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ANTIBIOTIC RESISTANCE: OVERVIEW AND MECHANISMS

Sonia Bindu Pandra*, David Banji, Otilia J. F. Banji, Ranjith Kumar Aavula and Swetha Merugu

Nalanda College of Pharmacy, Hyderabad road, Nalgonda, Andhrapradesh, India

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Correspondence to Author:

Sonia Bindu Pandra,

Department of Pharmacology,
Nalanda College of Pharmacy,
Hyderabad road, Nalgonda,
Andhrapradesh, India

ABSTRACT

Antibiotics have a well-documented efficacy in the treatment of established infections and as prophylactic agents in medically compromised patients. Antibiotics use is suggested to be a major risk factor for development of antibiotic resistance. Resistance to antimicrobials is emerging at an alarming rate that has reduced treatment options for nearly every pathogen infecting humans. Many bacteria now display a variety of mechanisms that help protect them during antimicrobial exposure. These include production of β-lactamases and cephalosporinases, alterations in penicillin-binding proteins, multidrug efflux pumps, transferable resistance to vancomycin, and mutations in genes encoding DNA gyrase. This article gives information about how bacteria develop antibiotic resistance, new era of antimicrobial therapeutics and new strategies to eliminate antibiotic resistance.

INTRODUCTION: Antibiotics have proven to be a dynamic category of drugs in the fight against infectious bacteria [1]. Antibiotic resistance is one of the greatest current challenges to the effective treatment of infections. Furthermore, there is every indication that antibiotic resistance will become an even greater challenge in the future [2]. Antibiotic resistance occurs due to changes in bacteria in some way that reduces or eliminates the effectiveness of drugs, chemicals or other agents designed to cure or prevent the infection [3]. Increasing antibiotic resistance threatens our ability to effectively treat bacterial infections.

Antibiotic therapy enhances the growth of existing drug-resistant bacteria and the exchange of resistance mechanisms between bacteria and selects for resistance mutations. Antibiotic resistance, a well-known phenomenon in nature .It assumes significant public health importance when it gets amplified many folds due to human misuse and neglect. In the present age the threat has become global due to rapid spread of organisms from one part of the world to another. It is no longer a problem of the developing countries alone. Today even after all the advances in therapeutics and the availability of a large number of antibiotics, a person can die in a developed country also due to infection with resistant bacteria [4].

The astonishing effects of antibiotics, the occurrence of antibiotics, and the considerable resources spent on antibiotics globally are convincing reasons for concern about ensuring adequate and proper use of these powerful agents. Antibiotics are largest by any therapeutic agents often accounting for 15-30% of total drug expenditure. The history of antibiotic resistance coincides with the history of antibiotics themselves. Antibiotic resistance first became challenging shortly after Penicillin gained extensive use in the 1940s [5]. The period of late 1940s and early 1950s saw the discovery and introduction of broad spectrum antibiotics such as Streptomycin, Chloramphenicol and Tetracycline and the age of antibiotic chemotherapy came into full being. By the late 1980s even Methicillin resistant Staphylococcus aureus had become prevalent in many hospitals and difficult to treat [6, 7]. Until recently Vanomycin was a dependable drug for the treatment of infections caused by multidrug resistant Enterococci but Vanomycin resistance began to emerge in the mid 80s. A study by Gaynes reported that Vanomycin resistance had increased more than 20 fold from 1989 to 1995. For a number of years, Penicillins were the drug of choice to treat Gonorrheae but in 1976, the plasmid mediated Beta lactamase of *E. coli* was found in *Neisseria gonorrhoeae* isolates in Africa and Asia [8]. Development of antibiotic resistance was first reported in animal models in 1940s [9] and subjectively reported among patients in the 1970s [10].

Antibiotics are given to human for the treatment and prophylaxis of human disease, 90% Antimicrobial drugs are used in out patients and reminder in hospitals. The Center for Disease Control and Prevention in USA has estimated that some 50 million of 150 prescriptions for antibiotic are written for out patient every year are unnecessary. The overuse of antibiotic use contributes to the development of resistance. The frequency of resistance of some of the antibiotics is as follows;

Ampicillin > tetracycline > sulpha- trimethoprim> streptomycin > chloramphenicol > cephradine> kanamycin > nalidixic acid [11].

