A review on drug resistance patho-mechanisms in ESKAPE bacterial pathogens

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Abstract

The escalating incidence of nosocomial infections stemming from ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) bacterial pathogens presents a formidable clinical challenge globally, affecting both the developed and developing nations. These pathogens, which are distinguished by their robust antibiotic resistance mechanisms, pose a significant threat to the public health. Their ability to evade traditional antimicrobial treatments underscores the urgent need for novel therapeutic stratifies or alternative approaches to mitigate their negative impact. Understanding the intricate mechanisms underpinning antibacterial resistance in ESKAPE bacteria is paramount for developing effective interventions. Enhanced insights into these mechanisms will facilitate the prediction of resistance patterns among the multidrug-resistant pathogens, thereby guiding the development of targeted therapies and preventive measures. Consequently, comprehensive efforts are needed aiming at unraveling the intricacies of antibacterial resistance in ESKAPE pathogens that are imperative to safeguarding the public health. The aim of the present review was to highlight the patho-mechanisms of ESKAPE bacteria towards the different antibiotics and genes involved in multi-drug resistance.

Keywords: Antibacterial resistance, ESKAPE, Nosocomial infection, Multidrug resistance, Pathophysiology
1. Introduction

Health-associated infections (HAI) are complex and widespread infections affecting hospitalized patients. The term "nosocomial" refers to infections acquired during the process of receiving health care that were absent at the time of admission. These infections, also known as healthcare-associated infections, are often linked to prolonged hospital stays and can lead to severe health issues, including death (Monegro et al., 2024). Nosocomial infections originate from both of endogenous and exogenous sources and are transmitted directly or indirectly among patients, healthcare workers, contaminated objects, visitors, and households. According to the National Healthcare Safety Network (NHSN) and the Centers for Disease Control and Prevention (CDC), nosocomial infections have been categorized into 30 different forms across 50 specific sites of infection. Common infection sites include gastroenteritis, meningitis, respiratory infections, surgical and soft tissue infections, and urinary tract infections (UTIs) (O’Leary et al., 2024). In 2011, a survey in the United States reported approximately 722,000 cases of hospital-acquired infections (HAIs), resulting in the deaths of 75,000 individuals suffering from nosocomial infections (Genovesi, 2023). The use of antibiotics for clinical infections is an advanced technique in human medicine. However, the effectiveness of antibiotics has diminished over time due to the emergence of antibiotic-resistant pathogens (Murugaiyan et al., 2022). According to CDC, antibiotic-resistant pathogens cause more than two million infections in the U.S. every year, with these numbers expected to increase tenfold by 2050. Globally, multidrug-resistant (MDR) bacteria are now responsible for 15.5% of healthcare-associated infections (HAIs), driven by factors such as hospital infection exposure, self-medication, and overuse of antibiotics (Mulani et al., 2019). Based on their modes of action, antibacterial drugs that exhibit resistance against bacteria have been divided into classes. These strategies include blocking formation of bacterial cell walls, depolarizing cell membrane, blocking synthesis of proteins, blocking synthesis of nucleic acids, and blocking metabolism of bacteria (Makarewicz et al., 2021). The actions of these antibiotics include depolarizing the cell membranes (e.g., lipopeptides), inhibiting protein synthesis (e.g., Chloramphenicol, tetracyclines, lincosamides, streptogramins, and aminoglycosides), inhibiting the nucleic acid synthesis (e.g., levofloxacin, norfloxacin, and ciprofloxacin), and inhibiting the metabolic pathways (e.g., Sulfonamides) (Baran et al., 2023). The main factors influencing antibacterial resistance include improper use of antibiotics for animals and humans, overprescription of antibiotics by physicians, patient self-medication, non-compliance with recommended treatments, and prolonged antibacterial therapy among hospitalized patients (Ayukekbong et al., 2017). Bacterial resistance is classified into two categories: intrinsic and acquired resistance. Intrinsic resistance occurs when elements within the bacteria contribute to antibiotic resistance, independent of previous antibiotic exposure and not resulting from horizontal gene transfer (Mancuso et al., 2021). Acquired resistance, on the other hand, results from the transfer of genetic material that confers resistance, often through horizontal gene transfer. Acquired resistance arises from the presence of genes transferred between bacteria via exogenous DNA and/or from mutations in the bacteria's indigenous genes (Mc Carlie et al., 2020). Examples of bacterial pathogens with intrinsic resistance to different antibiotics include Enterococci (aminoglycosides, cephalosporins, and lincosamides), Staphylococcus aureus (daptomycin, fluoroquinolones, and linezolid), Klebsiella spp. (ampicillin, quinolones, and tigecycline), Acinetobacter spp. (ertapenem, ampicillin, and amoxicillin), Pseudomonas spp. (tetracyclines, ertapenem, and ß-lactams), and Enterobacter spp. (cephalosporin, fluoroquinolones, clindamycin, and ertapenem) (Denissen et al., 2022).

