Archives of Pharmaceutical Sciences Ain Shams University 2023; Vol. 7(1): 97-128

OPEN ACCESS

Microbiology & Immunology

Review Article

New Insights on the Carbapenem-resistant Gram Negative-associated-Infections: Challenges and Opportunities

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ABSTRACT

Gram-negative bacterial (GNB) infections represent a worldwide serious public health challenge, especially with the increased global spread of carbapenem resistance (CR) among these pathogens. There are different forms of CR including, intrinsic and acquired mechanisms, one of the most significant of which is carbapenemase production. In the last decade, the widespread plasmid-mediated carbapenemase production, on top of the chromosomally encoded carbapenemases- already abundant since the 1990s- further complicated the situation and necessitated urgent intervention to further understand and tackle this issue. In this review, the phenotypic and genotypic methods for the detection of different types of carbapenemase have been discussed. Also, the different control measures and strategies that should be applied in an attempt to control the massive spread of GNB infections especially in healthcare facilities, have been elaborated on in this article. The challenges of GNB-associated infection in terms of the emergence of resistance to carbapenems, the last line of defense against GNB, and the continuing spread of this resistance left us with almost no options for treatment as well as their complication on the host. On the other hand, we explore the various opportunities for their control such as the development of new classes of antimicrobials and the structural modification of existing ones. It is also inevitable to explore novel treatment options including the association of antimicrobial agents with non-antimicrobials, inhibition of quorum sensing, bacteriophage therapy, photodynamic therapy, and monoclonal antibodies for treatment and prevention.

Keywords: *Antimicrobial resistance; Gram-negative pathogens; Carbapenem-resistance; Carbapenemase; Extensively-drug resistant; Carbapenem.*

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Citation | Mabrouk SS, Abdellatif GR, Abu Zaid AS, Aboshanab KM, 2023. New Insights on the Carbapenem-resistant Gram Negative-associated-Infections: Challenges and Opportunities. Arch Pharm Sci ASU 7(1): 97-128

DOI: 10.21608/aps.2023.206876.1118

Print ISSN: 2356-8380. **Online ISSN**: 2356-8399.

Received 27 April 2023. **Accepted** 22 May 2023.

Published by: Ain Shams University, Faculty of Pharmacy

1. GNB-Associated Infections Epidemiology

1.1. Community-acquired infections (CAIs)

Urinary tract infections (UTIs) represent a group of the most common infectious illnesses in both the community and hospital settings, potentially responsible for high morbidity levels **[1]**. Previous research identified *Escherichia (E.) coli* (46.4% to 74.2%), *Klebsiella* spp. (6%-13.45%), and *Proteus* spp. (4.7%-11.9%) as the most common GN uropathogens associated with community UTIs **[2]**. Furthermore, additional research revealed *Pseudomonas* (*P.) aeruginosa* as an emerging opportunistic pathogen linked to 10.7%-25% of community-acquired UTIs **[3]**.

Community-acquired pneumonia (CAP), another common infectious disease treated by clinicians, is regarded as a significant cause of

hospitalization, high healthcare costs, morbidity, and mortality, particularly in elderly and immunocompromised patients worldwide. The admission of the disease differs from mild cases that can be treated at home to severe cases that need intensive care unit (ICU) therapy. A recent study found that 230 of 427 patients with CAP had pleural effusion, the most common complication noticed, along with 32% respiratory complications, 23% septic shock, 16% cardiac, 0.6% neuroseriousssues, and (0.3%) cholestatic jaundice, all of which need serious health attention. CAP is typically acquired through the inhalation or aspiration of pulmonary pathogens with an incidence ranging from 0% to 9% for GNB and 0% to 5% for *P. aeruginosa* [4]. Patients with chronic alcoholism and those who have bronchiectasis or cystic fibrosis are more likely to develop CAP from *K. pneumoniae* and *P. aeruginosa* respectively. Additionally, *E. coli* infection that results in UTIs or bacteremia may less frequently lead to CAP **[5]**.

1.2. Healthcare-associated infections (HCAIs) and hospital-acquired infections (HAIs)

Healthcare-associated infections (HCAIs) have become a significant problem for individuals who had prior contact with the healthcare service within one year and have been infected within 48 h of hospital admission. The USA National Healthcare Safety Network determined that 40% of HCAIs were thought to be associated with GNB including, *P. aeruginosa, A. baumannii, K. pneumoniae*, and *Enterobacter* species **[6]**. Additionally, the problem has been made worse by the fact that about 20% to 40% of healthcare facilities have reported at least one isolate from the previously stated bacteria with multiple drug resistance patterns. The highest incidence of multidrug resistance (MDR) was found in *A. baumannii* (44-78%), followed by *K. pneumoniae* or *K. oxytoca* (15%), while the lowest incidence was found in *E. coli* and *Enterobacter* spp., which represented less than 5% of the total **[7]**.

Hospital-acquired infections are most frequently associated with invasive medical devices or surgery. UTIs are the most common, while blood stream infections (BSIs) and lower respiratory tract infections are the most lethal. The majority of HAIs in intensive care units (ICU) about 60% and nearly one-third of all HAIs are caused by GNB **[8]**. Hospital-acquired pneumonia (HAP) is still one of the most frequent life-threatening HAIs and the issue is getting worse as about 10% to 20% of patients develop ventilator-associated pneumonia (VAP) after 48 hours which will ultimately lengthen hospital stays and increase mortality rates [9]. According to research, nosocomial pneumonia microbiology is still complex, The GNB mostly *Pseudomonas* spp., *Klebsiella* spp., *Acinetobacter* spp., *E. coli*, and other Enterobacteriaceae are among the most important pathogens causing VAP **[7]**.

2. The challenges and current circumstances with the emergence of antibiotic-resistant GNB

According to data from the National Healthcare Safety Network, between the turn of the new millennium and 2011, CR increased four-folds from 1.2% to 4.2% among *Enterobacter* isolates and ten-fold from 1.6% to 10.4% among *Klebsiella* isolates. The USA reported that acute care and long-term care hospitals were associated with at least one Carbapenem-resistant Enterobacteriaceae (CRE) infection of about 4% and up to 18% in the first half of 2012 **[10]**. During the period from 2011 to 2017, about 2306 out of 3836 Enterobacteriaceae isolates were determined to be CR according to the Egyptian HAI surveillance program; *Klebsiella* (85.1%), *E. coli* (10.2%) and *Enterobacter* (4.7%) were the most frequently recorded pathogens for CRE cases, respectively.

Additionally, the average incidence of HAI caused by CRE was 3.7 per 10,000 patient-days **[11]**.

It is unabated that GNB antimicrobial resistance is still expanding. A summary of antibiotic resistance among 18 pathogens with significant health implications was provided by the Centers for Disease Control and Prevention (CDC) in 2019. Out of the 16 antibiotic-resistant bacteria, nine were GNB and seven were Gram-positive. The CDC classified the threat level of antibiotic resistance in this report for the first time into three categories: urgent, serious, and concerning. Urgent threats have a significant impact due to the major risks identified across many criteria. Despite the fact that urgent threats may not be currently spreading, they have the potential and hence demand quick action to identify infection and limit transmission. CR *Acinetobacter* and CRE were the first and fourth urgent threat levels tracked by the CDC, respectively. The serious threats included Extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, MDR *P. aeruginosa*, drug-resistant nontyphoidal Salmonella, drug-resistant Salmonella serotype Typhi and drug-resistant Shigella. While the concerning threats included Erythromycin-resistant group A *Streptococcus* and Clindamycin-resistant group B *Streptococcus*. Furthermore, the number of hospitalized patients caused by CR *Acinetobacter* and CRE was estimated to be 8,500 and 13,100, respectively, with attributable healthcare costs of 281 million dollars and 130 million dollars, and estimated deaths of 700 and 1,100, respectively. The CDC recognized other GNB, including MDR *Acinetobacter* spp., *P. aeruginosa*, ESBLs, drug-resistant non-typhoidal *Salmonella/Salmonella typhi*, and *Shigella*, as a serious concern that required sustained and quick intervention to resolve **[12]**.

The annual incidence and mortality rates for MDR *A. baumannii* were estimated to be 7,300 and 500, respectively; 6,700 and 440 for MDR *P. aeruginosa*, 26,000 and 1,700 for ESBLs, respectively **[13]**. Along with CRE, CR non-fermenter (NF), GNB such as *Acinetobacter* spp. and *Pseudomonas* spp. are expanding in healthcare facilities. According to data on antibiotic sensitivity from various geographic locations, up to 87% of *Acinetobacter* spp. isolates were imipenem-resistant while up to 45% of *Pseudomonas* spp. isolates were resistant to the same drug. Additionally, the meropenem antimicrobial resistance pattern was also noted by about 43% and 20% in *Acinetobacter* spp. and *Pseudomonas* spp., respectively **[14]**.