Causes of Antibiotic Resistance: Antibiotic resistance was only caused by the failure of prescribed drug regimens and human errors also contribute to the development of antibiotic-resistant bacteria.

1. Misuse of antibiotics occurs in medicine, agriculture, and household products. The magnitude of the problem is significantly increased by the possibility of bacteria to transfer resistance determinants horizontally and by the mounting increase in the use (over-use and misuse) of antibiotics, which has created an enormous selective pressure towards resistant bacteria. Antibiotics are used in livestock production to combat disease and improve animal performance. Feed based antibiotics consistently benefit production, increasing the ability of farms to maintain profitable margins, reducing effects of animal wastes on the environment, and diminishing pathogen carriage. However, the evolution and transfer of antibiotic resistance elements in bacteria has caused some groups to recommend restricting or banning agricultural use of antibiotics [12].

2. Anomalous combinations can produce on drug-resistant microbes e.g., Mechanisms of genetic exchange between bacterial species, the mere coexistence of these two particular bacteria helped to bring about drug resistance in *S. aureus*. *It* was shown to that S.aureas acquire vancomycin resistance genes through cohabitation with the vancomycin-resistant bacteria, *Enterococcus faecalis*, in the wound of a hospitalized patient [13].

3. The enhanced transmission of resistant factors or the increased efficiency with which resistance genes are exchanged causes antibiotic resistance. Expression of drug resistance carried by the bacteria possesses resistance-transfer (R) factors which are of two types: fi- (negative fertility inhibition) and fi+ (positive fertility inhibition). Resistance-transfer factors (R factors) are episomes isolated from members of the Enterobacteriaceae that transmitted mechanism of expression of drug resistance [14].

   An efficient mode of operandi of organisms to escape the lethal action of drugs is by the alteration of resistant gene expression. Modulation of gene expression can occur at the transcriptional or translational level following mutations or the movement of mobile genetic elements and may involve induction by the antibiotic. In the latter case, the antibiotic can produce a triple activity: as an antibacterial agent, as an inducer of resistance to itself, and as an inducer of the dissemination of resistance determinants. Mating experiments of multiple-resistant coliforms with an E. coli K-12 donor sustained the capability of transferring its resistance.

4. The reservoir hypothesis states that each antibiotic has a threshold level that is required to induce and maintain antibiotic resistance. During antibiotic treatment, there is a decline in the populations of susceptible bacteria however some of their thrive for creating a reservoir of antibiotic-resistant bacteria [15].

5. Low-level antibiotic resistance is a gateway to high-level clinically relevant resistance and may be associated with increased virulence, resistance to unrelated compounds and more successful in vivo survival in Staphylococcus aureus [16].

6. The strain of Streptococcus cristatus, exhibits it resistance to doxycycline and erythromycin although it was sensitive to doxycycline before treatment. This strain acquired a novel conjugative transposon CTn6002 which was sequenced and found to be a complex element derived in part from Tn916 and an unknown element during treatment [17].

Mechanism of Antibiotic Resistance: There are two basic mechanisms by which organisms develop resistance to antimicrobial agents.

Genetic Mechanisms of Transmission: The development of antibiotic resistance tends to be related to the degree of simplicity of the DNA present in the microorganism becoming resistant and to the ease with which it can acquire DNA from other microorganisms. For antibiotic resistance to develop, it is necessary that two key elements combine: the presence of an antibiotic capable of inhibiting the majority of bacteria present in a colony and a heterogeneous colony of bacteria where at least one of this bacterium carries the genetic determinant capable of expressing resistance to the antibiotic.

Resistance to antibiotics can be natural (intrinsic) or acquired and can be transmitted horizontally or vertically. Natural form of antibiotic resistance is caused by a spontaneous gene mutation in the lack of selective pressure due to the presence of antibiotics and is far much less common than the acquired one. For the most part, however, the micro-ecological pressure exerted by the presence of an antibiotic is a potent stimulus to elicit a bacterial adaptation response and is the most common cause of bacterial resistance to antibiotics [18].

