Antibiotic Resistance: An Update

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Abstract

Antibiotic resistance has emerged as one of the major threats to the community, especially in developing countries. In the past few decades, there have been several reports suggestive of irrational and inappropriate use of antibiotics in the healthcare sector. As a result of which, there was a considerable increase in development of resistance among the infective microbes. The spread of resistance among the microbes was mainly through food, animal products, ecology and most importantly, through the healthcare professionals who deal with the antibiotics. Microorganisms have adapted several defense mechanisms in the recent past to combat antibiotics for their survival and existence, some of which have been highlighted in this review. Several strategies and policies have been formed by regulatory authorities all over the world to overcome antibiotic resistance. In India, in the year 2011, "National policy for containment of antimicrobial resistance" was implemented by the Ministry of Health & Family Welfare to establish a standard antibiotic policy. One of the recent strategies is the development of Antibiotic Stewardship program (ASP) which has gained its prospects by significantly reducing the development of resistance in many centers after its implementation. In India, ICMR is actively playing its role in the implementation of ASPs in many tertiary care centers across the country. Despite all these measures antibiotic resistance continues to be the major hindering factor in the delivery of standard healthcare to the community. This review will discuss the various mechanisms of resistance adopted by microbes, the rapid development of resistance to newer drugs, measures to overcome it and various health policies to tackle the antibiotic resistance. A team of experts, including Clinical Pharmacologists, Clinical Microbiologists and Infectious disease Physicians with the collective support of Government and Pharmaceutical industries through their constant efforts may greatly help in preventing the antimicrobial resistance in the future. However, there should be stringent regulations to prevent the inadvertent use of antibiotics for saving the community from the further emergence of resistance to antibiotics.

Keywords: Antibiotics, Resistance, types of antibiotics resistance, update

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Introduction

Infectious disease is one of the leading causes of morbidity and mortality all over the world. The discovery of 'Penicillin' was the most important milestone of the antibiotic era. Later there were several developments in the medical arena, which led to the rapid decrease in the morbidity and mortality in the community. Antibiotic resistance has emerged as one of the major threats to the medical fraternity, especially in developing countries. In the past few decades, there have been several reports suggestive of irrational and inappropriate use of antibiotics in the health care sector. (1)

The burden of antibiotic resistance according to a recent report by the U.S. Center for Disease Control and Prevention estimated "at least 2million people acquired serious infections from resistant pathogens and annual deaths count to approximately 23,000 in USA". (2) It is also known that the burden of infectious diseases is relatively high in India when compared to the rest of the world. In India, "around 5% of GDP is spent on health and a major chunk of public expenditure was mostly for medicines". The growth of resistant organisms may lead to the longer periods of stay in hospitals, which may cause a profound impact on productivity as well as the economy of the country. Antibiotic resistance caused by the health care workers may grossly be due to overt misuse or inappropriate use of antibiotics while providing health care to the community. (3)

Acquisition and Transfer of resistance

Antibiotic resistance can occur because of the two main factors: one which is dependent on humans and the other factor which is closely related to the use of antibiotic itself. Human dependent factors can be a poor socioeconomic status, migration of people in different areas all over the world, lack of knowledge of the health care professionals, use of antibiotics in agricultural practices and poultry. Recent studies estimate that "global antimicrobial consumption will increase by 67% from an estimated 63,151 tons in 2010 to 105,596 tons in 2030". Much of this rise in consumption is expected to come from "China, India, Russia, South Africa, and Brazil"; countries where meat consumption is growing due to rising incomes. "11–14 million kilograms of antibiotics" are used annually in the production of poultry animals. Almost half of these are used for non-therapeutic purposes, which is significantly more than the estimated 1.4 million kilograms of antibiotics used in human medicine. Many classes of antibiotics are used in animal production, including "β-lactams, sulfonamides, tetracyclines, streptogramins,macrolides, lincosamides, polyethers, quin oxalines, elfamycins, glycolipids, arsenics, and polypeptides". Antibiotic dependent factors are inadequate dose/duration of treatment, improper antibiotic selection, acquisition of resistance by a genetic mutation and transfer of resistance between the microbes. (1,4)

