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WHAT DO WE KNOW SO FAR ABOUT GES CARBAPENEMASES, AND WHAT THREAT DO THEY POSE?

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Abstract. Carbapenemases, classified as bacterial enzymes, have the ability to hydrolyze carbapenems – important broad-spectrum antibiotics. This work attempts to summarize the information on the diversity of Guiana Extended-Spectrum (GES) subgroup of carbapenemases, and highlights the serious threat posed by infections caused by bacteria capable of producing these enzymes. The structure, functional characteristics, classification of different types of GES carbapenemases and diagnostic methods are discussed in detail. There are 59 GES-type carbapenemases, which have different amino acid sequences of the protein chains as well as activity against various antibiotics. Currently, bacterial strains with antibiotic resistance of the GES type are treated with: cefiderocil belonging to the cephalosporins, eravacycline belonging to the tetracyclines, lefamulin belonging to the pleuromutulins, colistin, fosfomycin, nitrofurantoin, tobramycin, amikacin, imipenem with relebactam, meropenem with waborbactam, ceftazidime with avibactam and plazomycin. In addition, the following drugs are under study: durlobactam with sulbactam, taniborbactam and cefepime with enmetazobactam This paper aims to summarize the current knowledge on GES-type carbapenemases, their diagnosis and treatment.

Keywords: antimicrobial resistance, carbapenemases, Guiana Extended-Spectrum, carbapenemases, carbapenemase-producing strains

1. Introduction

Gram-negative bacteria continue to pose a severe threat to the health and lives of people around the world due to their constantly increasing drug resistance. Carbapenems, strong antibiotics from the beta-lactam group used to treat severe infections caused by these microorganisms, have been named antibiotics of last resort. However, carbapenemase enzymes produced by bacteria can inactivate carbapenems, thus significantly complicating the effective treatment of infections (Chmielewska and Leszczyńska 2019).

Carbapenemases are bacterial enzymes that hydrolyze the β-lactam bond present in carbapenems. These beta-lactamases also can deactivate penicillins, cephalosporins, and monobactams, which shows how broad their spectrum of action is (Queenan and Bush 2007).

Until the early 1990s, all carbapenemases were described as species-specific, but this view was incorrect because different classes of enzymes began to be detected in various bacterial species. The genes encoding these bacterial enzymes are often found on plasmids, which makes them easy to transfer. Plasmids can be transferred by conjugation, which promotes the rapid spread of resistance. Moreover, carbapenemases belong to different classes of β-lactamase enzymes, reflecting their evolutionary diversity, which results from selective pressure induced by antibiotics, leading

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Table I
A comparison of the main features of selected GES-type carbapenemases. Some of the GES-type antibiotic resistances are ESBL-type enzymes, which do not have carbapenemase properties. Some of them do have carbapenemase activity.

Enzyme	Mutation	Gene location	Microrganism	Year and country of identification	Referencesm
GES-1	A170G	Plasmid	*K. pneumoniae*	1998, France	Poiler *et al.* 2000
GES-2	G170N	Plasmid	*P. aeruginosa*	2000, South Africa	Poiler *et al.* 2001
GES-4	G170S	Plasmid	*K. pneumoniae*	2002, Japan	Queenan and Bush 2007
GES-5	G170S	Chromosomal	*P. aeruginosa*	2007, Spain	Viedma *et al.* 2009
GES-6	G170S	Plasmid	*K. pneumoniae*	2004, Greece	Queenan and Bush 2007
GES-11	G243A	Plasmid	*A. baumannii*	2008, France	Moubareck *et al.* 2009
GES-14	G170S, G234A	Plasmid	*A. baumannii*	2008, described in Belgium	Mabrouk *et al.* 2017
GES-16	Gln38Glu, G170S	Plasmid	*S. marcescens*	2011, Brazil	Escandón *et al.* 2017
GES-18	G170S, V80I	Plasmid	*P. aeruginosa*	2010, Belgium	Bebrone *et al.* 2013
GES-20	A165S	Chromosomal	*P. aeruginosa*	2011, Mexico	Garza-Ramos *et al.* 2015

to the acquisition and modification of resistance genes. Thus, what was once a problem of clonal dispersal has now become an interspecies problem on a global scale (Queenan and Bush 2007).

2. Carbapenemases classification

Carbapenemases represent the most diverse family among beta-lactamases. The classification of β-lactamases considers two criteria: a functional one, based on enzymatic activity, developed by Bush and Jackoby (2010), and a molecular one, based on amino acid homology, developed by Ambler et al. (1991). The first system distinguishes four functional groups (marked with numbers from 1 to 4), and carbapenemases occur mainly in group 2f, susceptible to inhibition by β-lactam inhibitors, and group 3, including metallo-β-lactamases (MBLs), which are inhibited by ethylenediaminetetraacetic acid (EDTA), but are not susceptible to β-lactam inhibitors. The second division, showing the evolutionary relationship of β-lactamases, distinguishes four groups differing in molecular structure (marked with letters A to D). The hydrolytic mechanisms involving serine are present in classes A, C, and D, while carbapenemases from class B have zinc in their active sites (Mammeri *et al.* 2005; Paterson and Bonomo 2005).