Susceptible bacteria can acquire resistance to antimicrobial agents by either genetic mutation or by accepting antimicrobial resistance genes from other bacteria. The genes that codify this resistance are normally located in specialized fragments of DNA known as transposons (sections of DNA containing ‘‘sticky endings’’) which allow the resistance genes to easily move from one Plasmid to another. The presence of complex and unique DNAfragment in transposons called ‘‘integron’’, a site capable of integrating different antibiotic resistance genes and thus able to confer multiple antibiotic resistance to a bacteria. Integrons have been identified in both gram-negative and gram-positive bacteria, and they seem to confer high-level multiple drug resistance to bacteria that carry and express them. Once a genetic mutation occurs and causes a change in the bacterial DNA, genetic material can be transferred among bacteria by several means. Bacteria also develop resistance through the acquisition of new genetic material from other resistant organisms. This is termed horizontal evolution, and may occur between strains of the same species or between different bacterial species or genera. The most common mechanisms of genetic transfer are conjugation, transformation and transduction [19].

Conjugation: Conjugation is the most important and prevalent mechanism of transmission of resistance in bacteria. This mechanism is normally mediated by plasmids (circular fragments of DNA) that are simpler than chromosomal DNA and can replicate independently of the chromosome. The mechanism of transmission of plasmids among bacteria is via the formation of a ‘‘pilus’’ (a hollow tubular structure) that forms between bacteria when they are next to each other, thus connecting them temporarily and allowing the passage of these DNA fragments [20].

Transformation: Transformation is another form of transmission of bacterial resistance genes and takes place when there is direct passage of free DNA (also known as ‘‘naked DNA’’) from one cell to another. The ‘‘naked DNA’’ usually originates from other bacteria that have died and broken apart close to the receiving bacteria. The receiving bacteria then simply introduce the free DNA into their cytoplasm and incorporate it into their own DNA [21].

Transduction: Transduction is a third mechanism of genetic transfer and occurs with the use of a ‘‘vector’’, which are mostly ‘‘bacteriophages’’. The virus containing the bacterial gene that codifies antibiotic resistance (the ‘‘resistant DNA’’) infects the new bacterial cell and introduces this genetic material into the receiving bacteria. Most times,

the infecting bacteriophage also introduces to the receiving bacteria its own viral DNA, which then takes over the bacterial replication system forcing the cell to produce more copies of the infecting virus until the bacterial cell dies and liberates these new bacteriophages, which then go on to infect other cells [22].

