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REVIEW ARTICLE

Antimicrobial Resistance: Then and Now

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ABSTRACT

Antibiotics have been considered as one of the significant discoveries of last century. But the problem that came attached with is the accelerating increase in antibiotic resistance in both hospitals and communities. The genetic makeup of microbes has profited from the overuse of antibiotic to utilize all the sources of resistant genes through horizontal gene transmission producing various mechanisms of resistance. Alexander Fleming upon assenting the 1945 Nobel Prize stated that "It is not difficult to make microbes resistant to penicillin in the laboratory by exposing them to concentrations not sufficient to kill them. There is a danger that an ignorant man may easily under dose himself and by exposing his microbes to non-lethal quantities of the drug and make them resistant". This review discusses the multifaceted views of development of antibiotic resistance, history, superbug and superresistance and resistance data observed in the last few years with an overt conclusion showing undeniable methods to overcome the discussed problem, glaringly striking that it is time to act.

Keywords: Antibiotics, Antibiotic Resistance, History, Mechanism of Resistance, Status in India, Superbug, Superresistance.

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INTRODUCTION

History of antibiotics

Antimicrobials are one of the most successful forms of chemotherapy in the history of medicine. Various molds, plant materials and extracts were used in treating infections by some of the earliest civilizations. Nevertheless, until the 20th century, infections that we now consider mellow to treat – such as pneumonia and diarrhea, were the number one cause of human death in the developed world.

It was in the early 20th century that scientists began to observe antibacterial chemicals in action. Paul Ehrlich, a German Jewish physician, observed that certain chemical dyes colored some bacterial cells but not others. He concluded that, according to this principle, it must be possible to create substances that can kill certain bacteria selectively without harming other cells. In 1909, he discovered that a chemical called Arsphenamine was an effective treatment for syphilis and this is how it became the first modern antibiotic. Ehrlich stated his discovery as 'chemotherapy' –using chemicals to treat a disease. The term 'antibiotics' was used for the first 30 years later by the Ukrainian-American microbiologist Selman Waksman. He, during his lifetime was responsible for the production of more than 20 different antibiotics and have also introduced procedures that drove the production of other antibiotics.

In the year 1928, Sir Alexander Fleming recognized penicillin which was a molecule produced by a kind of mold that halted the replication of certain types of bacteria. Fleming was working on a disease-causing bacterial culture; it was then he observed that spores of green *Penicillium notatum* in one of the culture plates. He then observed that, on the spots where the mold was growing those spots were bacteria free. He then isolated the mold and grew it in a fresh culture plate and observed that the mold has created bacteria free zones. Fleming found that *P. notatum* was very impactful in killing and preventing the growth of *Staphylococcus*. He also observed that Penicillin was effective at low concentrations and was less toxic than the disinfectant that was used at that time.

The first ever sulfonamide and the first systemically active antibacterial drug - Prontosil, was developed by a research team headed by Gerhard Domagk in 1932 in Germany, for which Domagk received the 1939 Nobel Prize in Physiology or Medicine.[1]

After it was confirmed that penicillin would treat wounds, collaborating with British pharmaceutical companies made sure that bulk production of penicillin could be promised. Researchers at Oxford University proved helpful in mass production of penicillin. Howard Florey and Ernest Chain were awarded the 1945 Nobel Prize in Medicine along with Alexander Fleming for the bulk production of first ever antibiotic.

Antibiotics being a major advancement in the field of medicine, has also played an important role in saving many lives.[2] They have successfully prevented and treated infections that can occur in patients who are

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receiving chemotherapy; who have chronic diseases like diabetes, renal disease, or rheumatoid arthritis; or patients who have undergone complex surgeries like organ transplants, joint replacements, or cardiac surgery.[3]

Antibiotics have proved instrumental in increasing the life span of people by altering the treatment outcome of bacterial infections. In 1920, the expected life span of people in the U.S. was only 56.4 years, now the average American life expectancy has increased up to almost 80 years.[4] Antibiotics have shown same advantageous impact all around the world. In under developed and developing countries where in sanitation conditions are not up to the mark, antibiotics have helped in increasing the life expectancy, also have improved the treatment outcomes of infections arising due to food and water borne and other poverty-related factors.[5]

Mechanism and origin of antimicrobial resistance

The issue of antibiotic resistant was infrequent just after the discovery of antibiotics by Alexander Fleming. However, Fleming cautioned that inappropriate use of the antibiotics would lead to the genesis of resistant bacteria. The development of resistance was seen few decades after the antibiotics were discovered.