Mechanisms of anti-microbial resistance

The microbes which are constantly being exposed to pressure, chemical agents (such as antibiotics), stress, etc., tend to adopt different ways to overcome such situations by evolving their own mechanisms to combat such stressful environment. The transfer of antibiotic resistance can be through three ways, i.e. from one person to another person by bacteria; between the 2 bacteria via plasmids and within the bacterium through transposons. The transfer of resistance between the bacteria is termed as horizontal transfer and it involves three different methods. (i) Conjugation is a method of gene transfer by a direct

cell to cell contact between the bacteria by forming a sex pilus or bridge. In this conjugation process, even multiple resistance genes can be transferred in a single process. This type of resistance phenomenon is observed in some Enterococcus species and gram-negative bacilli. (ii) Transduction is the acquisition of the genetic material (DNA) from a bacteriophage which is observed in the resistance mechanisms adopted by the strains of staphylococci and streptococci. (iii) "Transformation is the uptake and incorporation into the host genome by homologous recombination of free DNA released into the environment by other bacterial cells". This forms the molecular basis of penicillin resistance in Pneumococci and Neisseria sp. (5) The transfer of resistance within the bacterium is termed as vertical transfer and drug resistance may be acquired by the passage of resistant trait vertically to daughter cells. This function is carried out by transposons, gene cassettes, and integrons. "Transposons are stretches of DNA that can be transposed from a plasmid to a chromosome or vice versa". For instance, resistant gene (r gene) transposon contained within the plasmids may code for the enzyme responsible for the integration of the r-gene into another plasmid which gets separated after this process. Thus, during the replication of the plasmids, r-gene is vertically transferred to both the plasmids. Gene cassettes and integrons are also the mobile genetic elements responsible for the transfer of multidrug resistance genes through vertical transfer.(6)

Fig 1: **biochemical mechanisms involved in antibiotic resistance**
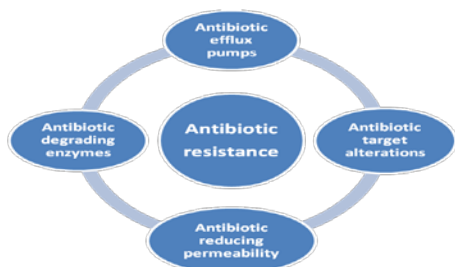
Antibiotic degrading enzymes

Beta-lactamase is one of the important enzymes responsible for the development of resistance to the beta-lactam antibiotics including cephalosporins. Staphylococci mostly produce this enzyme and such resistance is transferred by the process of conjugation. Semisynthetic penicillins suchas methicillin and betalactamase inhibitorslike monobactam and sulbact am were used to treat such resistant strains. But the problem of methicillin resistance is troublesome because beta-lactamases produced by the resistant strains are not inducible but constitutive in nature. This will prevent the entry of the drug into the membrane-associated target sites as the enzyme does not inactivate the drug in the surrounding medium rather it will remain attached to the cell wall. Chloramphenicol acetyltransferase, an enzyme which is produced by both Gram-positive and Gram-negative bacteria are responsible for the inactivation of chloramphenicol. Some alterations in the enzyme pathways are dihydrofolate reductase and dihydropteroate synthetase in trimethoprim and sulfon amide resistances respectively. (5,6)

Antibiotic reducing permeability

In gram-negative bacteria, the outer membrane is impermeable to larger molecules and permeable to smaller molecules through the porin channels. Thus, if there is any mutation or absence of porin channel, it will lead to reduced entry of the drug into the bacteria conferring resistance. One such example is Trypanosoma brucei in which melarsoprol is actively taken up by "trypanosome P2 protein transporter". If P2 transporter is absent or mutant, it will cause decreased intake of drug to the pathogen and the development of resistance. (6)