2. ESKAPE pathogenic bacteria
Antimicrobial-resistant (AMR) infections have been classified as an emerging threat to human health by the CDC and World Health Organization (WHO). The WHO has identified twelve bacterial species urgently needing new antibiotics (Salam et al., 2023). Among these, ESKAPE bacteria are of top priority. Healthcare-associated infections (HAIs) are primarily caused by ESKAPE pathogens, which resist the biocidal action of antibiotics. These bacteria frequently cause severe illness in the immunocompromised patients due to their treatment resistance mechanisms (Benko et al., 2020).

3. Antibacterial resistance mechanisms of ESKAPE pathogens

Antimicrobial resistance is primarily mediated by antimicrobial resistant genes (ARGs), which are inherited in chromosomal DNA, plasmids, and/or transposons (Mei et al., 2024). Antibacterial resistance can be either intrinsic (innate) or acquired. Intrinsic resistance originates from the pathogen’s genome, making it naturally resistant to certain antibiotics. Acquired resistance results from events such as acquisition of foreign genes through several mechanisms; mainly conjugation, transformation involving plasmids and transposons, gene integration via integrons, and transduction by bacteriophages. Bacteria can also become resistant by a variety of cellular gene alterations and mechanisms, such as drug alteration or deactivation, changes to drug binding sites, permeability changes in cells, and production of biofilms (Nguyen et al., 2022).

3.1. Antibiotic inactivation/alteration

The development of enzymes that degrade or neutralize antibiotics is a common method by which ESKAPE bacteria exhibit drug resistance. These enzymes are divided into two categories: those that deactivate the antibiotic's active site and those that covalently alter the drug's structural components to prevent interaction with the bacterial target site (Gauba and Rahman, 2023).

3.2. Production of β-lactamase enzymes

During the discovery and purification of penicillin, β-lactamases were discovered. β-lactamase enzymes are categorized into the Ambler scheme (Bush-Jacoby system) based on their amino acid sequence, dividing them into four main classes (A, B, C, and D). Classes A, C, and D use serine at their active sites, including cephalosporinases, penicillinases, carbapenemases, and extended-spectrum β-lactamases (ESBLs). The majority of β-lactam antibiotics, including penicillins, cephalosporins, oxyimino, monobactams,cephamycins, and carbapenems, as well as β-lactamase inhibitors, such as clavulanic acid and tazobactam, are rendered inactive by β-lactamase enzymes. The ambler class A comprises the following: KPC (Klebsiella pneumoniae carbapenemase), TEM (Temoniera), SHV (Sulhydryl reagent variable), and CTX-M (Cefotaxime-Munich) (Timsit et al., 2022). Klebsiella pneumoniae and Enterobacter spp. typically harbor TEM (Temoniera) enzymes that hydrolyze the cephalosporins and penicillins (Bonomo, 2017). The SHV-1 enzyme, commonly found in Klebsiella pneumoniae, exhibits high mutation rates, expanding the potential for antibiotic resistance (Castanheira et al., 2021). Carbapenemases such as KPC-1 are prevalent in Klebsiella pneumoniae, showing resistance to multiple antibiotics (Ramadan et al., 2022). Metallo-β-lactamases (MBLs) in Ambler class B require Zn<sup>2+</sup> as a cofactor and hydrolyze most β-lactam antibiotics except aztreonam. Ambler class D enzymes hydrolyze oxacillin, with several members such as OXA-11, OXA-14, and OXA-16 (Laws et al., 2019).

