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Emerging threats and strategies to combat antibiotic resistance: A review

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Abstract

An Antibiotic resistant bacterium is an emerging global health concern that results in numerous fatalities and significant economic repercussions annually. Two categories these bacteria Multidrug-resistant bacteria (MDR), Extremely Drug-Resistant Bacteria (XDR) exhibit resistance to a wide range of medicines. XDR can spread through direct contact with contaminated surfaces, hence becoming particularly dangerous in healthcare environments. The environment and genetic settings of the bacteria are responsible for causing alterations in the gene expression leading to phenotypic resistance (natural bacterial evolution). Due to this it becomes difficult to control the emergence and impacts of antibiotic resistance. In such type of cases it becomes very important to have a deep understanding of resistance determinants in bacterial populations, resistance mechanisms or how bacteria acquire antibiotic resistance genes (ARG) to find or develop new medicine or treatment to deal with such antibiotic resistance bacteria. Thus, there is an urgent need to understand distribution of resistance determinants in bacterial populations, elucidate resistance mechanisms, and determine environmental factors that promote their dissemination. This comprehensive review describes the major known self and acquired resistance mechanisms and therapeutic alternatives to deal with such bacteria.

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Introduction

The global health crisis of antibiotic resistance is a growing concern that results in numerous fatalities and significant economic repercussions annually. This complex issue encompasses genetic and environmental factors. Recent studies show that in 2019, drug-resistant diseases caused 1.27 million fatalities worldwide, with the potential to escalate to 10 million by 2050 if no action is taken. Besisdes these, antibiotic resistance could lead to a yearly global GDP decrease of $3.4 trillion and drive an additional 24 million individuals into extreme poverty within the next decade (UNEP, 2022). Antibiotic resistance affects all regions of the world. In the United States alone, antibiotic-resistant infections resulted in over 2.8 million infections and more than 35,000 deaths in 2019 (CDC, 2019). The European Union reports 25,000 deaths and 2.5 million extra hospitalization days annually due to antibiotic resistance (CDC, 2022). India recorded over 58,000 infant deaths within a year caused by antibiotic-resistant bacterial infections (CDC, 2022). Similarly, Thailand faces more than 38,000 deaths and 3.2 million hospitalization days each year due to antibiotic resistance (CDC, 2022).

The emergence and spread of antimicrobial resistance pose a global threat. The presence of superbugs, such as bacteria resistant to multiple or all antibiotics, is particularly alarming (WHO, 2023). Combatting antibiotic resistance is hindered by the excessive and inappropriate utilization of antibiotics, which facilitate the development of resistance (WHO, 2023). Factors such as inadequate prescription practices, patient non-compliance with antibiotic courses, unnecessary use of antibiotics in agriculture, and substandard infection prevention practices all contribute to exacerbating the problem (CDC, 2022). The escalating global consumption of antibiotics is also a concerning trend (Klein *et al.*, 2023). AMR has been divided by the WHO into three tiers based on the level of observed resistance. The following are these levels:

Antimicrobial resistance (AMR)

Level 1: Resistant bacteria are prevalent, but they are still treatable with widely available drugs.

Level 2: Multidrug-resistant bacteria (MDR) - Treatment is more difficult when germs are resistant to many widely used antibiotics (Table 1)

Level 3: Extremely drug-resistant bacteria (XDR) - These bacteria are resistant to a wide range of medicines, including some of the most potent last-resort remedies. As a result, there aren't many effective treatments available.

AMR prevalence varies greatly between geographical locations and bacterial species. For instance, a study in India discovered that more than 70% of *Escherichia coli* isolates had at least some common antibiotic resistance, whereas a study in the US discovered that about 22% of *Klebsiella pneumoniae* isolates had MDR characteristics (CDC, 2021; Kant *et al.*, 2019).

AMR is a risk since it can render many conventional antibiotics ineffective, making the management of infectious diseases more challenging and raising the risk of morbidity and mortality. AMR has substantial negative effects on the economy as well since it drives up healthcare expenses through longer hospital stays, greater usage of more expensive medications, and lost productivity. By 2050, it is anticipated that AMR would have cost the world economy more than $100 trillion annually (Review on Antimicrobial Resistance, 2016). Here are a few instances of AMR bacteria and their resistance mechanisms.

Methicillin-resistant MRSA (Staphylococcus aureus)

It is challenging to treat infections brought on by MRSA since this particular bacterium is resistant to methicillin and other similar medications. The mecA gene, which encodes for a different penicillin-binding protein that is unaffected by methicillin, was acquired, and this led to the creation of MRSA (Otto, 2013).MRSA is especially dangerous in healthcare environments, such as hospitals and long-term care institutions, because it can spread through direct contact with contaminated surfaces or skin. Additionally, MRSA can spread infections in the community, particularly among weaker immune systems or crowded housing communities (Tong *et al.*, 2015). MRSA is particularly dangerous to these groups.

Carbapenem-resistant Enterobacteriaceae (CRE)

CRE (Carbapenem-resistant Enterobacteriaceae) is a type of bacteria that exhibits resistance to carbapenem antibiotics, which are typically employed as a last-line treatment for severe illnesses. The emergence of genes responsible for producing carbapenemases, enzymes that degrade carbapenem drugs, is the underlying cause of CRE development (Logan & Weinstein, 2017). Transmission of CRE occurs through direct contact, contaminated surfaces, or medical equipment, making them particularly perilous within healthcare facilities. Furthermore, the ability of CRE to disseminate within the community contributes to its status as a significant public health concern (Guh *et al.*, 2020).

Vancomycin-resistant Enterococci (VRE)

The VRE bacterial group is resistant to vancomycin, a popular treatment for gram-positive bacterial infections. Genes for vancomycin resistance, like vanA and vanB, were acquired as the VRE evolved (Arias & Murray, 2012).Because they can be spread by direct contact with contaminated surfaces or medical equipment, VRE are a particular concern in hospital settings. Besides, it should be emphasized that VRE, which is resistant to Vancomycin, can spread infections to the general population, particularly to those with compromised immune systems or those who are highly vulnerable (Uttley *et al.*, 1988).

The CDC's approximations on the occurrence of MDR bacteria leading to sickness and fatalities in the USA - nearly 2.8 million and 35,000 respectively on a yearly basis. Because they restrict the number of available treatments, make infection management more challenging, and raise the risk of morbidity and mortality, MDR bacteria are particularly harmful. MDR bacteria can also spread quickly in healthcare facilities, providing a risk to patients who are already weak or are having surgery or intensive care, for example. Additionally increasing the danger of infection and transmission, MDR bacteria can spread across the community (Liu *et al.*, 2021). Here are some examples of MDR bacteria and their pathways of resistance.

Pseudomonas aeruginosa

Pseudomonas aeruginosa is a common cause of a variety of infections. It causes bloodstream infections, urinary tract infections, and pneumonia etc. This bacterium is resistant to various antibiotics due to adoption of multiple methods. Some include the upregulation of efflux pumps that help to remove antibiotics from the cell, the production of enzymes that render antibiotics inactive, and mutations that alter the antibiotic's target site (Lyczak *et al.*, 2000). The acquisition of *P. aeruginosa* may not always happen due to contact with uncontaminated objects, medical supplies, or healthy individuals. Another mechanism by which this bacterium has become antibiotic resistant is by producing a biofilm, composed of communities of microorganisms which can survive on surfaces for extended periods of time and are impervious to antibiotics (Mulcahy *et al.*, 2014).