In Egypt, *Acinetobacter* spp. had demonstrated a sharp rise in resistance to carbapenems that was approximately (98%) along with its elevated levels of resistance to quinolones and aminoglycosides **[15]**. El-Mahdy and his colleagues noted that about 42.5% of *Pseudomonas* spp. collected from hospital-acquired infections in Egypt were carbapenem-resistant and among which 61.8% were carbapenemase producers **[16–18]**. This relatively high CR rate among recovered isolates poses a significant challenge to currently available therapeutic options. In addition, the management of NF GNB had become more challenging due to exerting either intrinsic or acquired resistance to various classes of antibiotics, including cephalosporins, aminoglycosides, and fluoroquinolones **[19]**. Presently, the resistance issue is still more concerning among MDR GNB, specifically enterobacterial species, *P. aeruginosa*, and *Acinetobacter* spp. **[20]**.

3. Significant risks of carbapenem-resistant GNB emergence

CRE infections, along with *Pseudomonas* spp. and *Acinetobacter* spp., represent a triple

danger to public health due to their high mortality, antibiotic resistance - which limits therapeutic options - and high transmission potential **[11]**. According to reports, the mortality rate of CRE infections ranges from 40% and 50% **[21]**. However, some minor clinical investigations have revealed that deaths could exceed 72% as a result of multiple factors, such as underlying illness, delays in treatment, and a lack of efficient therapy **[13]**. In February 2015, California's Ronald Regan Medical Center reported two deaths and an additional five cases of CRE infections. Furthermore, 179 patients received significant endoscopic contamination during the management of pancreaticobiliary disorders **[11]**. The risk of CRE is still alarming as a high mortality rate is observed in a variety of vulnerable populations, especially in children and patients with infected burns **[15]**. Therefore, efficient control measures and antimicrobial stewardship must be implemented to stop future CR GNB outbreaks **[22]**.

It is extremely concerning that GNB antibiotic resistance can express MDR or Extensively-drug resistant (XDR) phenotypes, especially in critically ill patients with serious comorbidities. Although the definitions for these types of resistance models do not require a CR, the CR phenotype is very popular for MDR and particularly for XDR isolates **[23, 24]**. Clinically, carbapenems were the preferred treatment for highly drug-resistant GNB expressing an ESBL phenotype, but the emergence of carbapenemase producers has reduced their clinical activity **[25]**.

Carbapenemases producing bacteria typically exhibit broad resistance to β-lactam class of antibiotics, which includes carbapenems, penicillin, and cephalosporins this is in addition to aminoglycosides and quinolones **[26]**. As a result, the carbapenemase enzyme production in CR GNB is the main contributing cause of MDR and is regarded as the last step before pan-drug resistance **[21]**. The most terrifying issue, both now and in the future, is going to be GNB, which is resistant to all of the anti-microbial agents that are frequently used at the facility pan drug resistance (PDR). Thus, it would be imperative to bring back the older classes and introduce newer antibiotics to combat CR. Fosfomycin, aminoglycosides (amikacin, gentamicin, and tobramycin), and polymyxins (colistin and polymyxin B) have been regarded as standard CRE core medications despite their efficacy, pharmacokinetics, and toxicity **[27]**. However, resistance to these drugs had quickly increased, and there were regretfully few effective treatment options left **[11]**.

At the beginning of the 1990s, the majority of carbapenemases discovered were chromosomally encoded, but during this decade, plasmid-mediated genes spread dramatically around the world **[20]**. Because they were found on transposable genetic elements, particularly IncF-type plasmids, transposons, and integrons, they facilitated the horizontal spread of resistant genes between different species **[22]**. Additionally, carbapenemase-producer plasmids among CRE frequently carry additional resistance determinants that increase resistance to multiple drug classes, making them Pan-drug resistant **[24]**. Lately, the appearance of plasmid-mediated mcr-1 and colistin resistance in CRE has been reported **[25]**.

4. Carbapenem resistance mechanisms among clinically relevant GNB

Resistance can be classified as either intrinsic or acquired. In the former method, microorganisms do not always contain drug target sites, have low drug permeability, or have resistance coding genes on the host's chromosome. The latter method comprises changes in antibiotic-targeted genes as well as the transfer of resistance determinants carried on plasmids, bacteriophages, transposons, and other

mobile genetic elements. In general, this exchange occurs via transduction, conjugation, and transformation mechanisms. Furthermore, the following processes commonly lead to antimicrobial resistance: drug inactivation; target modification; reduced cellular uptake; and increased efflux **[28, 29]**. Antibiotic resistance of clinically significant GNB has spread globally and has significant consequences. MDR GNB is becoming more widely recognized in Enterobacteriaceae, particularly *Klebsiella*, *E. coli*, and *Enterobacter*, as well as the nosocomial pathogens *Pseudomonas* and *Acinetobacter*. In the previously described GNB, ESBLs, and CR GNB currently exhibit the highest levels of antibiotic resistance. Most often, two of the main mechanisms for CR are either the production of carbapenemase or the production of derepressed cephalosporins (Amp C) or ESBL in conjunction with decreased permeability caused by mutation or loss of porin **[30]**. **Fig. 1** summarizes the different mechanisms of carbapenem resistance.

4.1. Carbapenemases Production

Among highly adapted GNB, the formation of β-lactamases that hydrolyze the β-lactam ring is regarded as the most significant resistance mechanism. Ambler molecular classification (classes A to D) and Bush-Jacoby (groups 1 to 4) are the two main classification systems used to categorize β-lactamases. The distinction between the two classifications is based on the homology of the amino acid sequence i.e., molecular structure, in the former case, and the substrate and its inhibitory activity, or functional activity, in the latter. Class A, C, and D are β-lactamase enzymes that have a serine residue at the active site, while zinc is essential for the function of Class B. Among nosocomial pathogens classes A, B, and D are of the utmost clinical significance **[31]**. The five main plasmid-encoded carbapenemases with their hydrolytic profiles are shown in **Table 1**.

Table 1. Plasmid-encoded carbapenemases [32]

Ambler class	Representative carbapenemase	Hydrolytic profile				Inhibitory profile
		1st and 2nd generation cephalosporin	3rd and 4th generation cephalosporin	Aztreonam	Carbapenem	
A	*blaKPC*	-	++	+	++	Boronic acid
B	*blaIMP*, *blaVIM*	++	++	++	-	EDTA, dipicolinic acid
	blaNDM	++	++	-	+	
D	*blaOXA-48*	±	±	±	+	No specific inhibitor is available

Abbreviations: *blaKPC*, the gene coding for Klebsiella pneumoniae carbapenemases (KPC); *blaNDM*, the gene coding for New Delhi metallo-β-lactamase (NDM); *blaVIM*, a gene coding for Verona integron-encoded metallo-β-lactamase (VIM); blaIMP, the gene coding for the imipenem-resistant Pseudomonas-type carbapenemases (IMP); *blaOXA-48*, the gene coded oxacillinase (OXA-48-like) types.