3.3. Aminoglycoside modifying enzymes

The generation of aminoglycoside modifying enzymes (AMEs), which are divided into aminoglycoside phosphotransferases (APHs), aminoglycoside nucleotidyl transferases (ANTs), and aminoglycoside acetyltransferases (AACs), is the basis for resistance to aminoglycosides in ESKAPE bacteria (Wang et al., 2022a). Geographical location, bacterial species, and antibiotic selection pressure all affect global distribution of the amin modifying enzymes (AMEs). Horizontal gene transfer contributes to the
spread of AMEs among ESKAPE pathogens (De Oliveira et al., 2020). Multiple or bi-functional AMEs enhance aminoglycoside resistance, with Staphylococcus aureus and Enterococcus spp., showing high resistance to gentamicin due to bi-functional AAC(6')-APH (2') enzyme (Gnanamani et al., 2017). The AAC(6')-Ib-cr enzyme is present in Acinetobacter baumannii, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Enterobacter spp., giving them resistance to ciprofloxacin and aminoglycosides (Ahmadian et al., 2021).

3.4. Modification of drug binding sites

By changing their target sites through mechanisms like target enzyme modification, ribosome target site modification, and cell wall precursor modification, resistant bacteria avoid the effects of antibacterial medications (Munita and Arias, 2016).

3.4.1. Target enzyme modification

Bacteria exhibit β-lactam antibiotic resistance by modifying penicillin binding protein (PBP) enzymes. Methicillin-resistant Staphylococcus aureus (MRSA) displays resistance due to the mecA gene, encoding PBP2a (Baig et al., 2018). PBP5 in Enterococcus faecalis and Enterococcus faecium also shows resistance to β-lactams. Fluoroquinolone resistance is another example of antibacterial resistance (AMR) affecting ESKAPE bacteria, which include ciprofloxacin and norfloxacin. Fluoroquinolone resistance arises from mutations in the gyrA, gyrB, and parC genes, affecting topoisomerase enzymes (Ruekit et al., 2022). Plasmid-mediated quinolone resistance (PMQR) also contributes to resistance in Klebsiella pneumoniae and Enterobacter spp. (Wang et al., 2022b).

3.4.2. Ribosomal target site alterations

Ribosomes play a crucial role in the development of aminoglycoside and linezolid resistance in ESKAPE species. Staphylococcus aureus and Enterococcus species exhibit higher susceptibility to linezolid as a result of methylation of 23S rRNA mediated by cfr and mutations in the genes producing 50S ribosomal subunit proteins and 23S rRNA (Han et al., 2022). Enzymatic methylation of 16S rRNA mediates acquired antibacterial resistance (AMR) in Gram-negative bacteria, leading to resistance against aminoglycosides (Gauba and Rahman, 2023). Staphylococcus aureus and Enterococcus spp. are resistant to the macrolide-lincosamide-streptogramin B (MLS) antibiotic due to the erm-encoded rRNA methyl-transferases (Miklasinska-Majdanik, 2021).

