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Review Article

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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, over-prescription 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 β-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 (Sulghydryl 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^{2+} 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 amino 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 some of the most problematic bacterial pathogens. Worldwide, one of the most often prescribed antibiotic classes is fluoroquinolones, 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 (MLSB) antibiotic due to the erm-encoded rRNA methyl-transferases (Miklasińska-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 charge existing on the cell surface, composition and metabolism of the phospholipids, and response of the bacterial membranes to stress. *Klebsiella pnemoniae*, *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

Mechanism	**Mediators involved in the mechanism**	**Enzymes/ Genes involved**	**References**
Antibiotic inactivation/ alteration	β- lactamase	TEM, SHV, CTX-M, KPCS. OXA, BLAz, MBL-imp, ampC, VIM, NDM-1	Tooke *et al*., (2019)
	Aminoglycosides	AACs- AAC(1), AAC(2), AAC(3), AAC(6)	Zhang *et al*., (2020)
		APH- APH(4), APH(6), APH(3'), APH(2'), APH(7'') APH (3')- IIIa	Goudarzi *et al*., (2020)
		ANT-ANT(6), ANT(9), ANT(4'), ANT(2''), ANT(3'')	Munita and Arias, (2016)
Modification of drug binding Site	Target Enzyme Modification	PBP, PBP5, PBP2a, mecA, gyrA, gyrB, QnrA, QnrB, QnrS	De Oliveira *et al*., (2020)
	Ribosomal target site alterations	23sRNA, 50sRNA, 16sRNA – ArnA- ArnH and NmpA cfr, erm, MGE's, bla_{oxa-23}, bla_{NDM} MLS_B, erm(A), erm(B), erm (C)	De Oliveira *et al*., (2020)
	Cell Wall Precursor Alterations	vanA, vanB, mgrB, phoPQ, pmrAB, crrAB, TCRs, ipxA, ipxD	Bray *et al*., (2022); Stogios and Savchenko, (2020)
Changes in cell permeability	Porins	oprD, ompk35, ompk36, A-KPC	Delgado-Valverde *et al*., (2024)
	Efflux pumps	Acr AB-ToIC, mexAB-oprM, MexAB-oprM, MexCD-oprJ, MexY-oprM AcrAB, AdeABC, AdeDE, AdeFGH, AdeIJK	Compagne *et al*.,(2023)

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 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 cephamycins, 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, norfloxacin, ofloxacin, and ciprofloxacin (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).

4.6. *Enterobacter* spp.

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

Pathogen	Characteristics	Resistance different antibiotic	Genes involved in drug resistance	References
Enterococcus faecium	Gram-positive, facultative anaerobic bacteria, resident of human intestinal tract	Penicillin Cephalosporin Vancomycin Tobramycin Kanamycin Gentamycin	aac (6')-li, ant (6)-la, aph (3')-llla, msrc, efmA, tet(m), pbp5 vanA, vanB,	Weber *et al*., (2020); Bush and Vazquez-Perteio, (2023)