Acinetobacter baumannii

Acinetobacter baumannii is found to be frequently associated with healthcare-associated infections. It is more evident in particular settings such as intensive care units. The bacterium has acquired Genes which is responsible for production of carbapenemases which is a kind of enzyme that breaks down carbapenem antibiotics. This resulted into emergence of antibiotic resistant bacteria, which is resistant to a variety of antibiotics, including carbapenems (Lee *et al.*, 2017). Potential transmission routes include person-to-person contact, contaminated surfaces, or medical equipment for *A. baumannii*. Another concern in healthcare facilities for this bacterium arises due its ability to persist on surfaces for extended periods of time (Fournier & Richet, 2006).

Escherichia coli

Escherichia coli is a bacterium that is frequently discovered in both people and animals' digestive tracts. However, some E. coli strains are challenging to treat due to their multi-antibiotic resistance. Extended-spectrum beta-lactamases (ESBLs) and carbapenemases are two examples of the antibiotic resistance genes that these MDR strains of *E. coli* have acquired (Ghafourian *et al.*, 2014). MDR E. coli can spread via contact with infected surfaces,

ingestion of tainted food or drink, or person to person contact. Additionally, according to Kaper *et al.* (2004), these germs can infect people in both public and medical settings.

Many kinds of infections like Bloodstream infections, pneumonia, and urinary tract infections are just a few of the illnesses that XDR bacteria can cause. The World Health Organisation (WHO) claims that XDR bacteria are to blame for significant mortality rates, with certain cases reporting death rates as high as 50–90%. Global public health authorities are concerned about the spread of XDR bacteria because it can result in higher medical expenses, lost productivity, and subpar health consequences. Additionally, XDR bacteria can quickly spread within medical facilities, providing a concern to patients who are already at risk, such as those who are undergoing surgery or those who are in intensive care units. . Here are some XDR bacteria examples and their mechanisms of resistance.

Klebsiella pneumoniae

The bacterium *Klebsiella pneumoniae*, commonly found in the human gut, is often responsible for hospital-acquired infections. The have ability to break down carbapenem antibiotics due to acquisition of plasmids. This acquired plasmid contains genes responsible synthesis of carbapenemases, which is a enzymes that break down carbapenem antibiotics. Some strains of *K. pneumoniae* have evolved resistance to numerous antibiotics, including the carbapenems (Navon-Venezia *et al.*, 2017). Person-to-person contact, contaminated surfaces, or medical equipment are all ways that XDR K. pneumoniae might spread. These bacteria are especially problematic in healthcare settings because they may survive on surfaces for long periods of time (Gupta *et al.*, 2011).

Mycobacterium tuberculosis-

Mycobacterium tuberculosis causes a bacterial infection known as tuberculosis (TB). It primarily affects the lungs. There are some strains of *M. tuberculosis* for which isoniazid and rifampin which are used as first line medicines are ineffective Also the 2020 report from the World Health Organisation reveals that fluoroquinolones, classified as second-line antibiotics and injectable antibiotics, are ineffective in treating these strains of *Mycobacterium tuberculosis*.A common route of spreading of this XDR M. tuberculosis is by Person-to-person contact, which is most common in densed, crowded and poorly ventilated areas. Additionally, due to the capacity of these bacteria to be able to live longer in the environment, it creates worry in areas with a high burden (World Health Organisation, 2020).

Enterobacteriaceae

Enterobacteriacea family responsible for common infections. *Klebsiella pneumonia* and *Escherichia coli* are also belong to this family. Enterobacteriaceae strains have developed resistance to different types of antibiotics, including the carbapenems (Endimiani *et al.*, 2011).The XDR Enterobacteriaceae can be transmitted by contaminated surfaces, person-to-person contact, or by usage of contaminated medical equipments. Additionally, according to Endimiani *et al.* (2011), these bacteria can spread diseases in both public and medical settings.

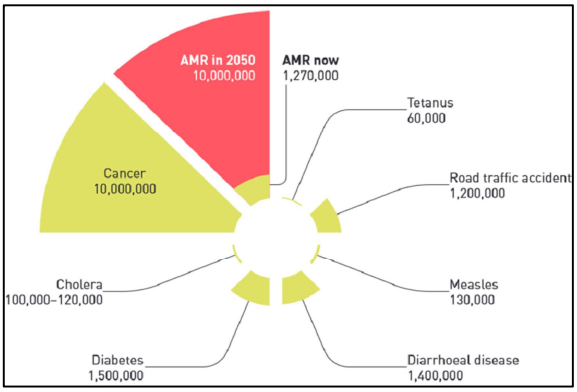

Fig. 1. Predicted mortality from AMR compared to common causes of death today (adapted from O'Neill 2016; Murray *et al.* 2022).

Inherit resistance

According to Davies and Davies (2010), inherent characteristics of some bacterial species or strains, such as impermeability of the cell wall or efflux pumps that can pump out drugs, allow them to naturally withstand the effects of antimicrobial agents. The existence of an impermeable outer membrane in Gram-negative bacteria, which serves as a barrier to

many antibiotics and prevents them from reaching their internal targets, is one of the most prevalent mechanisms of innate resistance in bacteria (Nikaido, 2003). Gram-negative bacteria, for instance, are innately resistant to the antibiotic vancomycin because it cannot pass through their outer membranes like Gram-positive bacteria can (Nikaido, 1996).

Table 1. List of different antibiotics with their released year and resistant bacteria developed for that antibiotics with identified year (U.S. Centers for Disease Control and Prevention, 2019).

Antibiotic Approved or Released	Year Released	Resistant Germ Identified	Year Identified
Penicillin	1941	Penicillin-resistant *Staphylococcus aureus*[21] Penicillin-resistant *Streptococcus pneumoniae* Penicillinase-producing *Neisseria gonorrhoeae*	1942 1967 1976
Vancomycin	1958	Plasmid-mediated vancomycin-resistant *Enterococcus faecium* Vancomycin-resistant *Staphylococcus aureus*	1988 2002
Amphotericin B	1959	Amphotericin B-resistant *Candida auris*	2016
Methicillin	1960	Methicillin-resistant *Staphylococcus aureus*	1960
Extended-spectrum cephalosporins	1980 (Cefotaxime)	Extended-spectrum beta-lactamase- producing *Escherichia coli*	1983
Azithromycin	1980	Azithromycin-resistant *Neisseria gonorrhoeae*	2011
Imipenem	1985	*Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae*	1996
Ciprofloxacin	1987	Ciprofloxacin-resistant *Neisseria gonorrhoeae*	2007
Fluconazole	1990 (FDA approved)	Fluconazole-resistant Candida	1988
Caspofungin	2001	Caspofungin-resistant Candida	2004
Daptomycin	2003	Daptomycin-resistant methicillin-resistant *Staphylococcus aureus*	2004
Ceftazidime-avibactam	2015	Ceftazidime-avibactam-resistant KPC-producing *Klebsiella pneumoniae*	2015

Efflux pumps, membrane-bound proteins that actively pump medicines out of the bacterial cell, are another route of innate resistance in bacteria (Li and Nikaido, 2004). These pumps can give resistance to several kinds of medications and can either be produced constitutively or activated by the presence of antibiotics (Blair *et al.*, 2015). For instance, Escherichia coli's efflux pump AcrAB-TolC may pump out a variety of antibiotics, making this bacterium naturally resistant to various medications (Li and Nikaido, 2004). Due to variations in the structure or functionality of their target proteins, some bacteria may also be innately resistant to particular types of antibiotics. For instance, certain bacteria have ribosomes with mutations or alterations that prevent the antibiotics from binding properly, making those bacteria naturally resistant to this class of medications (Roberts and Poole, 2014). As a result, macrolide antibiotics are proved to be ineffective agaist these bacteria. Overall, innate antibiotic resistance of bacteria is a complex process that can be caused by a number of mechanisms, such as altered target proteins, efflux pumps, and cell walls that are impermeable. Understanding the mechanisms of inherent resistance is crucial for creating new antibiotics that can get past these obstacles and enhancing the efficiency of already available medications.