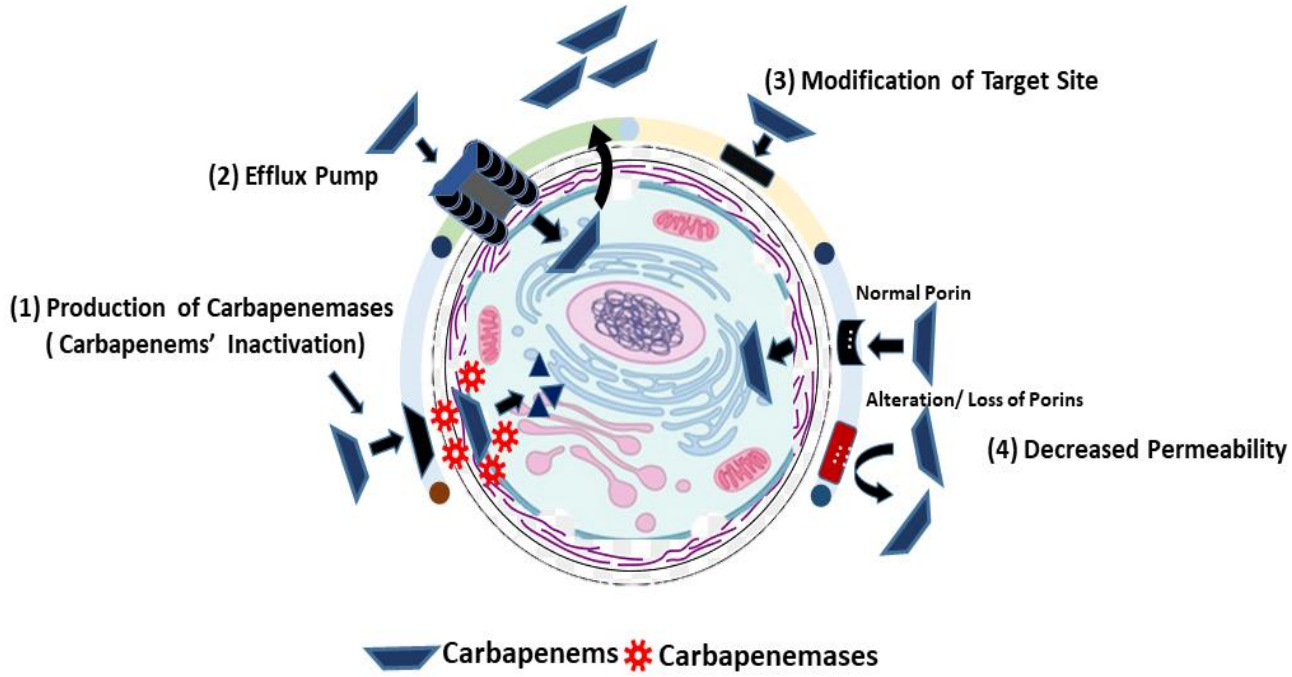

Fig. 1. Different mechanisms of carbapenem resistance.

blaNDM, the gene coding for New Delhi metallo-β-lactamase (NDM); *blaVIM*, a gene coding for Verona integron-encoded metallo-β-lactamase (VIM); blaIMP, the gene coding for the imipenem-resistant Pseudomonas-type carbapenemases (IMP); *blaOXA-48*, the gene coded oxacillinase (OXA-48-like) types.

Class A serine carbapenemases

Class A carbapenemase relates to functional group 2f which can hydrolyze almost all β-lactam antibiotics including carbapenem, cephalosporins, penicillin, and aztreonam. Nevertheless, tazobactam and clavulanate can inhibit them. Tazobactam and clavulanate can, however, inhibit them. Serine class A carbapenemases include different subtypes: some are chromosomally encoded as non-metallo carbapenemase (NMC-A); *Serratia marcescens* enzymes (SME) as well as imipenem hydrolyzing β-lactamase (IMI-1), while others are plasmid-encoded like *K. pneumoniae* carbapenemase (KPC); (IMI-2) and Guiana extended-spectrum (GES). In general, clavulanic acid partially inhibits class A carbapenemases, which can effectively hydrolyze carbapenems **[33]**.

Generally, SMEs are typically restricted to *Serratia marcescens* while IMI and NMC-A enzymes are occasionally found in *Enterobacter cloacae* (*Ent. cloacae*). The truth about being chromosomally encoded may shed light on why it is so infrequently reported globally **[34]**. In contrast, plasmid-mediated genes were widely accepted throughout the world. Of all the previously mentioned enzymes, KPC is the most popular with a global public health concern **[35]**. Although there were 23 variants found, KPC-2 and KPC-3 are still among the most abundant variants globally **[36]**. Other enteric bacteria such as *K. oxytoca*, *Ent. cloacae*, and NF GNB, which are similar to *P. aeruginosa* and *A. baumannii*, have been found to produce KPC-2 **[37]**. In the USA and Israel, nosocomial *K. pneumoniae* has

frequently been found to produce KPC-3. Furthermore, it has been reported that KPC producers are endemic in Greece, and the number of cases in Italy and France has also increased **[38]**.

Class B metallo β-lactamase

Metallo β-lactamases (MBLs) belong to a superfamily of enzymes with a wide range of catalytic diversity. Such enzymes can hydrolyze all β-lactam antibiotics excluding monobactams. According to the DNA sequence alignments, MBLs are further classified into three subclasses B1, B2, and B3. Despite the low degree of resemblance between determinants, this classification is supported by crystallographic analysis of the corresponding enzymes [39]. MBLs are reported for their ability to hydrolyze all β-lactams other than aztreonam, and their activity is inhibited by ethylene diamine tetra acetic acid (EDTA) but not clavulanic acid **[40]**.

The most prevalent members of MBLs family are Verona integron encoded metallo-β-lactamase (VIM), imipenemase (IMP), Sao Paulo metallo-β-lactamase (SPM), Seoul imipenemase (SIM), German imipenemase (GIM), and New Delhi metallo-β-lactamase (NDM-1). The first IMP-1 to be reported was in *S. marcescens* from Japan **[41]**. Subsequently, MBLs have been identified worldwide with high mortality rates ranging from 18% to 67% (Nordmann et al., 2011a). Additionally, outbreaks and single reports of VIM or IMP MBLs producers have been noted in numerous Mediterranean countries, including Egypt **[44]**.

In 2009, NDM-1 was first discovered among *K. pneumoniae* and *E. coli* isolates from a Swedish patient who has been medically treated in India [45]. The emergence of NDM-1 among *E. coli* was a major threat as this represents a real opportunity for patients to infect themselves with their resistant flora causing treatment failures **[37]**. Moreover, genetic studies have highlighted that these enzymes are encoded on highly transmissible plasmids along with 16S ribosomal methylases conferring resistance to all aminoglycosides, macrolides (esterases) quinolones (Qnr), and chloramphenicol antibiotics **[46]**.

Class D serine oxacillinases

Oxacillinases (OXA-β-lactamases) were originally named for their capacity to hydrolyze oxacillin and cloxacillin at a rate of greater than 50% compared to benzyl penicillin. Class D included OXA-type ESBL and OXA-type carbapenemase. The OXA carbapenemase involved OXA-23-like, OXA-24-like, OXA-48-like, OXA-51-like and OXA-58-like. OXA-48 is one of the major enzymes with strong hydrolyzing activity against penicillin and weak hydrolyzing activity against carbapenem and ESBLs **[31]**. However, its association with ESBLs boosted the CR **[47]**. Initially, The OXA-48 was reported in *K. pneumoniae* from Turkey. From that time on, strains producing OXA-48 have been broadly spread as a cause of nosocomial outbreaks in Mediterranean countries such as Egypt **[48]**.

4.2. Modification of target sites

Bacteria can escape the action of certain antibiotics by changing the targeted site of action. This escapism mechanism can be started against all classes of antimicrobial agents regardless of their mechanism of action. Modifications of target sites are often attributed to genetic mutations as a reaction to selective pressures in the presence of antimicrobials, nevertheless, modified targets may be acquired by genetic exchange **[49]**.

4.3. Porin-mediated Resistance and cephalosporinases production

Porins are outer membrane proteins (OMPs)

that can create pathways for molecules to move across lipid bilayer membranes within GNB; as a result, altering the structure of porins or porin loss can offer a defense against the pressure of antimicrobials. The intrinsic resistance amongst *A. baumannii* and *Pseudomonas* spp. can be contributed to the limited number and small size of porins compared to other different GNBs. Recently, the reduced expressions of mainly carbapenem-associated OMP (CarP) and Omp 33-36 have been included in CR among *A. baumannii* **[50]**. For *P. aeruginosa* strains, the loss of outer membrane porin (OprD) - a particular substrate from which carbapenems enter periplasmic space - will substantially reduce the susceptibility to carbapenems **[51]**.

AmpC β-lactamase enzyme overexpression coupled with porin loss and efflux mechanism can also result in CR **[52]**. AmpC β-lactamase is a class C cephalosporinase enzyme produced by different Enterobacteriaceae members. The enzyme is either encoded with plasmid or chromosomal-mediated genes. The majority of resistance in *Enterobacter*, *Serratia*, *Pseudomonas*, *Acinetobacter*, and *Citrobacter* spp. is frequently chromosomally mediated **[53]**.

The inducible AmpC enzyme's mutational overexpression grants resistance to third-generation cephalosporins like cefotaxime, ceftazidime, and ceftriaxone. In the case of *Enterobacter* spp. infections, the issue is particularly critical since isolates are typically resistant to most β-lactam antibiotics but carbapenems. Moreover, isolates that show high sensitivity towards third-generation cephalosporins can confer resistance after treatment **[54]**. Of particular concern in recent decades is the prevalence of plasmid-mediated AmpC genes among the majority of Enterobacteriaceae including *Klebsiella* spp., *Proteus mirabilis*, and *Salmonella* spp. which remain clinically significant leading to complicated treatment options **[55]**.

