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An Overview of Antibiotic Resistance: A Silent Threat for Global Public Health

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Abstract: ***The discovery of antibiotics is considered as milestone for Infectious disease improvement, but simultaneously developing resistance surpasses the benefit, resistance develops due to the overuse or misuse of antibiotics irrationally, it causes a decreased quality of life for patients, with a longer hospital stay, more expensive treatments, and furthermore resistance of last resort antibiotics. It is like arriving at the pre-antibiotic era again. The microorganisms that have developed resistance to available therapeutic agents are threatening to public health worldwide. ESKAPE (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter spp.) pathogens display multidrug resistance and virulence through various mechanisms and it is time to discover or design new antibiotics against ESKAPE pathogens, by knowing the mechanism one can decide the appropriate antibiotics to treat infection especially in combination strategy. In this review article, we focused more on mechanisms, which will help in deciding the appropriate therapy.***

Keywords: Antibiotic Resistance, Mechanism of Resistance, ESKAPE pathogens, Multidrug resistance

1. Background

Resistance occurs when bacteria, evolve with a different mechanism and no longer respond to antibiotics, which they are previously sensitive to, making infections harder to treat and increasing the risk of disease spread, severe illness, and death. As a result of resistance, antibiotics become ineffective to resistant microorganisms and infection becomes increasingly difficult to treat [1]. This natural evolutionary phenomenon, enhanced by the misuse of antibiotics and the global spread of AMR, mainly affects unhealthy and debilitated patients, giving rise to Multi-Drug Resistance organisms also known as superbugs. It inflicts a high cost on the health sector of all the countries [2]. According to one report from the European Union (EU), Iceland, and Norway, Antibiotic Resistance (ABR) has significant costs to society in terms of increased mortality, morbidity, use of healthcare resources, and intangible of affected individuals. Infection owing to multidrug-resistant micro-organisms results in 25,000 deaths and €1.5 billion per year in hospital and societal costs, Another report from the Centre for Disease Control and Prevention United States of America healthcare, indicates that nearly 23,000 people die each year as a direct result of infection due to resistant microorganisms, with associated hospital cost of more than $20 billion [3].

2. History of antibiotics development and resistance occurrence

1) Exposure to antibiotics in the pre-antibiotic era

At 350-550 CE, in the Sudanese Nubian population, Tetracycline traces were found in human skeletal. Anecdotes about the Red soil that contains Antibiotic-like properties found in Jordan, that used historically for treating skin infections. Remedies used for millennia in traditional Chinese medicine, for example, the discovery of the potent antimalarial drug qinghaosu (Artemisinin), extracted from Artemisia plants in the 1970s, used by Chinese herbalists for thousands of years as a remedy for many illnesses. Long-term history of exposure to Antibiotics and their use extensively may be one of the factors that contribute to the accumulation of Antibiotic resistance genes in the human population. The natural history of ABR genes can be revealed through the phylogenetic reconstruction and this kind of analysis suggests the long-term presence of genes conferring resistance to several classes of Antibiotics in nature before the Antibiotic Discovery Era [4].

Before the discovery of antibiotics, the knowledge of microorganisms and infectious illnesses was insufficient. Millions of people died as a result of the ineffective treatment methods and prevention of the spread of these infectious diseases, which frequently reached an epidemic level, for instance outbreak of the “plague” caused millions of deaths, due to scarcity of treatment [5]. German physician Robert Koch and French bacteriologist Louis Pasteur each carried out separate bacterial investigations in the second half of the 19th century. Robert Koch researched on *Mycobacterium tuberculosis* and established a connection between certain bacterial species and associated disease and Louis Pasteur focused on *Bacillus anthracis*. Observations by these two pivotal microbiologists have pushed microbiology and antibiotic development toward its modern era [6]

2) The antibiotic era

The beginning of the modern “Antibiotic Era” was associated with the names of ‘Paul Ehrlich & Alexander Fleming’. Ehrlich’s idea about a “Magic bullet” that selectively only targets disease-causing microbes and not the host cells. This idea led him to find a ‘drug against Syphilis’ in 1904. After synthesizing hundreds of compounds, they came across “Salvarsan-Arsphenamine” (6th compound of the 600th series, named compound 606) marketed by Hoechst AG, which showed significant promise for patients with syphilis with the limited clinical trial. Salvarsan enjoyed the status of the most

frequently prescribed drug until its replacement by penicillin in the 1940s [4]

The systemic screening approach by Paul Ehrlich became the mainspring of drug search strategies in the pharmaceutical industry and as a result, thousands of drugs were identified and translated into clinical practice, including a variety of antimicrobial drugs. This approach also led to the invention of "Sulfa drugs", namely Sulfonamidochrysoidine - Prontosil, synthesized by Bayer chemists in the 1930s. However, Prontosil has appeared to be a precursor of the active drug 'Sulfanilamide', and this active molecule has already been used in the dye industry for some years, and hence it's not patentable, as Sulfanilamide cheap to synthesize, off-patent and moiety was easy to modify, many companies started the mass production of sulfonamide derivatives. The benefaction of this oldest antibiotic on the market is possibly reflected in one of the most broadly disseminated cases of drug resistance called "Sulfa drug resistance" [4].

In 1899, the first antibiotic used in the hospital was discovered named "Pyocyanase" prepared from *Pseudomonas aeruginosa* (formerly *Bacillus pycyaneus*), Unfortunately, it was quite toxic for humans, and treatment with it was eventually abandoned. In 1928 Alexander Fleming discovered the "Penicillin" from the *Penicillium* genus, Fleming determined that Penicillin had antibacterial activity against, staphylococci and other gram-positive pathogens. He was also among the first, who cautioned about potential resistance to penicillin if used inappropriately [6]

The period between the 1950s and 1970s was indeed considered the Golden Era of the discovery of novel antibiotic classes, with no new classes of antibiotics discovered since then, and that's considered one of the disadvantageous factors for combating Antibiotic resistance. With a decline in discovery rate, mainstream approaches for the development of new drugs to fight emerging and re-emerging resistance of pathogens to antibiotics have been the modification of existing antibiotics or repurposing its use [4].

3) Current antibiotic discovery and resistance era

Despite living in the era of novel technologies in biomedical research, numerous untreatable infectious diseases are ranked as the main causes of human death worldwide. Increased antibiotic use in humans and animal production are the two major causes of the emergence of resistant bacteria in hospitals, human communities, and animal farms. Apart from it over-prescription of last resort antibiotics, lack of education in the community, and irrationally use of antibiotics without any indication are the most common risk factor for developing antibiotic resistance in the current era. It is a fact that there has been a marked halt growth of new antibiotics development and discovery after the golden era of antibiotic discovery, only a few of the novel antibiotics are discovered, and with development there is the issue of fast emerging resistance to many potent antibiotics. Dry pipeline for new discovery, fast emerging resistance, and irrational use of antibiotics would be the reasons of rising the MDR organisms [7]

Antimicrobial drug discovery is highly challenging, and the current rise in AMR is eroding the efficacy of available Antibiotics, in this era, we usually utilize the antibiotics discovered in the golden era of antibiotics discovery either by repurposing or adding adjuvant to tackle the resistance problem. Since 1960, only 4 new classes of Abx available for use in patients i.e., Quinolones, Lincosamides, oxazolidinones, and cyclic Lipopolypeptides. The reserved use of such novel drugs as last-resort measures has decreased the profit margins of the pharmaceutical industry, leading to the withdraw their research effort from antimicrobial drug discovery [8]