Class A includes *Klebsiella pneumoniae* carbapenemase (KPC), Sulfhydryl variable-5 (SHV-5), Sulfhydryl variable-38 (SHV-38), cefotaximase-33 (CTX-M-33), Imipenemase/Not Metallo-β-lactamase Carbapenemase-A (IMI/NMC-A), Broad-spectrum *Klebsiella* Carbapenemase-1 (BKC-1), *Serratia fonticola* Carbapenemase-1 (SFC-1), *Serratia marcescens* Enzyme (SME), Frankfurt Resistance-Imipenem (FRI), Formosa Lactamase Class C (FLC) and the Guiana Extended-Spectrum (GES) β-lactamases, which were initially identified as the ESBL family (Queenan and Bush 2007; Bonnin *et al.* 2021). ESBLs are extended-spectrum β-lactamases. The ESBL-producing strain is capable of hydrolyzing penicillins, cephalosporins (except cephamycins), and monobactams but is susceptible to carbapenems and β-lactamase inhibitors (Chmielewska and Leszczyńska 2019). Over time, GES variants were discovered and characterized by low but measurable hydrolysis of imipenem – an antibiotic belonging to the carbapenem group, ultimately separating a new subgroup (Queenan and Bush 2007). The name GES comes from where this enzyme was discovered – French Guiana (Poirel *et al.* 2000). The main features of selected GES-type carbapenemases are compared in Table I.

3. General characteristics of GES carbapenemases

GES carbapenemases are relatively rare compared to other carbapenemases, e.g. class A (KPC) or class B (MBLs). The bacteria most often found to be able to produce GES carbapenemases are *Pseudomonas aeruginosa, Acinetobacter baumannii,* and *Enterobacterales* (including *Klebsiella pneumoniae, Escherichia coli,* and *Serratia marcescens*). These bacteria are responsible for infections of the urinary tract and respiratory system, such as pneumonia, especially in hospitalized patients. Penetrating the bloodstream, they can cause bacteremia or sepsis. Infections caused by bacteria producing GES carbapenemases also include skin, surgical wounds, soft tissue infections, infections related to medical devices, and peritonitis.

Genes determining the resistance of GES-type carbapenemases are encoded on plasmids, and ESBLs are encoded on plasmids and integrons. Ellington et al. (2020) wrote that these genes are inherited by

horizontal gene transfer (conjugation, transduction, and transformation) between genera and species. The GES carbapenemase genotype may confer an ESBL-like resistance phenotype to bacteria with low resistance to ertapenem and meropenem. GES-type antibiotic resistance, or more specifically GES-1, was first discovered in 1998 in a strain of *Klebsiella pneumoniae* in France (Poirel *et al*. 2000). It was found to confer resistance to cephalosporins and penicillin, but not carbapenemase activity. There are many variants of GES, and it is worth noting that some variants confer resistance to carbapenems, unlike ESBLs. When describing resistance, it is worth mentioning that in the case of ESBL resistance, in addition to resistance to β-lactam antibiotics, there may also be resistance to other antibacterial drugs such as aminoglycosides, trimethoprim, sulfonamides, chloramphenicol, tetracyclines, and fluoroquinolones. The cause is that the genes encoding ESBL are located on transposons and plasmids close to other antibiotic resistance genes and are commonly co-transferred between bacteria (Mammeri *et al*. 2005; Paterson and Bonomo 2005; Hawkey *et al*. 2018; Pablo-Marcos *et al*. 2023).

4. Omega (Ω) loop

The Ω loop is a fragment of Ambler class A β-lactamases, which include GES carbapenemases. It significantly impacts the substrate selectivity of these enzymes because it is located in their active center. A component of this loop is Glu166, an amino acid playing a key role in the two-step catalytic cycle of β-lactam antibiotic hydrolysis (Egorov *et al*. 2019).

In addition to Glu166, the loop also includes Asn170 and both these amino acids are involved in the adhesion of the water molecule necessary for the deacylation of the antibiotic (Levitt *et al*. 2012).

5. GES-1

This variant was first discovered in *K. pneumoniae*, although it may also occur in other species. The active site of the GES-1 carbapenemase contains several key residues. These include serine (Ser70), which is a nucleophilic residue that attacks the beta-lactamase ring; lysine (Lys73), which stabilizes the transition state during hydrolysis; glutamate (Glu166), which is involved in the deacylation step of the enzymatic reaction; and serine (Ser130), which contributes to substrate binding and catalysis. The GES-1 active site lacks Asn170 (the ligand for hydrolytic water), which is replaced by glycine, making the enzyme unable to hydrolyze imipenem (Poirel *et al*. 2001; Smith *et al*. 2007).