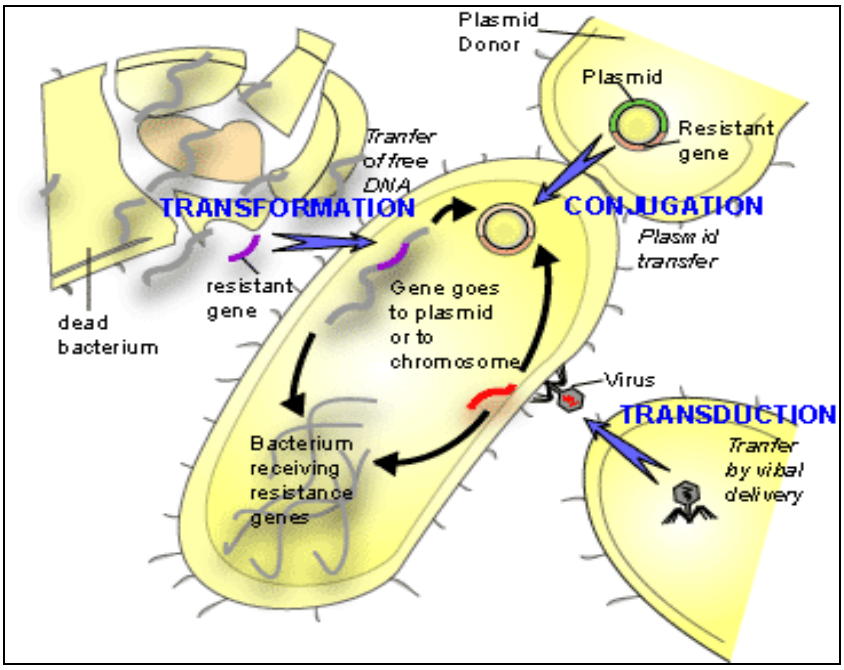

FIG. 1: MECHANISM OF GENE TRANSFER BY CONJUGATION, TRANFORMATION AND TRANSDUCTION AND FORMATION OF RESISTANT GENE

Biological Mechanisms of Resistance: Resistance can only develop if a gene is able to express itself and produce a tangible biological effect resulting in the loss of activity of the antibiotic. These biological mechanisms are many and varied but they can be summarized as follows.

Inactivation of antibiotics: The defence mechanisms within the category of antibiotic inactivation include the production of enzymes that degrade or modify the drug itself. Biochemical strategies are hydrolysis, group transfer, and redox mechanism.

Antibiotic inactivation by hydrolysis: Many antibiotics have hydrolytically susceptible chemical bonds (e.g. esters and amides). Several enzymes are known to destroy antibiotic activity by targeting and cleaving these bonds. These enzymes can often be excreted by the bacteria, inactivating antibiotics before they reach their target within the bacteria. The classical hydrolytic amidases are the β-lactamases that cleave the β-lactam ring of the penicillin and cephalosporin antibiotics. Many Gram-negative and Gram-positive bacteria produce such enzymes, and more than 200 different β-lactamases have been identified. β-Lactamases are classified into four groups on the basis of functional characteristics, including preferred antibiotic substrate. Clinical isolates often produce β-lactamases belonging to different functional groups. They can be both chromosomal and plasmid-encoded β-lactamases transferred from different bacteria [23].

Antibiotic inactivation by group transfer: The most diverse family of resistant enzymes is the group of transferases. These enzymes inactivate

antibiotics (aminoglycosides, chloramphenicol, streptogramin, macrolides or rifampicin) by chemical substitution (adenylyl, phosphoryl or acetyl groups are added to the periphery of the antibiotic molecule). The modified antibiotics are affected in their binding to a target .The chemical strategies (e.g.: O-nucleotidylation) [24]. These covalent modification strategies all require a co-substrate for their activity (ATP, acetyl-CoA, NAD+, UDP-glucose, or glutathione) and consequently these processes are restricted to the cytoplasm.

Antibiotic inactivation by Redox Process: The oxidation or reduction of antibiotics has been infrequently exploited by pathogenic bacteria. However, there are a few of examples of this strategy. One is the oxidation of tetracycline antibiotics by the TetX enzyme. Streptomyces virginiae, producer of the type- A streptogramin antibiotic virginiamycin M1, protects itself from its own antibiotic by reducing a critical ketone group to an alcohol at position [25].

Target modification: Receptor modification occurs when the intracellular target or receptor of the antibiotic is altered by the bacteria, resulting in the lack of binding and consequently the lack of antibacterial effect. Modifications in the structural conformation of penicillin-binding proteins (PBPs) observed in certain types of penicillin resistance [26], ribosomal alterations that can render aminoglycosides, macrolides or tetracycline's inactive, and DNA-gyrase modifications resulting in resistance to fluoroquinolones [27].

Efflux pumps and outer membrane (OM) permeability: The efflux pumps are the membrane proteins that export the antibiotics out of the cell and keep its intracellular concentrations at low levels. Reduced outer membrane (OM) permeability results in reduced antibiotic uptake. The reduced uptake and active efflux induce low level resistance in many clinically important bacteria [28].

The role of efflux systems in resistance: The multidrug efflux systems are composed of three protein components, an energy-dependent pump located in the cytoplasmic membrane, an outer membrane porin and a linker protein which couples the two membrane components together. This tripartite arrangement forms an efficient extrusion system for toxic molecules present in the cytoplasm, the cytoplasmic membrane or the periplasm, i.e. the region between the outer and cytoplasmic membranes. Four different antibiotic efflux systems have been described in *P. aeruginosa*: mexAB-oprM, mexXY-oprM, mexCD-oprJ and mexEF-oprN10. All classes of antibiotics except the polymyxins are susceptible to extrusion by one or more of the efflux systems. MexAB-oprM is responsible for extrusion of β-lactams, quinolones and a range of disinfectants. MexXY-oprM extrudes aminoglycosides and mexEF-oprN extrudes carbapenems and quinolones. The genes for the systems are present in all strains but they are not expressed at high levels. However, increased expression can result from mutation in regulatory genes such as mexR, which controls expression of the mexAB-oprM genes [29, 30].

