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Mechanisms of Antibiotic and Antimicrobial Resistance: An Overview

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ABSTRACT

Antibiotics represent one of the most successful forms of therapy in medicine. But the efficiency of antibiotics is compromised by a growing number of antibiotic-resistant pathogens. Antibiotic resistance, which is implicated in elevated morbidity and mortality rates as well as in the increased treatment costs. According to the World Health Organization (WHO), thousands of deaths caused by Salmonella sp., Escherichia coli, Staphylococcus aureus or Mycobacteria tuberculosis are due to failure in therapy caused by resistance to the chemotherapeutic agents. There is no doubt that antimicrobial agents have saved the human race from a lot of suffering due to infectious disease burden. Without antimicrobial agents, millions of people would have succumbed to infectious diseases. Man has survived the accidental wrath of microorganisms using antimicrobial agents and other mechanisms that keep them at bay. Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis. Microbial resistance to antimicrobial agents was not a new phenomenon for it had been constantly used as competitive/survival mechanisms by microorganisms against others. These mechanisms have been well documented. Factors that have led to the continued occurrence of bacterial resistance to antimicrobial agents include: over prescription of antibiotics, use of under dose, prescribers' irrational attitudes, patients' demands, inappropriate advertisements and use of antibiotics in agriculture. Microbial resistance to antibiotics can thus be minimized through proper enlightenment, more rational antibiotic selection during treatment and proper legislation.

INTRODUCTION

Antimicrobial resistance (AMR) is the ability of a microbe to resist the effects of medication previously used to treat them. This broader term also covers antibiotic resistance, which applies to bacteria and antibiotics [1-6].

Antibiotic Resistance

Antibiotic resistance is the ability of a microorganism to withstand the effects of an antibiotic. Today, almost all important bacterial infection in the India and throughout the world are becoming resistant to antibiotics. The consequences of the emergence of antimicrobial resistant bacteria include an increase in morbidity and mortality. The use of less effective e.g., bacteriostatic rather than bactericidal drugs, may be necessary. Hospitalization may be prolonged or the patient may be isolated as a result of his particular infection. Finally, the drugs may be more expensive or more toxic. The rational use of antibiotics is the key to controlling the spread of resistance [7, 8, 9, 41-56]. There are many important pathogens that are resistant to multiple antibiotic classes [10]. Use of antimicrobial agents selects for bacterial variants within a population that are less susceptible, or resistant, to the antimicrobial agent used, leading to a situation where the resistant variant predominates under such selective pressure. Furthermore, selection of resistance to a single antimicrobial agent often results in bacterial variants that harbor transferable multidrug resistance determinants. These selective pressure phenomena are thought to occur in areas where antimicrobial agents are extensively used, such as in human clinical medicine, agriculture, and in natural soil and aquatic environments. Therefore, antimicrobial use fosters bacterial drug resistance and dissemination of drug resistance determinants within populations[11]. Antibiotic-resistant bacteria that are difficult or impossible to treat are becoming increasingly common and are causing a global health crisis [3]. Development of antibiotic resistance by a bacterial strain is most typically due to antibiotic misuse consequentially leading to an ineffective therapy, which, for example, in economically disadvantaged countries can also occur due to inadequate access to drugs .[2] New resistance mechanisms are constantly being described, and new genes and vectors of transmission are identified on a regular basis. Antibiotic resistance is encoded by several genes, many of which can transfer between bacteria [3]. The multidrug efflux systems contribute significantly to the increased resistance to multiple antibiotics in bacteria [1].

In general, the reasons for increasing resistance levels include the following:

- The population of organisms that spontaneously acquire resistance mechanisms as a result of selective pressure either from antibiotic use or otherwise, the rate of introduction from the community of those resistant organisms into health care settings, and the proportion that is spread from person to person.
- Increased use of invasive devices and catheters.
- Ineffective infection-control practices, transfer of colonized patients from hospital to hospital. (7)
- Suboptimal use of antimicrobials for prophylaxis and treatment of infection.
- Noncompliance with infection-control practices.
- Prolonged hospitalization.
- Increased number and duration of intensive care-unit stays.
- Multiple comorbidities in hospitalized patients.
- Grouping of colonized patients in long-term-care facilities.
- Antibiotic use in agriculture and household chores, and increasing national and international travel[7].