6. Characteristics of individual GES carbapenemases

Currently, there are several dozen types of enzymes classified as GES. It should be noted that we classify some of the GES-type antibiotic resistances as ESBL-type enzymes, which do not have carbapenemase properties, but not all, some of them do have carbapenemase activity. The properties of GES enzymes that can inhibit the action of carbapenems are presented below. GES carbapenemases are classified based on the differences in amino acid composition. These differences are substitutions of one to three amino acids, determining substrate specificity. GES-type beta-lactamases, which, according to Ambler's classification, belong to class A, represent a large and diverse group of enzymes. As of 2023, they include as many as 54 lactamases, of which at least 20 are classified as carbapenemases, but not all have been fully characterized. Some of the main variants of the GES carbapenemases that are currently best known are described below (Tanabe *et al*. 2023). Due to the existence of a large number of GES-type carbapenemases and editorial limitations, the article presents those that are the best studied and provide the most helpful information (U.K. Health Security Agency, 2024).

6.1. GES-2

The carbapenemase active site of GES-2 exhibits a similar distinguishing pattern to that observed in GES-1, with serine (Ser70), lysine (Lys73) and glutamate (Glu166) all playing a comparable role. However, a critical mutation resulted in the conversion of glycine-to-asparagine (Asp170), which is responsible for increasing the enzyme activity (Poirel *et al*. 2001).

A characteristic feature of this carbapenemase is that it has a canonical asparagine at position 170, unlike GES-4, -5, and -6, which have a Gly170Ser substitution at this position. In this enzyme, a hydrolytic water molecule positions itself between Ser70 and Glu166 and is also bound to Asn170. The presence of GES-2, like GES-4, -5, and -6, causes a decrease in bacterial susceptibility to imipenem. Research conducted by Frase et al. (2011) showed that blocking this carbapenemase with tazobactam (at a concentration of 4 μg/ml) changed the MIC (minimum inhibitory concentration) for piperacillin from > = 128 μg/ml (resistance) to 1 μg/ml. Based on this, it can be concluded that tazobactam, in combination with carbapenems, can be used in therapy against bacteria with GES-2 resistance. The dissociation constant for the noncovalent complex of GES-2 and tazobactam was in the nanomolar range, indicating the high affinity of tazobactam for GES-2. Additionally, studies have shown that the mentioned inhibitor has a rapid onset of enzyme inhibition (Frase *et al*. 2011).

6.2. GES-4

The GES-4 carbapenemase active site exhibits a similar function to that of GES-1, with the presence of serine (Ser70), lysine (Lys73) and glutamate (Glu166). However, critical mutations have occurred, resulting in the conversion of glycine to serine (Ser170), which is responsible for increasing the enzyme activity. Additionally, the conversion of alanine to valine (Val173) affects the substrate specificity and efficiency of the enzyme (Wachino *et al.* 2004; Barlow and Tenover 2024).

Research conducted by Vourli et al. (2006) showed that after exposure *K. pneumoniae* 78–01 strain with GES-4 and SHV-5 resistance to clavulanic acid in combination with imipenem or ceftazidime, the susceptibility to these antibiotics was partially restored. The gene encoding GES-4 (*blaGES-4*), in the form of genomic cassettes, is located in the variable regions of class 1 integrons, which are carried by plasmids, and this enables horizontal transfer of this resistance between bacteria (Bebrone *et al*. 2013).

6.3. GES-5

The active center of the GES enzyme 5, a carbapenemase, is structured similarly to other class A β-lactamases. It contains a catalytic serine residue in its active site, crucial for its hydrolytic activity. This serine is part of a conserved sequence motif (Ser70-X-X-Lys73) that plays an essential role in the enzyme's ability to hydrolyze β-lactam antibiotics (Smith *et al*. 2012).

The study performed by Kotsakis et al. (2010) showed that this enzyme has the highest carbapenemase activity, which is associated with the presence of serine at position 170. The Gly170Ser substitution increases the ability to hydrolyze cefoxitin and imipenem but also causes a decrease in activity towards ceftazidime and aztreonam. The presence of serine at position 170 changes the structure of the enzyme, namely in the Ω loop and results in improved catalytic properties of the enzyme against carbapenems and cephamycin; it also increases the resistance to β-lactamase inhibitors (Poirel *et al*. 2018).

IR-GES-5 refers to an integron-associated GES-5 (Guiana Extended Spectrum) β-lactamase enzyme. The "I. R." typically stands for Integron-encoded Resistance, indicating that the gene encoding this enzyme is located within an integron, a genetic element in the bacterial genome. The association of GES-5 with IR-GES-5 enhances its ability to spread rapidly across different bacterial species, making it a concern in the treatment of bacterial infections. The active center of the IR-GES-5 enzyme, like other GES-type β-lactamases, possesses a catalytic serine residue (Ser70). The structure of the active site allows GES-5 to bind and hydrolyze carbapenems, which are often resistant to degradation by other β-lactamases (Labuschagne *et al*. 2008).

6.4. GES-6

The active center of the GES enzyme 6 (GES-6), like other GES-type enzymes, features a serine-based mechanism typical of class A β-lactamases. The key residues in the active site are Lys and Ser at the 104 and 170 positions, respectively. The active center also includes other residues, such as Lys73, Ser130, Glu166, and Asn170, which are involved in substrate binding, catalysis, and stabilization of the transition state (Kotsakis *et al*. 2010).