Now, the quick appearance and transmission of resistant bacteria is happening globally which is a serious issue. The antibiotic which was first discovered – penicillin, now doesn't work against most of the bacteria. Those microorganisms which are sensitive, requires high dose of penicillin, this has led to the synthesis of semi synthetic penicillin like ampicillin, methicillin, amoxycillin etc. which are widely used nowadays as bacteria show less resistance against these antibiotics. Other newer antibiotics and alternative therapies are also being practiced. The bacteria isolated from hospital-acquired infection are more likely to show resistance to antibiotics. It is due to the excessive exposure of antibiotics.

Today's major threats are Methicillin Resistant Staphylococcus aureus (MRSA), Vancomycin resistant enterococci (VRE), multi-drug resistant Mycobacterium tuberculosis (MDR-TB), etc. These bacteria have been found resistant to most of the commonly used antibiotics.

Bacteria which are resistant against multiple antibiotics are known as multidrug resistant (MDR) bacteria or superbugs. Every living organism makes efforts to survive. If an organism adjusts itself to a changing environment, it survives, and if not, it dies. When bacteria constantly come in contact with antibiotics, many bacteria establish a resistance mechanism. These bacteria have a better chance of survival than those compared with the vulnerable ones. Thus, antibiotic resistance is not an artificial occurrence.

If very few bacteria are antibiotic resistant in a large population of bacteria, the antibiotic, on its exposure, kills all the susceptible bacteria. This leads to a selective pressure for the survival of resistance bacteria (Figure 1). The resistant bacteria are now able to multiply rapidly giving rise to more number of resistant bacteria. In addition, resistant bacteria also transfer their resistant gene to susceptible bacteria. One resistant bacterium among millions of bacteria cannot cause harm, but problem arises when it transfers its resistant gene to other bacteria ultimately making resistant bacteria a majority.

Ways of gene transfer

Natural resistance – There are a number of ways of gene transfer, as depicted in Figure 2. Many bacteria, by the virtue of their nature are resistant to some antibiotics. For example, antibiotics inhibiting cell wall synthesis are useless for Mycoplasma as these organisms lack cell wall. Similarly, most Gram-negative bacteria have been found resistant to glycopeptide antibiotics like vancomycin. It is because these antibiotics are larger in size which cannot permeate through the small pores of outer membrane of the Gram-negative bacteria. Natural resistance against certain antibiotic is not generally considered as antibiotic resistant because the bacteria were never susceptible to that antibiotic.

Acquired resistance - If the antibiotic presence is not favorable, bacteria either be suppressed or develop a resistance. If bacteria are not resistant against antibiotics naturally, they may gain resistant gene from other bacteria. Bacteria can gain resistance either by mutation or alternatively via gene transfer from resistant bacteria.

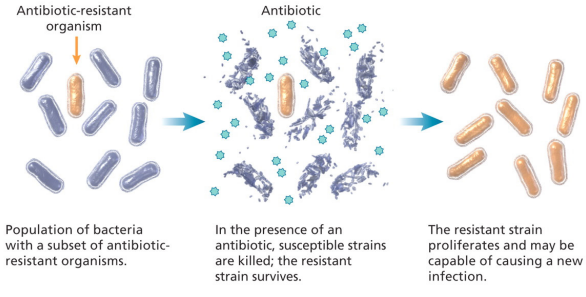

Figure 1: Selection Pressure

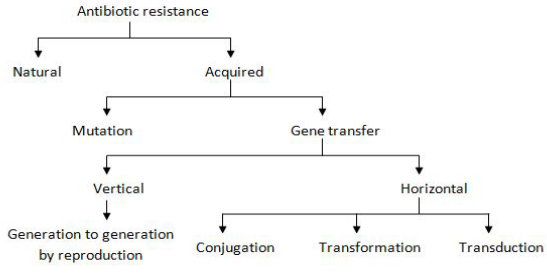

Figure 2: Various mechanism of development of antibiotic resistance.

Mutation - Mutation is a persistent heritable change of genes of an organism. Diverse mutations produce distinct forms of resistance. Resistant cells can be isolated from the cultures of bacteria which were susceptible to the antibiotic. This type of resistance is usually due to the mutation in chromosomal gene.