4.4. Antibiotic efflux

Efflux pumps are often able to determine several substrates because affinity is based not on chemical structures, but rather on physiochemical properties (e.g., hydrophobicity, aromaticity, or electric charge). This explains the prevalence of MDR efflux pumps, which may expel some structurally unrelated antibiotics along with other substances like naturally occurring host products involving bile salts and specialized host-defense molecules **[56]**. GNB including *Acinetobacter* spp. and *P. aeruginosa* are known for their efflux-mediated resistance to β -lactams **[49]**. In the presence of numerous hydrophobic small molecules, the structural basis of the inner membrane pump AcrB has been determined, which suggests that each ligand has a different binding mode, at least in this efflux pump component **[57]**. Another study revealed that 98 of the 298 Escherichia coli carbapenem-resistant isolates were shown to have efflux pump-mediated resistance. This demonstrated that the AcrAB pump plays a significant role in the development of resistance against the carbapenem class of antibiotics and is a crucial antibiotic resistance determinant in the tested bacterial pathogens **[58]**. Also, the resistance-nodulation-division-type efflux system AdeABC plays a crucial role among CR *A. baumannii* **[59]**.

5. Detection methods of GNB carbapenem resistance

To ensure proper infection control measures, the diversity and complexity of CR mechanisms, particularly carbapenemase production, calls for quick and precise methods of detection. The following paragraphs discuss the techniques available for phenotypic detection and molecular characterization of Enterobacteriaceae (CPE) and other non-fermenting CPOs.

5.1. Phenotypic screening of carbapenemase-producing GNB

Phenotypic screening of carbapenemase producers could be challenging since the elevated minimum inhibitory concentration (MIC) typically does not take this into account. Initially, KPC enzyme-producing isolates were not identified because the tested carbapenem's MIC was within the susceptible range. Conversely, isolates with different CR mechanisms, such as porin loss coupled with cephalosporins production, displayed high MIC. Therefore the clinical laboratory standard institute (CLSI), European Committee on Antimicrobial Susceptibility Testing (EUCAST), and CDC are in frequent states of adjusting breakpoints and cutoff values to avoid missing potential CPOs **[32]**.

Clinical Laboratory Standard Institute (CLSI) Guidelines

In 2009, the CLSI advised performing the modified Hodge test (MHT) to investigate Enterobacteriaceae with carbapenem MIC values between 2 µg/mL to 4 µg/mL and revealing resistance to all third-generation cephalosporins. However, the production of OXA-48, which may be sensitive to carbapenems other than ertapenem and third-generation cephalosporins, has complicated the implementation of this recommendation. In 2010, CLSI had reduced carbapenem breakpoints based on clinical outcome review, MIC distribution, and pharmacokinetics and drug dynamics. From 2015 until now, the CLSI published that carbapenemase-producing isolates usually exhibit intermediate (I) or resistant (R) patterns to one or more carbapenems. Since tested isolates have always been less sensitive to ertapenem, it is thought to be the most sensitive indicator of CPE. The current interpretive criteria also reveal that carbapenemase producers frequently exhibit resistance to one or more agents of third-generation cephalosporins. However, some SME or IMI-producing isolates are frequently sensitive to 3rd generation cephalosporins. The CLSI mandated in 2020 that all isolates that produce carbapenemase and have imipenem, meropenem MICs of 2–4 µg/mL or ertapenem MIC of 2 µg/mL should be examined using the Carba NP test, modified carbapenem inactivation method (mCIM), or a molecular assay producing isolates are frequently susceptible to 3rd generation cephalosporins **[60]**.

Screening chromogenic plate

Several chromogenic media have been marketed for the presumptive screening of carbapenemase producers in high-risk patients. The incorporation of chromogenic enzyme substrates, primarily glycosides that are hydrolyzed by bacterial enzymes to release pigment, is the main basis for the chromogenic media. Supercarba agar is a specialized medium that uses ertapenem to select CR characteristics, cloxacillin to inhibit AmpC, and zinc to simplify MBLs detection in Drigalski lactose agar **[61]**. An additional screening method called CHROM agar was created exclusively to identify KPC producers with MIC values of less than or equal to 4 µg/mL **[62]**.

Carbapenem inactivation method (hydrolysis method)

This method relies on carbapenem enzymatic hydrolysis in the presence of CPOs. Based on this methodology, several testing techniques, such as the Modified Hodge test (MHT), modified carbapenem inactivation method (mCIM), colorimetric assays, and mass spectrometry, were developed **[63]**.

Modified Hodge test (MHT)

For the identification of carbapenemase-producing Enterobacteriaceae (CPE), it is advised to conduct a phenotypic CLSI confirmatory test

of MHT or the cloverleaf test. The test is primarily used in developing nations where genotyping facilities are not always available. The test is based on the inactivation of carbapenem by carbapenemase-producing organisms by enabling the indicator organism to expand growth toward the disk and along the streaked tested organism **[64]**. Although MHT frequently has a high sensitivity that exceeds 90%, it does not provide details regarding the specific type of carbapenemase involved **[65]**. Additionally, false positive results may be found among isolates displaying CR other than the production of carbapenemase including the production of ESBLs or AmpC β-lactamases accompanied by porin loss **[66]**.

Modified carbapenem inactivation method (mCIM)

In 2020, CLSI suggested the phenotypic test-modified carbapenem inactivation technique (mCIM) for the detection of CPE utilizing easily accessible laboratory reagents. Briefly, a meropenem disk is momentarily submerged in a bacterial suspension of the tested strain for at least 4 h. The disk would subsequently be transferred to a plate inoculated with *E. coli* ATCC 29522 and then incubated overnight. The absence of an inhibition zone shows the development of carbapenemase production. The test has a sensitivity and specificity of over 99% for CPE **[60]**.

Blue-CARBA test

Blue- CARBA and Carba Nordmann-Poirel (Carba NP) are examples of colorimetric assays which rely on color change of pH indicator either bromothymol blue for the former test or phenol red for the latter test upon carbapenem hydrolysis. Blue-Carba is a Carba NP modification that is directly carried out on bacterial colonies instead of using bacterial extract. However, recent Carba NP modifications have also made it possible to use bacterial colonies **[67, 68]**.

Mass spectrometry

Recently, routine bacterial and fungal detection in clinical laboratories has been accomplished using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). The MALDI-TOF can also be used for quick identification of CPE via the determination of specific degrading carbapenem products following bacterial enzymatic hydrolysis **[69]**. The effectiveness of this approach for identifying CR in Enterobacteriaceae and *Pseudomonas aeruginosa* caused by carbapenemase production has been confirmed in earlier studies **[70, 71]**. Additionally, other studies have examined the application of quantitative MALDI-TOF for quick detection of resistance by comparing a correlation of microbial growth in the presence or absence of meropenem **[72]**. Moreover, the inclusion of specific carbapenemase inhibitors in the assay identifies the type of carbapenemase involved **[73]**. In addition to MALDI-TOF, the research settings employ other mass spectrometry systems such as liquid chromatography, ultra-performance liquid chromatography, and polymerase chain reaction electrospray ionization to detect carbapenemases **[67]**.

Inhibitor based approach

Inhibitor-based Tests depend on the ability to use specific substances that specifically inhibit the activity of carbapenemases. While KPC detection relies on the use of phenylboronic acid, MBLs phenotypic detection is primarily based on the use of various chelating agents such as EDTA, dipicolinic acid, 1, 10-phenanthroline, and thiol compounds (PBA) **[74]**.

The double disc synergy test, the combination disc test, and gradient diffusion strips are a few examples of inhibitor-based

approach tests that are frequently used in clinical laboratories. The idea of a synergy test depends on the use of a carbapenem disk close to a disk with an MBL inhibitor, hence the term double disk synergy (DDST). These chelating substances work by reacting with zinc rendering it inactive against β-lactam and therefore, the synergy pattern suggests MBLs production. On the other hand, KPC-producing isolates can be found using the inhibitory action of boronic acid and its derivatives, such as phenyl boronic acid and 3-amino-phenylboronic acid, which share structural similarities with β-lactam. The interpretation of the synergy effect is arbitrary and cannot be quantified **[74]**. To overcome the challenges associated with DDST interpretation, the combined disk test (CDT) relied on using a carbapenem disk either meropenem or imipenem along with. a combination of carbapenem and a carbapenemase inhibitor disk. The latter disk's potentiated activity above a predetermined cutoff value indicated the production of carbapenemase **[75, 76]**. Similar to CDT, Bio-Merieux has a variety of gradient diffusion E-test strips available for the detection of MBLs that contain double-sided carbapenem dilution and carbapenem combined with EDTA at a fixed concentration. A reduction of > 3-fold of the Carbapenem MIC in the presence of the inhibitor is always a marker for MBLs production **[75]**.