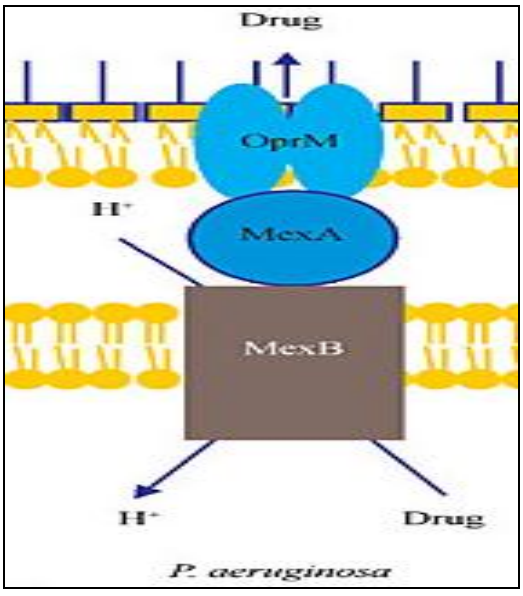

FIG. 2: EFFLUX SYSTEMS IN *P.AERUGINOSA*

Gram-negative bacteria possess an outer membrane consisting of an inner layer containing phospholipids and an outer layer containing the lipid A moiety of lipopolysaccharides (LPS). This composition of the outer membrane (OM) slows down drug penetration, and transport across the OM is achieved by porin proteins that form water-filled channels. Drug molecules can penetrate the OM employing one of the following modes: by diffusion through porins, by diffusion through the bilayer or by self-promoted uptake [31] e.g.: Pseudomonas aeruginosa is an opportunistic pathogen and displays high-level intrinsic and acquired multiple antimicrobial resistance. Mechanisms for such resistance are attributed by the between the broad-specific multidrug efflux pumps and the low outer membrane permeability [30]. The combination of restricted permeability of the outer membrane and the efficient removal of antibiotic molecules that do penetrate by the action of efflux pumps causes failure of antibiotics to accumulate within the organism.

