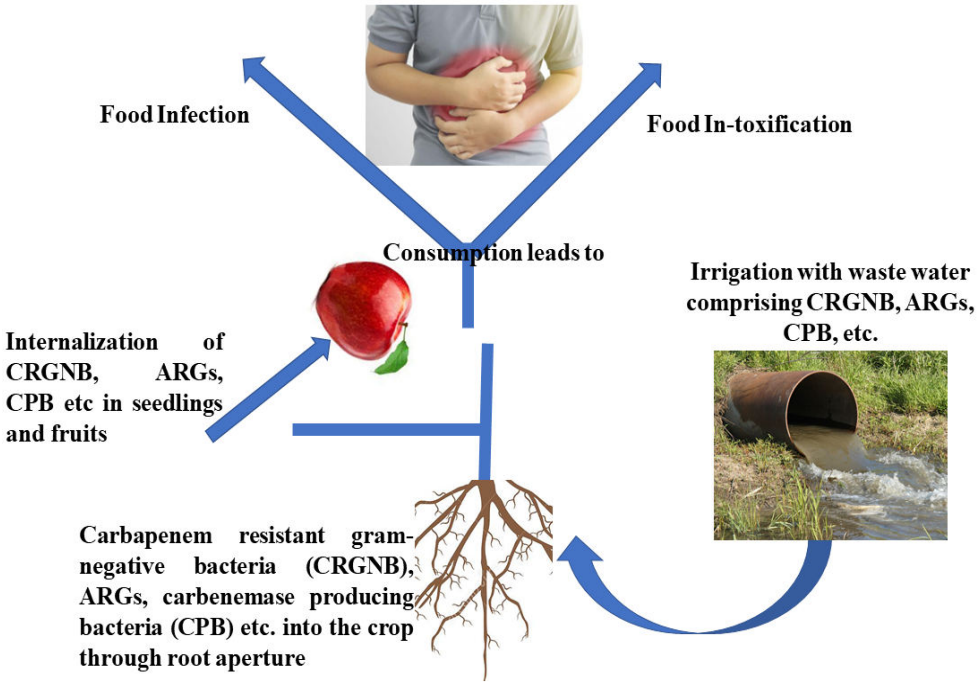

Figure 4. Uptake and internalization of CRGNB, ARGs, CPB to edible fruits.

Carbapenem inactivation method

Van der Zwaluw and colleagues originally presented carbapenem inactivation method (CIM), a unique phenotypical technique, in 2015. The CIM depends on in-vitro identification of CPO-mediated CI. Using this technique, microbial clusters are suspended in water comprising carbapenem discs as well as the setup is incubated for 2 hours. The meropenem disc was then placed onto Mueller-Hinton agar also called MHA, which has been injected with a strain of *E. coli* that is susceptible to carbapenem. If there is not a significant zone of inhibition, the target organism's production of the carbapenemase enzyme has rendered the meropenem disc inactive against it. A distinct inhabitation zone all around meropenem disc, which can be seen if the disc is still active, shows that the bacterium of attention was not a carbapenemase-producer (CP). The CIM was characterised as simple, inexpensive, extremely sensitive, and specific approach that is accessible to many clinical laboratories. In the procedure of the altered CIM, it is now advised by CLSI 2018 and is similar in price to MHT but was more penetrating and precise. The CIM was more reliable than Carba NP and MHT for identifying CP amongst *Enterobacteriaceae*, according to several comparative studies. The CIM displayed equivalent or superior sensitivity at detecting OXA-48, according to these trials. Though the CIM needs more period to culture target bacterium, it was easy, affordable, and doesn't call for specialised tools or knowledge. The CIM is better suitable for microbiology laboratory labs than other phenotypic techniques because of these characteristics.