In the current era, the resistance pattern of microorganisms from antibiotics is seen as MDR (Multidrug-resistant), XDR (Extensive drug-resistant), and PDR (Pandrug-resistance). According to experts MDR was defined as non-susceptibility to at least one agent in three or more antimicrobial categories, XDR was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and PDR was defined as non-susceptibility to all agents in all antimicrobial categories [9]. The CDC declared in 2013 that the human race is now in the "post-antibiotic era," and in 2014, the World Health Organization (WHO) warned that the antibiotic resistance crisis is becoming threat. Fifteen MDR bacteria have been declared a substantial threat to U.S. public health and national security by the IDSA and the Institute of Medicine, as well as the federal Interagency Task Force on Antimicrobial Resistance. In the current era mainly in Gram-positive pathogens most common resistance is seen in *S. aureus*, and Enterococcus, specifically in Methicillin-resistant staphylococcus aureus (MRSA) and Vancomycin-resistant enterococci. Apart from that resistance among Gram-negative pathogens are more concern, due to resistance to all available Abx observed in clinical settings, among them *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and ESBL-producing *Escherichia coli* are more prone to develop resistance. In 2019 The CDC assessed antibiotic-resistant bacterial infections according to seven factors: clinical impact, economic impact, incidence, 10-year projection of incidence, transmissibility, availability of effective antibiotics, and barriers to prevention. The threat level of each bacteria was then classified as "urgent," "serious," or "concerning". Threats that are urgent or serious require more monitoring and prevention activities, whereas those considered concerning require less action comparatively [10]. Table 1 represents the CDC classification of pathogens according to threat level, recently in 2024 World Health Organization has also revised the list of drug-resistant bacteria that are most threatening to human health. Bacterial Priority Pathogens List (BPPL) 2024, featuring 15 families of antibiotic-resistant bacteria grouped into critical, high and medium categories for prioritization. The list provides guidance on the development of new and necessary treatments to stop the spread of antimicrobial resistance (AMR) [11]. In conclusion, figure 1 displays the timeline of antibiotic discovery and resistance development.

Table 1: CDC Classification of Pathogens according to threat Level

Category	Pathogens
Urgent threats	Carbapenem-resistant Acinetobacter Carbapenem-resistant Enterobacteriaceae
Serious threats	ESBL-producing Enterobacteriaceae Vancomycin-resistant Enterococci Multidrug-resistant Pseudomonas aeruginosa Methicillin-resistant Staphylococcus aureus Drug-resistant Streptococcus pneumoniae
Concerning threats	Erythromycin-resistant group A Streptococcus Clindamycin-resistant group B Streptococcus

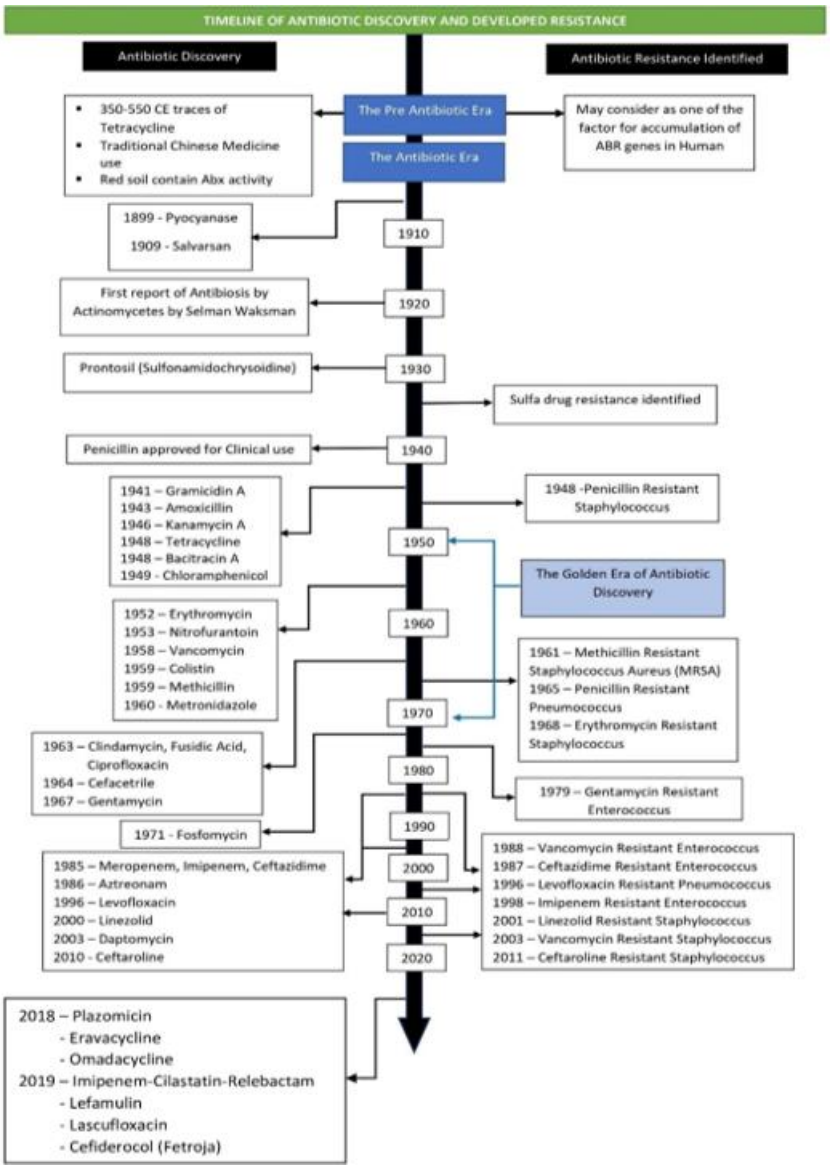

Figure 1: Timeline for Antibiotic Discovery and Development of Resistance

3. Mechanism of Antibiotic Resistance

3.1 Origin of Resistance

Antimicrobial Resistance is ancient and it is expected to result from the interaction of many organisms with the environment. Most antibiotics are naturally produced molecules and hence co-resident bacteria have evolved mechanisms to overcome their action to survive, so these microorganisms are often considered to be "Intrinsically" resistant to one or more antibiotics [12]**.** "Acquired Resistance" is resistance by the bacterial population that was originally susceptible to the antimicrobial agent. It can be the result of mutations in chromosomal genes or due to the acquisition of external genetic determinants of resistance, likely obtained from intrinsically resistant organisms present in the environment. Below Table 2 represents intrinsic resistance of certain micro-organisms for listed commonly used antibiotics.

Table 2: Intrinsic Resistance in Micro-Organisms

Microorganism	Intrinsic Resistance
Pseudomonas aeruginosa	Sulfonamides, Ampicillin, 1st, and 2nd Generation Cephalosporins, Chloramphenicol, Tetracycline
Enterococcus	Aminoglycosides, Cephalosporins, Lincosamides
Klebsiella spp.	Ampicillin
Escherichia coli	Macrolides
Acinetobacter spp.	Ampicillin, Glycopeptides
Bacteroides (anaerobes)	Aminoglycosides, Many B-Lactams, Quinolones
All Gram Positives	Aztreonam
All Gram-Negative	Glycopeptides, Lipopeptides
Stenotrophomonas maltophilia	Aminoglycosides, B-Lactams, Carbapenems, Quinolones

3.2 Antimicrobial Resistance

3.2.1 Genetic Basis – from an evolutionary perspective, bacteria use two major genetic strategies to survive antibiotic attack

1) Mutational Resistance – Mutation of Gene(s), often associated with the mechanism of action of the compound. In this, a subset of bacterial cells derived from a susceptible population develops mutations in their genes that affect the antibiotic activity, resulting in preserved cell survival in the presence of the antibiotic. Once a mutational resistance emerges, antibiotics eliminate the susceptible population of microorganisms and resistant bacteria predominate [12]. Figure 2 represents the pictorial of mutational resistance.