Self-Resistance Mechanisms In Producer Organisms- Bacteria that produce antibiotics have developed defences to guard against the negative effects of the antibiotics they produce. By preventing the bacteria from being killed by their own antibiotics, which might prevent them from generating and releasing additional antibiotics in the future, these self-resistance mechanisms are crucial. They frequently have several systems operating simultaneously in order to provide total defence against the physiologically active substances they create. It's interesting to note that the genes responsible for antibiotic biosynthesis are usually always grouped with the genes responsible for self-resistance and their expression is co-regulated (Mak *et al.*, 2014).

The key biochemical types of self-defense mechanisms present in producing organisms are highlighted in the following section, with examples for each category given.

Antibiotic Modification or Degradation-

Some bacteria that are resistant to antibiotics have developed ways to chemically alter antibiotics, making them useless against the bacteria. One of the ways is by synthesising enzymes. These enzymes alter the antibiotic's molecule's structure, thereby preventing its binding to the target or some of the enzymes completely destroy it. A transfer of functional groups, such as ADP-ribosyl, acetyl, phosphoryl, adenyl, and glycosyl are responsible for change in the structure of several antibacterial compounds (such as rifampicin, aminoglycoside, chloramphenicoletc) (Wright, 2005). Aminoglycoside antibiotic are modified by various aminoglycoside modification enzymes, such as O-adenyltransferases, N-and acetyltransferases, O-phosphotransferases, which are responsible for adenylylating , acetylating, or phosphorylating respectively. This is how bacteria inhibit the antibiotic from binding to the target site (the bacterial ribosome), which leads to resistance (Peterson and Kaur 2018). The production of aminoglycosides and the presence of modification enzymes in producer Streptomyces are not always directly correlated, despite the fact that these enzymes were originally discovered in the producer Streptomyces species in the early 1970s. As an example, some species may not manufacture antibiotics but still have modifying enzymes, and the opposite is also true. These enzymes may conduct different metabolic tasks rather than being directly implicated in producers' resistance, according to some theories (Benveniste and Davies, 1973; Martinez, 2018). Comparative sequencing analyses demonstrate that the AMEs are highly diverse and are encoded by a sizable number of unrelated genes, indicating that they may have evolved through different convergent pathways leading to a similar function, which supports this claim (Shaw *et al.*, 1993). Aminoglycoside-modifying enzyme genes are typically found in MGEs (plasmids and transposons), but they have also been thought to be a part of some bacteria's core genomes (chromosomes). According to Ramirez and Tolmasky (2010), this situation was observed with several aminoglycoside acetyltransferases in *Providencia stuartii*, *E. faecium*, and *S marcescens*.

Chloramphenicol, one of the broad-spectrum antibiotics is used for treatment of a number of bacterial diseases. However, some bacteria have developed the ability for synthesise of enzymes called chloramphenicol acetyltransferases (CAT). This enzyme alters the structure of the antibiotic molecule and thereby preventing it from attaching to its specific target inside the bacterial cell. One of the important roles played by CATs is in preventing chloramphenicol from binding to protein synthesing organelle i.e ribosomes. This is achieved by covalently linking the two hydroxyl groups of chloramphenicol with an acetyl group from acetyl-CoA. The activity of the mainky depends on the histidine residue present in its C-terminal region. Interestingly, CAT enzymes come in a variety of forms, including the type III Escherichia coli enzyme that has been studied extensively due to its connection with chloramphenicol.A Type B chloramphenicol acetyltransferase from the newly discovered pathogen Elizabethkingia anophelis NUHP1 is another illustration. Its structure is significantly comparable to other previously characterised Type B (CatB) proteins from Pseudomonas aeruginosa, Vibrio cholerae, and Vibrio vulnificus, which adopt a hexapeptide repeat fold 2. It does not resemble the structure of the traditional Type A CATs.

Generally the treatment of bacterial diseases such as tuberculosis, Leprosy, is done by using an antibiotic called rifampicin. Rifampicin is responsible for killing extracellular organisms, semidormant mycobacteria, and intracellular mycobacteria in tissues. Some bacteria produce an enzyme called rifampicin hydrolases, which rendered Rifampicin inactive by catalysing the hydrolysis of rifampicin. This lead to the emergence of antibiotic resistance. This is one method by which bacteria can render rifampicin inactive. By degrading the antibiotic molecules these enzymes make them inactive and thereby preventing

their binding to their targets in bacterial cells. *Nocardia farcinica* bacteria produce a rifampicin hydrolase that has been found to render rifampicin inactive in vitro (Qi-Yu *et al*., 2021). Apart from modification in rpoB gene, and activation of NorA and MepA pumps, the bacteria *Staphylococcus aureus*, also synthesing a rifampicin hydrolase known as Arr-2 that inactivate rifampicin in vitro and in vivo (Victor *et al*., 2015). Rifampicin inactivation via phosphorylation has also been seen in *Nocardia* spp., *Rhodococcus* spp., and *Bacillus* spp. (D'Costa and Wright, 2009). Finally, glycosyltransferases catalyse glycosylation at the 23-position of rifampicin, inhibiting effective target binding to RNA polymerase b by blocking hydrogen bonding with the 23-hydroxyl (Yazawa *et al*., 1993).

Clindamycin and lincomycin belonging to antibiotic class lincosamides works by preventing production of proteins by attaching to the bacterial ribosome. But in some bacteria lincosamides is inactivated, which has resulted the development of antibiotic resistance. Lincosamide nucleotidyltransferases add a nucleotide group to the lincosamide molecule. Hence the structure of lincosamide molecule changes. As a result it cannot bind to ribosome. For instance, the lincosamide nucleotidyltransferase Lnu (A) (lnu(A) to lnu (F) and linAN produced by the bacterium *Staphylococcus aureus* has been demonstrated to alter both clindamycin and lincomycin in vitro(Qi-Yu *et al*., 2021). *Streptococcus agalactiae* also produces a lincosamide nucleotidyltransferase called Lnu (B), that modify the structure of clindamycin in vitro by adding nucleotide group (Victor *et al*.,2015).

According to Schwartz *et al.* (2016), the majority of the genes that have been described so far are found on plasmids, transposons, chromosomal DNA, or have been discovered as a gene cassette in a class 1 integron. Lincosamide nucleotidyltransferases have been split into two categories based on amino acid sequence homology in the context of the emergence of drug resistance. Proteins Lnu(A), Lnu(C), Lnu(D), and Lnu(E) belong to one group that exhibit similarities to the aminoglycoside nucleotidyltransferase ANT(20')-Ia (Petinaki *et al*., 2008). The b-subunit of the DNA polymerase shares sequence similarities with the second, smaller group, which also includes LnuB and LnuF. This link is in line with the theory that the nucleotide polymerases, which are present in all bacteria, gave rise to the nucleotidyltransferases Lnu (B) and Lnu (F) (Morar *et al*., 2009).