Gene transfer - Resistant gene may also be transferred from a single bacterium to the others. The conveyance of resistant genes occurs via vertical gene transfer, alternatively horizontal gene transfer takes place. Resistant gene can be transmitted from one origination to another by reproduction. This is process is known as vertical gene transfer. When bacteria transfer their resistant gene in a susceptible bacteria it generally happens by horizontal gene transfer. Bacteria may transfer resistant gene horizontally among equivalent species or even between different genus and species. It can occur through conjugation, transformation as well as transduction.

Conjugation is a mating process through which genes are transferred through the temporary fusion of mating partners. Thus, plasmid or a portion of chromosome which bears resistant genes could be transferred. Another method of transferring gene is transformation. When bacteria containing resistant genes dies, its DNA is released outside. Then another bacterium can receive that naked, "free" DNA from the environment.

Viruses (bacteriophages) are also a means for passing resistant genes between bacteria through the process called transduction. When a virus invades an antibiotic resistant bacterium, the resistant gene of the bacterium is loaded in crest of the virus. When virus invades another (susceptible) bacterium, it injects the resistant gene into that bacterium. This approach is known as transduction.

Superbug and Superresistance

Superbug means a microbe that shows very high morbidity and mortality because of the number of mutations that has occurred over a period of time, and has led to resistance against specifically those antibiotics that are used in the treatment against countering their infections. This superbug caused infection is difficult to treat, both for the patient as well as the physicians, as the treatment options are reduced because most of the antibiotics that work are resistant and for the patient it will involve increased duration of hospital stays as well as increased cost of the treatment.[6]

Table 1: *Staphylococcus aureus* ICMR AMR Data (2014)[9]

AMA	*'n'*	*No. (%) Resistant*
Cefoxitin	3221	1150(35.7)
Ciprofloxacin	3246	2066(63.3)
Clindamycin	3206	801(25)
Erythromycin	3318	1672(50.4)
Genatmycin	2402	428(17.8)
Linezolid	2456	6(0.2)
Muprocin	1588	30(1.9)
Penicillin	2861	2551 (89.2)
Teicoplanin	2508	0
Tetracycline	2860	1056(36.9)
Trimethoprim – Sulfamethoxazole	1666	761(45.7)
Vancomycin	3223	4(0.1)

Many of the microbes which are linked with human disease's outbreak has developed into multidrug-resistant (MDR) types because of regular use of antibiotic. To take an example MDR *M. tuberculosis* is one of the main microorganism that has been identified in voth rural and industrialized areas and can now be considered as a 20th-century variant of an old pathogen.[6] Other infections consist of nosocomial infections of *Acinetobacter baumannii, Burkholderiacepacia, Campylobacter jejuni, Citrobacterfreundii, Clostridium difficile, Enterobacter* spp., *Enterococcus faecium, Enterococcus faecalis, Escherichia coli, Haemophilusinfluenzae, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella* spp., *Serratia* spp., *Staphylococcus aureus, Staphylococcus epidermidis, Stenotrophomonasmaltophilia,* and *Streptococcus pneumoniae.*

a) Methicillin Resistant Staphylococcus aureus

MRSA was first observed fifty years ago.[7] From that time on till now the infection has spread worldwide in several countries like Europe, America and various Asia –Pacific regions. Currently it is the known as the most infamous superbug.[6] It is present in the nasal passage in 30% of population and it has earlier been linked to the simple skin infections like boils. This multidrug-resistant microorganism has appeared as one of the main nosocomial infection.[8] After the breakthrough of Penicillin, it was believed that *S. aureus* infections could be

Table 2: Enterobacteriaceae, ICMR AMR data (2014)[9]

	% Resistant		
AMA	*Escherichia coli*	*Klebsiella sp.*	*Enterobacter sp.*
Amikacin	24	54	44
Cefepime	79	88	80
Cefoperazone-Sulbactam	33	62	390
Cefotaxime	80	83	83
Ceftazidime	81	84	77
Ciplrofloxacin	81	65	48
Colistin	1	1	0
Gentamicin	46	65	56
Imipenem	18	35	26
Meropenem	35	53	38
Netilmicin	12	42	18

controlled; however, relieve from resistance was not long. The milestone finding and institution of methicillin in the year1959 were believed to be a certain protection towards the penicillinases, however methicillin-resistant *S. aureus* (MRSA) within a very short period of time has invariably resulted in many more multiantibiotic-resistant variants, therefore the name has now changed to multidrug-resistant *S. aureus*. Moreover, very recently, MRSA has now started affecting the community outside the hospital and has now become a main community-acquired (CA) microorganism, equipped with strengthened virulence and characteristics responsible for transmission (Table 1).[6]

b) Enterobacteriaceae sp.