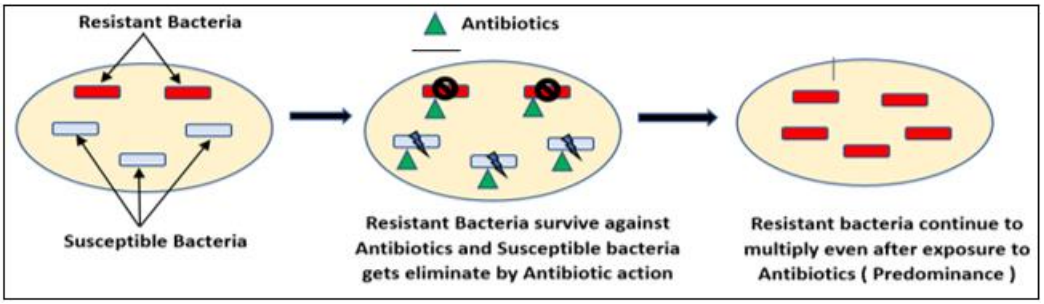

Figure 2: Schematic Diagram of Mutational Resistance

2) Horizontal Gene Transfer (HGT):

Bacterial evolution by the acquisition of foreign DNA material through HGT is one of the most important culprits for bacterial resistance. Mainly bacteria acquire external genetic material generally through three main strategies:

1] Transformation (Incorporation of naked DNA) – It is the simplest type of HGT, but from the clinical perspective it is not the most relevant. 2] Transduction (Phage mediated) – Bacterial DNA is moved from one bacteria to another by bacteriophage (virus). 3] Conjugation – The most common type that involves an emergency of resistance in the hospital environment, mostly occurs owing to bacterial cell-to-cell contacts and mostly happens in the gastrointestinal tract of humans under Antibiotics treatment. It utilizes the mobile genetic elements (MGEs) as a vehicle to share valuable genetic information. The most important MGEs are plasmids and transposons [12]**.** Figure 3 represents the different modes of horizontal gene transfer.

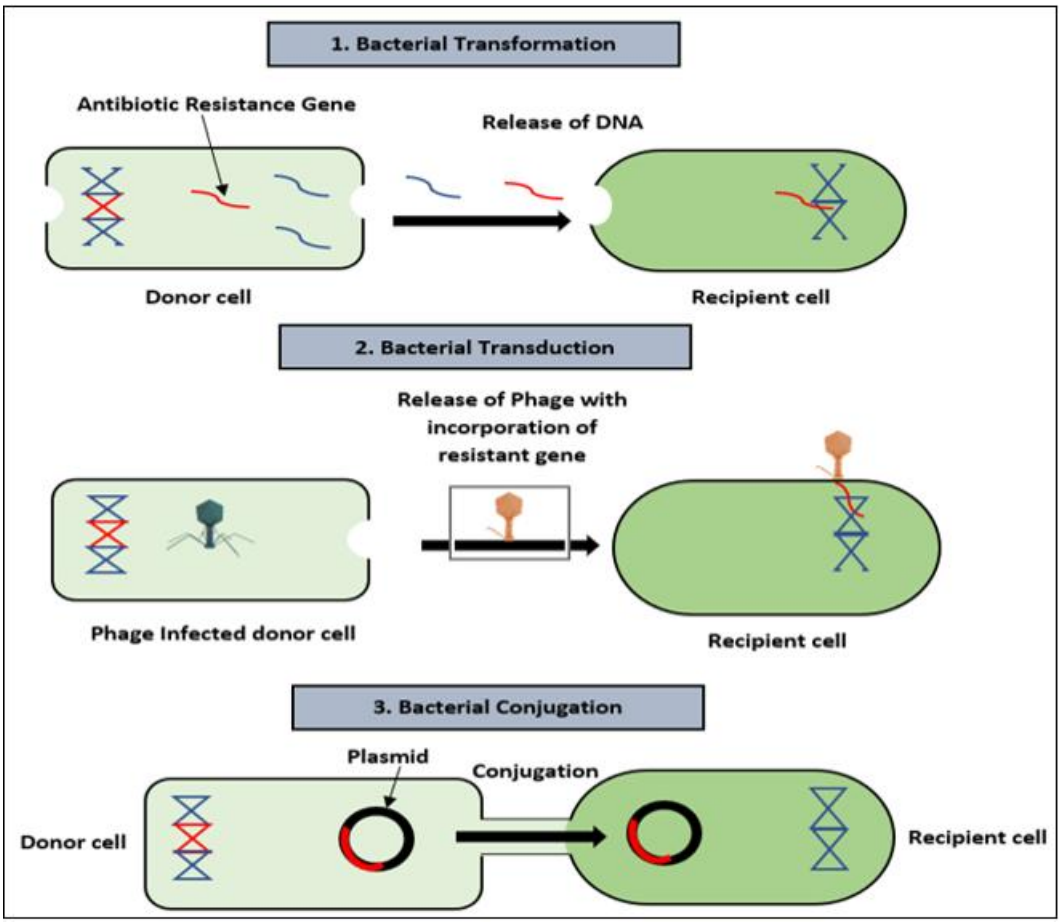

Figure 3: Schematic Diagram of Different Modes of Horizontal Gene Transfer

3.2.2 Mechanistic Basis

There are many mechanisms involved in bacterial resistance, among them, the following are the most frequently observed and show high frequency in clinical isolates [13].1] Modification of Antibiotic molecule, 2] Drug efflux**,** 3] Modification of drug target**,** 4] Limiting the drug uptake, 5] Biofilm formation. Figure 4 represents a schematic diagram of the different mechanisms of antibiotics.

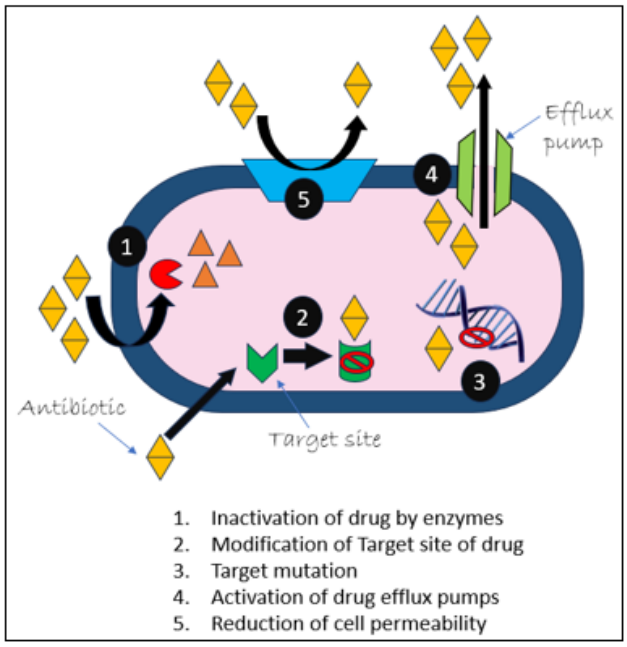

Figure 4: Represents A Schematic Diagram of Different Mechanisms of Antibiotics

In which intrinsic resistance may involve by drug inactivation, drug efflux, and limiting drug uptake, whereas Acquired resistance mechanism is involved in drug inactivation, drug efflux, and modification of drug targets, Owing to structural changes and many other reasons, there is variation in the type of resistance, mainly Gram-negative bacteria (GNB) involve in all the mentioned mechanism, and Gram-positive bacteria (GPB) less commonly use limiting drug uptake, due to lack of Lipopolysaccharide (LPS) outer membrane, and also GPB don't have a capacity for a certain type of drug efflux mechanisms[10].