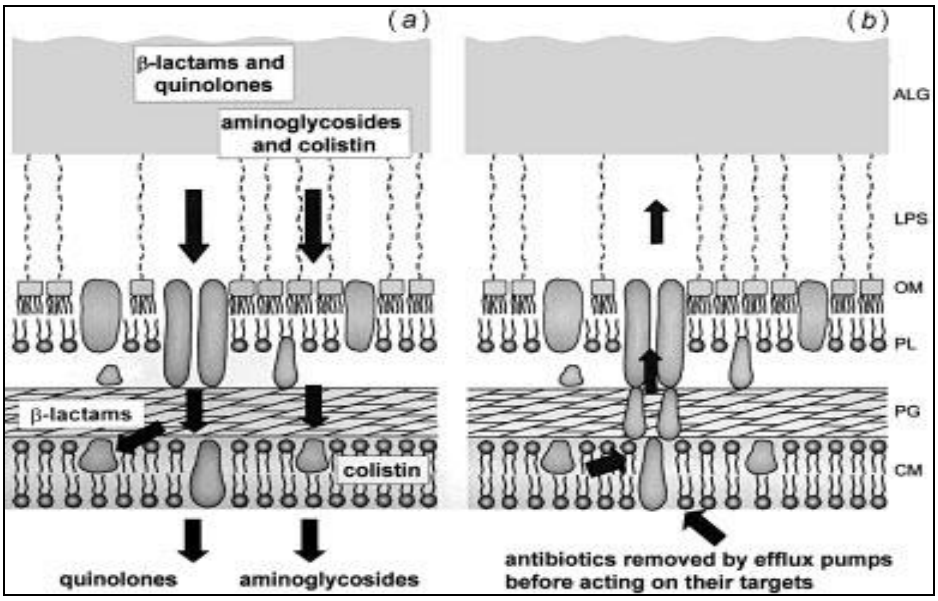

FIG. 3: SCHEMATIC REPRESENTATION OF THE ARRANGEMENT OF COMPONENTS IN THE CELL WALL OF *P. AERUGENOSA*

CM=cytoplasmic membrane; OM=outermembrane; PG=peptidoglycan; LPS=lipopolysaccharide; ALG=alginate. (a) The pathways for penetration of β-lactams and quinolones through porin channels in the outer membrane. Amino glycosides and colistin promote their own uptake by interacting with the LPS on the outer face of the OM. (b) how efflux systems reverse the diffusion of antibiotics across the OM. The efflux pumps comprise three components: an energy-dependent pump in the CM, a porin in the outer membrane and an adapter protein joining the two membrane components. Antibiotics which have entered the cell are collected from the cytoplasm, the cytoplasmic membrane or the periplasm and expelled from the cells through the porin of the known pumps of *P. aeruginosa*, the Mex AB-OprM efflux system plays the most important role in drug resistance and shows the broadest substrate ranges that include most conventional classes of antibiotics. An OM protein acts as barriers against the movement of antibiotics into the cytoplasm and channels for the removal of antibiotics out of cells.

Detection:

PCR using specific primers or DNA microarray: PCR using specific primers or DNA microarray was used for screening for resistance genes with around 300 nucleotide probes representing 7 classes of antibiotic resistance genes. The DNA based assay involves detection of specific conserved regions of the *mec*A, *bla*Z (methicillin and penicillin resistance), *aac* (6_) - *Ie-aph* (2_) (aminoglycoside resistance), *erm*A and *erm*C genes (MLS_B resistance), and the *msr*A gene (macrolide and streptogramin B resistance). The

microarray uses a variable sequence region of the 16S rRNA gene to broadly differentiate between Staphylococcus aureus and other coagulase-negative staphylococci (CoNS) [32].

New era of antimicrobial therapeutics: The new avenues of therapeutic treatment for prophylaxis. Here the most believable approaches are described:

- Bacterial interference - inoculate hosts with nonpathogenic bacteria.
- Bacteriophage therapy - Bacteriophages are viruses that infect bacteria and it take over the host's protein-making machinery [33].
- Bacterial vaccines - Development of bacterial vaccines has become an increasingly popular with the unraveling of complete genomic sequence and the understanding of virulence regulatory mechanisms [34].
- Cationic peptides - These diverse peptides are natural compounds that posses both hydrophobic and hydrophilic characteristics.
- Cationic peptides have several mechanisms of action, all of which involve interaction with the bacterial cell membrane leading to cell death [35].

New strategies to eliminate antibiotic resistance: There are three main parts to this strategy:

1. The emphasis on those drugs whose consumption has been shown to correlate strongly with resistance should be reduced during prescribing.
2. Development of new formulations of those drugs whose pharmacodynamics parameters are better suited to deal with highly resistant strains; and
3. Encouragement of the use of antibiotics with the maximal capability of bacterial eradication. We believe such a strategy would reduce the spread of resistance.

- *Staphylococcus aureus* infection is due to nasal carriage of this organism has eliminating by nasal mupirocin. Nasal mupirocin has might help in prevention and controlling outbreaks of methicillin-resistant S. aureus. Eradication of nasal S. aureus with mupirocin has been shown to be effective in preventing postoperative infections in patients undergoing cardiothoracic surgery and in preventing infections in patients undergoing hemodialysis [36].

- The antibacterial activity of the new penem antibiotic Men 10700 against a total of 740 gram-positive and gram-negative clinical isolates, in comparison to imipenem, meropenem, cefotaxime, ceftriaxone, ciprofloxacin and gentamicin. Men 10700 have shown a wide spectrum of antibacterial activity, with a potent activity both against gram-positive species and gram negative species [37].