Destruction of the antibiotic molecule-

The creation of hydrolytic enzymes, such as b-lactamases and macrolide esterase, which result in antibiotic degradation due to irreversible structural arrangements, is the other method of inactivating antibiotic compounds. According to Bush (2013), hydrolytic enzymes are primarily encoded on MGEs and are easily transferred. The hydrolytic enzymes b-lactamases are catagorized into four classes (A, B, C, and D) according to the Ambler classification system (King *et al*., 2016) depending on their amino acid sequence and utilisation of serine or zinc ion as their primary catalytic domain. Classes A, C, and D of hydrolyting contain serine in the active catalytic site enzymes whereas class B enzymes which are metallo-b lactamases (MBLs) contain zinc in the active site (Bush 2013). Antibiotics bind to its specific target present on the the bacterial cell wall. Antibiotics such as cephalosporins and penicillins contain beta-lactam ring in its structure. Beta-lactamases break this ring and thereby preventing its binding to its target. Many bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) become antibiotic resistant due to this mechanism. Anothere mechanism that leads to the ability of antibiotic resistance is by synthesizing enzymes that alter the structure of antibiotic molecule. For example certain bacteria produce Aminoglycoside-modifying enzymes (AMEs), which is responsible for chemical alteration of aminoglycoside medicines like gentamicin and kanamycin. By blocking the antibiotics from attaching to the bacterial ribosome, where they are required to stop protein synthesis, these changes may render them ineffective (Victor *et al*., 2015).

TEM and SHV genes synthesise Class A beta-lactamases in bacteria like *Escherichia coli* and *Klebsiella pneumoniae.* This class A beta-lactamases hydrolize Penicillins and some cephalosporins antibiotics.

Another category of beta-lactamases i.e Class B beta-lactamases, also known as metallo-beta-lactamases (MBLs), generally found in both Gram-positive and negative bacteria. But MBLs can hydrolyze these beta-lactam antibiotics such as carbapenems also. MBLs require zinc ions for their catalytic activity. IMP, VIM, and NDM are examples of MBLs (Victor *et al.*, 2015). Class C subset of beta-lactamases frequently encoded by genes like Amp C, and is typically present in Gram-negative bacteria. These enzymes can hydrolyze antibiotics such as cephalosporins and certain penicillins. The production of ampC beta-lactamases is stimulated when the bacteria containing the gene ampC such as bacteriaceae and *Pseudomonas aeruginosa* is exposed to beta-lactam drugs (M. Hassan *et al.*, 2012). Although gram positive bacteria are generally not pathogenic but *Enterococcus faecalis*, *Staphylococcus aureus* which are responsible for causing cystitis, pyelonephritis and catheter-associated UTI, endocarditis synthesise Class D beta-lactamases. Genes mecA and vanA are responsible for this synthesise. Class D beta-lactamases can hydrolyze penicillins and certain cephalosporins. The different types of beta-lactamases and their capacity to confer resistance to a variety of beta-lactam medicines, created significant complications in treating bacterial infections. To formulate new drugs and therapy, detailed knowledge and research of different kinds of beta-lactamases, the genes that synthesise is required.

Decreased antibiotic penetration-

Bacteria adopt different mechanisms to minimize the amount of antibiotics entering into their cells. Some bacteria modify their outer membrane or cell wall, to become antibiotic resistane by minimizing the amount of antibiotic inside the cell. Gram-negative bacteria's outer membrane is heavily reliant on porin proteins. They create channels through which nutrients and other chemicals, such as antibiotics, can enter the cell. Antibiotics' capacity to pass through the outer membrane and reach their target, however, is diminished since some resistant bacteria have developed ways to alter their porins or lower their expression. The alteration of the porin channels' dimensions or form is one frequent method. The size of molecules that can flow through the channel has been restricted by the evolution of smaller-than-normal porins in some resistant bacteria. In order to make it more challenging for some antibiotics to connect to the channel and pass through the membrane, some bacteria have altered the structure of their porins. Regulating the expression of porin channels that are present in outer membrane or cell wall many bacteria defend themselves against antibiotics such as *Pseudomonas aeruginosa* bacteria. It is responsible for causing infections in hospitalised patients (Lister *et al.*, 2009). They prevent the entry of antibiotics by lowering the production of specific porins, and enable the bacteria to live and grow in the host e.*g P. aeruginosa* can develop resistance to numerous antibiotics (Lister *et al.*, 2009).

Another illustration is *Klebsiella pneumoniae*, which is frequently responsible for infections connected to hospital settings, including bloodstream infections and pneumonia (Navon-Venezia *et al.*, 2017). By creating porin variations that restrict the uptake of specific antibiotics, such as carbapenems, K. pneumoniae can develop resistance to a variety of antibiotics (Navon-Venezia *et al.*, 2017). Since gram-positive bacteria lack an outer barrier, limiting access to medications is less common. Due to the high lipid content of the outer membrane of mycobacteria, hydrophobic drugs—such as rifampicin and fluoroquinolones have better access to the cell than hydrophilic ones do (Piccaro *et al.*, 2015; Reygaert, 2018).

Antibiotic Efflux-

Efflux pumps present on the cell surface of bacteria, extrude substances out of the cell. Some bacteria over express these effluxes pumps thereby lower the concentration of antibiotics inside cell and prevent their accumulation to hazardous levels. This ultimately gives rise to antibiotic resistance in bacteria. Both Gram positive and gram negative bacteria become resistant to a wide variety of antibiotics, including beta-lactams, tetracyclines, and fluoroquinolones by adopting this mode of resistance (Blair *et al.*, 2015). In *Escherichia coli* the AcrAB-TolC efflux pump system is responsible for its ability of antibiotic resistance.

AcrAB-TolC systems in *Escherichia coli* extrude a wide variety of structurally varied antibiotics, such as beta-lactams, fluoroquinolones, tetracyclines, chloramphenicol, and macrolides (Nikaido and Pages, 2012). Three proteins make up this system: TolC, an outer membrane channel protein, periplasmic membrane fusion protein AcrA, and inner membrane transporter AcrB (Hassan *et al.*, 2013). There are three proteins involvd in this system, AcrB, TolC and AcrA. The main drug-binding protein, known as AcrB, is the main drug-binding protein. It also interacts with and transport antibiotics through the inner membrane. TolC produces a channel that spans the outer membrane and enables the extrusion of antibiotics from the periplasmic region into the extracellular environment. AcrA acts as an adapter protein that links AcrB and TolC (Nikaido and Pages, 2012). Several transcriptional regulators, including the multidrug resistance regulator A (MarA), the SoxRS transcription factor, and the AcrR repressor, control the expression of the AcrAB-TolC system (Blair *et al.*, 2015). The AcrAB-TolC system can be upregulated by mutations that increase the expression or activity of these regulators, which can increase antibiotic resistance (Nikaido and Pages, 2012). Antibiotic resistance can also increase as a result of mutations in the acrAB genes themselves. For instance, mutations that change AcrB's conformation or interfere with AcrB and AcrA's interaction can increase antibiotic efflux (Nikaido and Pages, 2012).

In conclusion, the AcrAB-TolC efflux pump system is a substantial contributor to antibiotic resistance in gram-negative bacteria, particularly *E. coli*. Its ability to expel a diverse range of structurally dissimilar antibiotics, coupled with its intricate regulatory circuitry, underscores the significance of this resistance mechanism in the emergence and dissemination of antibiotic-resistant bacteria. This is a critical issue that demands attention and resolution (Blair *et al.*, 2015).