3.2.2.1 Modification of Antibiotic Molecule:

There are mainly two ways in which bacteria inactivate drugs 1) By transfer of a chemical group to the drug or 2) By Destruction of the drug

1) By transfer of chemical group to the drug or chemical alteration of the antibiotics:

The production of enzymes that are capable of introducing chemical change to antibiotic molecules is a well-known mechanism of Acquired Abx resistance In both GNBs and GPBs. Most of the Abx affected by these enzymatic modifications exert a mechanism of action by inhibiting Protein synthesis at the Ribosomal level. Bacteria may produce enzymes that can attach various chemical groups to the drugs. This modifies the drug molecule and prevents it from binding to its target in the bacterial cell. Transfer of phosphoryl, acetyl, and adenyl groups to the compound is the most effective method of drug inactivation by chemical group

transfer [14]. The most frequent biochemical reaction catalyses by these type of Enzymes includes 1) Acetylation (Aminoglycosides, Streptogramins, and Chloramphenicol), 2) Phosphorylation (Aminoglycosides and Chloramphenicol) 3) Adenylation (Aminoglycosides and Lincosamides). For instance, AMEs (Aminoglycoside Modifying Enzymes) and CATs (Chloramphenicol Acetyltransferases) [10].

2) By destruction of antibiotic molecule:

Drug inactivation by the destruction of antibiotic molecule contains well-characterized enzyme i.e beta-lactamase**,** It works against beta-lactam antibiotics such as Cephalosporins, Penicillins, Monobactam, Carbapenems, which contains beta-lactam ring as a core structure, bacteria secretes beta-lactamase enzymes that degrade the beta-lactam ring of antibiotics and makes them inactive[10].Classically beta-lactamases are classified using two main classification systems 1) The Ambler classification and 2) Bush Jacoby Classification, Table 3 represents the classification various beta lactamase enzyme with list of antibiotics that are inactivated and possible inhibition. Hydrolysis of beta-lactam antibiotics by beta-lactamases is the most common mechanism of Resistance, and Clinically important for GNB, because Penicillins, Cephalosporins, Carbapenems are included in the preferred treatment regimens for many Infectious diseases, presence and characteristics of these enzymes play a critical role in the selection of appropriate therapy [15].

Table 3: Represent classification of various Beta-Lactamase enzymes

Ambler Classification	Example	Inactivate	Inhibited By	Representative Enzymes
A	• Penicillinase • Cephalosporins • Narrow Spectrum Beta-Lactamase • Extended Spectrum Beta-Lactamase • Carbapenemases	• Penicillin (except Temocillin) • 3rd Generation Oxyiminocephalosporins (Ceftazidime, Cefotaxime, Ceftriaxone) • Aztreonam • Cefamandole • Cefoperazone • Cephamycin • Carbapenem	Beta Lactamase Inhibitors (BLI) • Clavulanic Acid • Sulbactam • Tazobactam	• TEM • SHV • CTX-M
B (Jacoby's group 3)	Metallo-Beta Lactamases (MBLs)	Resistance to all beta Lactams including Penicillins, Cephalosporins, Carbapenems	Resistance to all BLI, except combination with Aztreonam	• IMP • VIM • NDM-1
C	Cephalosporinase	Low level resistance to narrow spectrum Cephalosporins Inactivates Aztreonam, all Penicillins, most of the Cephalosporins	Resistance to most of the BLIs, except Avibactam	• AmpC Beta-Lactamase
D (Jacoby's group 2d)	Oxacillin hydrolysing enzymes (OXA)	Most of 3rd generation Cephalosporins (ESBLs) Carbapenems	Resistant to all BLIs, Except OXA-18 type	OXA-11, OXA-14, OXA-16, OXA-23, OXA-48, OXA-58 etc.

3.2.2.2 Drug Effulx

Bacteria consist of efflux pumps in the membrane that facilitate the transport of various substances including the efflux of toxic substances out of the cell. Bacteria produce specific efflux pumps that extrude the antibiotics out of the cell and develop resistance. The first described efflux pump was found in the early 1980s, which efflux Tetracycline out of *E.coli* cytoplasm since then many classes of efflux pumps have been discovered in both GNBs and GPBs[12]. Bacteria possess chromosomally encoded genes for efflux pumps that contribute to intrinsic resistance and some are induced or overexpressed under certain environmental stimuli or in presence of a suitable substrate. Although a high level of resistance is usually seen via a mutation that modifies the transport channels [10].

The efflux pump system may be substrate-specific (i.e. for particular antibiotics, such as *tet* determinants for Tetracycline and *mef* genes for Macrolides in pneumococci) or maybe broad substrate specificity, which is usually found in Multidrug Resistance (MDR) bacteria. Efflux pumps mediated resistance affects many antibiotic classes including protein synthesis inhibitors, Fluoroquinolones, Beta-Lactams, Carbapenems, and Polymyxins [10].

There are five major families of Efflux pumps classified, based on their structure and energy source:

1) ATP Binding Cassette (ABC) family, 2) Multidrug and Toxic compound Extrusion (MATE) family 3) Small Multidrug Resistance (SMR) family 4) Major Facilitator Superfamily (MFS) 5) Resistance Nodulation cell Division (RND) family. Figure 5 represents the schematic diagram of different efflux pumps.

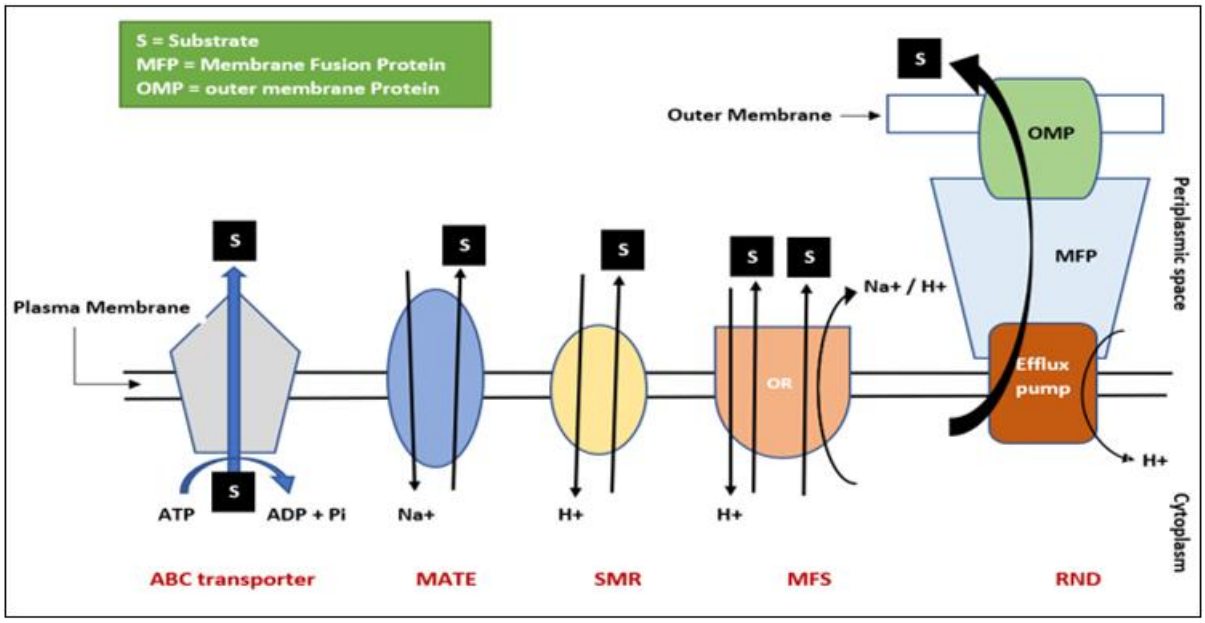

Figure 5: General Structure of Main Efflux Pump Families

Most of these Efflux pump families are single-component pumps, which transport substrates across the cytoplasmic membranes. Table 4 represents the different characteristics and possible affected antibiotic examples of various efflux pump families. The RND family are Multi-component pumps, found mainly in GNBs, whose function is associated with a periplasmic membrane fusion protein (MFP) and an outer membrane protein (OMP-porin) to efflux substance out of the entire cell envelope. It may be possible that other efflux family members act with cellular components as multi-component pumps in GNBs, for instance, one Member of the ABC family, MacB works as a tripartite pump (MacAB-TolC) to extrude Macrolides, and one member of MFS, EmrB works also as a tripartite pump (EmrAB-TolC) to extrude Nalidixic acid in *E.coli*. Efflux pumps found in GPBs may confer intrinsic resistance majorly, because of encoded on the chromosome, these pumps include members from MFS and MATE families and mostly extrude Fluoroquinolones. In GNBs, efflux pumps are widely distributed and may come from all five families, but most of the clinically significant pumps belong to the RND family, while in GPBs most efflux pumps found are from the MFS family [10].