Mechanisms of Action of Antimicrobial Agents (12, 41-56)

Table No:1

I. Inhibition of Cell Wall Synthesis	➢ Peptidoglycan synthesis ➢ Block Cross-linking of sugar chains
2. Inhibition of Protein Synthesis	➢ Blocking Sites of the Ribosome ➢ Preventing Initiation ➢ Preventing Peptidyl Transfer ➢ Premature Termination ➢ Preventing Ribosomal Translocation
3. Inhibition of Metabolic Enzymes	➢ Folic acid metabolism Mycolic acid synthesis
4. Inactivation of membranes	➢ Destruction of the cell electrochemical gradient ➢ Damaging cytoplasmic membranes
5. Inhibition of *mRNA polymerase*	-
6. Inhibition of DNA replication	➢ Binding *DNA gyrase* causing supercoiling of the DNA

1. PROBLEM OF RESISTANCE [13]

The development of resistance is a normal evolutionary process for microorganisms, but it is accelerated by the selective pressure exerted by widespread use of antibacterial drugs. Resistant strains are able to propagate and spread where there is non-compliance with infection prevention and control measures. [14].

- ✓ Resistance varies with setting, e.g. hospital vs. community
- ✓ Infectious resistance - infection may be in abscess or at intracellular location
- ✓ Resistance varies with geographical location
- ✓ Multiple antibiotic resistance - *S. Aureus*, *S. Pneumoniae*, Tuberculosis

Regulation of Resistance Genes

In the absence of erythromycin, stem-loop structure forms in the mRNA, which buries the Ribosome Binding Site (RBS) and the start codon. Thus, in the absence of the antibiotics, the drug resistance gene is not expressed. However, low concentrations of erythromycin cause the RBS and start codon to be exposed, causing a translation of the drug resistance gene, erm, resulting in the expression the gene [15].

2. Biology of antibiotic resistance [10,1,11,13,15,36-40,41-56]

Table No:2

❖ **Biochemical aspects**	❖ **Genetic aspects**
I. Enzymatic inactivation of the drug results from the metabolic degradation of the drug into a form that is rendered ineffective in inhibiting bacterial growth	I. Mutations - spontaneous mutations ✓ Frequency of spontaneous mutations is 10^{7} -10^{9}. Low frequency is unrelated to presence of antibiotic.
II. Antibiotic inactivation – 1.hydrolysis – 2.group transfer -3. redox process (e.g. Bacterial resistance to aminoglycosides can be due to plasmid-encoded aminoglycoside-modifying enzymes. Similarly, β-lactamase production is the most common mechanism of resistance to penicillin's and other β-lactam drugs	✓ Not a major reason for massive sudden emergence of drug resistance ✓ Mutations rarely lead to complete resistance I. Hypermutators - adaptive mutagenesis I. Horizontal gene transfer - plasmids -

III. Target modification - peptidoglycan structure alteration - protein structure interference - DNA synthesis interference. IV. Alteration of the drug target results in the inability of the drug to bind to its biological target, thus rendering the drug unable to kill the bacteria. e.g. aminoglycosides. Bacteria can also transport antimicrobial drugs out of the cell through efflux pumps. Resistance to numerous drugs, including fluoroquinolones, macrolides, tetracyclines and beta-lactam antibiotics, is mediated by this mechanism. V. OM permeability changes. Active efflux of drugs from bacteria results in the intracellular dilution of drugs, making the extruded drugs unavailable for their inhibitory action VI. Drug permeability reduction mechanisms prevent cellular entry of drug into the inside of the bacterial cell; VII. Target bypass VIII. Ribosome protection IX. Biofilm formation[16,31]	(conjugative) transposons - integrons