One of the main efflux pumps behind *Pseudomonas aeruginosa*, a Gram-negative bacterium frequently found in healthcare facilities multidrug resistance (MDR), is the MexAB-OprM system. MexA, MexB, and OprM make up the three proteins that make up this efflux pump system. MexA and MexB are found in the bacterium's inner membrane, and OprM is found in the outer membrane. A tripartite efflux pump system made up of these proteins may transport a variety of antibiotics and other harmful substances out of the bacterial cell. MexB, an integral membrane protein, and MexA, a periplasmic membrane fusion protein, work together to create a useful pump. MexB is the energy transducer that powers the efflux of antibiotics and other chemicals out of the bacterial cell via the proton motive force. OprM creates a pore in the bacterium's outer membrane that permits the effluxed substances to leave the cell. MexAB-OprM system made a wide variety of antibiotics, including beta-lactams, fluoroquinolones aminoglycosides, and tetracyclines, ineffective. Antibiotics and other hazardous substances cause this efflux pump mechanism to be activated, which increases the efflux of these substances out of the bacterial cell. The MexAB-OprM system participates in quorum sensing, biofilm formation and other cellular functions in addition to its function in antibiotic resistance. Bacteria communicate with one another and coordinate their actions in response to environmental cues through a process called quorum sensing. Bacterial populations known as biofilms are frequently resistant to antibiotics and other stresses because they are immersed in a matrix of extracellular polymeric materials. Targeting the MexAB-OprM efflux pump system as a method of preventing antibiotic resistance in *P. aeruginosa* has been the subject of several research. Utilising efflux pump inhibitors, which are substances that can hinder the efflux pump system's function and raise the concentration of antibiotics inside cells, is one strategy.

The creation of new antibiotics that are not substrates for the MexAB-OprM system is an alternative strategy. To completely comprehend the mechanisms underpinning efflux pump-mediated antibiotic resistance in *P. aeruginosa*, additional study is required. However, the development of efficient inhibitors or new medicines remains a hurdle.

Gram-negative pathogens are more likely than Gram-positive bacteria to exhibit efflux pump-mediated antibiotic resistance (Nikaido 1996). In contrast, the QacA protein (Qac 14 quaternary ammonium compound) pumps out hydrophobic disinfectant compounds (Neyfakh *et al*., 1993). For instance, the NorA, -B, and -M proteins of *S. aureus* are MFS transporters, which can efflux fluoroquinolones and ciprofloxacin.

ATP-binding cassette (ABC) efflux pumps, made up of an ATP-binding protein, a membrane-spanning protein, and a substrate-binding protein found in bacteria. This ABC efflux pumps play roles in efflux of numerous substrates, including antibiotics, from the bacterial cell thereby creating antibiotic resistant bacteria. Another efflux pump i.e the ABC efflux pumps draw substances from the bacterial cell, including antibiotics, using the energy of ATP hydrolysis. The hydrolysis of ATP, which is connected to the movement of the substrate across the membrane, produces the energy needed for efflux.

The study of the MsbA transporter in Escherichia coli demonstrated how the ABC efflux system works and lead to antibiotic resistance. In *Escherichia coli*, MsbA extrude out various antimicrobial substances, such as macrolides, tetracyclines, and aminoglycosides. Apart from the antimicrobial substances, MsbA transporter is also involved in the export of lipopolysaccharides, which are crucial parts of the bacterial cell wall.

The Sav1866 transporter in *Staphylococcus aureus* is a further instance of the ABC efflux system contributing to antibiotic resistance. Fluoroquinolones and macrolides are just a couple of the antibiotics that this transporter is involved in releasing. In addition, it has been demonstrated that the Sav1866 transporter is involved in the efflux of toxins, which may increase the pathogenicity of the bacteria. In conclusion, an important mechanism through which bacteria can develop antibiotic resistance is the ABC efflux pumps. These pumps can render antibiotics ineffective by pumping them out of the bacterial cell before they reach their intended targets. The ABC efflux pumps can also pump out other substrates including toxins and lipopolysaccharides, which can increase the bacterium's overall pathogenicity.

By modification

Target alterations or modifications can potentially result in the development of antibiotic-resistant microorganisms. This happens when the antibiotic's target is changed in some way, rendering it less vulnerable to its effects. The modification of bacterial ribosomes, which can prevent antibiotics from attaching to them and preventing protein synthesis, is a typical illustration of target modification. For example, target binding site of macrolide antibiotics like erythromycin in *Streptococcus pneumonia* get altered due to mutations in the genes that code for ribosomal proteins. As a result an antibiotic become unable to suppress protein synthesis at the ribosomes and becomes responsible for antibiotic resistance capacity of the bacteria. Experiments have also shown that fluoroquinolone-resistant *Escherichia coli* and other bacterial species have mutations in the genes producing DNA gyrase and topoisomerase IV, which are targets of fluoroquinolone antibiotics. These changes may change the target enzymes' structure, decreasing the antibiotics' ability to attach to them and making them useless. *Staphylococcus aureus* synthesise beta-lactamases, that break the beta-lactam ring of penicillin thereby make it inactive. Also certain bacteria have altered their cell walls by adding certain groups of chemicals, such as by adding D-alanine to the peptidoglycan layer, thereby preventing penicillin from binding to its target spot.

Acquired resistance

According to Davies and Davies (2010), acquired resistance of bacteria to antibiotics refers to the capacity of bacteria to develop antibiotic resistance through genetic alterations or acquisition of resistance genes. Multiple processes, such as chromosomal mutations, horizontal gene transfer, and gene amplification, might result in acquired resistance. One of the most frequent routes of acquired resistance in bacteria is by Chromosomal mutation. Due to Chromosome mutation, the bacterial target site gets altered, uptake of drug is reduced, or extrusion of drug is increased (Blair *et al*., 2015).

By reducing the binding of antibiotics like erythromycin to the gene encoding the bacterial ribosome confer resistance to these medications (Roberts and Poole, 2014). Another way of acquired resistance is horizontal gene transfer that includes the three primary pathways i.e Conjugation, transformation, and transduction mediates transfer of resistance genes between bacterial cells (Davies and Davies, 2010). Bacterial cells uptake free DNA from the environment by transformation and transduction process. Conjugation and transduction involve transfer of DNA by bacteriophages involving cell-to-cell interaction. The proliferation of extended-spectrum beta-lactamases (ESBLs) in Enterobacteriaceae is one instance of acquired resistance through horizontal gene transfer. Many beta-lactam antibiotics are hydrolyzed by ESBLs, making them useless (Pitout and Laupland, 2008). Overuse of antibiotics, which can provide a selective pressure for the emergence and dissemination of resistance strains, has been related to the expansion of ESBL-producing bacteria (Pitout and Laupland, 2008). Another mechanism of acquired resistance is gene amplification, due to which a particular gene is duplicated or its proportion in genome is increased. The ole of gene amplification in antibiotic resistance is that, this process makes duplication of genes that encode antibiotic resistance. As a result expression of the resistance gene is increased that resulted in higher levels of antibiotic resistance (Blair *et al*., 2015). For exammple, in *E. coli,* due to amplification of the gene responsible for efflux pump AcrAB-TolC, resulted in increased production of the pump that lead to extrusion of antibiotic substance from the cell and gives greater levels of antibiotic resistance (Nikaido, 2009). In conclusion a variety of mechanisms, such as chromosomal changes, horizontal gene transfer, and gene amplification are involved in acquired antibiotic resistance in bacteria.