Table 4: Represent characteristics of different efflux pump families

Family	Characteristics	Example
ABC family (ATP Binding Cassette)	• Uptake and Reflux transport system • Utilize ATP as energy source • 6 Transmembrane segments (TMS) • Very few found in clinically significant bacteria	➢ Vibrio cholerae contain VcaM pump that transport Fluoroquinolones and Tetracycline
MATE family (Multidrug And Toxic Compound Extrusion)	• Utilize Na+ as energy source • 12 TMS	➢ Most of efflux Fluoroquinolones and some Aminoglycosides ➢ Most of found in GNBs
SMR family (Small Multidrug Resistance)	• Utilize Proton-Motive Force (H+) • Hydrophobic in nature and efflux mainly Lipophilic cations • 4 TMS • Very narrow substrate range	➢ Confer resistance to Beta-Lactams and Aminoglycosides ➢ Staphylococcus epidermis contains SMR pump that transport Ampicillin, Erythromycin, Tetracycline ➢ E.coli contains EmeR pump that transport Vancomycin, Erythromycin, Tetracycline
MFS family (Major Facillator Superfamily)	• Catalyse transport via substrate/cation (Na+/H+) symport or substrate/H+ antiport • 12/14 TMS • Nearly 50% of Efflux pumps in E.coli are MFS pumps	➢ Acinetobacter baumannii has separate MFS for Erythromycin (SmvA pump) & Chloramphenicol (CraA & CmlA) ➢ E.coli has separate MFS for Macrolides (MefB), Fluroquinolones (QepA) & Trimethoprim (Fsr) ➢ Broader specificity are seen in Staphylococcus aureus for Fluoroquinolones and Chloramphenicol (NorA pump) and LmrS pump for Linezolid, Erythromycin, Chloramphenicol and Trimethoprim
RND family (Resistance Nodulation cell Division)	• Catalyse substrate by efflux via substrate/H+ Antiport system by the help of MFP and OMP • Complex multi-component pumps • 12 TMS • Found in numerous GNBs	➢ Pseudomonas aeruginosa MexAB-oprM pump confer intrinsic resistance to Beta-Lactams, Chloramphenicol, Tetracycline, Trimethoprim, sulfamethoxazole & some Fluroquinolones ➢ E.coli AcrAB-TolC pump confer resistance to Penicillins, Chloramphenicol, Tetracycline, Macrolides and Fluroquinolones

3.2.2.3 Modification of Drug Target:

For the bactericidal or bacteriostatic activity of antimicrobial drugs, they get bind to the bacterial cell targets. There are multiple components in the bacterial cell, which are targeted by antimicrobial drugs for the activity. Many targets may be modified by bacteria to acquire resistance to those antimicrobial drugs, some the examples of them are listed below;

- Some GPBs acquired resistance to Beta-lactams, in which the mechanism of resistance involve is via alteration in structure or number of PBPs (Penicillin-binding proteins). PBPs are transpeptidases involved in the development of peptidoglycan cell wall. A change in number i.e. increase in PBPs that decrease the drug binding ability or a decrease in PBPs with normal drug binding, further impacts the activity of a drug. A change in the structure of the target side may decrease the ability of the drug to bind or completely inhibit drug binding at the target side. For instance, PBP2a is the alteration of PBP in *Staphylococcus aureus* by the acquisition of the *mecA* gene
- The Glycopeptides (Vancomycin) also act by inhibiting cell wall synthesis and the Lipopolypeptides (Daptomycin) work by depolarizing the cell membrane. As GNBs have thick LPS layer, they possess intrinsic resistance to these antibiotics. Resistance to Vancomycin becomes a major issue in the *Enterococci* (VRE-Vancomycin Resistance Enterococci) and *Staphylococcus aureus* (MRSA-Methicillin Resistance Staphylococcus Aureus), Resistance occurs may be due to the acquisition of *van* genes, which results in a change in the structure of peptidoglycan precursor that causes a decrease in binding ability of Vancomycin. Similarly, in Daptomycin, which requires the presence of Calcium for binding to the target and initiating activity. Mutation in genes like *mprF* changes the charge of the cell membrane surface to positive, which leads to inhibiting the bonding of Calcium and hence decreasing the activity of Daptomycin.
- Resistance to Antibiotics that target Ribosomal subunits may occur via one of the following mechanisms; 1) Ribosomal mutation (Aminoglycoside and Oxazolidinones) and 2) Ribosomal subunit methylation (Aminoglycoside, Oxazolidinones, Macrolides, Streptogramins) most commonly involve the *erm* genes or 3) Ribosomal protection (Tetracyclines). These all mechanisms interfere with the ability of the antibiotics to bind with the ribosome.
- Resistance to Antibiotic that targets the nucleic acid synthesis (Fluoroquinolones), acquired resistance via modification of DNA gyrase (In GNBs – via *gyrA* gene) or Topoisomerase IV (In GPBs – via *grlA* gene), their mutations cause a change in the structure of gyrase and topoisomerase, which decrease or eliminate the ability of antibiotics to bind with their respected components
- Resistance to drugs that inhibit the metabolic pathways, resistance acquires via mutations in enzymes, such as a mutation in DHPS (Dihydropteroate synthase – target side for Sulfonamides activity) and DHFR (Dihydrofolate reductase – target side for Trimethoprim), which involves in folate synthesis pathway in bacteria, or/and mutation also acquires by overproduction of mutated/resistant DHPS and DHFR enzymes. Mutation of these enzymes is most often located in/near the active site and results in structural changes of enzymes that interfere with drug binding, while still allowing the natural substance to bind and continue the metabolic process in Bacteria [10].

3.2.2.4 Limiting the Drug Uptake

Gram-negative bacteria have a membrane outside the cell wall, the outer membrane that is made up of a lipid bilayer, which consists of Lipopolysaccharide, owing to its hydrophobicity, the passage of hydrophilic compound become difficult giving these bacteria innate resistance to a certain group of large antimicrobial agents [16]. Bacteria that lack of cell wall, such as mycoplasma and related species, are therefore intrinsically resistant to all drugs that target the cell wall including Beta-Lactams and Glycopeptides [10]. Due to difficulty in the passage of hydrophilic compounds bacteria consist of porins or outer membrane porins (OMPs). Which are proteins that facilitate the passage of hydrophilic compounds across the LPS membrane. Many factors affect drugs' ability to pass through the porins, such as size, shape, and charge These are some typical porins such as OmpF, OmpC, and OmpE. Each of the bacterial species produces specific porins, and the loss or impairment of one or more OMPs is a common contributing factor to developing resistance. There are two main ways in which porin changes can limit the drug uptakes 1) A reduction in the number of porins present and 2) the Mutation of the porin channel that changes the selectivity for antibiotic action. As Gram-positive bacteria do not possess an outer membrane, and hence restricting drugs access is not as prevalent [10]. Some of the examples that develop resistance by limiting the drug uptake mechanism are listed below;