Staphylococcus aureus	Gram-positive, commensal bacteria, resident of human nasal mucosa	Methicillin Penicillin Oxacillin Quinolones Tetracycline Vancomycin	mecA, fexA, lnu(A), (F), optrA, ermA, ereA-b, ter(A), aac (3')-Ia, aadA1, aph(3')-II,	Mlynarczyk-Bonikowska *et al.*, (2022)
Klebsiella pneumoniae	Gram-negative, opportunistic bacteria, resident of gastrointestinal tract of humans.	Amikacin cephalosporin ceftotaxime meropenem ciprofloxacin carbapenems	bla_{KPC}, bla_{NDM-1}, bla_{TEM}, $bla_{CTX-M-15}$, bla_{OXA-4b}, IntI, IntII, IntIII	Surleac *et al.*, (2020); Cireşă *et al.*,(2024)
Acinetobacter baumannii	Gram-negative, aerobic, opportunistic pathogen, resident of urinary tract and lungs.	Norfloxacin Ofloxacin Ciprofloxacin Imipenem Chloramphenicol Nalidixic acid	TEM, SHE, GES, AdeA, tet(A), carO, omp22-23, Oxa-51, KPC, CARB, PER	Vrancianu *et al.*,(2020); Ahuatzin-Flores *et al.*, (2024)
Pseudomonas aeruginosa	Gram-negative, opportunistic pathogen resident of urinary tract and lungs.	Cefoxitin Imipenem, Polymyxins Piperacillin tazobartum meropenem carbapenem	mexR, nfxB, cmrA, fstI, opB, glpT, fusa1, ampD, parS, gryA, gyrB	Balasubramanian *et al.*, (2012); Edward *et al.*, (2023)
Enterobacter spp.	Gram-negative, encapsulated bacteria, resident of blood, urinary tract and respiratory tract.	Cephalosporin aztreonam carbapenem imipenem etrapenem meropenem	ampC, NDM-1, qnr, orqep, gyrA, gyrB, parC, Imp-1 aac (6')-1	Balasubramanian *et al.*, (2012); Tamma *et al.*, (2023)

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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Author's Contributions

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5. References

Abbas, R.; Chakkour, M.; Zein El Dine, H.; Obaseki, E.F.; Obeid, S.T.; Jezzini, A. et al. (2024). General overview of *Klebsiella pneumonia*: Epidemiology and the role of siderophores in its pathogenicity. Biology. 13(2): 78. https://doi.org/10.3390/biology13020078

Abdi, S.N.; Ghotaslou, R.; Ganbarov, K.; Mobed, A.; Tanomand, A.; Yousefi, M. et al. (2020). *Acinetobacter baumannii* efflux pumps and antibiotic resistance. Infectious Drug Resistance. 13: 423-434. https://doi.org/10.2147/IDR.S228089.

Abebe, A.A. and Birhanu, A.G. (2023). Methicillin Resistant *Staphylococcus aureus*: Molecular mechanisms underlying drug resistance development and novel strategies to combat. Infection and Drug Resistance. 16: 7641-7662. https://doi.org/10.2147/IDR.S428103

Ahmadian, L.; Bazgir, Z.N.; Mohammad, A.; Valadan, R. and Goli, H.R. (2021). Role of Aminoglycoside Modifying Enzymes (AMEs) in resistance to aminoglycosides among clinical isolates of *Pseudomonas aeruginosa* in the North of Iran. Biomed Research International. 1-10. https://doi.org/10.1155/2021/7077344

Ahuatzin-Flores, O.E.; Torres, E. and Chávez-Bravo, E. (2024). *Acinetobacter baumannii*, a multidrug-resistant opportunistic pathogen in new habitats: A Systematic Review. Microorganisms. 12(4): 644. https://doi.org/10.3390/microorganisms12040644

Annavajhala, M.K.; Gomez-Simmonds, A. and Uhlemann, A.C. (2019). Multidrug-Resistant *Enterobacter cloacae* complex emerging as a global, diversifying threat. Frontiers in Microbiology. 10: 44. https://doi.org/10.3389/fmicb.2019.00044

Arredondo-Alonso, S.; Top, J.; Corander, J.; Willems, R.J.L. and Schurch, A.C. (2021). Mode and dynamics of vanA-type vancomycin resistance dissemination in Dutch hospitals. Genome Medicine. 13: 1-18. https://doi.org/10.1186/s13073-020-00825-3

Asif, M.; Alvi, I.A. and Rehman, S.U. (2018). Insight into *Acinetobacter baumannii*: Pathogenesis, global resistance, mechanisms of resistance, treatment options, and alternative modalities. Infection and Drug Resistance. 11: 1249-1260. https://doi.org/10.2147/IDR.S166750

Ayukekbong, J.A.; Ntemgwa, M. and Atabe, A.N. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. Antimicrobial Resistance & Infection Control. 6(1): 47. https://doi.org/10.1186/s13756-017-0208-x