Spontaneous mutation-

Bacteria can become resistant to antibiotics by a process known as spontaneous mutation, in which genetic changes arise naturally during DNA replication (Blair *et al*., 2015). Genes that govern antibiotic uptake and efflux can also get mutated, as can genes that encode antibiotic targets, such as ribosomal proteins or enzymes necessary for cell wall formation (Davies and Davies, 2010). Antibiotic resistance spontaneous mutation frequency ranges from 10^{-8} to 10^{-9}. Accordingly, one out of every 10^{-8}–10^{-9} bacteria will mutate to become resistant (Davies and Davies 2010). These mutations happen at random throughout the genome and are frequently accelerated by mutagenic chemicals. They are linked to unchecked errors made during DNA replication. The majority of these mistakes are harmful to the host bacterium and do not persist at the cellular or population level. However, they might take over the cell if they offer an evolutionary advantage through vertical transmission (Martinez and Baquero 2000).

Due to the rapid growth rate of bacteria and the high number of cells produced, resistance quickly develops in a bacterial population even though mutation is an uncommon occurrence (Coculescu 2009). The resistance genes are directly passed to all progeny during DNA replication once they have evolved. The rate and pattern of antibiotic usage (selection pressure) affects the selective environment of antibiotics, which causes the wild types (nonmutants) to perish and the resistant mutants to grow. Mutations in the gyrA and parC genes render fluoroquinolones unable to bind to its target DNA gyrase and topoisomerase IV. This causes the bacteria to be antibiotic resistant. For example Fluoroquinolone resistance,that develop due to mutation in gyrA and parC genes is found methicillin-resistant *Staphylococcus aureus* (MRSA) (Blair *et al*., 2015). Rifampicin inhibits bacterial growth by inhibiting bacterial RNA polymerase. However mutations in the rpoB gene, which normally encode RNA polymerase beta subunit, changes the drug's binding site to it and thereby lead to resistance. For instance, rifampicin resistance in Mycobacterium tuberculosis is frequently linked to rpoB gene alterations (Blair *et al*., 2015). Isoniazid, a significant antibiotic used to treat tuberculosis, works by preventing the formation of mycolic acids to target the mycobacterial cell wall. Resistance to isoniazid can be brought on by mutations in the katG gene present in bacteria is responsible for synthesising a

catalase-peroxidase enzyme which is necessary for the drug's activation. But due to mutation in katG, it becomes unable to synthesise catalase-peroxidase enzyme properly. This lead to antibiotic resistance for instance, it has been found that *Mycobacterium tuberculosis* is linked to isoniazid resistance due to mutations in the katG gene (Blair *et al*., 2015). Penicillins and cephalosporins are antibiotics that include beta lactam ring in its structure. These antibiotic inhibit the formation of peptidoglycan cell wall layer, thereby inhibiting bacterial growth. But due to mutations in the genes encoding the beta-lactamases,its activity is enhanced that break down the beta-lactam antibiotics, by which resistance to beta-lactams can develop. Mutation in blaSHV gene is associated with beta-lactam resistant Escherichia (Davies and Davies, 2010). Methicillin is used for the treatment of infections caused by *Staphylococcus aureus*. mecA gene produces a penicillin-binding protein whose affinity for methicillin is lowered by mutation in this mecA gene. Due to this bacteria become resistant to methicillin. For example, methicillin resistance in MRSA is linked to changes in the mecA gene (Blair *et al*., 2015).

Acquisition of new genetic material from another source

Horizontal gene transfer (HGT) is an important process by which bacteria acquire new genetic material from a different source (Beceiro *et al*., 2013). This process of gene transfer plays an important role for the emergence of antibiotic resistance in bacteria. According to Gupta and Birdi (2017), the transfer of mobile genetic elements (MGEs), or genetic material, between individual bacteria of the same species or even between distinct species can occur by the process of HGT. There are three ways by HGT can occur: transformation, transduction, and conjugation.

Transformation is the process by which bacteria can take up free DNA from their surrounding and integrate into their own genome. Bacteria can acquire antibiotic resistance genes by this process. This lead to emergence of antibiotic-resistant strains. For example, the process of transformation is responsible for transfer of the penicillin-binding protein (PBP) genes from other bacteria that are already resistant to penicillin to a non-resistant bacteria, lead to emergence of new Penicillin-resistant *Streptococcus pneumonia*. To take up genes of penicillin-binding protein (PBP), the bacteria need to express those genes involved in DNA absorption and recombination. recA gene is responsible for the recombination of extracellular DNA with the bacterial chromosome, and comABCDE and comX, are responsible for creation of competence factors that enhance the uptake of extracellular DNA. *S. pneumoniae* may develop penicillin resistance after incorporating these genes into its genome (Claverys and Havarstein, 2017). Tetracycline-resistant another example of transformation leading to antibiotic resistance is *Escherichia coli*. Tetracycline resistance is usually conferred via the tet genes, which generate tetracycline efflux pumps that may remove tetracycline from cells. These genes are on plasmids, which *E. coli* can change into carriers of them. If the plasmid encoding the tet genes is incorporated into the *E. coli* genome, tetracycline resistance may arise (Mazel, 2006).

Transduction

Transduction is the process in which bacteriophages (viruses that infect bacteria) facilitates transfer of genetic material between bacteria. When bacteriophages attack bacteria, it takes up DNA from that bacterium and delivered it to other bacteria. If that taken up DNA contain antibiotic resistant genes then, when the bacteriophage will deliver that DNA it will result into development of antibiotic resistant bacteria owing to the acquisition of genes for antibiotic resistance. This process is called as transduction. During the process of replication, it has been found that some part of the bacterial DNA may be packaged into fresh bacteriophage particles in place of the phage DNA. When this bacteriophage containing some part of bacterial DNA infects a new host cell, the whole phage DNA conating both phage and bacterial DNA transduced bacterial DNA gets transferred to that newly infected cell. Due to transfer of the blaKPC-2 gene responsible for carbapenem resistance by bacteriophage has resulted into a carbapenem-resistant strain of *Klebsiella pneumonia* (Chen *et al*., 2017).

In this instance, sustained inheritance of the resistance characteristic was made possible by the integration of the blaKPC-2 gene into the bacterial chromosome. According to a study, a tetracycline-resistant strain of *Staphylococcus aureus* was created when a transposon harbouring the gene for tetracycline resistance, tetM, was transferred by transduction (Boyd *et al.*, 2000). The tetM gene was incorporated into the bacterial chromosome in this instance, enabling persistent inheritance of the resistance characteristic.

Due to the high density of bacteria, phages, and plasmids in the environments like hospital effluents, sewage drains from communities, wastewater treatment plants, abattoir waste, and aquaculture are thought to be prime locations (referred to as "hotspots" below), where exchange events occur (Graham *et al.*, 2011; Subirats *et al.*, 2016). Additionally, recent research has demonstrated that the sub-inhibitory concentrations of toxic substances, metals, and antibiotics in these hotspots represent a selective pressure that may induce transformation and transduction, potentially increasing HGT through these two mechanisms (Pal *et al.* (2015), Gullberg *et al.* (2014) and Gullberg *et al.* (2011).

Conjugation

The process of conjugation involves the physical interaction of two bacterial cells to transfer genetic material. Plasmids, which are compact, circular, extra-chromosomal DNA molecules capable of independent replication from the bacterial chromosome, are transferred during this procedure. Conjugation is a key process by which antibiotic resistance is disseminated throughout bacterial populations because plasmids frequently carry genes that confer antibiotic resistance. Conjugation is a particularly effective method of transmitting antibiotic resistance because plasmids can transfer numerous antibiotic resistance genes at once. For instance, a study discovered that a multidrug-resistant strain of *E. coli* was created as a result of the conjugative transfer of a plasmid containing several antibiotic resistance genes, such as blaTEM-1 and sul2 (Coque *et al.*, 2008). In addition to encoding a conjugative transfer system, the transmitted plasmid contained instructions for transferring the resistance genes to other bacteria in the community. Conjugation can transfer resistance genes found on transposons or integrons in addition to plasmids. While integrons are genetic platforms that can ensnare and incorporate resistance genes into their DNA, transposons are mobile genetic elements that can migrate within or across chromosomes.