3.4.3. Cell wall precursor alterations

The Gram-positive glycopeptide resistance is one way through which ESKAPE bacteria demonstrate antibacterial resistance (AMR). Glycopeptides target the D-Ala-D-Ala peptidoglycan precursor residues present in the outer cell wall of sensitive Gram-positive bacteria, which in turn blocks production of the bacterial cell walls (Uddin et al., 2021). In Enterococci, glycopeptide resistance is mediated by coordinated van gene clusters. The development of modified peptidoglycan precursors (D-Ala-D-Ala termini are transformed to either D-Ala-D-lactate or D-Ala-D-Seine) displays subdued glycopeptide binding. By producing D, D-carboxypeptidases, the natural D-Ala-D-Ala precursors are removed from the host cell (Arredondo-Alonso et al., 2021). Staphylococcus aureus and Enterococci have shown daptomycin resistance against Gram-positive bacteria, where this resistance may be caused by the change existing on the cell surface, composition and metabolism of the phospholipids, and response of the bacterial membranes to stress. Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa have shown polymyxin resistance through remodeling of the outer membrane lipopolysaccharide lipid A structures (Nguyen et al., 2022).

3.5. Changes in cell permeability and drug efflux

Porins are a type of protein present in the outer membranes of Gram-negative bacteria. In
**Pseudomonas aeruginosa**, porins facilitate the passage of several hydrophilic compounds, including antibiotics (Prajapati et al., 2021). Resistance to imipenem develops as a result of decreased drug influx into the cells caused by a decreased level of porin protein OprD (Bo et al., 2024). By losing the outer membrane proteins such as OmpK35 and Omp36 and producing new resistant enzymes, primarily AmpC lactamase and carbapenemase KPC (Klebsiella pneumoniae carbapenemase), Klebsiella pneumoniae bacteria display resistance to lactams (Li et al., 2023). Efflux pumps are bacterial membrane proteins that act as efflux pumps for certain antibiotics, removing them from the intracellular compartments. These efflux pumps remove drugs from the bacteria rapidly, so the drug concentrations are not high enough to produce antibacterial effects. The majority of efflux pumps are multidrug transporters that increase multidrug resistance and release a range of medications. ATP-binding cassette (ABC) family, main facilitator superfamily, multidrug and toxic chemical extrusion family, resistance nodulation division (RND) family, and small multidrug resistance family are five members of the efflux pump family (Huang et al., 2022). The common type of efflux pump existing in the MDR Gram-negative bacterial phenotype belongs to the resistance nodulation division (RND) superfamily. *Pseudomonas aeruginosa* contains four potent RND superfamily efflux pumps, which are resistant to carbapenems, fluoroquinolones, and aminoglycosides (De Gaetano et al., 2023).

### 3.6. Biofilm formation

Biofilm is an aggregate of microorganisms within a self-formed exopolysaccharide to which the microbial cells adhere on living or non-living surfaces (Rather et al., 2021). Bacteria producing biofilms have shown higher antibiotic tolerance compared to their planktonic state. Antibacterial therapy is not effective in inhibiting biofilms. As mentioned by Sharma et al., (2023), the survey conducted by the National Institutes of Health (NIH) in 2022 demonstrated that more than 80 % of microbial infections are caused by biofilm formation (Sharma et al., 2023). The key mechanism of bacterial antibiotic resistance is through biofilm formation, which embeds the bacteria in an exopolysaccharide matrix. The extracellular matrix acts as a mechanical and biochemical barrier, altering the bacterial environment to include low O\(_2\), low pH, high CO\(_2\), and low water availability, which reduces the antibiotic effectiveness. Kumar Oli et al., (2022) reported that under such circumstances, eradicating the bacteria using conventional antibiotics is challenging. Even the lack of vitamins increases antibiotic resistance as presented in Table (1).