Table NO:3 Examples : SPECIFIC MECHANISMS OF RESISTANCE[13,17,15,29,32-40]

A. Inactivation of drug by enzymes - usually plasmid-mediated 1. Penicillins & Cephalosporins - b-lactamases (penicillinases) 2. Aminoglycosides - phosphorylation, adenylation, or acetylation 3. Chloramphenicol – acetylation 4. Macrolides - erythromycin esterase
B. Alteration of membrane permeability 1. Common mechanism in Gm – bacteria. Change in porins or transport proteins. 2. Examples - tetracyclines, b-lactams, aminoglycosides, quinolones
C. Efflux pumps – Active transport pump to remove antimicrobial agent [25]

1. Active transport or efflux pumps tetracyclines, quinolones, and macrolides out of bacteria
D. Alteration of intracellular target site 1. Macrolides - methylation of 23S ribosomal RNA, blocking erythromycin binding 2. Aminoglycosides - altered protein in 30S ribosome
E. Alteration of intracellular target enzyme 1. Trimethoprim - production of dihydrofolate reductase with high Km 2. b-lactams - alteration in penicillin binding proteins with less affinity or decreased production of PBPs. 3. Rifampin - altered DNA-dependent RNA polymerase 4. Quinolones - modified DNA gyrase and topoisomerase IV
F. Overproduction of target enzyme 1. Sulfonamides - increased levels of *Dihydropteroate synthetase* 2. Trimethoprim - increased levels of DHFR
G. Auxotrophs that bypass blocked step 1. Sulfonamides – Resistant auxotrophs can utilize exogenous folic acid. 2. Trimethoprim - loss of *Thymidylate synthetase*. Resistant bacteria take up thymidine and produce thymidylate via salvage pathways.

Quinolone resistance is affected by mutations in topoisomerase IV (*DNA gyrase*), which reduces the binding affinity of the drug to its target. Resistance in GNB has also been ascribed to resistance genes target. Resistance in GNB has also been ascribed to resistance genes (from plasmids) which produce proteins that bind to the topoisomerase and so "protect it" from the action of the drug.

Aminoglycoside resistance can result from modifications of the structure of the bacterial ribosome.

Penicillins: Methicillin resistance, in MRSA, results from the production of an additional transpeptidase(PBP2' or PBP2a) which is not susceptible to additional *transpeptidase* (PBP2, or PBP2a) which is not susceptible to inhibition by penicillins.

Sulfonamide antibacterials are rendered ineffective in many cases as the bacteria have evolved an alternative route to folic acid which does not need the dihydropteroate

Chloramphenicol resistance: is most often due to enzymic acetylation of the drug by chloramphenicol *acetyltransferase* (CAT).

According to WHO report 2013, bacteria of international concern are-

Table No:4 (Adapted from WHO report 2013 and modified):

Bacteria	**Types of resistant to:**
1. *Escherichia coli*	third-generation cephalosporins, including resistance, conferred by extended-spectrum beta-lactamases (ESBLs), and to fluoroquinolones
2. *Klebsiella pneumonia:*	carbapenems
3. *Staphylococcus aureus:*	beta-lactam antibacterial drugs (methicillin, methicillin-resistant S. aureus [MRSA]);
4. *Streptococcus pneumonia:*	resistance or non-susceptibility to penicillin (or both);
5. Non-typhoidal Salmonella (NTS):	to fluoroquinolones
6. Shigella species:	to fluoroquinolones
7. *Neisseria gonorrhoeae*:	decreased susceptibility to third-generation cephalosporins. In gonococci, chromosomally mediated resistance is generally slow to emerge and disseminate[18].
8. enterococci (VRE)	Vancomycin resistant

According to WHO report, the resistance for the most of antimicrobial drugs are developed for the diseases like:[5-57]

Malaria (The estimated annual cost of containment operations in areas of artemisinin resistance is US$ 10 – 20 per person at risk), HIV, Candidiasis, Influenza, Tuberculosis.