Baig, S.; Johannesen, T.B.; Overballe-Petersen, S.; Larsen, J.; Larsen, A.R. and Stegger, M. (2018). Novel SCC *mec* type XIII (9A) identified in an ST152 methicillin-resistant *Staphylococcus aureus*. Infection, Genetics and Evolution. 61: 74-76. https://doi.org/10.1016/j.meegid.2018.03.013

Balasubramanian, D.; Schneper, L.; Merighi, M.; Smith, R.; Narasimhan, G.; Lory, S. et al. (2012). The regulatory repertoire of *Pseudomonas aeruginosa* AmpC ß-Lactamase regulator AmpR includes virulence genes. PLoS ONE. 7(3): e34067. https://doi.org/10.1371/journal.pone.0034067

Baran, A.; Kwiatkowska, A. and Potocki, L. (2023). Antibiotics and bacterial resistance- A short story of an Endless Arms Race. International Journal of Molecular

Sciences. 24(6): 5777. https://doi.org/10.3390/ijms24065777

Benko, R.; Gajdacs, M.; Matuz, M.; Bodo, G.; Lazar, A.; Hajdu, E. et al. (2020). Prevalence and antibiotic resistance of ESKAPE pathogens isolated in the emergency department of a Tertiary care teaching hospital in Hungary: A 5-Year Retrospective Survey. Antibiotics. 9(9): 624. https://doi.org/10.3390/antibiotics9090624

Bhonchal Bhardwaj, S. (2020). Enterococci: An important nosocomial pathogen. IntechOpen. https://doi.org/10.5772/intechopen.90550

Bo, L.; Sun, H.; Li, Y.D.; Zhu, J.; Wurpel, J.N.D.; Lin, H. et al. (2024). Combating antimicrobial resistance: The silent war. Frontiers in Pharmacology. 15: 1347750. https://doi.org/10.3389/fphar.2024.1347750

Bonomo, R.A. (2017). β-Lactamases: A focus on current challenges. Cold Spring Harbor Perspectives in Medicine. 7(1): a025239. https://doi.org/10.1101/cshperspect.a025239

Bose, P.; Chowdhury, G.; Halder, G.; Ghosh, D.; Deb, A.K.; Kitahara, K. et al. (2024). Prevalence and changing antimicrobial resistance profiles of *Shigella* spp. isolated from diarrheal patients in Kolkata during 2011–2019. PLOS Neglected Tropical Diseases. 18(2): e0011964. https://doi.org/10.1371/journal.pntd.0011964

Boyd, S.E.; Livermore, D.M.; Hooper, D.C. and Hope, W.W. (2020). Metallo-β-Lactamases: Structure, function, epidemiology, treatment options, and the development pipeline. Antimicrobial Agents and Chemotherapy. 64(10): e00397-20. https://doi.org/10.1128/AAC.00397-20

Bray, A.S.; Smith, R.D.; Hudson, A.W.; Hernandez, G.E.; Young, T.M.; George, H.E. et al. (2022). MgrB-dependent colistin resistance in *Klebsiella pneumoniae* is associated with an increase in host-to-host transmission. mBio. 13(2): e0359521. https://doi.org/10.1128/mbio.03595-21.

Bush, L.M. and Vazquez-Pertejo, M.T. (2023). Enterococcal infections. MSD manual professional version. Accessed 21 May 2024. https://www.msdmanuals.com/en-kr/professional/infectious-diseases/gram-positive-cocci/enterococcal-infections.

Castanheira, M.; Simner, P.J. and Bradford, P.A. (2021). Extended-spectrum β-lactamases: An update on their characteristics, epidemiology and detection. JAC-Antimicrobial Resistance. 3(3): dlab092. https://doi.org/10.1093/jacamr/dlab092.