Immunochromatographic assays

The principal carbapenemase enzymes from bacterial cultures can now be swiftly and precisely detected using a variety of immunochromatographic (IC) techniques. These assays capture carbapenemase antigens using antibody-antigen binding-based technology. A novel IC assay to identify MBL producers in *P. aeruginosa* and *Acinetobacter* was developed in 2011 by Kitao et al. This quick assay, which was in line with PCR data, identified every IMP-producer. Furthermore, commercialised versions of IC tests like the KPC-k-set and OXA-48 K-set tests, for instance, use monoclonal antibodies to collect epitopes unique to KPC and OXA-48-based enzymes utilising colloide gold-based nanoparticles inked to a nitrocellulose-basedmembrane in a adjacent flow device [34]. The KPC-k-set as well as OXA-48 K-set

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study are assessed by Glupczynski et al. The quick recognition of KPC as well as OXA-48 enzymes showed good performance with great precision, according to these investigators. In a different investigation, 82 isolates of enterobacteria that were resistant to carbapenems were subjected to the OXA-48 K-set study. These scientists decided that OXA-48 K-set accurately and quickly recognized every OXA-48 producer within 10 minutes. The IC assay's capability to identify few allelic variants of OXA-48, including OXA-204, -244, -181 and -232, is an additional intriguing feature. Immunochromatographic tests were helpful as a quick and affordable screening tool for nations has higher levels of OXA-48 and KPC endemicity.

Nonphenotypic-based methods

Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry

Depending on molecular weight of various chemical components found in microbial and fungal cells, matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS) was frequently employed to recognize numerous infections. Numerous clinical laboratories have recently started using MALDI-TOF to find signs of antibiotic resistance, like the action of beta-lactamase enzyme in GNB. When the recovered proteins of microorganisms are treated (usually for 2-4 hours) with carbapenems, the detection of beta-lactamase activities by means of this technique depends on determining the breakdown products of certain β-lactams. The first MALDI-TOF carbapenemase test was released and it was validated using 58 *P. aeruginosa* and *Enterobacteriaceae* only formed carbapenemases. This method offers a sensitivity and specificity of 96.67% and 97.87%, despite reports of false-positive and false-negative outcomes in *P. aeruginosa*. Due to its difficulty in picking up OXA-48-like enzymes, a different MALDI-TOF carbapenemase test showed a lower sensitivity. The study also claimed that, an introduction of ammonium bicarbonate raised the sensitivity of the reaction mix to 98 percent. Numerous reports also discussed the usage of MALDI-TOF for the straight identification of carbapenemase-producers from blood cultures. A second MALDI-TOF method depends on the identification of protein related with the plasmid that confers CR was introduced in 2014. Only CRE separates that the source of an epidemic at the National Institutes of Health (NIH) Clinical Center in 2011 were found to contain the blaKPC carbapenemase-carrying pKpQIL plasmid using this method. Since many clinical laboratories have MALDI-TOF apparatuses, MALDI-TOF carbapenemase may seem more appealing for repetitive labor, but other clinical laboratories, especially those in impoverished nations, still find these procedures to be too expensive. Additionally, MALDI-TOF assays struggle to identify carbapenemase, like many phenotypic carbapenemase detection techniques. Surprisingly, false-negative consequences observed and linked to extended incubation periods. These longer incubation times may cause the degradation products to further decompose, eventually becoming a very light mass. A MALDI-TOF assay may be unable to detect the products after such decompositions. Finally, there is no denying the value of MALDI-TOF as a microbiological identification tool. Expert users must, however, employ MALDI-TOF settings those advised for microbial documentation when using it frequently for carbapenemase detection [35].

Molecular recognition of carbapenemase genes

Phenotypic approaches were not as accurate as molecular methods in identifying bacteria that produce carbapenemase. These technologies comprise WGS, hybridization-based methods, and polymerase chain reaction (PCR)-based procedures.