The receiving bacterial cell may pick up several resistance genes as a result of the conjugational transfer of transposons or integrons. For example the tet(M) gene is carried by the tn916 transposon, which also carries the ermB and aphA-3 resistance genes. ErmB confers resistance to macrolides, lincosamides, and streptogramin B antibiotics.

In study by Donskey *et al* in 1999, found that conjugation with Tn916 transposnon resulted in the formation of multidrug-resistant *Enterococcus faecalis* strains. tet(M) gene, which is responsible for a ribosome protective protein, also provide tetracycline resistance capacity. For example by using the conjugative transfer of a plasmid containing tet(M)tetracycline-resistant strains of *Listeria monocytogenes* were created (Shen *et al.*, 2019).

By biofilm production

Bacteria create the sticky, polymeric material known as the biofilm matrix. Because of its potential thickness, antibiotics may have a hard time penetrating it and reaching the microorganisms inside. Metabolic changes are frequent in biofilms. Therefore it becomes difficult for the antibiotics to target the bacteria's metabolic pathways. Hence, bacteria become less vulnerable to antibiotics. In order to interact with one another, bacteria use a technique called quorum sensing. Quorum sensing in biofilms can cause the expression of genes that support antibiotic resistance. This is so that the bacteria within the biofilm can organise their efforts and cooperate to develop greater resistance to drugs.

Proteins called efflux pumps have the ability to expel antibiotics from cells. Bacteria in biofilms frequently have higher efflux pump expression, which can aid in their ability to eliminate antibiotics before they have a chance to work.

Table 2. Active inhibitors of efflux pumps, with specification of substrates, bacterial species and efflux pumps inhibited.

Inhibitor	Substrates	Bacterial Species	Efflux Pumps Inhibited
Verapamil	β-lactams, aminoglycosides, fluoroquinolones	Gram-positive and Gram-negative bacteria	AcrAB-TolC, NorA, MexAB-OprM
Quinupristin /dalfopristin	Macrolides	Gram-positive bacteria	MshA, LmrA
Rifampicin	Rifampicin	Gram-positive and Gram-negative bacteria	NorA, MexAB-OprM
Tigecycline	Tetracyclines	Gram-positive and Gram-negative bacteria	AcrAB-TolC, NorA, MexAB-OprM
Moxifloxacin	Fluoroquinolones	Gram-positive and Gram-negative bacteria	AcrAB-TolC, NorA, MexAB-OprM

Therapeutic Alternatives to drug resistant bacteria

Combating the growing issue of antibiotic resistance necessitates the discovery of therapeutic substitutes for drug-resistant microorganisms. The creation of novel antibiotics, combination medicines, and alternative therapeutic modalities are some of the techniques that researchers have been investigating to solve this problem.

By applying bacteriophages

Applying bacteriophages, known as viruses that target and infect bacteria specifically, is one possible strategy. In phage therapy, certain viruses called bacteriophages are used to target and eradicate bacterial illnesses. It is effective against both curable and antibiotic-resistant bacteria, which are posing a serious danger to world health. Phages are naturally occurring bacterial predators that have the ability to identify and infect particular bacterial strains without causing harm to the human host or the typical microbiota. Treatment with phages can be done either alone. But administration of phages in conjunction with antibiotics and other medications increase the effectiveness of the phage treatment. It contributes a lot in reducing the emergence of resistance1. In recent years, life-threatening illnesses caused by multidrug-resistant bacteria, which were not responsive to traditional therapies, showed encouraging response to phage therapy. People infected with *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus aureus* were successfully treated with personalized phage cocktails according to several case reports 23. Currently, many clinical trials are ongoing to assess the security and effectiveness of phage therapy for a number of diseases, including osteomyelitis, chronic lung infections, urinary tract infections, and diabetic foot ulcers 23. The nature and life cycle of the phages used determine the phage therapy's mechanism i.e Lysogenic and lytic phages.

Lysogenic phages are those that integrate their DNA into the bacterial genome and proliferate alongside the host cell, as opposed to lytic phages, which infect and kill bacterial cells by rupturing their cell walls. Lysogenic phages are associated with lower risk infecting bacteria with latent infection and also there is less chance of transferring harmful genes to them then lytic phages. For this reason Lysogenic phages are chosen for therapeutic reasons over lytic phages.

Some of the genes involved in this process are: gp5 (tail fiber), gp17 (tail tube), gp27 (baseplate), gp28 (tail sheath), gp49 (head protein), gp50 (head protein), gp51 (head protein), gp52 (head protein), gp53 (head protein), gp54 (head protein), gp55 (head protein), gp56 (head protein), gp57 (head protein), gp58 (head protein), gp59 (packaging ATPase), gp60 (connector protein), gp61 (connector protein), gp62 (connector protein), gp63 (connector protein), gp64 (connector protein), gp65 (connector protein), gp66 (connector protein), gp67 (connector protein), gp68 (connector protein), gp69 (connector protein), gene 32 (single-stranded DNA binding protein), gene 43 (DNA polymerase), gene 45 (sliding clamp), gene 62 (sliding clamp loader subunit), gene 44/62/63/45/54 complex (replisome complex). Lambda phages have the ability to infect *Escherichia coli*, both in lytic or lysogenic mode. Some of its genes involved are: J (tail fiber), H (tail tube), GpU/GpV/GpW/GpX/GpY /GpZ/GpQ/GpR/GpS/GpT/GpU' complex.

By using antimicrobial peptides (AMPs)

Pathogenic microorganisms such as bacteria, virus, fungi etc. are eradicated or prevented by some multifunctional peptides known as antimicrobial peptides (AMPs). Humans and other living beings' innate immune systems are comprised of these small molecules, AMPs. In comparison to traditional antibiotics, AMPs have a number of advantages, including broad-spectrum efficacy, minimal toxicity, excellent specificity, and a low propensity for resistance development.

AMP acts through generation of pores or channels in microbial membranes leading to leakage of cytoplasmic contents and result in cell death. crucial cellual procedures such as transcription, translation, and cell division are obstructed by these AMPs and causes disruption of DNA, RNA, proteins, and ribosomes. The process is controlled by various genes, some of those are pmrA and pmrB, phoP, phoQ, arn BCADTEF, mprF, adeABC, mprA and mprB, aprA and colicins that controls the alteration of lipopolysaccharide (LPS), outer membrane of Gram-negative Bacteria. Bacteria can lessen their negative charge and the binding of cationic AMPs by adding aminoarabinose or phosphoethanolamine to LPS, enzymes that help produce aminoarabinose from UDP-glucosamine. The PMRA-PMRB system controls it, a two-component mechanism that controls how LPS is altered in response to low magnesium or high AMP levels. Additionally, it regulates the expression of other genes linked to AMP and antibiotic resistance, a membrane protein that increases the outer leaflet of the membrane's positive charge and repels cationic AMPs by transferring lysyl-phosphatidylglycerol (LPG) to it. Additionally, it facilitates phosphatidylglycerol's (PG) flip to the inner leaflet, lowering its negative charge; RND stands for resistance-nodulation-cell division. Efflux pump that allows the cell to expel AMPs and other antimicrobials. The adeRS two-component system controls it, an alkaline protease that can degrade some AMPs, such as LL-37 and lactoferricin B, two-component system that regulates the expression of proteases, such as aprA, that can degrade AMPs respectively and colicins generated by some *Escherichia coli* strains that can eradicate other *Escherichia coli* strains or similar species. They work by attaching to certain receptors on the bacterial surface, creating membrane holes, or concentrating on internal components. Bacteria can contend with one another for resources and habitats by generating colicins.