RESISTANCE TO ANTIFUNGALS:

Antifungal drug resistance to candidiasis contributes to a burden for patients and the health-care system. Resistance to fluconazole, a common antifungal drug, varies widely by country and species. Resistance to the newest class of antifungal agents, the echinocandins, is emerging in some countries [14]. Resistance of the yeast *Candida albicans* to fluconazole has manifested itself in a wide variety of ways: Point mutations in the ERG11 gene (encoding *lanosterol 14α-demethylase*) Reduced drug affinity for the target enzyme Over-expression of

enzyme, as revealed in increased ergosterol synthesis, Alterations in other enzymes of the ergosterol biosynthetic pathway Production of various sterols supporting growth; Cross-resistance to other azoles and amphotericin B (AmB) ,Overexpression of CDR and MDR genes encoding efflux pumps Reduced drug accumulation in the cell[19].

RESISTANCE TO ANTI-INFLUENZA:

Widespread resistance to adamantanes currently circulating A (H1N1) and A (H3N2) viruses have left neuraminidase inhibitors as the primary antiviral agents recommended for influenza prevention and treatment, but resistance to these drugs is a growing concern.

RESISTANCE TO ANTI-HIV:

HIV drug resistance causes ART failure. Therefore, minimizing the emergence of HIV drug resistance and its transmission is critical to ensure the continued effectiveness of ART, in view of the need for lifelong treatment, the limited treatment options available, and the fact that second-line and salvage treatment regimens are considerably more expensive, less patient-friendly and have more side-effects than WHO-recommended first-line regimens. With the expanded availability and use of ART, resistance to ARV drugs is slowly increasing.

RESISTANCE TO ANTI-TB:

There were an estimated 450 000 new MDR-TB cases in 2012, about half of which were in India, China and the Russian Federation. Extensively drug-resistant TB (XDR-TB) has been reported by 92 countries. The average proportion of MDR-TB cases which have XDR-TB is estimated to be 9.6% .[14].

TYPES OF RESISTANCE [32-40]:

1. Natural and Acquired Resistance

Intrinsic resistance – i.e. inherent or natural resistance (e.g. Chlamydia do not have peptidoglycan and so are not susceptible to β-lactams[19]. Antibiotic resistance can be divided into natural resistance and acquired resistance. Acquired resistance refers to bacteria that are usually sensitive to antibiotics, but are liable to develop resistance. Acquired resistance is often caused by mutations in chromosomal genes, or by the acquisition of mobile genetic elements, such as plasmids or transposons, which carry the antibiotic resistance genes.

Natural resistance means that the bacteria are 'intrinsically' resistant. [15] Mechanisms of acquired resistance are: naturally acquired, serial passage, direct plating. [20,25-27]

2. Genetic and Phenotypic Resistance

Genetic resistance is due to chromosomal mutations or acquisition of antibiotic resistance genes on plasmids or transposons. Phenotypic resistance is due to changes in the bacterial physiological state, such as the stationary phase, antibiotic persisters, and the dormant state [15].

FACTORS THAT PROMOTE MICROBIAL RESISTANCE TO ANTIBIOTICS:

Patients' demand, Prescribers, Drug advertisement, Dispensing prescribers, Use of antibiotics in agriculture[4]. Socioeconomic consideration in developing countries like, 1. Unskilled health practitioners, 2. Misuse of antibiotics, 3.Poor-quality drugs, 4.Antibiotics widely available through non-professional channels, 5. Poor surveillance and compliance, 6. Poverty and poor hygiene[21].The agricultural industry has also had an impact on the development of antibiotic resistant bacteria. Animals that are raised for human consumption are treated with antibiotics such as fluoroquinolones and tetracyclines[28].