Compared to GES-1, GES-6 has more significant activity against carbapenems and ceftolozane and reduced susceptibility to β-lactamase inhibitors (except avibactam). It is worth noting that when ceftolozane was combined with tazobactam, the MIC decreased slightly compared to the value for ceftolozane without the inhibitor, demonstrating the reduced effectiveness of the inhibitors. However, the activity of GES-6 towards imipenem was higher than in GES-1, which confirmed the involvement of Ser170 in the higher activity of the enzyme. It should be noted that the substrate profile of GES-6, in some sense, reflects MBLs with activity against carbapenems and some resistance to inhibitors (Poirel *et al*. 2018).

Botelho et al. (2015) showed that the blaGES-6 gene in the *P. aeruginosa* strain is accompanied by the *aacA7* gene encoding aminoglycoside acetyltransferase type 1, conferring resistance to amikacin, netilmicin, and tobramycin.

6.5. GES-11

This enzyme was first discovered in 2008 in France in *Acinetobacter baumannii* (Moubareck *et al*. 2009). Substitution of the glycine at position 243 in GES-11 was associated with increased activity toward aztreonam, as had been observed for GES-9. GES-11 did not have a substitution of the Gly170 residue, resulting in increased hydrolysis of imipenem as in GES-2, GES-4, GES-5, and GES-6. Research conducted by Moubareck et al. (2009) showed that expression of the blaGES-11 gene in porin-deficient cells may lead to resistance to imipenem. The active site of GES-11 is similar to other serine carbapenemases, including serine at position 70 (Ser70), lysine at position 73 (Lys73), glutamate at position 166 (Glu166) and glycine at position 170 (Gly170). The lack of substitution of the Gly170 residue increases the hydrolytic properties of this enzyme. GES-11 has not been fully classified, and research is still ongoing on whether it belongs to β-lactamases or carbapenemases (Moubareck *et al*. 2009).

6.6. GES-14

The active site of GES-14, considering key amino acid positions, is similar to other carbapenemase-active GES variants: Ser 70, Lys 73, Glu 166, Gly 170. The exact sequence of the amino acids surrounding these residues defines the enzyme's active site. Unlike GES-11 carbapenemase, GES-14 contains additional hydrogen bonds in the active site formed by oxygen in the side chain of Ser170 with the carboxyl group of Glu166. It is worth noting that GES-11 has a serine at position 170 and, similarly to the previous variants, it shows activity towards carbapenems. Another feature of the amino acid chain of this enzyme is that it has an alanine at position 243, which confers increased resistance to classic β-lactamase inhibitors. Ala243 also makes the enzyme effective against aztreonam, ceftazidime, and cefotaxime (Moubareck *et al*. 2009).

IR-GES-14 enzyme is a variant of the GES-type β-lactamases, specifically associated with integrons, which enhances GES-14's ability to spread antibiotic resistance genes. Catalytic serine residue (Ser70) is crucial for the enzyme's ability to hydrolyze β-lactam rings. It acts as a nucleophile in the hydrolysis reaction. The active site of GES-14, like other GES enzymes, is flexible enough to accommodate a wide range of β-lactam antibiotics (Bonnin *et al*. 2011).

6.7. GES-16

This enzyme was first identified in *S. marcescens* in Brazil. The carbapenemase activity of GES-16, like that of other GES variants, is mainly determined by the presence of specific amino acids in its active site. These residues typically include Ser 70, Lys 73, Glu 166 and Gly 170. Based on changes in the amino acid chain, i.e. Gln38Glu and Gly170Ser, GES-16 and GES-5 have been distinguished. In a comparative study regarding the activity of GES-16 against imipenem, ertapenem, and meropenem conducted by Streling et al. (2018), it has been shown that this enzyme has the highest effectiveness against imipenem (compared to other carbapenems). GES-16, apart from hydrolyzing carbapenems, also inhibits the action of other antibiotics, i.e. penicillin, cephamycin, and cephalosporins, but importantly, it does not hydrolyze aztreonam (Escandón *et al*. 2017; Streling *et al*. 2018).

6.8. GES-18

The amino acid sequence in the GES-18 active center is similar to GES-1 and GES-2; the substitution of Gly170Ser and Val80Ile causes a change in the location of the hydrolytic water molecule and the amino acid essential in hydrolysis – glutamic acid (position 166), which may partially explain the differences in enzyme specificity and action. Like GES-5, it has low effectiveness against ceftazidime. Also, it hydrolyzes imipenem and cefotaxime with similar kinetic parameters, while the difference concerns the presence of Val80Ile (in GES-18), but this change does not significantly affect the substrate profile. It is worth noting that GES-18, unlike GES-1, is less susceptible to classic inhibitors, i.e. clavulanic acid and tazobactam (Bebrone *et al*. 2013).