- In Enterococci, the fact that polar molecules have difficulty in cell wall penetration, gives intrinsic resistance to Aminoglycosides
- Gram-positive bacteria, such as *Staphylococcus aureus* recently develop resistance to Vancomycin, still it is an unexplainable mechanism that allows bacteria to produce thickened cell wall, which makes it difficult for the drug to enter the bacterial cell and hence provides intermediate resistance to Vancomycin (VISA)
- Member of *Enterobacteriaceae* is known to become resistant, owing to a decrease in the number of porins, sometimes it is also observed that it stops the production entirely of certain porins. It mainly confers the resistance to Carbapenems [16].
- Mutation that causes the changes within the porin channel have been observed in *Enterobacter aerogenes* (Now, *Klebsiella aerogenes*), which become resistant to Imipenem and certain Cephalosporins [10].
- Loss of OprD porins in *Pseudomonas aeruginosa* confers resistance to Imipenem and Meropenem, in another species loss of OmpF can lead to Multidrug Resistance (MDR) organisms. *Pseudomonas aeruginosa* also reduces the production of porin as the mechanism of resistance, which makes it low susceptible to Beta-Lactams
- In some strains, it is possible to see porin exchanges, which promote a reduction or loss of affinity to Antibiotics with these proteins, which further lose their ability to overcome from the outer membrane and enter the cell, for example, it is observed in OmpK35 to OmpK36 in *Klebsiella pneumonia* [17].

Limiting the drug uptake by mutation or change in several porins channels in the bacterial cell results in an increase in

MIC (Minimum Inhibitory Concentration) to hydrophilic antimicrobials and reduce the choice of antibacterial therapeutics in clinical practices. Many studies observed that selective pressure exerted by prolonged use of Abx is an important factor in the appearance of MDR bacteria and modification of porins is an important factor in this process

3.2.2.5 Biofilm Formation

Apart from the above main mechanism of resistance, another exclusively seen phenomenon in bacterial colonization is the formation of biofilm by the bacterial community [10]. Biofilms are complex microbial communities living as a thin layer on biotic or abiotic surfaces, established in a matrix of extracellular polymeric substances created by the biofilms themselves [18]. The major component of the matrix is secreted extracellular polymeric substances, which mainly consist of polysaccharides. proteins, lipids, and extracellular DNA from the microbes, there are three key steps involved in the formation of biofilm. The first step is Adhesion, which occurs as cells reach a surface and anchor to the site. The second step is Growth and Maturation, which happens as the microbes start to generate the exopolysaccharide that developed the matrix and then matures from microcolonies to multi-layered cell bunches. The final step is Detachment, which occurs in two types: Active and Passive. Active detachment is initiated by bacteria themselves, for instance by quorum sensing and enzymatic degradation of the biofilm matrix, while passive detachment is caused by external forces, like a fluid shear, scarping and human intervention. For pathogenic organism, the formation of a biofilm protects the bacteria from the attack of the host immune system, and protect antimicrobial agents. Biofilm matrix makes it difficult for antimicrobial agents to reach the bacteria, thus to be effective, a much higher concentration of drugs is needed [16]. Matrix of biofilm provides a mechanical and biochemical shield that provides conditions needed to attenuate the activity of drugs (Sessile – slow metabolic rate, slow cell division, low O2, low pH, high Co2, low water availability). Under this condition, it is difficult to eliminate bacteria using conventional antibiotics. Antimicrobials that target growing and dividing bacterial cells have little effect on bacteria. [10, 16].

Moreover, micro-organisms within the biofilm can interact with each other's, as well as with the environment, whenever bacteria experience nutrient scarcity, they could become tolerant to antibiotics, and this may explain the apparent greater antibiotics resistance of cells in the deep layer of biofilm. It's observed that bacteria extracted from the biofilm and grown in media, recover their full susceptibility, which indicates that resistance from biofilm formation is phenotypic, not genotypic. An important remark about biofilm is that it is likely facilitated by HGT by proximal bacterial cells, hence sharing antimicrobial resistance genes is potentially easier for these bacterial communities. The most common pathogens found in biofilms in a clinical setting *are Staphylococcus aureus, Pseudomonas aeruginosa, Acinetobacter baumannii, and Klebsiella pneumonia* [16, 18].

4. Mechanism of Resistance in Eskape Pathogens

Healthcare-associated (Nosocomial) infections, can be caused by various microbes such as bacteria, viruses, fungi, parasites, and other pathogens. The transmission of this infection could be due to numerous reasons, such as interpersonal communication between patient to patient, healthcare professionals to patients, contaminated equipment, lack of proper sterilization measures, industrial utilization aspects, etc., Nosocomial infection may contribute to the burden on patients, by increase length of hospital stay (LOS), increase the treatment-related cost (to treat patient with MDR organism, higher antibiotics are utilized, which further increase the burden to patient and payer eventually), higher mortality due to treatment failure and if recovered it may decrease further patient's quality of life (QOL). To combat this future reason for the epidemic, the Infectious Disease Society of America (IDSA) has complied with the bacterial pathogens responsible for most of the hospital-acquired infections referred to as "ESKAPE" pathogens, they can Escape from the biocidal activity of antibacterial drugs. ESKAPE is an acronym, given by Louis B Rice, the group of six pathogens, encompassing both gram-positive and gram-negative species, consisting of gram-positive species - ***E****nterococcus faecium,* ***S****taphylococcus aureus, and gram-negative species –* ***K****lebsiella pneumonia,* ***A****cinetobacter baumannii,* ***P****seudomonas aeruginosa,* ***E****nterobacter* spp., These pathogens are a common cause of life-threatening nosocomial infection amongst critically ill and immunocompromised individuals and are characterized by potential Multidrug Resistance (MDR) mechanism[16, 19]. In 2017 World Health Organization (WHO) included ESKAPE pathogens in the inventory of 12 bacteria, which demanded the development of new antimicrobials urgently. WHO classified the bacteria into three categories according to the priority of combat, which are Critical priorities, High priorities, Medium priorities[20]. Figure 6 represents the priority-wise categorization of antibiotics, which was released in 2017, recently in 2024 WHO has released new priority list in which, the critical group consists of: Carbapenem-resistant *Acinetobacter baumannii* (CRAB), 3rd-generation cephalosporin-resistant and Carbapenem-resistant Enterobacterales, the high group consist of Fluoroquinolone-resistant *Salmonella* Typhi, and *Shigella* spp., Fluroquinolone-resistant Non-typhoidal *Salmonella*, 3rd generation cephalosporin and/or Fluroquinolone-resistant *Neisseria gonorrhoeae*, Vancomycin resistant *Enterococcus faecium*, Carbapenem-resistant *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus* (MRSA) and Medium group consist of Macrolide-resistant Group A Streptococci and *Streptococcus pneumoniae*, Ampicillin-resistant *Haemophilus influenzae* and Penicillin-resistant Group B Streptococci[11].