Chen, Y.T.; Zhou, C.; Yang, Z.B. and Li, G.B. (2023). Chapter 16- Benzoxaborole: a privileged scaffold for drug discovery. Academic Press. 335-355. https://doi.org/10.1016/B978-0-443-18611-0.00038-3.

Cireșa, A.; Talapan, D.; Vasile, C.C.; Popescu, C. and Popescu, G.A. (2024). Evolution of antimicrobial resistance in *Klebsiella pneumoniae* over 3 years (2019-2021) in a tertiary hospital in Bucharest, Romania. Antibiotics. 13: 431. https://doi.org/10.3390/antibiotics13050431.

Compagne, N.; Vieira Da Cruz, A.; Muller, R.T.; Hartkoorn, R.C.; Flipo, M. and Pos, K.M. (2023). Update on the discovery of efflux pump inhibitors against critical priority Gram-negative bacteria. Antibiotics. 12: 180. https://doi.org/10.3390/antibiotics12010180.

De Gaetano, G.V.; Lentini, G.; Fama, A.; Coppolino, F. and Beninati, C. (2023). Antimicrobial resistance: Two-component regulatory systems and multidrug efflux pumps. Antibiotics. 12(6): 965. https://doi.org/10.3390/antibiotics12060965.

De Oliveira, D.M.P.; Forde, B.M.; Kidd, T.J.; Harris, P.N.A.; Schembri, M.A.; Beatson, S.A. et al. (2020). Antimicrobial resistance in ESKAPE pathogens. Clinical Microbiology Reviews. 33(3): e00181-19. https://doi.org/10.1128/CMR.00181-19.

Delgado-Valverde, M.; Portillo-Calderon, I.; Alcalde-Rico, M.; Carmen, C.; Hidalgo, C.; Toro Esperon, C. et al (2024). Activity of imipenem/relebactam and comparators against KPC-producing *Klebsiella pneumoniae* and imipenem-resistant *Pseudomonas aeruginosa*. European Journal of Clinical Microbiology and Infectious Diseases. 43: 445–457. https://doi.org/10.1007/s10096-023-04735-1.

Denissen, J.; Reyneke, B.; Waso-Reyneke, M.; Havenga, B.; Barnard, T.; Khan, S. et al. (2022). Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection, and risk to human health. International Journal of Hygiene and Environmental Health. 244: 114006. https://doi.org/10.1016/j.ijheh.2022.114006.

Edward, E.A.; El Shehawy, M.R.; Abouelfetouh, A. and Aboulmagd, E. (2023). Prevalence of different virulence factors and their association with antimicrobial resistance among *Pseudomonas aeruginosa* clinical isolates from Egypt. BMC Microbiology. 23: 161. https://doi.org/10.1186/s12866-023-02897-8.

Fergestad, M.E.; Stamsas, G.A.; Morales Angeles, D.; Salehian, Z.; Wasteson, Y. and Kjos, M. (2020). Penicillin-binding protein PBP2a provides variable levels of protection toward different β-lactams in *Staphylococcus aureus* RN4220. Microbiology-Open. 9(8): e1057. https://doi.org/10.1002/mbo3.1057.

Gargvanshi, S.; Vemula, H. and Gutheil, W.G. (2021). Effect of vancomycin on cytoplasmic peptidoglycan intermediates and van operon mRNA levels in VanA-type vancomycin-resistant *Enterococcus faecium*. Journal of Bacteriology. 203(16): 1-12. https://doi.org/10.1128/JB.00230-21.

Gauba, A. and Rahman, K. M. (2023). Evaluation of antibiotic resistance mechanisms in Gram-negative bacteria. Antibiotics. 12(11): 1590. https://doi.org/10.3390/antibiotics12111590.

Genovesi, G. (2023). Infection control today exclusive: The challenge of Health Care Associated Infections (HAIs) and evolving prevention: A focus on hand hygiene. Infection Control Today. https://www.infectioncontroltoday.com/view/infection-control-today-exclusive-challenge-health-care-associated-infections-hais-evolving-prevention-a-focus-on-hand-hygiene.