Polymerase Chain Reaction

Many molecular approaches for identifying carbapenem resistance rely on PCR. They serve as a reference approach to verify or correct issues with few outcomes from phenotype recognition techniques. Both approaches, multiplex-PCR or uni-plex-PCR, rely heavily on the intensification of a particular target area in chromosomal DNA. In the situation of real time-

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PCR that can produce results in less than an hour, PCR methods are used immediately on clinical specimens or on microbial colonies with turn-around periods of 4-6 hours. Many different multiplex-PCR methods are created for both commercial assays and laboratory-developed tests (LDTs). For the identification and separation of the alleles encoding the 5 separate families of acquired MBL genes, presented a multiplex-PCR approach. The detection and assurance of allelic discernment of 5 groups of MBL genes, like the IMP and VIM variations, were successfully accomplished using this method. Another multiplex-PCR experiment in 2011 to identify 11 carbapenemase genes utilising three distinct multiplex reactions. The most clinically significant carbapenemase genes, as well as less significant "minor" carbapenemase genes, all belong to various classes of genes and found it to be effective performance against a variety of control and clinical strains of CPGNB 65 has increased over the past ten years. This is because multiplex real-time PCR can simultaneously detect and differentiate numerous carbapenemase types created the first multiplex real time-PCR technique for identifying MBL-encoding genes. These carbapenemases were classified based on the melting peak temperatures and various amplicon sizes (Tm). The detection of all clinical isolates carrying MB was very good with this technique. To detect six different carbapenemase types which already been found in *Enterobacteriaceae* in a single process, another internal multiplex real time-PCR approach was presented. The assay was run on 30 renowned isolates containing those six carbapenemase genes, and the results were 100% consistent with those of earlier genotyping tests. Seven diverse laboratories assessed this assay that leads to 100 percent sensitivity and specificity. The mainstream of the leading carbapenemase genes seem to be detectable using this assay. Recent research has demonstrated that numerous commercial multiplex PCR-based methods have sensitivities among 97 and 100% percent. These profitable tests are helpful resources for detecting carbapenemase in surveillance cultures that can lead to the early adoption of contagion control strategies [36]. It is obvious that PCR-based techniques were quick, reliable, and effective for identifying the most prevalent carbapenemase families. Nevertheless, most of existing PCR-based approaches cannot identify novel carbapenemase categories or novel variants of well-known kinds, additionally classy and requiring specialised knowledge and tackle to execute the tests. approaches based on hybridization (microarrays). A hybridization-based method called a microarray format enables the identification of many genes in a single reaction. Microarrays are tiny DNA patches with a specific DNA sequence, often known as DNA chips (called probes). To detect just probe-target hybridization, the DNA of microbes is hybridised with balancing base pairs of probes in slides. For the identification and characterization of several bacterial species, microarrays have frequently been used [37]. Numerous carbapenemase genes have also been found and identified using this approach, created a microarray assay in 2010 to distinguish seven distinct carbapenemase categories. In this procedure, multiplex PCR is used to simultaneously amplify the beta-lactamase genes, and then oligonucleotide probes are hybridised with tagged DNA. The Verigene gram-negative blood culture nucleic acid test (BC-GN) is just a few of the recently developed commercial microarray-based assays. The scientists decided that this microarray study precisely recognized all the carbapenemase genes which is in the species. For instance, the Verigene (BC-GN) was created to identify the six important beta-lactamase types as well as the eight most prevalent microbial infections straight in blood cultures. Studies that tested the Verigene (BC-GN) assay concluded that it has outstanding sensitivity and specificity [36, 37]. A microarray test has a benefit over PCR-based approaches in that it may identify hundreds or even thousands of targets, while PCR only target about 4-5 DNA sequences per test. Despite this benefit, microarrays have a few drawbacks that prevent their widespread usage in medical

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laboratories, like being labour- and cost-intensive [36]. The below figure 5 projected plan for the recognition of carbapenemases in investigative labs.