### 4. ESKAPE bacteria

#### 4.1. Enterococcus faecium

Enterococci are Gram-positive, facultative anaerobic bacteria present in the normal flora of the intestinal tract of animals and humans, which are generally harmless to humans and are considered unimportant medically (Yun et al., 2023). Enterococci were considered as bacteria with low pathogenicity, infecting immunocompromised patients. They cause several infections such as urinary tract infections, bacteremia, or endocarditis. Enterococcus spp., are important bacterial genera, with a 12 % frequency in hospital infections (Bhonchal Bhardwaj, 2020). Enterococcus faecium accounts for 20 % of human infections, but this virulent strain results in a 30 % mortality rate due to nosocomial infections (Gargvanshi et al., 2021). Vancomycin shows resistance by binding to D-Alanyl-D-Alanine moieties in the bacterial peptidoglycan (PG) precursors that exist on the terminal cell membrane, thus inhibiting cell wall biosynthesis (CWB). Van A and Van B type vancomycin gene-resistant Enterococcus (VRE) strains are the most common types, where D-Ala-D-Ala is replaced by D-Ala-D-lactate (Lac). The vancomycin resistance gene cassette codes for seven proteins, vanR, vanS, vanH, vanA, vanX, vanY, and vanZ, which replace D-Ala-D-Ala with D-alanyl-D-lactate termini (Gargvanshi et al., 2021). Vancomycin binds to D-Ala-D-Lac weakly compared to the normal dipeptide product, making vancomycin binding affinity much less (Jubeh et al., 2020).
Table 1: Diversity of mechanisms, mediators, and genes involved in the development of drug resistance in ESKAPE pathogenic bacteria

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Mediators involved in the mechanism</th>
<th>Enzymes/ Genes involved</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antibiotic inactivation/ alteration</td>
<td>β-lactamase</td>
<td>TEM, SHV, CTX-M, KPCS, OXA, BLAz, MBL-imp, ampC, VIM, NDM-1</td>
<td>Tooke et al., (2019)</td>
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<td></td>
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<td>Aminoglycosides</td>
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<td></td>
<td></td>
<td>AACs- AAC(1), AAC(2), AAC(3), AAC(6)</td>
<td>Zhang et al., (2020)</td>
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<td></td>
<td></td>
<td>APH- APH(4), APH(6), APH(3′), APH(2′), APH(7′) APH (3′)- IIIa</td>
<td>Goudarzi et al., (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ANT-ANT(6), ANT(9), ANT(4′), ANT(2′′), ANT(3′′)</td>
<td>Munita and Arias, (2016)</td>
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<tr>
<td></td>
<td></td>
<td>Ribosomal target site alterations</td>
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<tr>
<td></td>
<td></td>
<td>23sRNA, 50sRNA, 16sRNA – ArnA- ArnH and NmpA cfr, erm, MGE’s, blaраз-23, blaNDM MLSB, erm(A), erm(B), erm (C)</td>
<td>De Oliveira et al., (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cell Wall Precursor Alterations</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>vanA, vanB, mgrB, phoPQ, pmrAB, crrAB, TCRs, ipxA, ipxD</td>
<td>Bray et al., (2022); Stogios and Savchenko, (2020)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Changes in cell permeability</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>oprD, ompk35, ompk36, A-KPC</td>
<td>Delgado-Valverde et al., (2024)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Efflux pumps</td>
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</tbody>
</table>
Since *Enterococcus faecium* can produce aminoglycoside-modifying enzymes (Amino modifying enzymes (AMEs), such as aminoglycoside nucleotidyl transferases (ANTs), aminoglycoside acetyltransferases (AACs), and aminoglycoside phosphotransferases (APHs), it is classified as a MDR bacterium. This bacterium is intrinsically resistant to aminoglycosides, including tobramycin, kanamycin, and gentamicin (Thacharodi and Lamont, 2022). Point mutations in the *gyrA* and *parC* genes, which encode subunits A of DNA gyrase and topoisomerase IV, respectively, result in high levels of fluoroquinolone resistance. Additionally, the efflux transporter NorA produces resistance to fluoroquinolones (Bose et al., 2024).