- Multidrug resistance pumps (MDRs) protect microbial cells from both synthetic and natural antimicrobials. Amphipathic cations are preferred substrates of MDRs. Berberine alkaloids, which are cationic antimicrobials produced by a variety of plants, are readily extruded by MDRs. Several Berberis medicinal plants producing berberine were found also to synthesize an inhibitor of the NorA MDR pump of a human pathogen Staphylococcus aureus. The inhibitor was identified as 5′-methoxyhydnocarpin (5′-MHC), a minor component of chaulmoogra oil, a traditional therapy for leprosy. 5′-MHC is an amphipathic weak acid and is distinctly different from the cationic substrates of NorA. 5′-MHC had no antimicrobial activity alone but strongly potentiated the action of berberine and other NorA substrates against S. aureus. MDR-dependent efflux of ethidium bromide and

berberine from S. aureus cells was completely inhibited by 5′-MHC. The level of accumulation of berberine in the cells was increased strongly in the presence of 5′-MHC, indicating that this plant compound effectively disabled the bacterial resistance mechanism against the berberine antimicrobial [38].

- Some antibiotic resistance modifying compounds from plants seen in **table 1** [39].

TABLE 1:

COMPOUND	PLANT SOURCE	ANTIBIOTICS POTENTIATED	REFERENCE
Ferruginol, 5-Epipisiferol	*Chamaecyparis lawsoniana*	Oxacillin, Tetracycline, Norfloxacin, Tetracycline	Smith *et al*., (2007)
2, 6-dimethyl-4-phenylpyridine-3, 5-dicarboxylic acid diethyl ester	*Jatropha elliptica*	Ciprofloxacin, Norfloxacin, Pefloxacin, Acriflavine and Ethidium bromide	Marquez *et al*., (2005)
Carnosic acid carnosol	*Rosmarinus officinalis*	Erythromycin	Oluwatuyi *et al*., (2004
Ethyl gallate	*Caesalpinia spinosa*	β-lactams	Shibata *et al*., (2005)
Lycopus europaeus	Tetracycline and Erythromycin	Tetracycline and Erythromycin	Gibbons *et al*., (2003)
Epicatechin gallate, Epigallocatechin gallate	Camellia sinensis	Norfloxacin, Imipenem, Panipenem, β-lactams	Gibbons *et al*., (2004), Hu *et al*., (2002), Zhao *et al*., (2001

- Overcoming glycopeptides resistance will require innovative approaches such as;

  a. Generation of new antibiotics or otherwise to inhibit the action of resistance elements in various bacteria.

  b. Understanding of chemical complexity of the glycopeptides, so as to successfully exploit targets [40].

- Nucleoside analogs represents a promising alternative for combating pathogenic bacteria .The pyrimidine-based nucleoside analogs, like 3'- azido- 3'- deoxythymidine (AZT) and 2', 2'- difluoro- 2'deoxycytidine (gemcitabine), are specifically activated by the endogenous bacterial deoxy-ribonucleoside kinases, leading to cell death but in deoxy- ribonucleoside kinase-deficient Escherichia coli strains become highly susceptible to nucleoside analogs when they express recombinant kinases from Staphylococcus aureus or Streptococcus pyogenes.The recombinant S. aureus deoxyadenosine kinase efficiently phosphorylates the anticancer drug gemcitabine in vitro [41].

CONCLUSION: Antibiotic resistance is an important healthcare problem that can demonstrate marked variability locally, regionally, nationally and globally. Prudent use of antibacterial drugs-using the appropriate drug at the appropriate dosage and for the appropriate duration- is one important means of reducing the selective pressure that helps resistant organisms emerge. Restricted permeability and efflux are common components of the resistance phenotype for β-lactams, aminoglycosides and quinolones and are essentially fundamental properties of the organism. The strategies to overcome antibiotic resistance such as development of inhibitors of resistant enzyme as co-drugs improve the delivery or otherwise enhance the accessibility of antibiotics to their sites of action (liposomal preparation of hydrophobic antibiotic). All the alternative

strategies to overcome resistance require expanded knowledge of the molecular mechanisms of antibiotic resistance, their origins and evolution, and their distribution throughout bacterial populations and genomes.

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