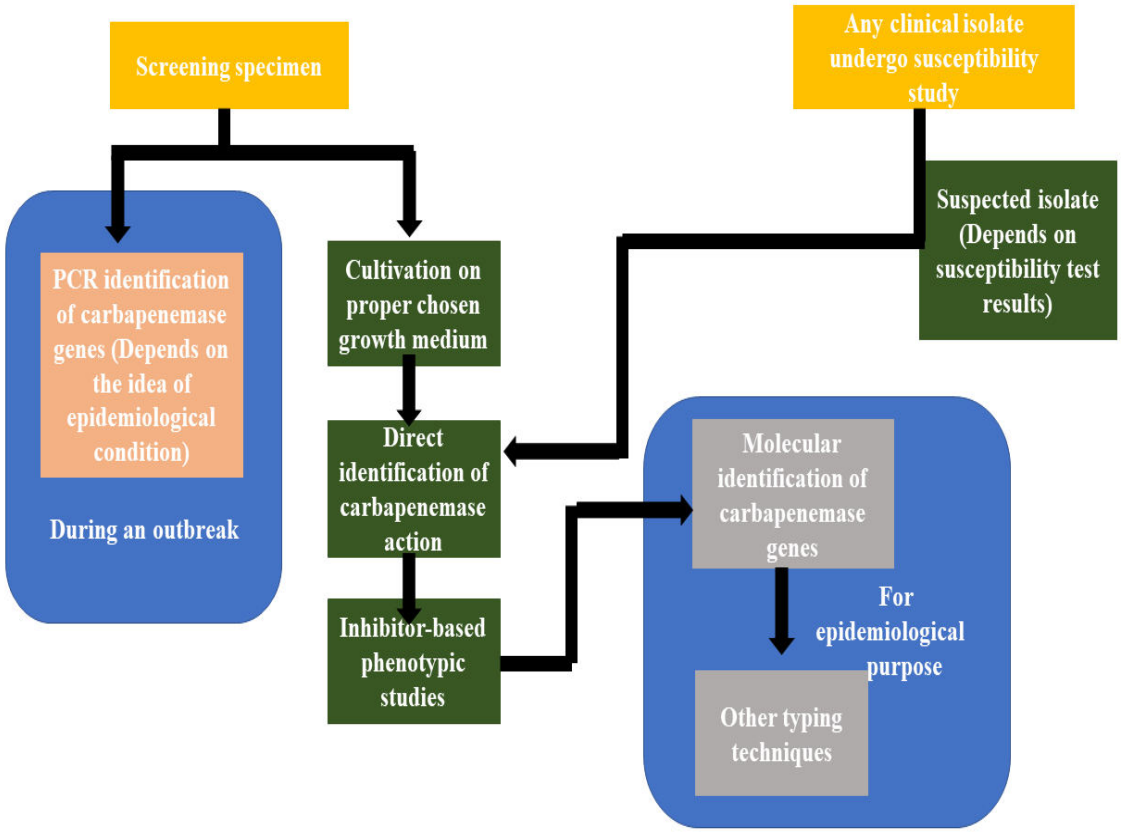

Figure 5. Proposed workflow for the recognition of carbapenemases in investigative labs.

Whole-genome sequencing

The deoxy-nucleotide triphosphates (dNTPs) as well as chain-terminating di-deoxynucleotide triphosphates (ddNTPs), which were tagged with four diverse fluorophores, are used to amplify DNA through a DNA polymerase enzyme in the sequencing stages that Sanger created [38]. Both capillary electrophoresis and mass spectrometry can be used to analyse the resultant fragments. In some clinical laboratories, Sanger sequencing has been utilised to look at the sequencing of numerous AR genes. However, for larger-scale initiatives like WGS, standard Sanger sequencing needs more labor, costly, laborious, and ineffective [38]. Next-generation sequencing (NGS) technology known as WGS is the most complete molecular technique for affordably providing all of the genetic data about a target organism. NGS is preferable than the Sanger approach because it can quickly and inexpensively analyse DNA along the complete genome. *E. coli*, *Salmonella enterica* followed by methicillin-resistant Staphylococcus aureus (MRSA), subject of epidemiological investigations using WGS [39]. Additionally, by making it possible to quickly identify all recognized resistance modes, like carbapenemases, WGS has developed into a crucial tool for the creation of novel antibacterial drugs. Additionally, WGS can detect additional host defenses such porin mutations and different plasmid-borne resistance genes. When new resistance mode or interesting virulency determinants remain discovered, WGS information might serve as a starting point for further research [40]. Nevertheless, WGS is still luxurious and requires automatic procedures for data interpretation.