Antibiotic target alterations

The protein target of an antibiotic may undergo either single point or multiple point mutations which result in a conformational change in the site where an antibody would come and bind. As a result of which there may be reduced affinity to the particular antibiotic. Some examples are as follows: (i) fluoroquinolones- mutations in QRDR gene that codes for DNA gyrase A and DNA topoisomerase IV (primarily parC subunits); (ii) aminoglycosides- mutation in 30s binding site; (iii) erythromycin- mutation in 50s binding site; (iv) rifampicin - chromosomal mutation in the gene encoding DNA-dependent RNA polymerase.(2,6)

Antibiotic efflux pumps

This is the mechanism adopted by microbes to efflux the drugs entering the microbial cells out of it which also includes the susceptible drugs. "Five major systems of efflux pumps are as follows:

a. The multidrug and toxic compound extruder (MATE)

b. The major facilitator superfamily (MFS) transporters

c. The small multidrug resistance (SMR) system

d. The resistance nodulation division (RND) exporters

e. ATP binding cassette transporters (ABC) transporters" (6)

Pfmdr 1 gene: A point mutation in this particular gene has led to the development of Plasmodium falciparum resistance to antimalarial drugs.

Tetracyclines and macrolides efficacy were also affected by these efflux pump mechanisms.

Emerging drug resistance

Meta-analysis of the drug susceptibility results of various laboratories in India reveals an increasing trend of development of resistance to commonly used antibiotics in microbial pathogens like "Salmonella, Shigella, Vibrio cholera, Staphylococcus aureus, N. meningitides, Klebsiella, Mycobacterium tuberculosis, HIV, Plasmodium, and others". Some of the newer strains Klebsiella sp. have been classified under Ambler into 4 categories viz. "Class A- Klebsiella pneumonia carbapenemase (KPC) {20 variants have been reported, of which KPC-2 & KPC-3 remain the most commonly identified variants}; Class B- New Delhi Metallo-beta-lactamases (NDM-1), Verona integrin-encoded metallobetalactamases (VIM), Imipenemase Met allo-proteinase (IMP) {NDM-1 is the common variant reported world-wide}; Class C- KPC-1(uncommon); Class D- Carbapenem-hydrolyzing oxacillinase-48 (OXA-48)".(7,8) In this NDM-1 was the descendant from India and it was reported in the year 2008 which later had its spread across the Indian sub-continent. In India, Ciprofloxacin-resistant Salmonella enteric serovar typhi, Pseudomonas aeruginosa and Acinet obacter baumannii resistant to ceftazidime, cefepime and ciprofloxacin have been reported. "A recent study in Sikkim, India found out that MRSA isolates were around 31.21%" from the collected samples. "Community acquired-MRSA (CA-MRSA) has an incidence of around 10%"and the beneficial part is that these strains are not multi-drug resistant and are treatable. Although Vancomycin Resistant Enterococcus (VRE) is being on a rise in the western countries, there are no much data on high incidence of VRE in India. Recently triclosan-resistant Staphylococcus strains reported, in which the mechanism postulated for triclosan resistance is by the acquisition of an additional fabl allele derived from Staphylococcus hemolyticus (sh-fabl).(3,8,9)

Adaptive resistance (ADR)

Adaptive resistance occurs as a result of concentration gradients, as well as exposure of a microbe to the sub-

inhibitory concentration of antibiotics which are being used both in humans and animal husbandries. In a broad term there are two forms of AdR: 1. Fast & Transient Mechanisms (FTM); 2. Slow & Stable Mechanisms (SSM); In this FTM occurs at shorter time scales in which the low concentrations of susceptible antibiotic is given to the organism in culture and the cells in the culture medium will mount a fast, transient and unspecific response to cope with such lower dosages. While in case of SSM due to exposure of higher concentration of antibiotics over a longer period of time may lead to the development of more efficient and permanent resistance mechanisms to the antibiotics. The factors postulated for the FTM are Noise, DNA methylation, and elevated rates of point mutation whereas the factors responsible for SSM are Genetic duplication, gene amplification, sequential & cumulative gene mutations. Genetic capacitance was also suggested as one of the factor contributing to AdR. The clinical implication of AdR is to give an antibiotic at a higher and faster rate as possible, so that it will entirely kill the whole microbial population thus preventing the development of resistance among the microbes.(10)