5.2. Carbapenemase-producing bacteria's molecular characterization

The development of molecular tools triggered a revolution in the treatment of contagious diseases by providing a plethora of data regarding the disease's origin, virulence factors, and resistance determinants that influence disease severity **[77]**. Compared to a culture-based method, the molecular test of carbapenemase genes based on nucleic acids has improved sensitivity and saved time and effort. Furthermore, without the need for cultivation, common carbapenemase genes could be detected directly from positive blood cultures, rectal swabs, and stool samples **[24]**. Based on the benefits previously listed, molecular methods are regarded as the gold standard for quick carbapenemase characterization. The most widely used molecular assays for CPE detection include the polymerase chain reaction, microarray, isothermal amplification technology, and whole genome sequencing **[44]**.

Polymerase chain reaction (PCR)

PCR is currently the most widely used molecular tool to identify and detect carbapenemase genes. PCR is either monoplex (single) or multiplex method. In the former, an interest target is amplified, whereas, in the latter, multiple interest targets can be simultaneously amplified by using multiple pairs of primers. The most prevalent carbapenemase genes, including KPC, NDM, IMP, VIM, and OXA, can be targeted and quantitated using a variety of commercial real-time PCR assays with 100% sensitivity, including xpertcaba R Hyperplex superbug ID and Check-Direct CPE **[78]**. Despite the benefits of the PCR technique over phenotypic tests that have already been mentioned, its major disadvantage is that it is unable to identify novel, unidentified carbapenemase genes that are constantly undergoing new variants. In the end, these techniques are relatively expensive and require highly skilled microbiologists **[76]**.

Microarray

In microarray technology, several DNA probes are used to hybridize with DNA of interest, including resistance genes. Microarray technology allows the multiplexing of various carbapenemase genes with an improved ability to detect closely related variants. To detect carbapenemases, numerous microarrays

including the Verigene, Biofire, and Checkpoints have been created. The Verigene is a nearly fully automated GNB blood culture that makes it simple to identify the five main carbapenemase genes along with CTX-M of ESBLs **[79]**.

Whole genome sequencing

Whole genome sequencing has been one of the most promising tools for rapid pathogen identification in clinical microbiology laboratories over the past ten years. Bioinformatics tools and advancements in DNA sequencing technology have made it possible to analyze and quickly identify antibiotic resistance genes **[80]**. Additionally, metagenomic sequencing showed large reservoirs of antibiotic-resistance genes that exist in natural environments like soil or surface water. Furthermore, the discovery of novel secondary metabolites with antimicrobial properties may also be facilitated by genome sequencing methods. Recent research has emphasized the value of whole genome sequencing in the study of genomic epidemiologies, with a focus on the spread of significant MDR *K. pneumoniae* and mcr-1 colistin-resistant genes. Whole genome sequencing is being used more frequently in clinical microbiological laboratories for epidemiological purposes by lowering costs and speeding up analysis **[81]**.

6. Infection control strategies and antibiotic stewardship

6.1. Infection control strategies

As a result of the current state of antibiotic therapy, infection prevention strategies continue to be our best chance at preventing the spread of these concerning species. The cornerstone of preventative control measures is good hand hygiene and standard precautions, along with interventions that are customized to the resources available and the situation. The world health organization (WHO) has just released guidelines for controlling and preventing CR in healthcare facilities amongst Enterobacteriaceae, *A. baumannii*, and *P. aeruginosa* **[82]**.

Hand hygiene

Maintaining good hand hygiene is essential for preventing the spread of microbial pathogens. Therefore, everyone, especially the healthcare staff, needs to practice proper hand hygiene whenever and wherever it is possible to avoid infection and prevent antibiotic resistance. As long as hands are not soiled, proper hand hygiene can be preserved by using soap, water, and appropriate alcohol-based hand rubs **[83]**. Since several studies have shown that CR GNB rates have considerably decreased since using this technique, the impact of hand hygiene strategies should not be ignored **[84]**.

Potential carriers screening

The screening of infected or colonized cases is one of the most essential infection preventive measures. Based on the epidemiology of the reflecting population, screening should be carried out. In low prevalence settings, testing rectal swabs sent for culture is advised to protect high-risk patients, such as those hospitalized abroad or in endemic institutions. Fast detection is crucial for CRE asymptomatic carriers as well because they serve as a reservoir for transmission. However, high-prevalence institutions, especially those in endemic regions or those that have experienced an outbreak, should fully consider active screening, advanced isolation, and contact precautions for all risk patients **[83]**.

Patient isolation and contact precautions

To prevent the spread of infectious agents within the patient's environment, contact precautions are a crucial component of infection control strategies. According to the WHO guidelines, contact precautions could be achieved by using personal protective equipment such as gowns or gloves, patient movement regulation,

and patient isolation **[82]**. Patient isolation and cohorting which include grouping infected patients with the same infectious agent together to decrease the number of secondary cases and control outbreaks in various settings **[85]**. According to one study, the median carrying period is three months **[86]**. However, other research has shown a prolonged period of up to a year **[87]**.

Feldman and colleagues conducted a prospective study on patients who had positive KPC screening test results. Within 30 days, 75% of patients continued to be positive, but after 6 months, less than 30% of patients did. The study associated catheterization, poor functional status, recent acquisition (4 months), and extended hospitalization with the prevalence of positive screening cultures **[88]**. Therefore, many studies have recommended patient isolation in a single room for restricting CRE transmission **[89]**.

Environment cleaning

Cleaning procedures received more consideration because it was thought that some outbreaks may have been caused by environmental contamination with CRE **[90]**. Sodium hypochlorite bleaching wipes have been used for routine cleaning of any high-touch surfaces to increase the effectiveness of environmental cleaning in an outbreak setting. In addition, patient rooms and equipment were decontaminated with hydrogen peroxide vapor to reduce the environmental bioburden by MDR organisms **[91]**. Recently, WHO guidelines strongly urged patient zone environmental clean-up protocols to be followed immediately for better results **[82]**.

Other advanced infection control measures

Most of the infection control measures mentioned above were used in hospitals in middle- and high-income nations. However, in developing nations with limited hospital resources, hand hygiene with common precautions combined with ongoing compliance audits for infection control measures should be implemented **[3]**. In addition, other infection control methods such as daily chlorhexidine baths for patients and visitation restrictions have also been used to decolonize patients and stop the spread of CRE in various outbreaks **[3]**.

Applying antimicrobial stewardship for preventing CRE emergence

The proper use of antibiotics is essential for patient safety as well as issues related to public health, and it is now recognized as a global priority. The goals of hospital-based "Antibiotic Stewardship Programs (ASPs)" are to raise patient care standards and lower antimicrobial resistance. The ASPs use an integrated approach that includes careful selection of an appropriate antimicrobial agent with appropriate dose adjustment, administration route, and optimal length of therapy. Additionally, reducing and minimizing antibiotic therapy once the results of the susceptibility testing are known will support efficient ASPs **[31, 92]**.

Considering the urgent need to improve antibiotic use in hospitals, the CDC advised in 2014 that all acute care hospitals adopt ASPs. The fundamental components of ASPs depend on leadership dedication and drug knowledge to track and report patterns of antibiotic use and resistance among patients and staff. More information is needed to determine the actual impact of using such programs on the emergence or even persistence of CRE in infected or colonized patients **[3]**. Recently, Horikoshi and his colleagues reported the benefits of using an ASP that existed for the past six years along with a further decrease in the use of carbapenems.

Additionally, *P. aeruginosa* resistance, hospitalization length, and infection-related mortality were all reduced **[93]**.

7. Treatment strategies

Effective treatment for infections caused by carbapenem-resistant bacteria is currently hampered by the lack of randomized clinical trials. Although polymyxins, tigecycline, and aminoglycosides were considered the drugs of choice for infections caused by these bacteria. Nevertheless, recent studies estimate that the resistance rate to these antibiotics exceeds 35% **[94]**. The different approaches used to control carbapenem-resistant Gram-negative-associated infections are displayed in **Fig. 2**.