Extended-spectrum beta-lactamase (ESBL)-generating Enterobacteriaceae possess a broad-spectrum beta-lactamase enzyme which gives them the liberty to be resistant against Penicillin and Cephalosporin.[10,11] ESBL-producing Enterobacteriaceae is responsible for 26,000 HAIs and 1,700 mortality cases each year.[12] Some of the ESBL-producing Enterobacteriaceae have been found resistant to almost all antibiotics in Penicillin as well as Cephalosporin (Table 2).[10]

Carbapenem-resistant Enterobacteriaceae (CRE) are the class of bacterium that have shown resistance to "all or nearly all" antibiotics, as well as carbapenems, which are considered as the last resort for any treatment in cases of pathogens which are drug resistant. drug-resistant pathogens.[10,11,13] Enzyme which is called as New Delhi metallo-beta-lactamase (NDM-1) is present in some gram-negative Enterobacteriaceae bacteria (notably *Escherichia coli* and *K. pneumoniae*) which turns them resistant to almost all beta-lactams, including carbapenems.[13]

c) M. tuberculosis

Tuberculosis is found to affect around one-third of the world population is an exemplary human pathogen. The anti-TB drugs are the customary treatment regimen successfully treating the disease but develops a multidrug resistance throughout the world. Tuberculosis is found to be resistant to front-line treatment by spontaneous mutation have spread in a great speed across the world.

d) Clostridium difficile

Clostridium difficile is majorly responsible for causing infection in intestine. A new hyper virulent strain has been identified which is gram positive in nature transmitted by different personnel, equipment presents in the hospital and as aerosol. The infection resulted due to the direct use of antibiotic such as cephalosporin, the newer penicillin's and fluoroquinolones which is responsible for the depletion of gram-negative strains, thus increasing the infection.

Status in India

The crude infectious disease mortality rate in India today is 416.75 per 100,000 persons[19,20] and is twice the rate prevailing in the United States when antibiotics were introduced (roughly 200 per 100,000 persons).[21] A mix of poor public health systems and hospital infection, high rates of infectious disease, inexpensive antibiotics, and rising incomes is coming together to increase prevalence of resistant pathogens and is increasing the burden of untreatable neonatal sepsis and health-care-associated infections.[22]

Antibiotic use is a major driver of resistance. In 2010, India was the world's largest consumer of antibiotics for human health at 12.9 x 10^9 units (10.7 units per person). The next largest consumers were China at 10.0 x10^9 units (7.5 units per person) and the US at 6.8 x10^9 units (22.0 units per person).[23] Seventy-six percent of the overall increase in global antibiotic consumption between 2000 and 2010 was attributable to BRICS countries, i.e., Brazil, Russia, India, China, and South Africa.[23] In BRICS countries, 23% of the increase in the retail antibiotic sales volume was attributable to India, and up to 57% of the increase in the hospital sector was attributable to China.

Overall, ampicillin and co-trimoxazole use is declining in India while quinolone consumption is high and increasing. Rates of carbapenem use per capita are low compared to other antibiotics in 2000 but had risen to over 10 million standard units by 2010.[23]

The scale-up in antibiotic use in India has been enabled by rapid economic growth and rising incomes, which have not translated into improvements in water, sanitation, and public health, although evidence exploring this key issue is anecdotal.[24] Antibiotics continue to be prescribed or sold for diarrheal diseases and upper respiratory infections for which they have limited value.[25,26] India's large population is often blamed for the easy spread of resistant pathogens, but population densities in India are lower than those in parts of Indonesia or China. The main problem is that India lags on basic public health measures. Immunization rates (as measured by diphtheria-tetanus-pertussis [DPT3]) coverage in India (72%) lag behind those in Brazil (95%), China (99%), and Indonesia (85%). The percentage of the population with access to improved sanitation facilities in India (36%) was far lower than the percentage in Brazil (81.3%), China (65.3%), and Indonesia (58.8%).[27] Under the Swacch Bharat Abhiyan (Clean India Program), the government has committed to providing toilets and improving sewage systems, but these measures will take time to implement.

Health system factors are also at fault. Doctors routinely receive compensation from pharmaceutical

companies and pharmacists in exchange for antibiotic prescriptions.[28] Infection control in hospitals is poorly monitored and could be improved. A point prevalence study in a large tertiary care hospital in India found an overall health-care-associated infection prevalence of 7%, with a third of these being surgical site infections.[29] Half of all patients were receiving antimicrobials.