Combined antibiotic tolerance

Combined antibiotic tolerance emerges when microbial population at a sufficiently high density can survive an antibiotic dose that would be a lethal dose to a low density population. Quorum sensing is one of the important mechanisms involved in the development of combined antibiotic tolerance. Quorum sensing allows the bacteria to sense its critical concentration on exposure to an antibiotic and in response it will activate certain genes which are responsible for the production of the protective enzymes. Rhamnolipid is one such protective enzyme produced by pseudomonas aeruginosa by quorum sensing signal. However, this quorum sensing is inhibited by

azithromycin, ceftazidime and ciprofloxacin in Pseudomonas aeruginosa strains.(11)

Challenges faced

Contamination of the hospital waste effluents and the antimicrobial residues from the meat production factories may exert selection pressures in microbes to develop not only resistance but also transmission of resistant genes to other microbes. Large scale animal production facilities generate huge volumes of animal waste that is contaminated with veterinary antibiotics. Many classes of antibiotics are used in animal production including β-lactams, sulfonamides, tetracyclines, streptogramins, macrolides, lincosamides, polyethers, quinoxalines, elfamycins, glycolipids, arsenicals, and polypeptides.

In India, in public health sector there is inadequate supply of the required antibiotics and also the quality of drugs pose a great challenge in combating the infectious diseases. In a private sector there are inadequately trained physicians which results in over use and misuse of antibiotics leading to rapid emergence of multi drug resistant pathogens. Self-medication and poor compliance are also the hindering factor in delivery of proper healthcare to the community. Moreover there is no national database for the surveillance of antibiotic usage and the development of resistance in the community.(3,4)

Table 1 Drugs and mechanism of resistance (5,6)

S.no	Drugs	Mechanism of resistance
1.	Beta lactams	Beta lactamase (inactivating enzyme)
2.	Tetracyclines	Efflux pump mechanisms (decreased concentration in the cell
3.	Chloramphenicol	Chloramphenicol acetyl transferase
4.	Fluoroquinolones	Altered DNA gyrase

		enzyme
5.	Sulfonamides	Reduced activity of folate synthetase
6.	Aminoglycosides	Inactivating enzyme and altered binding site (30s)
7.	Macrolides	Decreased permeability through efflux pumps and altered binding site (50s)
8.	Rifampicin	Chromosomal mutation in the gene encoding DNA-dependent RNA polymerase
9.	Isoniazid	Deletion mutation in KAT-G that codes for catalase peroxidase and mutation in INH-A
10.	Fusidic acid	Chromosomally determined decreased affinity of the target site or a plasmid-encoded decreased permeability to the drug

Strategies to overcome resistance

Recently phytochemicals have attracted significant attention in the therapy of infectious diseases largely by two ways that they can be used as one of the scaffolds in the development of new drugs and also their ability to influence biofilm without exerting selection pressure on microbes. The molecular modeling of Antimicrobial peptides (AMPs) from transcriptome is one of the newer prospects in therapy.

Six distinctive newer classes of AMPs have been reported and these AMPs may play a potential role in various fields such as medicine, agricultural and food industries to combat antimicrobial resistance. Several newer strategies have been adopted to tackle the carbapenem resistance.

"Ceftazidime-avibactam combination, fosfomycin, tigecycline, and minocycline" are the few combinations recently used to treat Carbapenem Resistance Enterobacteriaceae (CRE). Other drugs which are in "pipeline are meropenem-vaborbactam; imipenem-relebactam; plazomycin and eravacyclesine".(11,12)