6.9. GES-20

This type of enzyme was first identified in 2011 in a strain of *P. aeruginosa* in Mexico (Garza-Ramos *et al*. 2015). The *blaGES-20* gene has two single nucleotide substitutions, translating into amino acid chain changes. Studies have shown that GES-20 resistance often co-occurs with OXA-2 (oxacillinase-2). GES-20-producing isolates studied by Recio et al. (2022) showed the replacement of aspartic acid with serine (position 165). In the place that encodes leucine, a sequence change resulted in the STOP codon (position 237), thus shortening the amino acid chain, translating into resistance to CZA (ceftazidime/avibactam).

7. Identification of GES carbapenemases

Identification of GES carbapenemases called "minor class A carbapenemases" poses a particular challenge due to the low level of carbapenem hydrolysis that characterizes these enzymes (Bonnin *et al*. 2021), which contributes to an increase in the percentage of false-negative tests in phenotypic methods (biotyping, serotyping, assessment of drug susceptibility profiles, protein analysis methods). Biochemical tests only enable the detection of carbapenem resistance but without determining the specific type of resistance. Therefore, it is necessary to confirm the result by molecular tests. They mainly involve the amplification of nucleic acids using multiplex diagnostics such as PCR (Polymerase Chain Reaction), LAMP (Loop-mediated Isothermal Amplification), or RPA (Recombinase Polymerase Amplification) (Ortiz-Cartagena *et al*. 2023). They detect the presence of known carbapenemases genes on plasmids, porin channel mutations, or efflux pump mutations.

However, these methods, despite the possibility of accurate and simultaneous identification of individual GES carbapenemase genes, are quite limited due to the need for specialized equipment, costs, and reduced speed; additionally, it is possible to detect only known genes, which significantly limits the spectrum of gene detection (Tenover 2021).

Laboratories often use a combination of phenotypic and genetic tests against the risk of false results.

Diagnostics increasingly seek alternative methods to identify bacterial enzymes that hydrolyze the β-lactam bond in the carbapenem molecule. These include commercially available the EntericBio CPE test (Serosep Ireland), a multiplex real-time PCR reaction. In research conducted by Vanstone et al. (2018), this test showed both 100% specificity and susceptibility.

The CIM (Carbapenem Inactivation Method) test is a diagnostic tool used to detect carbapenemases' activity in Gram-negative bacteria. This test is a phenotypic test, which employs an indirect method for the detection of carbapenem production. The presence of resistance is determined by the interpretation of the enzymatic hydrolysis of a meropenem disc following exposure to strain producing carbapenemases, including GES-5, OXA-372, GIM-1 (German Imipenemase-1), FRI-1 (Florence Imipenemase-1), SME-1/-2 (*Serratia marcescens* Enzyme-1), NMC-A (Non-metallo Carbapenemase-A) and IMI-1/-2/-3. The test also detects the following carbapenemases: KPC-2, GES-5, SME-1/-2 (*Serratia marcescens* Enzyme-1), NMC-A (Non-metallo Carbapenemase-A), IMI-1/-2/-3. A positive result is indicated by the growth of the indicator *E. coli* strain on the Muller-Hinton medium. As this test detects a multitude of different carbapenemases, it is not feasible to ascertain with absolute certainty the specific resistance that has been identified (Aguirre-Quiñonero *et al*. 2017; Bonnin *et al*. 2021). In a study involving 124 *Enterobacteriaceae* strains, Aguirre-Quińonero et al. (2017) evaluated the CIM assay for its effectiveness in detecting different types of carbapenemases, including GES-6. While the test demonstrated efficacy in detecting carbapenemases of the KPC, NDM, VIM, IMP and OXA-48 types, it exhibited relatively lower sensitivity for GES-6 (79.3%). Of the 22 strains with the gene encoding GES-6, only eight were positive for CIM, while 11 exhibited a false negative result. This result may be attributable to the low hydrolytic level of GES-6. Nevertheless, despite the necessity for additional confirmation tests to identify GES antibiotic resistance, this test's simplicity and low cost render it a valuable tool. One modification of the CIM test, the rapid Carbapenem Inactivation Method (rCIM) assay, was found to facilitate rapid detection of carbapenemase activity, including, but not limited to, GES-5. This assay employed a nephelometer to accelerate the detection of carbapenemases (Muntean *et al*. 2018).

The MAST Carba PacE test is a colorimetric test based on the hydrolysis of a chromogenic cephalosporin analogue. A change in colour from yellow to orange or red is observed in the presence of an enzyme belonging to the carbapenemases. A study by Rezzoug et al. (2023) revealed that the MAST Carba PacE test exhibits insufficient sensitivity towards GES-type carbapenemases (the test did not detect any of the strains tested that produce GES-type enzymes), leading to the conclusion that the test is not effective in detecting this resistance.

Lateral flow immunoassays (e.g. NG-Test CARBA-5) represent a rapid and straightforward method for identifying carbapenemases, with a detection time of less than 15 minutes. The test exhibits high sensitivity and specificity in detecting carbapenemases in *Enterobacterales* strains, rendering it an efficacious diagnostic instrument within the hospital environment. In NG-Test CARBA 5 studies, the test demonstrated a sensitivity of 98% and a specificity of 100% for *Enterobacterales*, indicating its high compatibility with molecular methods. The test is valued for its rapidity and ease of use, crucial for managing life-threatening infections (Mende-Sotelo *et al*. 2023).