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Conclusion

Despite significant improvements in therapeutic expertise and services over the past 20 years, antibiotic resistance has sharply developed globally. Therefore, the World Health Organization (WHO) and many managements have made the expansion of new approaches to address this impending threat an urgent priority. These tactics comprise stricter regulations on antibiotic usage to minimise the spread of MDR infections by 2050. As a result, few researchers have advised that if AR continues, the biosphere could reoccurrence to the pre-antibiotic era. Unquestionably, the action of MDR contagions as well as the prevention of the supper of such separates require the quick identification and characterisation of antibiotic-resistant bacteria utilising effective methods. Today, many clinical laboratories around the world find it extremely difficult to identify and characterise CROs. The numerous phenotypic and non-phenotypic techniques for CRO detection were covered in this paper. Regarding a single best practise, no agreement has yet been established. Considerations for choosing the best discovery group for investigative or screening determinations include speed, affordability, accessibility, usability, and notch of correctness. Additionally, when choosing and using a method, it's crucial to take into account the many types of fighting elements, the occurrence of resistant microbes, the location, and patient group. Phenotypic techniques are most appealing for tedious usage in medical laboratory sceneries since they are easy to use and inexpensive, like culture-based techniques and screening media. However, they still need lengthy incubation periods and have questionable sensitivity and specificity. Despite the limited sensitivity of some biochemical tests, including Carba NP and OXA-48 tests were occasionally used due to their speed and ease of use. Because they can accurately and quickly identify carbapenemase-producers from clinical specimens, immunochromatographic tests have recently attracted increased attention. Because of its variations in CR activity amongst carbapenemases which might be perceived by various techniques, utilising two or more phenotypic detection approaches in addition to one can increase the identification of CROs. Because most molecular procedures can be completed quickly and with a higher degree of sensitivity and specificity, they have become the method of choice. The straight uncovering of CPOs from medical samples as well as the ability to definitively pinpoint the precise mechanism of carbapenem resistance are two further benefits of molecular approaches over phenotypic ones. These benefits are especially beneficial for both outbreak investigations and surveillance and epidemiological purposes. However, some of these molecular technologies are regarded as reference techniques for verification and additional characterisation of existing carbapenemases because they were not able to find new, undiscovered resilient genes. They were only permitted for usage in reference labs. A thorough investigation of the entire genome is possible thanks to the clear information produced by whole-genome sequencing. We anticipate that WGS may eventually develop into a very potent instrument for both epidemic analyses and genetic characterisation of AR genes in common medical situations. Nevertheless, WGS has a high upfront cost and calls for a publicly accessible database and an automated data interpretation system. It might take several years, especially in developing nations, before WGS is routinely used in clinical laboratories.

References

1. Eichenberger, E.M.; Thaden, J.T. Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics 2019, 8, 37.
2. Chung, P.Y. The emerging problems of Klebsiella pneumoniae infections: Carbapenem resistance and biofilm formation. FEMS Microbiol. Lett. 2016, 363, fnw219.