Gnanamani, A.; Hariharan, P. and Paul-Satyaseela, M. (2017). *Staphylococcus aureus*: Overview of bacteriology, clinical diseases, epidemiology, antibiotic resistance and therapeutic approach. In: Enany, S. and Crotty Alexander, L.E. (Editors). Frontiers in *Staphylococcus aureus*. Intech Open. https://doi.org/10.5772/67338.

Goudarzi, G.; Shakib, P.; Azadpour, M. and Nazer, M.R. (2020). Distribution of aac(6')/aph(2''), aph(3')-IIIa, and ant(4')-Ia genes among clinical nasal sources for *Staphylococcus aureus* strains isolated in Korramabad. Iran Crescent Journal of Medical and Biological Sciences.7(3): 429-433. https://sid.ir/paper/776256/en.

Han, X.; Zou, G.; Liu, J.;Yang, C.; Du,X.; Chen, G. et al. (2022). Mechanisms of linezolid resistance in *Staphylococcus capitis* with the novel mutation C2128T in the 23S rRNA gene in China. BMC Microbiology. 22: 1-8. https://doi.org/10.1186/s12866-022-02616-9.

Huang, L.; Wu, C.; Gao, H.; Xu, C.; Dai, M.; Huang, L. et al. (2022). Bacterial multidrug efflux pumps at the frontline of antimicrobial resistance: An overview. Antibiotics. 11(4): 520. https://doi.org/10.3390/antibiotics11040520.

Impey, R.E.; Hawkins, D.A.; Sutton, J.M. and Soares da Costa, T.P. (2020). Overcoming intrinsic and acquired resistance mechanisms associated with the cell wall of gram-negative bacteria. Antibiotics. 9(9): 623. https://doi.org/10.3390/antibiotics9090623.

Jubeh, B.; Breijyeh, Z. and Karaman, R. (2020). Resistance of gram-positive bacteria to current antibacterial agents and overcoming approaches.

Molecules. 25(12): 2888. https://doi.org/10.3390/molecules25122888.

Kumar Oli, A.; Javaregowda, P.; Jain, A. and Kelmani, C. (2022). Mechanism involved in biofilm formation of *Enterococcus faecalis*. In: Das, T. (Editor), Focus on Bacterial Biofilms. Intech-Open. https://doi.org/10.5772/intechopen.103949.

Kunz Coyne, A.J.; El Ghali, A.; Holger, D.; Rebold, N. and Rybak, M.J. (2022). Therapeutic strategies for emerging multidrug-resistant *Pseudomonas aeruginosa*. Infectious Diseases and Therapy. 11(2): 661-682. https://doi.org/10.1007/s40121-022-00591-2.

Kyriakidis, I.; Vasileiou, E.; Pana, Z.D. and Tragiannidis, A. (2021). *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens. 10(3): 373. https://doi.org/10.3390/pathogens10030373.

Laws, M.; Shaaban, A. and Rahman, K.M. (2019). Antibiotic resistance breakers: Current approaches and future directions. FEMS Microbiology Reviews. 43(5): 490-516. https://doi.org/10.1093/femsre/fuz014.

Li, S.; Feng, X.; Li, M. and Shen, Z. (2023). *In vivo* adaptive antimicrobial resistance in *Klebsiella pneumoniae* during antibiotic therapy. Frontiers in Microbiology. 14: 1159912. https://doi.org/10.3389/fmicb.2023.1159912.

Makarewicz, M.; Droada, I.; Tarko, T. and Duda-Chodak, A. (2021). The Interactions between polyphenols and microorganisms, especially gut microbiota. Antioxidants. 10(2): 188. https://doi.org/10.3390/antiox10020188.

Mancuso, G.; Midiri, A.; Gerace, E. and Biondo, C. (2021). Bacterial antibiotic resistance: The most critical pathogens. Pathogens. 10(10): 1310. https://doi.org/10.3390/pathogens10101310.