Preserving β lactam efficacy

Due to the significant role of β-lactam antibiotics in combating bacterial infections, the emergence of β-lactamase-mediated resistance has become a major concern. Overcoming this mechanism of resistance presents a significant challenge, but two strategies have emerged as effective approaches to preserve the efficacy of β-lactams against bacterial infections. These strategies include:

1. Development of β-lactam compounds that can evade enzymatic inactivation by β-lactamases.
2. Development of β-lactamase inhibitors that restore the activity of β-lactam antibiotics by preventing their degradation.

These approaches aim to ensure the continued effectiveness of β-lactam antibiotics by either modifying the antibiotic structure or utilizing inhibitors to counteract the action of β-lactamase enzymes. In this review we will focus only on beta lactamase inhibitors.

The first-generation beta-lactamase inhibitors, including clavulanic acid, displayed a modest level of inhibitory activity but lacked sufficient specificity and were easily broken down by particular -lactamases. The clavulanic acid--lactamases complex dissociates slowly, which makes enzymatic suppression of the complex particularly effective and reflects the effectiveness of its use in conjunction with other antibiotics, such as amoxicillin.

Sulbactam and tazobactam are examples of second-generation beta-lactamase inhibitors that have enhanced inhibitory activity against a wider variety of lactamases. These inhibitors can bind to the active site of -lactamases in a competitive manner because they share the same -lactam ring structure as -lactam

antibiotics. They shield -lactam antibiotics against deterioration by blocking -lactamases from hydrolyzing them by occupying the active site.

In order to solve the drawbacks of earlier inhibitors and provide improved inhibitory activity against a variety of -lactamases, including extended-spectrum -lactamases (ESBLs) and some carbapenemases, third generation beta-lactamase inhibitors, such as avibactam and relebactam, were developed. Serine lactamases from Classes A and C, as well as lactamases from Class D (which includes OXA-48), were all highly active against by avibactam. As a result, avibactam constituted a considerable advancement over the hitherto available commercial -lactamase inhibitors, which were essentially restricted to Class A enzyme inhibition and lacked appreciable activity against Class A KPC carbapenemases [251].

During the inhibition of beta lactamase various genes are targeted.

1. TEM genes: According to Paterson and Bonomo (2005), these genes produce TEM-type beta-lactamases, which are frequently found in Gram-negative bacteria like *Escherichia coli* and *Klebsiella pneumoniae.*

2. SHV genes: These genes produce beta-lactamases of the SHV-type, which are also common in Gram-negative bacteria and frequently linked to the synthesis of extended-spectrum beta-lactamases (ESBLs) (Paterson and Bonomo, 2005).

3. These genes produce beta-lactamases of the CTX-M type, which are an increasingly significant source of ESBL-mediated resistance in the Enterobacteriaceae (Cantón *et al.*, 2012).

4. AmpC genes: According to Jacoby (2009), these genes produce AmpC beta-lactamases, which are frequently seen in Enterobacteriaceae and can confer resistance to a variety of beta-lactam drugs.

5. Carbapenemase genes: These genes translate for the carbapenemase enzymes that cause carbapenem resistance and present a serious clinical problem, such as KPC, NDM, and OXA enzymes (Nordmann *et al.*, 2011).

Table 3. Some genes involved in beta lactamase inhibition in microorganisms and their mechanisms of inhibition and their discoverer name.

Gene	Mechanism of inhibition	Discoverer name
blaI	Represses the expression of blaZ, which encodes a penicillinase	Novick *et al.*, 1989
blaR1	Senses the presence of beta-lactams and activates blaI	Novick *et al.*, 1989
mecR1	Senses the presence of beta-lactams and activates mecI, which represses the expression of mecA, which encodes a PBP with low affinity for beta-lactams	Hiramatsu *et al.*, 1991
mecI	Represses the expression of mecA	Hiramatsu *et al.*, 1991[1]
ampR	Regulates the expression of ampC, which encodes an AmpC beta-lactamase	Lindberg *et al.*, 19772
ampD	Inhibits the expression of ampC by degrading its inducer	Lindberg *et al.*, 1977
ampG	Transports the inducer of ampC into the cytoplasm	Jacobs *et al.*, 1994
ampE	Inhibits the expression of ampC by binding to its promoter region	Juan *et al.*, 2006
clavulanic acid biosynthesis genes (e.g. cas1, cas2)	Produce clavulanic acid, a beta-lactamase inhibitor that binds irreversibly to the active site of beta-lactamases	Reading and Cole, 1977
blip gene	Encodes BLIP, a protein that binds non-covalently to class A beta-lactamases and inhibits their activity	Mark *et al.*, 1994

Besisdes these Alternative therapies, some other ways like stem cell-AMPs, CRISPR-Cas, probiotics, and nanobiotics should be explored to combat antibiotic-resistant bacteria.

CRISPR-Cas

CRISPR-Cas system is a novel way to address the issue of drug-resistant bacteria. CRISPR-Cas system employ RNA-guided DNA cutting mechanism, thereby targeting and eliminating the specifically, this system can be employed to re-sensitize drug-resistant bacteria to antibiotics by targeting and eliminating antibiotic resistance genes containg plasmids. This mechanism of CRISPR-Cas system makes it equivalent to antibiotic-sensitive bacteria in regards of susceptibility to antibiotics.

Inducing bacterial death

CRISPR-Cas system can be used to induce bacterial death by altering the expression of essential genes or virulence factors.

Reverting bacterial resistance to antibiotics

CRISPR-Cas systems can be engineered for the rapid and reliable detection and effectively kill bacteria or even revert bacterial resistance to antibiotics

Targeted microbiome engineering

CRISPR-Cas systems enable tools to understand and engineer targeted microbiome. A more innovative approach is necessary to treat and diagnose The infection caused due to the increase in the number of multi-drug resistant strains. Therefore CRISPR-Cas based medicine poses a critical role in the diagnosis and treatment of infectious diseases

Conclusion

An antimicrobial and multidrug resistant bacterium poses the most serious global public health and economic threat in this century. Developing antibiotic resistance varies across regions and bacterial species and is influenced by genetic and environmental factors. The rise of antibiotic resistance is due to inappropriate and overuse of antibiotics. Bacteria are capable of resisting antibiotics through several mechanisms including modifications to the outer membrane, overexpression of efflux pumps, and alterations in antibiotic targets. Mutations in genes control antibiotic uptake, efflux, or target binding which alteres antibiotic targets. Chromosomal mutations, horizontal gene transfer, and gene amplification are mechanisms of acquired resistance. Understanding these biochemical and genetic basis of resistance mechanisms is vital for developing effective strategies to combat antibiotic resistance and hopefully lead to better treatment options for infective diseases, and development of antimicrobial drugs that can withstand the microorganisms attempts to become resistant. Addressing this issue requires a wide range of approaches, including proper antibiotic use, effective infection prevention measures, and investment in research and development. To explore the diverse range of self-resistance mechanisms in bacteria and their genetic basis research is necessary. Concerning animal health and public health antibiotics should be used appropriately. Continual research and surveillance are essential to preserve the efficacy of antibiotics in treating bacterial infections.

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