The modified Hodge test (MHT) is a phenotypic test for detecting GES-type carbapenemases in bacteria belonging to the *Enterobacteriaceae* family. Regrettably, this test has low sensitivity and a high incidence of false positives, resulting in its limited use for detecting carbapenemases. The principal advantages of this test are its cost-effectiveness, ease of implementation in standard medical laboratories and simplicity of performance (Ramana *et al*. 2013). Another modification of this assay, involving the addition of Triton-X-100 (Triton Hodge assay), has been shown to have good sensitivity in detecting carbapenemases such as GES-5, SME-1 and NMC-A (Pasteran *et al*. 2016).

8. Future perspectives in the rare carbapenemases detection

Therefore, there is an urgent need to look for methods that would be equally susceptible and specific, and at the same time fast, simple, and cheap, in short, methods that would not require DNA extraction. In a study conducted by Concha Ortiz-Cartagena et al. (2023), an assay based on LAMP CRISPR-Cas13a (Clustered Regularly Interspaced Short Palindromic Repeats) has been adapted. It is not a commonly available test used in diagnosing GES carbapenemases, but due to its advantages, it seems particularly valuable and worth comments. It enables the detection of OXA-48 and GES carbapenemases in *Enterobacterales* and *Pseudomonas* spp. This technique is free from purification and concentration of nucleic acids. It allows the detection of *blaOXA-48* and *blaGES* genes responsible for carbapenem resistance. As the authors claim, this test costs less than EUR 10 per reaction, takes less than two hours to complete, and is 100% specific and susceptible to identifying both OXA-48 and GES carbapenemases. It is easily accessible because it does not require specialized equipment or trained personnel. It is currently one of the fastest

and most effective tests on the market, and it can be routinely introduced in clinical microbiology laboratory tests to detect multidrug-resistant pathogens.

9. Treatment of infections caused by carbapenem-resistant pathogens:

9.1. New drugs in the treatment of carbapenemases-producing strains

In 2024, the World Health Organization (WHO, 2024) published a list of antibiotic-resistant bacteria that pose a considerable threat to public health to indicate the direction for research and development initiatives. WHO has classified bacteria into three risk groups – see Table II.

Bacterial strains exhibiting GES-type antibiotic resistance are treated analogously to other carbapenem-resistant strains. Currently, there are no pharmaceutical agents available that are specifically designed to target this resistance. The following are examples of drugs that can be employed in the treatment of infections caused by carbapenem-resistant bacteria with GES-type resistance, among others.

Given the increasing antibiotic resistance, scientists should develop new drugs that could be used to treat multidrug-resistant strains that threaten humans. A recently developed drug is a siderophore cephalosporin – cefiderocol, which binds to iron, and its action is compared to the mechanism of a "Trojan horse". Iron is an essential element for the synthesis of bacterial DNA, energy production, and other processes necessary for life. Thus, once this drug binds to iron, it is absorbed by bacteria and then binds to PBP (penicillin-binding protein), its target (Wernicki 2018; European Medicines Agency 2020a).

Other new drugs are eravacycline, a tetracycline, and lefamulin, the first pleuromutilin approved for human use. Lefamulin works by inhibiting the synthesis of bacterial proteins – it blocks the 23S rRNA molecule of the 50S ribosomal subunit (European Medicines Agency 2020b). Eravacycline, a third-generation tetracycline, changes the conformation of ribosomes, preventing protein elongation. This drug has been approved for the treatment of complicated abdominal infections caused by strains producing GES carbapenemases (and NDM /New Delhi metallo-β-lactamase/, VIM /Verona integron-encoded metallo-β-lactamase/, OXA) (European Medicines Agency 2024a). It is also worth noting that eravacycline is in phase III clinical trials for use in complicated urinary tract infections. *In vitro* studies conducted by Grossman et al. (2015) showed its promising activity against *E. coli*. If, in *in vivo* studies, the effectiveness of this tetracycline on the biofilm formed by the bacteria mentioned above is confirmed. In a second phase III clinical trial, it was discovered that the administration of eravacycline at a dose of 1.5 mg/kg body weight via intravenous infusion every 24 hours, commencing on day 3 with a gradual reduction in dosage to 200 mg administered every 12 hours, was comparable to the use of levofloxacin in the treatment of complicated UTIs (Zhanel *et al*. 2016).