Mc Carlie, S.; Boucher, C.E. and Bragg, R.R. (2020). Molecular basis of bacterial disinfectant resistance. Drug Resistance Updates. 48: 100672. https://doi.org/10.1016/j.drup.2019.100672.

Mei, L.; Song, Y.; Liu, X.; Li, K.; Guo, X.; Liu, L. et al. (2024). Characterization and implications of IncP-2A plasmid pMAS152 harboring multidrug resistance genes in extensively drug-resistant *Pseudomonas aeruginosa*. Microorganisms. 12(3): 562. https://doi.org/10.3390/microorganisms12030562.

Miklasinska-Majdanik, M. (2021). Mechanisms of resistance to macrolide antibiotics among *Staphylococcus aureus*. Antibiotics (Basel, Switzerland). 10(11): 1406. https://doi.org/10.3390/antibiotics10111406.

Mlynarczyk-Bonikowska, B.; Kowalewski, C.; Krolak-Ulinska, A. and Marusza, W. (2022). Molecular mechanisms of drug resistance in *Staphylococcus aureus*. International Journal of Molecular Sciences. 23: 8088. https://doi.org/10.3390/ijms23158088.

Monegro, A.F.; Muppidi, V. and Regunath, H. (2024). Hospital-acquired infections. In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK441857/.

Mulani, M.S.; Kamble, E.E.; Kumkar, S.N.; Tawre, M.S. and Pardesi, K.R. (2019). Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A Review. Frontiers in Microbiology. 10: 539. https://doi.org/10.3389/fmicb.2019.00539.

Munita, J.M. and Arias, C.A. (2016). Mechanisms of antibiotic resistance. Microbiology spectrum. 4(2): 4.2.15. https://doi.org/10.1128/microbiolspec.VMBF-0016-2015.

Murugaiyan, J.; Kumar, P.A.; Rao, G.S.; Iskandar, K.; Hawser, S.; Hays, J.P. et al. (2022). Progress in alternative strategies to combat antimicrobial resistance: Focus on Antibiotics. Antibiotics. 11(2): 200. https://doi.org/10.3390/antibiotics11020200.

Nguyen, A.H.; Hood, K.S.; Mileykovskaya, E.; Miller, W.R. and Tran, T.T. (2022). Bacterial cell

membranes and their role in daptomycin resistance: A review. Frontiers in Molecular Biosciences. 9: 1035574. https://doi.org/10.3389/fmolb.2022.1035574.

O'Leary, E.N.; Neuhauser, M.M.; McLees, A.; Paek, M.; Tappe, J. and Srinivasan, A. (2024). An update from the National Healthcare Safety Network on Hospital Antibiotic Stewardship Programs in the United States, 2014-2021. Open Forum Infectious Diseases. 11(2): ofad684. https://doi.org/10.1093/ofid/ofad684.

Oliveira, D.; Borges, A. and Simões, M. (2018). *Staphylococcus aureus* toxins and their molecular activity in infectious diseases. Toxins. 10(6): 252. https://doi.org/10.3390/toxins10060252.

Prajapati, J.D.; Kleinekathofer, U. and Winterhalter, M. (2021). How to enter a bacterium: Bacterial porins and the permeation of antibiotics. Chemical Reviews. 121(9): 5158-5192. https://doi.org/10.1021/acs.chemrev.0c01213.

Qin, S.; Xiao, W.; Zhou, C.; Pu, Q.; Deng, X.; Lan, L. et al. (2022). *Pseudomonas aeruginosa*: Pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal Transduction and Targeted Therapy. 7(1): 199. https://doi.org/10.1038/s41392-022-01056-1.

Ramadan, R.A.; Bedawy, A.M.; Negm, E.M.; Hassan, T.H.; Ibrahim, D.A.; ElSheikh, S.M. et al. (2022). Carbapenem-resistant *Klebsiella pneumoniae* among patients with ventilator-associated pneumonia: Evaluation of antibiotic combinations and susceptibility to new antibiotics. Infection and Drug Resistance. 15: 3537-3548. https://doi.org/10.2147/IDR.S371248.