3. Viau, R.; Frank, K.M.; Jacobs, M.R.; Wilson, B.; Kaye, K.; Donskey, C.J.; Bonomo, R.A. Intestinal carriage of carbapenemaseproducing organisms: Current status of surveillance methods. Clin. Microbiol. Rev. 2016, 29, 1–27.
4. Lee, C.-R.; Lee, J.H.; Park, K.S.; Kim, Y.B.; Jeong, B.C.; Lee, S.H. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: Epidemiology, genetic context, treatment options, and detection methods. Front. Microbiol. 2016, 7, 895.
5. Lee, C.-R.; Park, K.S.; Lee, J.H.; Jeon, J.H.; Kim, Y.B.; Jeong, B.C.; Lee, S.H. The threat of carbapenem-resistant hypervirulent Klebsiella pneumoniae (CR-HvKP). Biomed. Res. 2018, 29, 2438–2441.
6. De Rosa, F.G.; Corcione, S.; Cavallo, R.; Di Perri, G.; Bassetti, M. Critical issues for Klebsiella pneumoniae KPC-carbapenemase producing K. pneumoniae infections: A critical agenda. Future Microbiol. 2015, 10, 283–294.
7. Meletis, G. Carbapenem resistance: Overview of the problem and future perspectives. Ther. Adv. Infect. Dis. 2016, 3, 15–21.
8. Alhazmi,W.A. Review on the Carbapenem Resistance Mechanisms of Klebsiella pneumoniae. J. Pharm. Res. Int. 2021, 33, 352–357.
9. Chen, L.; Mathema, B.; Chavda, K.D.; DeLeo, F.R.; Bonomo, R.A.; Kreiswirth, B.N. Carbapenemase-producing Klebsiella pneumoniae: Molecular and genetic decoding. Trends Microbiol. 2014, 22, 686–696.
10. Pitout, J.D.; Peirano, G.; Kock, M.M.; Strydom, K.-A.; Matsumura, Y. The global ascendency of OXA-48-type carbapenemases. Clin. Microbiol. Rev. 2019, 33, e00102-19.
11. Dortet, L.; Poirel, L.; Nordmann, P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. BioMed Res. Int. 2014, 2014, 249856.
12. Nordmann, P.; Poirel, L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. Clin. Microbiol. Infect. 2014, 20, 821–830.
13. Nodari, C.S.; Gales, A.C.; Barth, A.L.; Magagnin, C.M.; Zavascki, A.P.; Carvalhaes, C.G. Detection of OXA-370 directly from rectal swabs and blood culture vials using an immunochromatographic assay. J. Microbiol. Methods 2017, 139, 92–94.
14. Al-Zahrani, I.A. Routine detection of carbapenem-resistant Gram-negative bacilli in clinical laboratories: A review of current challenges. Saudi Med. J. 2018, 39, 861.
15. Mellouk FZ, Bakour S, Meradji S, et al. First detection of VIM-4-producing Pseudomonas aeruginosa and OXA-48-producing Klebsiella pneumoniae in Northeastern (Annaba, Skikda) Algeria. Microb Drug Resist. 2017;23(3):335–344.
16. Bakour S, Olaitan AO, Ammari H, et al. Emergence of colistin- and carbapenem-resistant Acinetobacter baumannii ST2 clinical isolate in Algeria: first case report. Microb Drug Resist. 2015;21(3):279–285.
17. Sefraoui I, Berrazeg M, Drissi M, Rolain JM. Molecular epidemiology of carbapenem-resistant Pseudomonas aeruginosa clinical strains isolated from western Algeria between 2009 and 2012. Microb Drug Resist. 2014;20(2):156–161.
18. Bouguenoun W, Bakour S, Bentorki AA, Al Bayssari C, Merad T, Rolain JM. Molecular epidemiology of environmental and clinical carbapenemase-producing Gram-negative bacilli from hospitals in Guelma, Algeria: multiple genetic lineages and first report of OXA-48 in Enterobacter cloacae. J Glob Antimicrob Resist. 2016;7:135–140.
19. Yousfi M, Touati A, Mairi A, et al. Emergence of carbapenemase producing Escherichia coli isolated from companion animals in Algeria. Microb Drug Resist. 2016;22(4):342–346.
20. Pantel A, Richaud-Morel B, Cazaban M, Bouziges N, Sotto A, Lavigne JP. Environmental persistence of OXA-48-producing Klebsiella pneumoniae in a French intensive care unit. Am J Infect Control. 2016; 44(3):366–368.