Carbapenems can also be used in combination with carbapenemase inhibitors against carbapenemase-producing strains. However, bacteria can cope with this by producing enzymes not susceptible to inhibitors. An example is GES-5 type resistance, characterized by the

Table II
World Health Organization (WHO) bacterial priority pathogens list

Bacteria	Priority group	Resistance type
Enterobacterales	critical	carbapenem-resistant
Enterobacterales		third-generation cephalosporin-resistant
Acinetobacter baumanii		carbapenem-resistant
Salmonella Typhi	high	fluoroquinolone-resistant
Shigella spp.		fluoroquinolone-resistant
Enterococcus faecium		vancomycin-resistant
Non-typhoidal *Salmonella*		fluoroquinolone-resistant
Neisseria gonorrhoeae		third-generation cephalosporin and/or fluoroquinolone-resistant
Staphylococcus aureus		methicillin-resistant
Pseudomonas aeruginosa		carbapenem-resistant
Group A Streptococci	medium	macrolide-resistant
Streptococcus pneumoniae		macrolide-resistant
Haemophilus influenzae		ampicillin-resistant
Group B Streptococci		penicillin-resistant

decreased susceptibility to carbapenemase inhibitors. Therefore, new inhibitors are looked for. For example, the following combinations have recently been developed: relebactam used in combination with imipenem, vaborbactam used in combination with meropenem (Hayden *et al.* 2020) and ceftazidime with avibactam (Vázquez-Ucha *et al*. 2020). These three drug combinations have proved safe and effective and should be considered an alternative treatment for infections caused by carbapenem-resistant pathogens (Bouchet *et al*. 2020).

Relebactam is a DBO (diazobicyclooctane) inhibitor of a second generation (non-β-lactam molecules). It binds to the active site of serine β-lactamases, which effectively inhibits class A (including GES) and C β-lactamases. The drug restores the activity of β-lactams despite bacteria being resistant to these drugs (Bouchet *et al*. 2020).

Imipenem, in combination with relebactam, has broad activity against many Gram-negative bacteria, including Enterobacterales, *P. aeruginosa*, and *Bacteroides* spp. (belonging to anaerobic bacteria) producing enzymes that inhibit the action of carbapenems. The combination of imipenem and relebactam also shows effectiveness against multidrug-resistant strains resistant to, e.g. fluoroquinolones (Bouchet *et al*. 2020).

Another important inhibitor is vaborbactam, which is a derivative of boronic acid. Studies have shown that the combination of vaborbactam and meropenem is effective in the treatment of UTIs, nosocomial pneumonia, ventilator-acquired pneumonia, intra-abdominal infections, or bloodstream infections associated with carbapenem-resistant bacteria. Microbiological experiments have shown that adding vaborbactam to meropenem restores the minimum inhibitory concentration to a level comparable to the wild strain of *Enterobacterales* (Vázquez-Ucha *et al*. 2020).

When describing new drugs against carbapenemase-producing strains, the combination of ceftazidime and avibactam should also be mentioned. Avibactam is the first synthetic DBO showing activity against clinically important resistance mechanisms, such as GES, KPC, SHV, CTX-M and OXA-48. The mechanism of action of avibactam, which is based on binding to the active site of the bacterial enzyme, is reversible. After deacylation, an unchanged drug is released, which can inhibit another β-lactamase (including carbapenemase) (Vázquez-Ucha *et al*. 2020).

9.2. Drugs against GES carbapenemases producing strains in development

GES carbapenemases represent a significant challenge in the treatment of bacterial infections due to their ability to hydrolyze a range of antibiotics, including penicillins, cephalosporins, monobactams and carbapenems (Bonnin *et al*. 2011). Carbapenems are among the most important antibiotics used to treat multidrug-resistant infections. Developing new drugs against bacterial strains is currently a topic of significant interest. Many drugs are currently under investigation, including durlobactam + sulbactam, taniborbactam and cefepime+enmetazobactam. These drugs have demonstrated activity against strains resistant to carbapenems, including strains with GES-type resistance (Soszyńska-Morys 2023; European Medicines Agency 2024a). Table III shows drugs under investigation for the treatment of infections with carbapenemase-producing strains of the GES type. The table provides information on the active substances, their class, spectrum of action, testing phases, and additional information on their efficacy and therapeutic areas.

Table III
Drugs against GES carbapenemases producing strains in development (Soszyńska-Morys 2023; European Medicines Agency 2024a)

Substance	Class	Spectrum	Research phase	Additional information
durlobactam + sulbactam	Inhibitor of β-lactamases of classes A, C and D, according to Ambler	*Acinetobacter baumanii*	Phase 3 clinical trials, completed	The combination shows greater activity against MDR
taniborbactam	Non-β-lactam inhibitor of β-lactamases of classes A, B, C, and D, according to Ambler	*P. aeruginosa, Enterobacterales*	Phase 3 clinical trials of taniborbactam with cefepime in UTIs, completed	Enables the use of cefepime against carbapenem-resistant strains
cefepime + enmetazobactam	β-lactam (cephalosporin) + β-lactamase inhibitor	ESBL-producing bacteria, Enterobacterales resistant to 3rd generation cephalosporins, Carbapenem-resistant *K. pneumoniae*	CHMP issued a marketing authorization for a medicinal product containing cefepime and enmetazobactam (2024)	Therapeutic area: pyelonephritis, UTI, HAP and VAP