Ramirez, D. and Giron, M. (2024). Enterobacter Infections. In StatPearls. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK559296/.

Rather, M.A.; Gupta, K. and Mandal, M. (2021). Microbial biofilm: Formation, architecture, antibiotic resistance, and control strategies. Brazilian Journal of Microbiology. 52(4): 1701-1718. https://doi.org/10.1007/s42770-021-00624-x.

Roy, S.; Chowdhury, G.; Mukhopadhyay, A.K.; Dutta, S. and Basu, S. (2022). Convergence of biofilm formation and antibiotic resistance in *Acinetobacter baumannii* infection. Frontiers in Medicine. 9: 793615. https://doi.org/10.3389/fmed.2022.793615.

Ruekit, S.; Srijan, A.; Serichantalergs, O.; Margulieux, K.R.; Mc Gann, P.; Mills, E.G. et al. (2022). Molecular characterization of multidrug-resistant ESKAPE pathogens from clinical samples in Chonburi, Thailand (2017–2018). BMC Infectious Diseases. 22(1): 695. https://doi.org/10.1186/s12879-022-07678-8.

Salam, M.A.; Al-Amin, M.Y.; Salam, M.T.; Pawar, J.S.; Akhter, N.; Rabaan, A.A. et al. (2023). Antimicrobial resistance: A growing serious threat for global public health. Healthcare (Basel, Switzerland). 11(13): 1946. https://doi.org/10.3390/healthcare11131946.

Sharma, S.; Mohler, J.; Mahajan, S.D.; Schwartz, S.A.; Bruggemann, L. and Aalinkeel, R. (2023). Microbial biofilm: A review on formation, infection, antibiotic resistance, control measures, and innovative treatment. Microorganisms. 11(6): 1614. https://doi.org/10.3390/microorganisms11061614.

Shi, J.; Cheng, J.; Liu, S.; Zhu, Y. and Zhu, M. (2024). *Acinetobacter baumannii*: an evolving and cunning opponent. Frontiers Microbiology. 15: 1332108. https://doi.org/10.3389/fmicb.2024.1332108.

Stogios, P.J. and Savchenko, A. (2020). Molecular mechanisms of vancomycin resistance. Protein Science. 29(3): 654-669. https://doi.org/10.1002/pro.3819.

Surleac, M.; Czobor Barbu, I.; Paraschiv, S.; Popa, L.I.; Gheorghe, I.; Marutescu, L. et al. (2020). Whole genome sequencing snapshot of multi-drug

resistant *Klebsiella pneumoniae* strains from hospitals and receiving wastewater treatment plants in Southern Romania. PLoS ONE. 15(1): e0228079. https://doi.org/10.1371/journal.pone.0228079.

Tamma, P.D.; Aitken, S.L.; Bonomo, R.A.; Mathers, A.J.; van Duin, D. and Clancy, C.J. (2023). Infectious diseases society of America 2023 guidance on treatment of Antimicrobial resistant Gram-negative infections. Infectious Diseases Society of America. 428:1-53. https://doi.org/10.1093/cid/ciad428.

Thacharodi, A. and Lamont, I.L. (2022). Aminoglycoside-Modifying Enzymes are sufficient to make *Pseudomonas aeruginosa* clinically resistant to key antibiotics. Antibiotics. 11(7): 884. https://doi.org/10.3390/antibiotics11070884.

Timsit, J.F.; Wicky, P.H. and De Montmollin, E. (2022). Treatment of severe infections due to metallo-betalactamases Enterobacterales in critically Ill patients. Antibiotics. 11(2): 144. https://doi.org/10.3390/antibiotics11020144.