In the year 2003, WHO started Antibiotic Resistance Surveillance and Control in the Mediterranean Region project (ARMed project), The Global Antimicrobial Resistance Surveillance System was launched by WHO in 2015 to support a standardized approach to combine the clinical, laboratory and epidemiological data on resistant microbial pathogens which pose threats to the health globally (2). In India, a large number of new initiatives have been launched to combat the problem of antibiotic resistance. These include " India Clen (Indian Clinical Epidemiology network) which has generated some quality data on antibiotic resistance in pneumococcus, H.influenza across the country; IIMAR (Indian Initiative for Management of Antibiotic Resistance) launched in March 2008, with WHO support, by a consortium of NGOs to promote prudent use of antimicrobials, INSAR (Indian Network for Surveillance of Antimicrobial Resistance) a network of 20 laboratories in the private as well as public sector across the country to generate quality data on AMR, organization by the ICMR of an expert group meeting in December 2009 and an Indo-Swedish workshop held at New Delhi on 2 February 2010 to discuss a joint strategy for containment of AMR.(13). "National policy for containment of antimicrobial resistance 2011" by Ministry of Health and Family Welfare, Government of India address the intervention strategies required and the steps for formulation and implementation of a standard antibiotic policy".(14)

One of the recent developmental strategies to combat antimicrobial resistance is the initiative of Antibiotic

Stewardship Program (ASP) globally. It is recommended that "core team should include a clinical pharmacologist, infectious disease physician, clinical microbiologist, an informatics specialist, a hospital epidemiologist and an infection control specialist". Antibiotic Stewardship program ensures to stress upon advocacy of the safe use of antimicrobials, which shall be strengthened, with periodic review of antimicrobial guidelines and implementation locally in each of the health care setups.(15,16) In India, in the year 2013 ICMR setup a steering committee for guiding Antibiotic Stewardship Program across the country. Two core strategies of ASP are front-end strategy and back-end strategy. In front end strategy antibiotics are made available only after the approval process like formulary restrictions and pre-authorization by the ASP committee whereas in back-end strategy antibiotics are reviewed after its administration which is a form of prospective audit and feedback.(17,18)

Strategies to improve antibiotic implementation policies

Viral syndromes should never be treated as acute bronchitis even on patient demand; antibiotic mono therapy is preferred and restriction of the use of broad spectrum antibiotics deemed to have a greater role in the reduction of antibiotic resistance in the community. "An effective Infection Prevention and Control Program would have the following components: 1. Infection Control Committee with its defined role and constituents; 2. Infection Control Core Team for day to day working with defined roles; 3. Infection Control Manual with policies, guidelines, recommendations and working protocols including activities and practices under the program with Hand hygiene and Standard Precautions being the mainstay; 4. Should incorporate Antimicrobial Stewardship programs" (17,18)

Summary

Infectious diseases are the most important causes of the morbidity and mortality to the community. In the antibiotic era starting from the discovery of penicillins which was then followed by the discovery of numerous antibiotics, microbial pathogens adopted various trends to combat such antibiotic resistance. Several ways of transfer of these resistant strains both through horizontal transfer by means of Conjugation, Transduction and Transformation and by vertical transfer by means of transposons, gene cassettes have made infective microbes stay on the battlefield for a longer time. Antimicrobial resistance is also acquired by: alteration of the binding site of antibiotics, enzyme degradation & alternate enzyme pathways, development of efflux pumps, reduction of membrane permeability. In recent decades serious threat is towards the development of the MRSA, KPC, VRE strains and also the development of newer strains like NDM-1significantly hamper the health prospects of the community. Moreover, the knowledge of adaptive resistance and combined antibiotic tolerance help us in handling the antibiotics in a judicious manner. In addition to this improper usage of antibiotics in food and animal husbandries led to the emergence of resistance. The role of phytochemicals, antimicrobial peptides may be of greater use in reducing the emergence of resistance in future. Several policies by WHO on antibiotic resistance notably Antibiotic Stewardship Program and the necessary national policies by the ICMR and the development of antibiotic guidelines are the major steps in preventing antimicrobial resistance to the community.(11,13,15,16)

Conclusion

Antimicrobial resistance being one of the important threats to the community, stringent regulations in the use of antibiotics is definitely needed in this situation.

However, several strategies to overcome resistance have been implemented but it is the role of healthcare professionals, government, public and private health sectors to deliver standard care to the community. The knowledge of antibiotic resistance need to be highlighted and development of resistance among infective microbes has to be prevented.

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