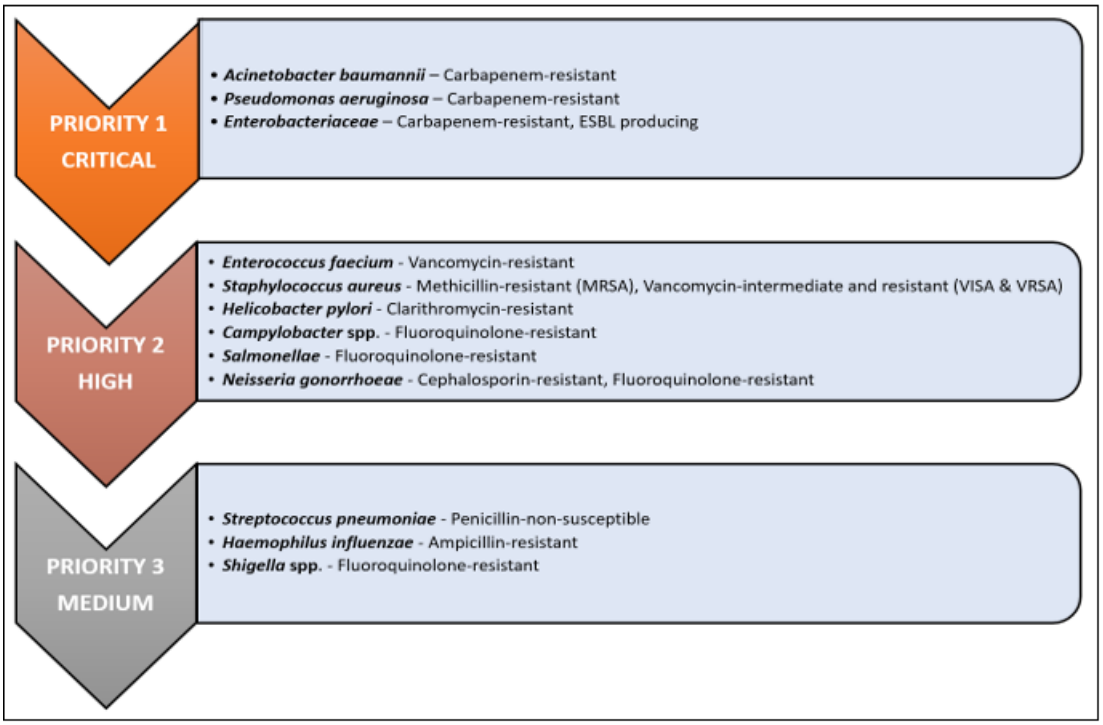

Figure 6: Who Priority Wise Categorization of Pathogens – 2017

4.1 Resistance in Eskape Pathogens

4.1.1 *Enterococcus faecium*

- *Enterococcus* species are formerly classified as part of genes *Streptococcus*, they are gram-positive facultative anaerobes, found in pairs or chains, their normal habitat is gut of humans and animals, among them, *Enterococcus faecium* and *Enterococcus faecalis* are more clinically relevant, the most infection caused by enterococcus are endogenously acquired, but cross-infection may occur in host patients. Over the past decades, some reports have revealed a rise in Ampicillin and Vancomycin-resistant enterococcal infections in healthcare facilities. In the Netherlands average number of invasive Ampicillin resistant enterococcal escalated from 10 infections in 1999 to 50 infections in 2005. Rates of antimicrobial resistance amongst enterococci are particularly concerning, and especially nowadays for incidence of Vancomycin resistance Enterococcus (VRE), mainly associated with *E. faecium* are rising[16], WHO classified it as PRIORITY-2 Highly required newer antibiotics development, currently, Vancomycin-resistant E. faecium (VRE*fm*) multilocus sequence type (ST), pertaining to clonal complex 17 (CC17) are responsible for a significant burden of Hospital-Acquired infection, they're highly prevalent in the gut microbiome of wild and domesticated animals, although Community-Associated infection caused by CC17 strains are uncommon. The entry of VRE*fm* into the bloodstream of the hospitalized patient is typically occurred by Antibiotics exposure, which makes VRE*fm* become the predominant species in the Gastrointestinal tract [17, 19].
- The duration of prior antibiotics exposure is strongly associated with a subsequent risk of VRE infection. The management of patients infected with VRE is complicated by the excess cost and disruption resulting from the need for isolating rooms, contact precautions, and proper maintenance room cleaning. The treatment of significant infection relies upon second-line antibiotic therapy i.e., Tigecycline and Daptomycin, which are often associated with increased cost, diminished efficacy, and a greater risk of toxicity, in comparison to first-line Antibiotic therapies. Most studies have indicated that VRE infection is associated with increased mortality and LOS, especially in case of VRE causes a bloodstream infection [8].

4.1.2 *Staphylococcus aureus*

- Gram-positive cocci bacterium, with cells arranged in grape-like structure, with non-fastidious growth requirement, *S. aureus* is part of the normal skin flora, especially of the nose and perineum of humans and animals. The carriage rate of *S. aureus* is high in the general population and transmission can occur by direct contact or airborne routes. Generally, an infection caused by *S. aureus* responded well to penicillin treatment, but owing to excessive use of these led to the emergence of Beta-lactamase producing *S. aureus* isolates in 1948[19].
- Report of Methicillin-Resistant *Staphylococcus aureus* first identified in 1961, due to widespread usage of Penicillin. Strains of MRSA that are community-acquired (CA-MRSA) are typically associated with skin & soft tissue infections, whereas strains of MRSA that are acquired from Hospital (HA-MRSA) are associated with severe pneumonia and bloodstream infection, although the division between CA-MRSA and HA-MRSA strains is becoming vague [8].
- In most cases, the first-line treatment of MRSA is Vancomycin and Teicoplanin, however owing to the selective pressure of these antibiotic has induced some strains to become intermediate susceptible to Vancomycin *in vitro*, with cases of clinical Vancomycin Intermediate and Vancomycin-Resistant *S. aureus* (VISA and VRSA, respectively) becoming more common (WHO PRIORITY-2 HIGH). VISA was first identified in Japan in the mid-1990s, now different

strains of it, are emerged in other countries such as Asia, the USA, Europe, and unfortunately, most isolates of VISA are also less susceptible to Teicoplanin, with term Glycopeptide intermediate S. aureus used to identify. VRSA is a particular concern, due to the interspecies exchange of resistance genes from VRE. VRSA isolates contain both *Van-A* and *mec-A* resistance determinants of VRE and MRSA, which results in resistance to multiple antibiotics, including Methicillin and Vancomycin [21]

4.1.3 *Klebsiella pneumoniae*

- Gram-negative bacillus, non-fastidious and usually encapsulated, *Klebsiella pneumoniae* is a member of the family Enterobacteriaceae, which are most often found in infection of healthcare settings. Infection may be endogenous or may be acquired by direct patient contact with an infected host. In recent years, many *K. pneumoniae* strains have acquired a huge variety of Beta-lactamase enzymes, which can destroy the Beta-lactam ring of Penicillin, Cephalosporins, and Carbapenems, among them Carbapenem-resistant *K. pneumoniae* (CRKP) are particularly more concern, challenging to treat and clinically prominent CRE, encoded as bla_{KPC} [16].
- Cephalosporins and Carbapenems have been the cornerstone to treating serious infections caused by Enterobacter, such as *K. pneumoniae*, but efficacy has been compromised by the widespread acquisition of genes encoding enzymes, like ESBLs and Carbapenemases, which causes the resistance to these critical drugs and high rates of mortality associated with resistance [8]
- In addition, the emergence of the NDM-1 enzyme in *K. pneumoniae* (encoded by bla_{NDM-1}), has increased the proportion of CRKP and may pose a threat to other antibiotics such as Beta-Lactams, Aminoglycosides, and Fluoroquinolones**.** Effective antimicrobial options are often lacking and 2nd line treatment is often associated with the risk of toxicity and safety concerns, such as for Aminoglycosides, Polymyxins, and Tigecyclines [8]. Even if several intensive infection control practices are used, outbreaks of Carbapenemase-mediated multidrug-resistant (MDR) strains are only reduced and cannot be completely eradicated hence effective treatment is needed to overcome these pathogens [16].