Tooke, C.L.; Hinchliffe, P.; Bragginton, E.C.; Colenso, C.K.; Hirvonen, V.H.A.; Takebayashi, Y. et al. (2019). β-Lactamases and β-Lactamase inhibitors in the 21st Century. Journal of Molecular Biology. 431(18): 3472-3500. https://doi.org/10.1016/j.jmb.2019.04.002.

Tuon, F.F.; Suss, P.H.; Telles, J.P.; Dantas, L.R.; Borges, N.H. and Ribeiro, V.S.T. (2023). Antimicrobial treatment of *Staphylococcus aureus* biofilms. Antibiotics (Basel). 12(1): 87. https://doi.org/10.3390/antibiotics12010087.

Turner, N.A.; Sharma-Kuinkel, B.K.; Maskarinec, S.A.; Hooven, T.A.; Rude, T.H.; Schultz-Cherry, S. et al. (2019). Methicillin-resistant *Staphylococcus aureus*: an overview of basic and clinical research. Nature Reviews Microbiology. 17(3): 203-218. https://doi.org/10.1038/s41579-018-0147-4

Uddin, T.M.; Chakraborty, A.J.; Khusro, A.; Zidan, B.R.M.; Mitra, S.; Emran, T.B. et al. (2021). Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future prospects. Journal of Infection and Public Health. 14(12): 1750-1766. https://doi.org/10.1016/j.jiph.2021.10.020.

Vrancianu, C.O.; Gheorghe, I.; Czobor, I.B. and Chifiriuc, M.C. (2020). Antibiotic resistance profiles, molecular mechanisms and innovative treatment strategies of *Acinetobacter baumannii*. Microorganisms. 8: 935. https://doi.org/10.3390/microorganisms8060935.

Wang, N.; Luo, J.; Deng, F.; Huang, Y. and Zhou, H. (2022a). Antibiotic combination therapy: A Strategy to overcome bacterial resistance to aminoglycoside antibiotics. Frontiers in Pharmacology. 13: 1-15. https://doi.org/10.3389/fphar.2022.1033734.

Wang, Y.; Ge, H.; Wei, X. and Zhao, X. (2022b). Research progress on antibiotic resistance of *Salmonella*. Food Quality and Safety. 6: fyac035. https://doi.org/10.1093/fqsafe/fyac035.

Weber, A.; Maechler, F.; Schwab, F.; Gastmeier, P. and Kola,A. (2020). Increase of vancomycin-resistant *Enterococcus faecium* strain type ST117 CT71 at Charite- Universitatsmedizin Berlin, 2008 to 2018. Antimicrobial Resistance Infection Control. 9: 109. https://doi.org/10.1186/s13756-020-00754-1.

Yun, S.E.; Choi, B.B.; Nam, S.H. and Kim, G.C. (2023). Antimicrobial effects of edible mixed herbal extracts on oral microorganisms: An in vitro study. Medicina. 59: 1771. https://doi.org/10.3390/medicina59101771.

Zack, K.M.; Sorenson, T. and Joshi, S.G. (2024). Types and mechanisms of efflux pump systems and the potential of efflux pump inhibitors in the restoration of antimicrobial susceptibility, with a special reference to *Acinetobacter baumannii*.

Pathogens. 13(3): 197. https://doi.org/10.3390/pathogens13030197.

Zhang, S.; Liao, X.; Ding, T. and Ahn, J. (2024). Role of β-Lactamase inhibitors as potentiators in antimicrobial chemotherapy targeting gram-negative bacteria. Antibiotics. 13: 260. https://doi.org/10.3390/antibiotics13030260.

Zhang, G.; Li, J.; Ai, G.; He, J.; Wang, C. and Feng, J. (2020). A new intrinsic aminoglycoside 6′-N-acetyltransferase subclass, AAC(6′)-III, in *Burkholderia pseudomallei*, *Burkholderia mallei* and *Burkholderia oklahomensis*. The Journal of Antimicrobial Chemotherapy. 75(5): 1352-1353. https://doi.org/10.1093/jac/dkaa011.