CHMP – Committee for Medical Products for Human Use, ESBL – Extended-Spectrum β-Lactamase, HAP – Hospital-Acquired Pneumonia, MDR – Multidrug Resistant Strains, UTI – Urinary Tract Infection, VAP – Ventilator Associated Pneumonia

Table IV
Summary of antibiotics that can be used against carbapenemase-producing strains (Rejestr produktów leczniczych 2012; 2014; 2015a; 2015b; Electronic Medicines Compendium 2024; European Medicines Agency 2018; 2020a; 2020b; 2022; 2024b; 2024c)

Substance	Spectrum	Indications
Colistin	*P. aeruginosa, K. pneumoniae, E. coli, A. baumanii*	Sepsis, lower RTI, UTI, RTI in CF patients
Fosfomycin	*K. pneumoniae, E. coli, Citrobacter* spp., *Proteus* spp.	Acute, uncomplicated cystitis; profuse, asymptomatic bacteriuria; UTI prevention before surgery and transurethral diagnostic procedures
Nitrofurantoin	*E. coli,* enterococci, staphylococci, *Citrobacter* spp., *Klebsiella* spp., *Enterobacter* spp.	Acute or recurrent lower UTI; inflammation of the renal pelvis (spontaneous or after surgery)
Tobramycin	*P. aeruginosa, Corynebacterium* spp., MSSA, *Citrobacter* spp., *Haemophilus* spp., *Salmonella* spp., *Shigella* spp, *P. vulgaris*	HAP (incl. severe pneumonia), exacerbations of lower RTI in CF patients, complicated and recurrent UTI; intra-abdominal infections; skin and soft tissue infections (incl. severe burns)
Amikacin	*P. aeruginosa, S. aureus, Citrobacter freundii, E. coli, K. pneumoniae, P. mirabilis, P. vulgaris*	HAP (incl. severe pneumonia), abdominal infections (incl. peritonitis and post-operative infections), complicated and recurrent UTI, skin and soft tissue infections and burns; bacterial endocarditis
Cefiderocol	*E. coli, K. pneumoniae, P. mirabilis, P. aeruginosa, Enterobacter cloacae complex*	Infections caused by aerobic Gram-negative bacteria, complicated UTI, pyelonephritis
Eravacycline	*E. coli, K. pneumoniae, S. aureus, E. faecalis, E. faecium, Streptococcus* spp.	Complicated intra-abdominal infections in adults
Lefamulin	*S. pneumoniae, S. aureus, L. pneumophila, M. pneumoniae, C. pneumoniae*	Community-acquired pneumonia (in case of ineffective treatment with recommended drugs)
Imipenem with relebactam	*E. coli, H. influenzae, K. pneumoniae, P. aeruginosa, S. mercescens,*	HAP, VAP; bacteremia in HAP, infections with aerobic Gram-negative bacteria in case of limited treatment options
Meropenem with vaborbactam	*E. coli, K. pneumoniae, Enterobacter cloacae complex, Citrobacter* spp., *P. aeruginosa, S. mercescens, S. aureus, S. epidermidis, S. agalacitiae, B. fragilis, C. perfringens, Prevotella* spp.	Complicated abdominal pneumonia, complicated UTI, pyelonephritis, HAP and VAP
Ceftazidime with avibactam	*C. freundii, E. cloacae, E. coli, K. oxytoca, K. pneumoniae, P. aeruginosa, P. mirabilis, S. mercescens*	Complicated intra-abdominal infection, complicated UTI, pyelonephritis, HAP, VAP, infections caused by aerobic Gram-negative microorganisms in adults and children > 3 months of age
Plazomycin	*E. coli, K. pneumoniae, P. mirabilis, E. cloacae*	UTI and pyelonephritis

CF – cystic fibrosis, CSF – cerebrospinal fluid, HAP – hospital-acquired pneumonia, incl. – including, MS – multiple sclerosis, MSSA – Methicillin-Susceptible *Staphylococcus aureus*, RTI – respiratory tract infection, UTI – Urinary tract infection, VAP – ventilator associated pneumonia

9.3. Summary of antibiotics that can be used against carbapenemase-producing strains

Table IV provides essential data on the antibiotics that can be employed to treat infections caused by carbapenemase-producing strains. The table offers comprehensive information on the active substances, their spectrum of action, and their indications for use. It serves as a valuable reference in daily medical practice to facilitate the selection of an appropriate antimicrobial treatment.

10. Conclusions

Individual GES carbapenemases have different amino acid sequences resulting from mutations in the bacterial DNA chain, thus allowing their differentiation by PCR and electrophoresis methods. Knowledge about specific resistance allows one to make the right treatment decisions. The use of appropriately selected drugs against GES will reduce the ineffectiveness of the therapy, which will prevent the spread and emergence of further resistance mechanisms. Currently, there are drugs against strains with GES resistance that are widely used, e.g. colistin or fosfomycin, as well as newly developed combinations of carbapenems with inhibitors (e.g. meropenem with vaborbactam) and many drugs under investigation. However, it should be noted that if rational antibiotic therapy is not followed, they will stop being active against some strains.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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