4.1.4 *Acinetobacter baumannii*

- Gram-negative coccobacillus, non-fermentative species, is widely distributed in the environment and readily contaminates the hospital environment, leading to high rates of cross-contamination in nosocomial infection. *A. baumannii* causes infection at a variety of sites, including respiratory and urinary tracts. These strains are frequently antibiotic-resistant and, hence, particularly problematic in managing the infection in surgical wards and Intensive care units [16]. Community-acquired pneumonia due to *A. baumannii* has been described in tropical regions of Asia and Australia among individuals with a history of alcohol abuse [8].
- The emergence of Carbapenemase-producing A. baumannii strains to carry imipenem Metallo- β-lactamases, encoded by bla_{IMP}, and oxacillinase serine β-lactamases, encoded by bla_{OXA}, has been reported. These strains show resistance to last-resort antibiotics like Colistin and Imipenem**.** Carbapenem-resistant *A. baumannii* (CRAB) is classified as PRIORITY-1 Critical, by WHO, which requires urgent new antibiotics to mitigate the infection, without proper action through adequate epidemiological surveillance and therapeutic development *A. baumannii* can potentiate a global epidemic [8, 19].

4.1.5 *Pseudomonas aeruginosa*

- *P. aeruginosa* is Gram-negative rod-shaped, facultative anaerobes that are part of normal gut flora, carriage rate of it is fairly low in the general population, but is higher in hospital inpatients. Infection caused by it is commonly associated with a severe respiratory infection, especially in an immunocompromised patient. *P. aeruginosa* shows longer viability in vivo and colonizes moist environments and therefore can be found in many healthcare settings, especially in the context of chronic wounds, ventilator support, urinary tract devices, where biofilm formation predisposes and be the reason for resistance [22].
- Many *P. aeruginosa* strains show an intrinsic resistance that decreased susceptibility to several Antibiotics, such as Carbapenems (especially Imipenem – by a combination of chromosomal AmpC production and porin changes) [WHO PRIORITY-1 CRITICAL], *P. aeruginosa* also produces the ESBLs and can harbour other Antibiotic resistance enzymes such as *K. pnemoniae* carbapenemase (KPC), bla_{VIM} and Imipenem Metallo beta-lactamase. The combination of these enzymes can lead to high resistance among *P. aeruginosa* isolates. The continuous increase of MDR isolates presents a complicated situation for the selection of appropriate therapy, fortunately, Colistin is still effective in many cases [18, 22].

4.1.6 *Enterobacter* species

- *Enterobacter* spp. are non-fastidious, Gram-negative rods that are sometimes encapsulated, they can cause opportunistic infection in immunocompromised patients and more often hospitalized patients and contain a wide range of resistance mechanisms [16]. Over the past 35 years, *Enterobacter aerogenes* (now named *Klebsiella aerogenes*) and *Enterobacter cloacae* (including *Enterobacter hormaechei*) have presented with significant threats to neonatal wards and patients in ICUs, particularly in a patient on ventilators. The emergence of these two Enterobacter spp. as clinically significant MDR pathogens has occurred in concurrent epidemic waves, moreover, pan-drug resistant *E. aerogenes* has also emerged, displaying resistance to the last-resort antibiotic colistin [8].
- In addition, *Escherichia coli* is emerging as resistant to many Antibiotics. E.coli is identified as the major cause of infection in the bloodstream and urinary tract infection (UTI) in both community and healthcare-associated settings, development of sepsis is the major manifestation of E.coli UTI, and Many E.coli strains contain ESBLs, and Carbapenems including VIM, OXA, MBL-1, and KPC. Most MDR strains expressed resistance to almost all available antimicrobials, except Tigecycline and Colistin**.** *E. coli* typically acquires resistance genes through HGT from other members of Enterobacterales and high rates of resistance are observed across Europe to Aminopenicillin,

Fluoroquinolones, Aminoglycosides, and 3rd generation Cephalosporins. In 2016, resistance to last-resort Polymyxins and colistin was identified in E.coli from a pig farm in China. AMR E.coli is currently one of the largest clinical burdens facing human and animal health [8, 18].

Global Burden of Antimicrobial Resistance

According to the recently published, 2022 The Lancet study on AMR, which provides the most comprehensive assessment of the Global burden of AMR in 2019, covers 204 countries and territories, the study estimates that the deaths of 4.95 million people were associated with drug-resistant bacteria, 1.27 million deaths were directly caused by AMR, the study also reports details of AMR burden for 23 bacterial pathogens, in which Escherichia coli is considered for the most deaths, and 88 pathogen-Drug combinations, in which Methicillin resistance Staphylococcus aureus (MRSA) attribute to the highest number of deaths (approximately more than 100000 deaths attributes to AMR in MRSA). The study also estimates the regional impact of AMR, in which the death rate attributable to resistance is highest in western Sub-Saharan Africa, at 27·3 deaths per 100000, and lowest in Australasia, at 6·5 deaths per 100000. The ESKAPE pathogens associated with resistance were responsible for 929000 deaths attributable to AMR, according to The Lancet study, the burden of resistant pathogens in India is displayed in Table 5 below; the study was performed in 2019, it may be possible that nowadays estimate the burden of resistance become worse, owing to COVID pandemic situation, AMR emerged as one of the leading public threats of the 21st century, unknowingly it may be ongoing pandemic situation AMR. World Health Organization (WHO), International Society Of Antimicrobial Chemotherapy (ISAC), Infectious Diseases Society Of America (IDSA), British Society For Antimicrobial Chemotherapy (BSAC), Indian Society of Medical Research (ICMR), etc., groups and researchers agree that spread of AMR is an urgent issue require serious global coordinated action plan to tackle with ongoing AMR issue with increase the Global fund contributes to only AMR issue [23, 24].

Table 5: Represent the burden of a resistant pathogen, particularly in INDIA by Lancet study

Isolated pathogen with resistance	Estimated Burden (%)
Carbapenem-Resistance *Acinetobacter baumannii*	≥ 80 %
3rd generation Cephalosporin Resistance *E.coli*	70 to <80 %
3rd generation Cephalosporin Resistance *Klebsiella pneumoniae*	70 to <80 %
MRSA	50 to <60 %
Fluoroquinolone Resistance *E.coli*	60 to <70 %
Carbapenem resistance *Klebsiella pneumoniae*	50 to <60 %

5. Conclusion and Future Perspective

Antimicrobial resistance (AMR) represents a critical global health threat, driven by a complex interplay of factors that undermine the effectiveness of antibiotics. Understanding the mechanisms of resistance is crucial for developing strategies to combat this issue. Effective management of AMR requires a multifaceted approach that addresses the primary risk factors associated with its spread. One of the significant risk factors is patient-related behaviors. Non-compliance with prescribed treatments, self-medication, and inappropriate demand for antibiotics can all contribute to the development and spread of resistant strains. Patients often do not complete their courses of antibiotics as directed, which can allow partially resistant bacteria to survive and proliferate. Additionally, self-medication and incorrect usage of antibiotics, driven by misinformation or the desire for quick relief, exacerbate the problem. Inappropriate antibiotic prescription practices further compound the issue. Prescribing antibiotics at incorrect doses, for insufficient durations, or without proper de-escalation of treatment can lead to resistance. The empirical use of broad-spectrum antibiotics, particularly when targeted or narrow-spectrum options would be more appropriate, increases selective pressure on bacterial populations, promoting resistance. Moreover, antibiotic misuse often occurs in cases of self-limiting illnesses or when antibiotics are prescribed for viral or protozoal infections, where they are ineffective. Such practices not only fail to address the underlying illness but also contribute to the broader problem of resistance. Transmission of resistant organisms within healthcare settings is another significant factor. Poor infection control practices, inadequate sanitation, and insufficient measures to prevent cross-contamination between patients can facilitate the spread of resistant bacteria. To effectively combat AMR, it is essential to focus on these risk factors. Strategies should include promoting patient education to enhance compliance and reduce self-medication, optimizing antibiotic prescribing practices, and reinforcing infection control measures within healthcare facilities. By addressing these factors comprehensively, we can mitigate the risk of AMR and preserve the efficacy of antibiotics for future generations.

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