Methods for the dissemination of antimicrobial resistance genes

1) **Clonal spread of a resistant strain**: Under the selective pressure of antibiotics a strain carrying antimicrobial resistance genes may be preferentially selected and transferred within a population. 2) **Plasmid transfer**: Plasmids carrying one or multiple antibiotic resistance genes can be transferred among different bacterial strains or species by conjugation or transduction.

3) **Free DNA**: Naturally transformable species such as the pneumococcus can acquire native DNA from the environment. Recombination events can then integrate this genetic material into the chromosome. This is believed to be the means by which pneumococci acquired penicillin resistance.

4) **Bacteriophage**: Transduction may be a means of transfer of both antimicrobial resistance genes as well as genes conferring virulence.[22].

Strategies to prevent AMR in healthcare settings [15, 23, 8]:

1) Assure and ensure adequate serum drug concentrations

2) Maintain heterogeneity of antimicrobial agents

3) Combination therapy

4) Narrow spectrum agents should be used whenever possible.

5) Limiting the Spread of Drug Resistant Bacteria

6) Development of New Antibiotics

7) Phage Therapy

8) Mobilization of Host Defence Mechanisms

9) The Use of Normal Bacterial Flora

10) Clinicians should be familiar with local antibiotic sensitivity profiles and should comply with the local antibiotic guidelines. A hospital antibiotic policy should be formulated based on local antimicrobial resistance data. Prescribers should be educated about the use of antibiotics, when not to use them and also the infection control strategies.

11) In the area of microbiologist: increase understanding of microbial physiology, ecology, genetics and resistance mechanisms; augment existing research infrastructure to support a critical mass of resistance mechanisms.[21]

12) Use of vaccines, proper duration of usage.

Some actions physicians and consumers can take to limit resistance -Table No:5 [24,2]

Physicians:	Consumers:
❖ Wash hands thoroughly between patient visits. ❖ Do not accede to patients' demand for unneeded antibiotics. ❖ When possible, prescribe antibiotics that target only narrow range of bacteria. ❖ Isolate hospital patients with multidrug-resistant infections. ❖ Familiarize yourself with local data antibiotic resistance.	❖ Do not demand antibiotics. ❖ Use soap and other products with antibacterial chemicals only when protecting a sick person whose defenses are weakened. ❖ Complete the whole course of antibiotic. ❖ Eliminate the use of antibiotics in animal feed.[22]

❖ Early treatment should be based on antibiotics that are most likely to succeed in a particular case and should be modified as necessary when the results of sensitivity tests are available.[4] ❖ Antibiotics must be prescribed according to the Antimicrobial Policy and detailed Antimicrobial Prescribing Guidelines. ❖ Where there is more than one case on a ward, the prescriber should consider avoiding cephalosporin use altogether in other patients on the ward. ❖ In an outbreak situation, the Infection Control Doctor (ICD), a Consultant Medical Microbiologist and the Antimicrobial Pharmacist will suggest interim alternative antibiotic prescribing guidelines on a ward/unit.[6]	

CONCLUSION

By referencing sources mentioned below, we come to across the facts that we totally depend on antibiotics for the treatment of infectious diseases, and they shouldn't be considered as mere commodities. Prudent use of antimicrobial agents is highly recommended for clinicians, veterinarians, ranchers, and farmers. Appropriate sanitation and hand-washing practices are extremely helpful for reducing the conditions that foster transfer of bacterial resistance determinants within populations, especially in the clinical settings. There is no perfect antibiotic, and once the most appropriate use of any new compound is identified, it is essential that prescription of the antibiotic be restricted to those users. Antibiotic resistance is growing global concern. Given the increasing knowledge of environmental reservoirs of resistance, it should now possible to have early warning of potential resistance mechanisms to new or old antibiotics and thus prepare for problems in the clinic. Finally, antibiotic stewardship must be implemented to reduce unnecessary antibiotic consumption in medicine and agriculture [41-57].

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