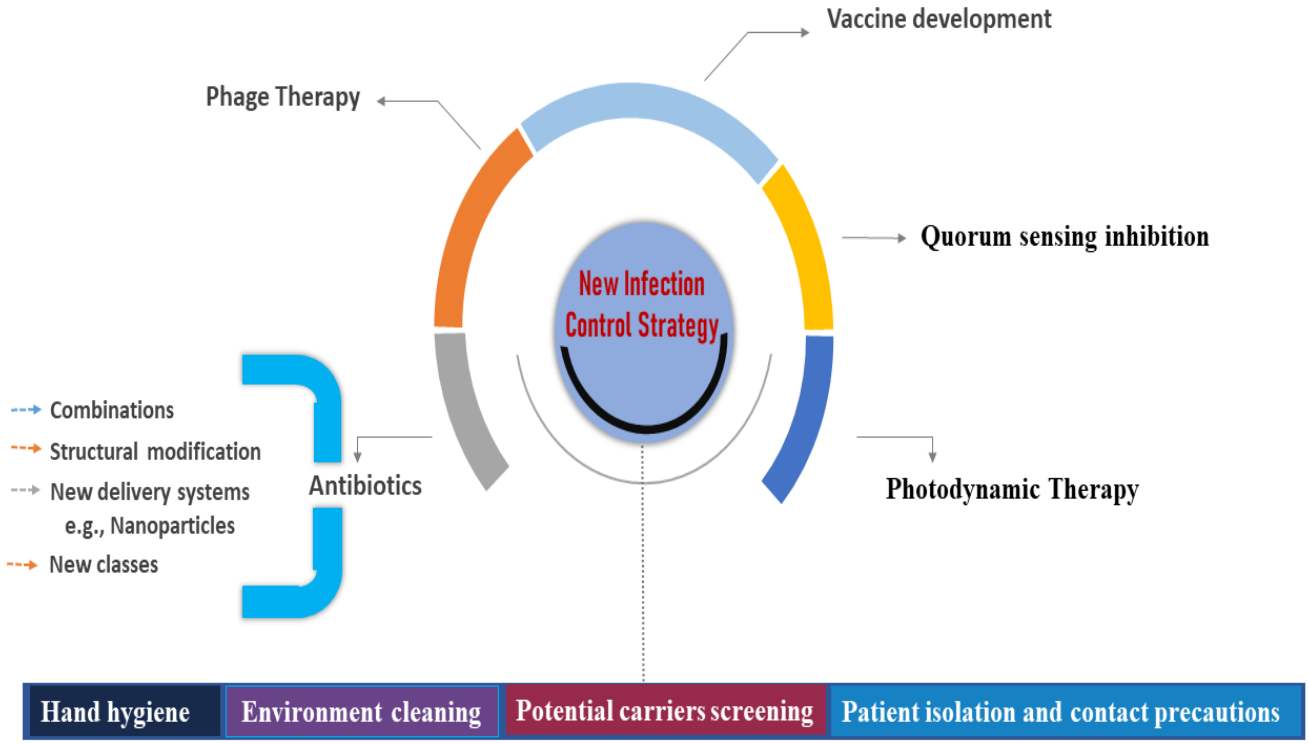

Fig. 2. Different approaches used to control carbapenem-resistant gram-negative-associated infections.

7.1. Monotherapy versus combination therapy

Because CPOs frequently reveal an MDR or even pan-drug resistant phenotype to the currently prescribed antibiotics, it might be advantageous for patient management to look for stagnant antimicrobial agents **[95]**. However, with notable treatment failures comes an increase in resistance to these drugs. It is strongly advised that various antimicrobial combinations with synergistic effects be tested due to the nature of MDR. Combination therapy can also increase effectiveness by broadening its spectrum, reducing resistance, and perhaps even reducing mortality rates **[96, 97]**. The evidence supporting combination therapy versus monotherapy for CR GNB involving Enterobacteriaceae, *Pseudomonas*, and *Acinetobacter* spp. is briefly summarized in the following paragraphs.

Polymyxins

Polymyxin B or colistin (polymyxin E) are

the most widely used polymyxin bactericidal antibiotics with cationic detergent properties for treating CR GNB. To achieve high plasma levels and a lower loading dose, the former differs from the latter by one amino acid (phenylalanine instead of D-leucine) **[98]**. According to Bergamasco and his colleagues, 67% of solid organ transplant recipients who had KPC-producing *K. pneumoniae* survived. In this study, all but one patient had received antibiotics, either polymyxin B alone or in combination with tigecycline or carbapenem in the previous 30 days **[99]**. Furthermore, even if the concentrations greatly exceed those that have been clinically achieved, in vitro growth and the emergence of monotherapy resistance may also take place. Although polymyxin B combination therapy with other antibiotics seems like a good option, there is no clinical evidence to support this claim **[100]**.

Colistin is administered as colistimethate sodium, an inactive pro-drug that must be transformed into an active form in the body. However, only a small portion is gradually converted, so for the first 12 to 24 h, a sufficient loading dose is needed to achieve therapeutic benefit. Additionally, polymyxins only effectively kill a small number of isolates with high MICs. The currently available dose scheme of colistin monotherapy is also not advised for isolates with MIC > 0.5 μg/mL. numerous studies, therefore, point to the significance of combination therapy **[101]**.

Zarkotou and his colleagues have conducted a cohort study to predict deaths in KPC-producing *K. pneumoniae* BSIs patients and the impact of appropriate antimicrobial therapy. They discovered that while four out of seven patients died from colistin monotherapy, none of the 14 patients who received colistin in combination with tigecycline, carbapenem, or gentamicin did. The emergence of resistance strains and nephrotoxicity should also be taken into consideration, even though prior studies demonstrated the role of colistin in combination therapy against KPC-producing *K. pneumoniae* strains **[102]**.

Carbapenems

At first glance, it might seem paradoxical to use carbapenems, but they are typically the most frequently prescribed adjuvant in the combination of the CRE drug control scheme. This was mainly because CRE shows a MIC range between 1-4 μg/mL that is close to or equal to sensitivity break points, especially for meropenem or doripenem **[103]**. The application of this method is therefore dependent on the determination of MIC, and it may be beneficial if the MIC of the infecting CRE is still relatively low, i.e. not exceeding 4-8 μg/mL **[101]**. Additionally, it had been suggested to use a double-carbapenem combination approach to treat KPC-producing bacteria. This strategy explains that ertapenem is easily hydrolyzed by KPC as well as doripenem which increased stability against KPC. Experimental data demonstrated the efficacy of a double carbapenem regimen for CP *K. pneumoniae* and colistin-resistant KPC-producing *K. pneumoniae* infections **[104]**. Additionally, adding colistin to a double carbapenem regimen was successful in eliminating bacteria within 24 h **[105]**.

Tigecycline

Tigecycline is a minocycline derivative of the glycylcycline class with in vitro activity against GNB and Gram-positive bacteria. It served as an adjuvant in combination therapy to treat CRE and CR *A. baumannii* infections **[106]**. However, tigecycline clinical experiences were particularly discouraging for serious infections like bacteremia and nosocomial infections **[107]**. Additionally, many clinicians continued to use tigecycline as their last resort treatment for CR

bacteria due to its suboptimal concentration in urine, blood, and the respiratory system [108]. Consequently, combination therapy and increased tigecycline dosage may provide a positive clinical result **[109]**.

Fosfomycin

Fosfomycin is an old broad-spectrum phosphonic acid derivative and is now an effective alternative against CR GNB. It is accessible in two pharmaceutical forms, orally or parenterally. The former formulation is known as fosfomycin tromethamine, which can quickly reach high urine levels and is hence frequently used in uncomplicated UTIs **[110]**. The latter is a fosfomycin disodium intravenous formulation that is commonly regarded as an adjuvant treatment for CRE **[111]**. Pontikis and colleagues investigated the effects of parenteral fosfomycin in combination with either colistin or tigecycline for XDR carbapenemase producers. Bacterial eradication was seen in 56.3% of cases, and fosfomycin resistance appeared in three of those cases **[112]**. There is an increasing interest that combination therapy can stop the emergence of such resistance because fosfomycin has a rapid potential to select resistant mutants during adjuvant therapy **[103]**. The emergence of resistance is particularly significant since fosfomycin has desirable pharmacokinetic properties that make it effective in cases of difficult and deep-seated infection **[113]**.