Over-the-counter access to antibiotics is a problem, but regulations to restrict access have to be balanced against the need to maintain access for the significant proportion of the population that lacks access to doctors. Lack of access to effective and affordable antibiotics still kills more children in India than does drug resistance.[30] However, to prevent over-the-counter (OTC) sales of important antibiotics, the Central Drugs Standard Control Organization (CDSCO) implemented Schedule H1 in India starting March 1, 2014. The H1 list includes 24 antibiotics, such as third- and fourth-generation cephalosporins, carbapenems, antituberculosis drugs, and newer fluoroquinolones. Antibiotics have previously been listed under Schedule H, which contained drugs that could be sold only with a valid prescription; almost all antibiotics were easily available over the counter in the country, leading to their rampant use. The stricter Schedule H1 specifies that the drugs covered by it carry a prominent Rx symbol in red and contain a box with red borders with a printed warning on their packaging. Moreover, drugs included in Schedule H1 can only be sold with the prescription of a registered medical practitioner and require that that pharmacist maintain a separate register with the patient's name, contact details of the prescribing doctor, and the name and dispensed quantity of the drug. The register has to be retained for at least three years and is subject to audit by the government. There have been some instances of enforcement of Schedule H1, with licenses of 213 retail pharmacies in the division canceled for "non-compliance to dispensing medicines without prescription and giving bill" in some parts of India.[31] Several important antibiotics, including gentamicin, piperacillin-tazobactam, linezolid, and tigecycline, are not included in Schedule H1. There is potential for OTC sales of these antibiotics by pharmacists to compensate for the restricted sales of stronger prescription antibiotics.

The problem of resistance is exacerbated by a wide range of fixed-dose combinations in the market, often without scientific or medical merit or evaluation. A recent study reported 48 fixed dose combinations and 22 loose antimicrobials for tuberculosis.[32] Loose antimicrobials come without packaging and do not mention the name of the drug, its manufacturer, the date of manufacture, or the date of expiry. There is poor clinician awareness of the rationality and dosing of fixed-dose combinations.[33] Incorporating principles of antimicrobial stewardship and appropriate use into undergraduate and postgraduate medical education can be implemented and is under consideration by the Government of India.

The overall burden of resistance is hard to assess for the general population but is likely focused on neonates and the elderly, both of whom are more prone to infections and vulnerable to ineffective treatment. Although accurate estimates of the overall burden of resistance are not available, it is estimated that 58,000 neonatal deaths are attributable to sepsis caused by drug-resistance to first-line antibiotics each year.[30]

Prevention of AMR

Each and every strain of bacteria isolated from clinical sample must be tested for minimum inhibiting concentration (MIC) and antibiotic sensitivity test (AST). This helps clinicians to choose correct antibiotic with correct dose and it reduces the chance of resistance. Combination of different nature of antibiotics helps to work against resistant bacteria. As these antibiotics act on different sites of bacteria, the bacteria are less likely to develop resistance. However, this may not be effective against MDR bacteria.

CONCLUSION

Some of the preventive ways are as follows:

Prevent infection - Infection can be prevented by immunization. Vaccines increase body's immune system and decrease resistant pathogens. Less use of IV line and catheter also helps to prevent infection in hospitals.

Diagnose and treat effectively - Patient's sample should be cultured in lab to isolate and identify the causative organism. Proper antibiotic should be prescribed. It is better to prefer narrow spectrum antibiotics which only target pathogenic bacteria.

Use antibiotic wisely - Patients should be given the right dose of antibiotics for right duration. Patients should be informed why full course of antibiotic is necessary. Focus should be given to treat the infection not the contamination.

Stop antibiotic therapy when unnecessary - If an antibiotic in use is found to be ineffective against the causative organism, it should be stopped. Sometimes antibiotics are prescribed prior to the culture reports; after finding negative result for bacteria the antibiotic should be stopped as infection may be caused by a virus.

Prevent transmission of pathogen - Patient should maintain proper hygiene and sanitization; hand washing should be promoted, direct contact with the patient

should be avoided to prevent the spread of communicable disease.

Antimicrobial stewardship Programs-AMS (Anti Microbial Stewardship) is the appropriate use of antimicrobials through strategies aimed at preventing their misuse and ensuring the best outcomes for people in health and care settings.[34]

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