### 4.2. *Staphylococcus aureus*

*Staphylococcus aureus* is a Gram-positive bacterium that normally resides in the nasal passages of healthy individuals. However, it can also act as a pathogen, causing a range of illnesses both in community and hospital settings. These infections include skin abscesses, food poisoning, pneumonia, sepsis, and toxic shock syndrome (Oliveira et al., 2018). Penicillin is an effective treatment for *Staphylococcus aureus* infection; however, abuse of this antibiotic has resulted in the rise of *Staphylococcus aureus* isolates that produce β-lactamase (Mancuso et al., 2021). Methicillin is semi-synthetic penicillin, initially developed in 1959 to combat penicillin-resistant *Staphylococcus aureus*. The first methicillin-resistant strain of *Staphylococcus aureus* was discovered in early 1961 (Turner et al., 2019). Methicillin, like other β-lactam antibiotics, binds to PBPs (penicillin-binding proteins) to prevent *Staphylococcus aureus* from growing (Fergestad et al., 2020). The genes responsible for developing resistance to methicillin in *Staphylococcus aureus* involve the *mecA* gene, which is a part of the mobile genetic elements in all methicillin-resistant *Staphylococcus aureus* (MRSA) strains (Abebe and Birhanu, 2023). *Staphylococcus aureus* has developed significant resistance to methicillin, leading to ineffectiveness of this antibiotic in treating infections caused by MRSA. To combat MRSA, vancomycin, a glycopeptide antibiotic, has been found to be effective. Apart from methicillin and vancomycin, other antibiotics like penicillin and quinolones are also used against *Staphylococcus aureus*. However, resistance to penicillin arises due to the production of the penicillinase, encoded by the *blaZ* gene carried on a plasmid (Tuon et al., 2023).

### 4.3. *Klebsiella pneumoniae*

*Klebsiella pneumoniae* is an opportunistic Gram-negative bacterium that infects hospitalized or immunocompromised people. It is a member of the *Enterobacteriaceae* family. This bacterium lives inside the digestive tracts of healthy people and animals. One-third of all Gram-negative infections are caused by *Klebsiella pneumoniae*. Both endogenous infection or direct interaction with an infected host can lead to infection. The multidrug-resistant (MDR) *Klebsiella pneumoniae* exhibits resistance to a wide range of medications, including β-lactam antibiotics, fluoroquinolones, and aminoglycosides. The β-lactams known as carbapenems are used to treat infections brought on by bacteria such as *Klebsiella pneumoniae* that produces extended-spectrum β-lactamase (ESBL). Additionally, *Klebsiella pneumoniae* is resistant to ciprofloxacin, cephalosporins, monobactams, cefotaxime, and meropenem (Abbas et al., 2024).