Aminoglycosides

Aminoglycosides have been used for more than 50 years to treat a variety of pathogens. Typically, gentamicin and to a lesser extent amikacin were used to show in vitro susceptibility to KPC and VIM enzymes **[94]**. According to the data examined by Tzouvelekis and his colleagues, aminoglycoside therapy is thought to be the most effective, whether used as a monotherapy or in combination with other treatments. It should be noted that while aminoglycoside combination therapy with carbapenem has a lower mortality rate, aminoglycoside monotherapy is particularly effective in UTIs with or without secondary bacteremia **[101]**. Additionally, recent research had demonstrated the great clinical effectiveness of aminoglycoside combination therapy against CRE **[114]**.

Rifampicin

Rifampicin is a rifamycin derivative, with a broad spectrum of activity against both Gram-positive and Gram-negative pathogens. Besides its ability to display rapid levels of resistance in vivo or in vitro if used alone, its potential role as an adjunct has been investigated. Polymyxins and rifampicin were combined to treat CR GNB, specifically MDR *A. baumannii* **[103]**. Although *A. baumannii* microbiological clearance was increased, clinical benefits in terms of improved patient survival were dubious **[115]**. According to a recent meta-analysis study, 72 % of CR *A. baumannii* showed decreased susceptibility to rifampin when used alone whereas 63% of isolates showed a synergistic effect by adding colistin **[116]**.

Aztreonam

Aztreonam is a monobactam antibiotic with a distinct activity among the clinically available β-lactam group because it is resistant to hydrolysis by CR GNB-producing MBL. Most pathogens that can produce MBLs can also produce other enzymes like ESBLs and AmpC that can render aztreonam inactive, raising questions about its clinical use. Aztreonam has been shown to have greater in vivo effectiveness than carbapenems against VIM-1-producing *E.* coli in the rabbit experimental model as four animals in the aztreonam group (26.7%) had culture-negative pus and no mortality was recorded. **[117]**. According to a recent study, colistin and

aztreonam work well together to treat MDR *P. aeruginosa* infections both in vitro and in vivo **[118]**.

7.2. Association of antimicrobial agents with non-antimicrobial agents

Despite the widespread use of antibiotics in the pharmaceutical industry, the misuse of these drugs accelerated the emergence of drug-resistant microorganisms. As a result, scientists have concentrated their efforts on developing a new strategy to combat this widespread bacterial resistance **[119]**.

Early in the 1960s, James W. Black developed the drug propranolol, which is now widely used for a variety of conditions, including hypertension, thyrotoxicosis, and antipsychotics. Numerous mechanisms of action for propranolol have been reported, including anti-proliferative, antiangiogenic, anti-lymphangiogenic, pro-apoptotic, and immune-modulating, with support from a variety of data sources to reduce cancer types and to improve oral bioavailability via bypass the drug among efflux transporter **[120]**.

In the case of GNB, using antihypertensive medications suggests that inhibiting the pumps may be a good way to not only combat this bacteria's resistance but also to make Gram-negative bacteria that are "intrinsically" resistant susceptible to a variety of medications. Alternately, cationic peptides can permeabilize the outer membrane, making bacteria more susceptible to antibiotics, particularly those that are lipophilic **[121]**. Recent reports indicate that propranolol has potent adverse effects on cell viability and growth, and it has been suggested as a potential treatment for cancer **[120]**.

Patients who have bacterial infections often experience fever and other types of pain, which necessitates combining treatment with non-antimicrobial agents like antipyretics and non-steroidal anti-inflammatory drugs (NSAIDs) to treat these symptoms. NSAIDs, like diclofenac, enhance ciprofloxacin's ability to decrease the MIC. In addition, it appears that the ability of non-antibiotic agents to increase or decrease the activities of some efflux pumps in Gram-negative rods is their most significant benefit, in addition to their therapeutic use **[122]**. Antipyretics and NSAIDs are widely used in conjunction with antimicrobial therapy, affecting microbe sensitivity to antimicrobial therapy by changing microbe hydrophobicity, impacting biofilm development, and interfering with drug transport and release **[123]**.

7.3. Introduction of novel therapeutic approaches to combat carbapenem resistance

Scientists had to come up with new preventive measures and treatment plans to deal with a world without antibiotics due to the global emergence of MDR GNB and the lack of new agents to meet the challenge of resistant strains. As a result, numerous contemporary and historical approaches have been researched to lead us to a brand-new era of antibacterial agents. In the following, we'll go over a few recent advancements in the fields of phage therapy, quorum sensing, photodynamic therapy, structural modification of carbapenems already on the market, and the development of new classes of therapeutic agents that exhibit enhanced activity against MDR pathogens.

Bacteriophage therapy

Phage therapy, or the use of viruses to treat bacterial infections, has a history that is much older than that of antibiotics **[124]**. Phage therapy relies on the use of bacteriophages, a type of naturally occurring antibacterial agent that can control bacterial populations by causing bacterial lysis. In the new millennium, a phage therapy development and genomics explosion had started to address phage therapy as a novel weapon for combating infectious diseases **[125]**. According

to earlier studies, phage therapy may have some advantages over traditional antibiotics, but a small number of adverse events cannot be completely ruled out. Bacteriophages are hosts that have a narrow spectrum of antibiotics, which reduces the risk of secondary infections brought on by antibiotic use. Furthermore, due to their in-situ replication, bacteriophages will grow to reach adequate densities at the infection site, which is why it is known as active therapy. Phage resistance is not as concerning as antibiotic resistance, despite the possibility that bacteria could develop phage resistance **[126]**.

Preparations with a variety of phages, whether they contain antibiotics, can prevent the emergence of phage resistance. Additionally, phage therapy's economic benefits appear promising despite the lengthy and significant treatment period. Despite the benefits already mentioned, there is still a matter of concern when phage therapy is used as a magic bullet. One of the most serious safety concerns is the potential for some phages, particularly temperate phages, to modify host bacteria and make them more pathogenic **[127]**.

A temperate phage is a lysogenic phage that can integrate its genome into bacteria instead of instantly killing the host; as a result, it should always be avoided when using phage therapy. The release of GNB endotoxins, on the other hand, is induced by lytic phages and may result in multiple organ failure **[128]**. Phage therapy may be a different option for treating bacterial infections brought on by MDR. Numerous studies have provided fresh perspectives on the potential application of lytic phage against MDR GNB, particularly *P. aeruginosa* and *A. baumannii* in treating wound infections in animal models **[129, 130]**. It is interesting to note that phage therapy has demonstrated promising outcomes in treating lung infections brought on by CR *A. baumannii* in mice without causing negative side effects **[131]**.

Quorum sensing inhibition

Quorum sensing is a bacterial cell-to-cell communication process that controls the expression of virulence genes, biofilm formation, and antibiotic resistance genes by producing, detecting, and responding to extracellular signaling molecules known as auto inducers. The three main steps of the system are signal detection, auto inducer detection, and auto inducer existence. The auto inducer detection step will stimulate auto inducer production, which encourages synchronization among the bacterial population **[132]**. Therefore, complex interactions, inhibition of quorum synthesis, and molecular degradation may be effective anti-virulence tools. In a recent study, a murine model was used to examine the effectiveness of a recombinant Ahl-1 lactonase formulated as a hydrogel to control the infection of an MDR *P. aeruginosa*-infected burn **[133]**. Theoretically, quorum-sensing inhibition strategies are considered an alternative to or addition to an antibiotic regimen for MDR pathogens because they do not target cell growth or create selective pressure for drug-resistant strains **[134]**.

Photodynamic therapy

Photodynamic therapy (PDT), also known as photodynamic inactivation (PDI), is a novel and optimistic method for eliminating pathogenic microorganisms, such as bacteria and fungi. The PDT is a non-thermal photochemical reaction that utilizes non-toxic dye photosensitizer and low-intensity visible light to produce cytotoxic species when oxygen is present. Gram-positive and GNB have different cell wall structures, and as a result, the former is more sensitive to PDI than the latter bacteria. However, the effectiveness of PDT will be increased by using photosensitizers with a cationic charge or by increasing the outer membrane's permeability

[135]. Unlike antibiotics, PDT does not cause the selection of resistant strains because reactive oxygen species can interact with a variety of structures **[136]**. PDT is therefore believed to have a potential future over traditional antimicrobial therapy for the treatment of MDR pathogens **[137]**.