21. Lee CR, Lee JH, Park KS, Kim YB, Jeong BC, Lee SH. Global dissemination of carbapenemase-producing Klebsiella pneumoniae: epidemiology, genetic context, treatment options, and detection methods. Front Microbiol. 2016;7:895.
22. Chen Y, Sun J, Liao XP, et al. Impact of enrofloxacin and florfenicol therapy on the spread of OqxAB gene and intestinal microbiota in chickens. Vet Microbiol. 2016;192:1–9.
23. Pailhoriès H, Belmonte O, Kempf M, et al. Diversity of Acinetobacter baumannii strains isolated in humans, companion animals, and the environment in Reunion Island: an exploratory study. Int J Infect Dis. 2015; 37:64–69.
24. Huber H, Zweifel C, Wittenbrink MM, Stephan R. ESBL-producing uropathogenic Escherichia coli isolated from dogs and cats in Switzerland. Vet Microbiol. 2013;162(2–4):992–996.
25. Adefurin A, Sammons H, Jacqz-Aigrain E, Choonara I (2011) Ciprofloxacin safety in paediatrics: a systematic review. Arch Dis Child 96(9):874–880.
26. Choi SH, Kim EY, Kim YJ (2013) Systemic use of fluoroquinolone in children. Korean J Pediatr 56:196–201.
27. Lutgring JD, Limbago BM (2016) The problem of carbapenemase producingcarbapenem-resistant-Enterobacteriaceae detection. J Clin Microbiol 54:529–534.
28. Berrazeg M, Diene SM, Medjahed L, Parola P, Drissi M, Raoult D et al (2014) New Delhi metallo-beta-lactamase around the world: an eReview using Google maps. Euro Surveill 19:20809Choi SH, Kim EY, Kim YJ (2013) Systemic use of fluoroquinolone in children. Korean J Pediatr 56:196–201.
29. Adam MA, Elhag WI (2018) Prevalence of metallo-β-lactamase acquired genes among carbapenems susceptible and resistant gram-negative clinical isolates using multiplex PCR, Khartoum hospitals, Khartoum Sudan. BMC Infect Dis 18:668–687.
30. Nordmann P, Dortet L, Poirel L (2012) Carbapenem resistance in Enterobacteriaceae: here is the storm. Trends Mol Med 18:263–272.
31. Zhang Y, Chen XL, Huang AW, Liu SL, Liu WJ, Zhang N et al (2016) Mortality attributable to carbapenem-resistant Pseudomonas aeruginosa bacteremia: a meta-analysis of cohort studies. Emerg Microbes Infect 5:e27.
32. Buehrle DJ, Shields RK, Clarke LG, Potoski BA, Clancy CJ, Nguyen MH (2017) Carbapenem-resistant Pseudomonas aeruginosa bacteremia: risk factors for mortality and microbiologic treatment failure. Antimicrob Agents Chemother 61:e01243–e01216.
33. Sanganyado E, Gwenzi W (2019) Antibiotic resistance in drinking water systems: occurrence, removal, and human health risks. Sci Total Environ 669:785–797.
34. Glupczynski Y, Evrard S, Ote I, Mertens P, Huang TD, Leclipteux T, et al. Evaluation of two new commercial immunochromatographic assays for the rapid detection of OXA-48 and KPC carbapenemases from cultured bacteria. J Antimicrob Chemother 2016; 71: 1217-1222.
35. Banerjee R, Humphries R. Clinical and laboratory considerations for the rapid detection of carbapenem-resistant Enterobacteriaceae. Virulence 2017; 8: 427-439.
36. Hrabak J, Chudackova E, Papagiannitsis CC. Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories. Clin Microbiol Infect 2014; 20: 839-853.
37. Lindsay JA. Staphylococci. In: Ivano de Filippis MM, editor. Molecular Typing in Bacterial Infections. New York (NY): Springer; 2013. p. 385-406.
38. Lupo A, Papp-Wallace KM, Sendi P, Bonomo RA, Endimiani A. Non-phenotypic tests to detect and characterize antibiotic resistance mechanisms in Enterobacteriaceae. Diagn Microbiol Infect Dis 2013; 77: 179-194.

ISSN 2515-8260 Volume 10, Issue 01, 2023

39. Leekitcharoenphon P, Nielsen EM, Kaas RS, Lund O, Aarestrup FM. Evaluation of whole genome sequencing for outbreak detection of Salmonella enterica. PLoS One 2014; 9: e87991.
40. Koser CU, Holden MT, Ellington MJ, Cartwright EJ, Brown NM, Ogilvy-Stuart AL, et al. Rapid whole-genome sequencing for investigation of a neonatal MRSA outbreak. N Engl J Med 2012; 366: 2267-2275.