### 4.4. *Acinetobacter baumannii*

One of the most well-known nosocomial infections of the ESKAPE bacterial pathogens category is *Acinetobacter baumannii*. It is an opportunistic, aerobic, Gram-negative, non-fermentative pathogen that is primarily found in surgical and intensive care units. *Acinetobacter baumannii* has a 60 % fatality rate in cases of severe infections and causes ventilator-associated pneumonia, bacteremia, urinary tract infections, and secondary meningitis (Asif et al., 2018). *Acinetobacter baumannii* shows both intrinsic
and community-acquired resistance to antibiotics. However, it shows low virulence, causing infections in patients with prolonged hospital stays, immunosuppression, and previous broad-spectrum antimicrobial therapy (Impey et al., 2020). The primary mechanism of antibiotic resistance in *Acinetobacter baumannii* is through inactivation of β-lactam antibiotics by β-lactamase enzymes. These enzymes are classified into four groups: A, B, C, and D, all of which have been identified in *Acinetobacter baumannii*. This species exhibits a high propensity for incorporating foreign DNA through a horizontal gene transfer. Consequently, *Acinetobacter baumannii* often possesses a substantial number of β-lactamases due to its ability to integrate exogenous genetic materials (Shi et al., 2024). β-lactamases of class A are inhibited by clavulanate, penicillins, and cephalosporins, and they cause β-lactam resistance. Class B β-lactamases, also known as class metallo-β-lactamases (MBLs), catalyze the hydrolysis of all β-lactam antibiotics, including carbapenems but not monobactam (Zhang et al., 2024). Class C β-lactamases are encoded by the ampC gene, which is resistant to cephemycins, penicillins, and cephalosporins (Chen et al., 2023). Class D β-lactamases or Carbapenem-hydrolyzing class D β-lactamases (CHDLs), also known as oxacillinases (OXA), can hydrolyze oxacillin. Among the 400 OXA enzymes identified, most of them have the ability to hydrolyze carbapenems (Vrancianu et al., 2020). *Acinetobacter baumannii* efflux system has chromosomally encoded genes showing resistance to various antimicrobial agents, where the chromosomally encoded efflux pump system is expressed constitutively, contributing to intrinsic resistance (IR) or leading to acquired resistance. The efflux pump subfamily of *Acinetobacter baumannii* is classified into four categories: MATE (multidrug and toxic compound extrusion family), SMR (small multidrug resistance transporters), MFS (major facilitator superfamily), and RND superfamily (resistance-nodulation-division superfamily). The AdeABC pump, which is one of the four efflux systems in the RND (resistance-nodulation-division) superfamily, is crucial for the resistance to antibiotics such as aminoglycosides (Abdi et al., 2020). The AdeRS pump is linked to the development of biofilms, and its activity demonstrates resistance to a number of antibiotics, including aminoglycosides, β-lactams, fluoroquinolones, tetracyclines, macrolides, and chloramphenicol. The AbeM efflux pump is a subcategory of the MATE family, which exhibits resistance to imipenem, gentamicin, doxorubicin, norfloxacain, ofloxacain, and ciprofloxacain (Zack et al., 2024). The major facilitator superfamily (MFS) plays an essential role in resistance to antibiotics. The tet(A) gene in *Acinetobacter baumannii* shows resistance to tigecycline. Another efflux pump from the MFS family, named AmvA, is resistant to different antibiotics, disinfectants, and dyes. The SMR (small multidrug resistance transporters) category includes AbeS, an efflux pump involved in resistance to several antibiotics and dyes (Vrancianu et al., 2020). Aminoglycoside-modifying enzymes are the major mechanism by which *Acinetobacter baumannii* confirms resistance to aminoglycosides. There are three types of aminoglycosides: mainly acetyltransferases, adenyl transferases, and phosphotransferases that are found on transposable elements (Kyriakidis et al., 2021). Porins create channels that enable movement of molecules across the outer bacterial membrane and play a crucial part in *Acinetobacter baumannii* antibiotic resistance, which is influenced by changes in the permeability of the membrane’s envelope. *Acinetobacter baumannii* develops carbapenem resistance due to reduced expression of several porins such as CarO, Omp22-33, Omp33-36, Omp37, Omp43, Omp44, and Omp47. Antibiotic resistance is influenced by alteration of the outer membrane proteins and elements of the envelope, including lipopolysaccharide (LPS) and peptidoglycans. *Acinetobacter baumannii* loses lipopolysaccharide (LPS), which reduces membrane integrity and promotes colistin resistance (Roy et al., 2022). In *Acinetobacter baumannii*, modification of the antibiotic target mechanism is mediated by overexpression of the penicillin-binding proteins showing resistance to imipenem or mutation of DNA.
gyrase, resulting in resistance to quinolone and tetracycline (Kyriakidis et al., 2021).