Structural modification of currently available carbapenems

Tebipenem is the first oral antibiotic to contain the active ingredient tebipenem pivoxil. It is formed by attaching a new side chain to the biapenem molecule's position 2C. In vitro studies of active metabolites have shown their broad spectrum and potent activity against microorganisms that cause UTIs and respiratory tract infections **[138]**. Tomopenem, also known as CS-023, is a broad-spectrum carbapenem antibiotic that can be used to treat HAP and has activity against both GNB and Gram-positive bacteria. Furthermore, with a low rate of spontaneous resistance, it demonstrates potent activity against MRSA, penicillin-resistant *Streptococcus pneumoniae*, ESBL-producing Enterobacteriaceae, and ceftazidime-resistant *P. aeruginosa* **[139]**. Trinems, formerly known as tribactams, had a structure similar to that of a carbapenem and a cyclohexane ring attached between carbons 1 and 2. Orally administered sanfetrinem is effective against bacteria like *Proteus vulgaris* and *Klebsiella oxytoca* that produce powerful Class A β-lactamase **[140]**.

Development of new classes of therapeutic agents

Although there has been significant progress in the clinical development of novel antimicrobial agents that target infections brought on by MDR GNB, there is still a major cause of concern in this area as unfortunately, the current antimicrobial agents under investigation did not encompass all clinically significant GNB **[141]**. In the subsequent paragraphs, we will examine the state of clinical development for newly discovered systemic antibacterial agents.

Eravacycline is a brand-new fluorocycline that resembles tigecycline structurally. Eravacycline was designed to prevent the tetracycline's typical efflux mechanism or to protect the ribosomal target site **[142]**. It has a broad spectrum of activity against Gram-negative and Gram-positive bacteria, but not against *Pseudomonas* species or *Burkholderia cenocepacia* **[143]**. Eravacycline's MIC90 against 9 Enterobacteriaceae species ranged from 0.5 to 2 μg/mL, and its activity was significantly inhibited by ESBLs, CR *E. coli*, and *K. pneumoniae*. Eravacycline also had an impact on *A. baumannii* and *St. maltophilia*, with MIC90s of 0.5 μg/mL and 4 μg/mL, respectively. Therefore, eravacycline is an antibiotic with a clinical activity that looks promising against MDR GNB **[144]**.

Plazomicin (ACHN-490) is a semi-synthetic aminoglycoside derived from sisomicin that is made to be resistant to the majority of clinically significant aminoglycoside modifying enzymes **[145]**. Plazomicin was found to have more potent MICs with in vitro activity of 0.5 to 2 μg/mL against ESBLs and carbapenemase producers, whereas amikacin and gentamicin reached 128 μg/ml and 256 μg/mL, respectively. However, due to the concurrent production of 16S ribosomal ribonucleic acid methyltransferase, it is ineffective against a large number of NDM-producing isolates. Plazomicin has an advantageous safety profile in comparison to colistin and other aminoglycosides. Plazomicin is consequently thought to be a new potential therapy for severe CRE infections **[146]**.

Ceftolozane/tazobactam and ceftazidime/avibactam were among the agents recommended for complicated UTIs and intra-abdominal infections. Ceftolozane is an anti-

pseudomonal cephalosporin with a high affinity for penicillin-binding proteins that also increases outer-membrane permeability and enhances stability against AmpC β-lactamase. Additionally, the combination of ceftolozane and the β-lactamase inhibitor tazobactam is effective against ESBL-producing Enterobacteriaceae and some CR *P. aeruginosa* isolates **[147]**. Ceftazidime-avibactam, which contains avibactam, a novel non-lactam lactamase inhibitor, is effective against a variety of CR GNBs, including some isolates of *P. aeruginosa*, but is ineffective against MBL producers **[148]**.

Avibactam has shown in vitro activity for Ambler class A (ESBL, KPC, and AmpC) and class C (AmpC), as well as some of class D (including OXA-48), and β-lactamase enzymes. However, there is no activity against the *A. baumannii*-producing MBLs (NDM, VIM, IMP) and OXA carbapenemases **[149]**. Although the Food and Drug Administration (FDA) of the United States has already approved ceftazidime/avibactam for the treatment of CRE infections, there are few clinical outcome data for this indication [150]. Avibactam and meropenem were approved by the FDA to treat difficult UTIs. Similar to avibactam, tazobactam was a powerful inhibitor of serine class A producers, such as resistant *Pseudomonas* spp. and *Acinetobacter* spp., as well as class C β-lactamases, with remarkable activity against KPC-producing bacteria **[151]**.

Relebactam is a brand-new non-lactam β-lactamases inhibitor that has been shown to have activity in vitro against β-lactamases of class A, including KPC, and -lactamases of class C **[152]**. Due to porin loss combined with AmpC expression, the combination of sulbactam and imipenem/cilastatin had shown clinical activity against KPC-producing *K. pneumoniae*, other CRE, and CR *P. aeruginosa* **[153]**.

Increasing the effectiveness of currently available carbapenems (new formulations/delivery systems)

Nanotechnology is a cutting-edge field that has a big impact on medical technology, including disease diagnosis, biomarkers, cell labeling, antimicrobial agents, and drug delivery. To get around the limitations of traditional systems, a lot of research has been done in recent years on the creation of new drug delivery systems with controlled and targeted delivery. As a result, drug delivery systems using nanoparticles (NPs) have gained potentiality and effectiveness. To achieve a controlled release of a pharmacologically active agent at a particular site, it is ideal for the design of NPs to focus primarily on controlling the particle size and surface properties **[154]**.

The NPs' ultra-small size and distinctive physicochemical properties allow them to enter the biological systems of both host cells and microbes **[155]**. Metals, metal oxides, and numerous biologically derived materials, such as polymeric NPs, are used to make the majority of antimicrobial nanoparticle carriers **[156]**.

The use of polymeric NP carriers in the delivery of antibiotics has gradually increased in recent years, with a focus on combating antimicrobial resistance and biofilm formation **[157, 158]**. Silver nanoparticles were coated with polyvinyl pyrrolidone to increase the antibacterial activity against the CR strain of *A. baumannii* **[159]**. Recently, Shaaban and his colleagues reported on the effectiveness of polymeric Imipenem loaded poly Ɛ-caprolactone (PCL) and polylactide-co-glycolide (PLGA) nano-capsules in destroying selected imipenem-resistant *k. pneumoniae* and *P. aeruginosa* clinical isolates **[160]**. Other studies highlighted the anti-bacterial activity of lipid-capped copper sulfide and zinc oxide nanoparticles against CR *A. baumannii* **[161, 162]**. Therefore, NPs can be seen as a

diverse array of hope for combating antibiotic resistance among clinically significant GNBs **[163]**.

Vaccines development

Developing new vaccines against pathogenic GNB computationally may be a promising area of study to lower antibiotic resistance **[164]**. Numerous vaccines are being developed that target clinically significant MDR GNB, such as Enterobacteriaceae, *P. aeruginosa*, and *A. baumannii*. A phase 1 trial for a promising biconjugate vaccine against the O antigen of *E. coli* and a protein-based vaccine is currently under development **[165]**. Other vaccines, mainly targeting K1 and K2, that target the capsular serotype of hypervirulent *K. pneumoniae* are still in the preclinical stage **[166]**. Monoclonal antibodies are a logical treatment and prevention option for sepsis brought on by the previously mentioned MDR GNB, and they are likely to become more common shortly **[167]**.

Conclusion

The threat to public health posed by the rise of CR has substantially increased globally. Bacterial resistance to carbapenems can be attributed to numerous mechanisms, such as decreased uptake, active carbapenem efflux, and inactivation via carbapenemases. Several molecular techniques, such as polymerase chain reaction, microarray, isothermal amplification technology, and whole genome sequencing, are now accessible for the detection of carbapenemase genes. Glycopeptides, fosfomycin, colistin, tigecycline, plazomicin, and novel tetracyclines like eravacycline are the last line of defense against infections caused by carbapenem-resistant bacteria. Hence, the administration of these last-resort antibiotics should be restricted to hospital intensive care facilities and only provided under rigorous medical monitoring to prevent antibiotic misuse or overuse.

Future perspective

Additional research is essentially required to determine the best course of action for serious Carbapenem Resistant Gram-negative-associated infections. Alternative approaches for combating such infections rather than those discussed in our review should be taken into consideration. More of the phage therapy, quorum sensing inhibition, and antibiotic combinations that showed promising results in vitro should immediately go for further clinical trials to ensure their safety and efficacy *in vivo*.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent to publish

Not applicable.

Availability of data and materials

The data generated or analyzed during this study are included in the main manuscript file.

Competing interests

The authors have no competing interests.

Funding Statement

No funding source was received.

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