4.5. *Pseudomonas aeruginosa*

*Pseudomonas aeruginosa* is a common Gram-negative environmental bacterium. It is an opportunistic human pathogen that can infect immunocompromised persons and cause life-threatening acute and chronic illnesses. *Pseudomonas aeruginosa* is linked to pneumonia brought on by a ventilator and nosocomial infections. Additionally, it contributes to a number of potentially fatal diseases in the intensive care unit, including endocarditis, septicemia, urinary tract infections, cystitis, pneumonia, and surgical wound infections. Furthermore, *Pseudomonas aeruginosa* has built-in resistance to many antibiotics (Qin et al., 2022). *Pseudomonas aeruginosa* infections have a natural ability to pose resistance to many antibiotics, causing the multidrug and pan-drug-resistant strains to increase worldwide. This bacterium is resistant to most of the commonly used antibiotics, including aminoglycosides, cephalosporins, fluoroquinolones, and carbapenems. *Pseudomonas aeruginosa* drug resistance is caused by an intrinsic resistance mechanism involving reduced outer membrane permeability and overexpression of the efflux pump. Acquired resistance in *Pseudomonas aeruginosa* is due to mutations in genes encoding porins, efflux pumps, penicillin-binding proteins (PBPs), and chromosomal β-lactamases, and acquisition of external resistance genes. This bacterium demonstrates resistance to aminoglycosides, carbapenems, fluoroquinolones, and β-lactams. The efflux pump hinders movement of antibiotics into and out of the cell membrane, contributing to this resistance mechanism (Kunz Coyne et al., 2022).


*Enterobacter* spp. are aerobic Gram-negative motile bacteria that belong to the *Enterobacteriaceae* family (Ramirez and Giron, 2024). These species are rod-shaped, non-fastidious, and occasionally encapsulated. Among these species is the *Enterobacter cloacae* complex (ECC), which is a group of bacterial pathogens that cause a wide range of diseases. The most prominent species among these bacterial pathogens are *Enterobacter cloacae* and *Enterobacter aerogenes*. Most commonly, *Enterobacter* spp., infect the urinary tract and respiratory tracts, and also cause bloodstream infections. They are opportunistic pathogens causing serious nosocomial infections in immunocompromised and hospitalized patients (Annavajhala et al., 2019). *Enterobacter* spp. are MDR strains that exhibit plasmid-encoded ESBLs (extended-spectrum β-lactamases) and carbapenemases, including metallo-β-lactamase, OXA, Verona integron-encoded metallo-β-lactamase, and KPC (*Klebsiella pneumoniae* carbapenemase), as shown in Table (2) (Boyd et al., 2020).

### Table 2. Compilation of the characteristics of ESKAPE bacterial pathogens, and their resistance profiles against a spectrum of antibiotics and associated specific genes

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Characteristics</th>
<th>Resistance antibiotic</th>
<th>Genes involved in drug resistance</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Enterococcus faecium</em></td>
<td>Gram-positive, facultative anaerobic bacteria, resident of human intestinal tract</td>
<td>Penicillin, Cephalosporin, Vancomycin, Tobramycin, Kanamycin, Gentamycin</td>
<td>aac (6′)-li, ant (6)-la, aph (3′)-Ia, msc, efmA, tet(m), pbp5, vanA, vanB,</td>
<td>Weber et al., (2020); Bush and Vazquez-Perteio, (2023)</td>
</tr>
</tbody>
</table>

Jain et al., 2024
Conclusion

Antibiotic resistance is the ability of microbial pathogens to resist antibiotics designed to inhibit their growth. It can be a natural phenomenon or the result of acquired resistance, where many genetic changes occur in the bacteria due to antibiotic exposure, overuse, and/or misuse of antibiotics. The World Health Organization (WHO) has grouped mainly six bacterial pathogens abbreviated as ESKAPE, which cause nosocomial infections in long-term hospitalized patients. The ESKAPE bacteria have developed antibiotic resistance through many mechanisms, including expression of genes responsible for this resistance. As almost all the bacterial pathogens have developed resistance against the available antibiotics, there is a necessity to discover new antibiotics. Understanding the different resistance mechanisms will provide new approaches and treatments for the emerging multidrug-resistant (MDR) bacterial pathogens.

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Conflict of interests

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