ANTIBIOTIC RESISTANCE IN CLINICAL MEDICINE

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ABSTRACT

Since the discovery of penicillin, antibiotics have been a cornerstone in modern medicine and significantly improved global health. And while decades of overuse, misuse and abuse of antibiotics in hospitals, and in general population, at an ever increasing rate, both when they are needed and when they are not, in human beings and animals have accelerated the emergence and spread of resistant bacteria. Indiscriminate use of antibiotics in veterinary practice, and in animal feeds led to emergence of drug resistant strains, that are transferred to humans. This emergence of drug resistance is a major problem worldwide in antibiotic therapy. Infections caused by resistant microorganisms often fail to respond to the standard treatment, resulting in prolonged illness, longer hospital stays, higher healthcare expenditures, and greater risk of death. This article reviews and focuses on various aspects of development and mechanisms of antibiotic resistance in bacteria, and preventive measures to fight the emerging antibiotic resistance threat.

KEYWORDS

Antibiotic resistance, Mutational drug resistance, Transferable drug resistance.

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INTRODUCTION

Strains of bacteria differ in their degree of susceptibility to antibiotics. The susceptibility of a strain of bacterium to any particular antibiotic may change with time. A strain of bacterium that had once been highly susceptible to an antibiotic may develop resistance, over time. Thus, antibiotic resistance is one of nature's never ending processes, but misuse of antibiotics in humans and animals is accelerating the process.[1-4] The biological development of antibiotic resistance is an ongoing process (biological resistance), which may not be detected by laboratory procedures. When antibiotic susceptibility has been lost to such an extent that the drug is no longer effective for clinical use; the bacterium is said to be achieved clinical resistance. Most of the laboratory methods used to detect resistance are meant to detect clinical resistance. Bacteria resistant to a certain antibiotic may also be resistant to other antibiotics (cross-resistance).[5,6]

Antibiotic resistance may also result from physical or chemical characteristics of the environment that either directly alter the drug or alter the microorganism's normal physiologic response to the drug (environment mediated drug resistance). The antimicrobial activity of several antibiotics are affected by pH. For example, the antimicrobial activity of erythromycin and aminoglycosides diminish with decreasing pH, whereas the activity of tetracycline decreases with increasing pH. Aminoglycoside activity is also affected by the concentration of cations, such as Mg++ and Ca++ in the environment. It is most commonly seen with Pseudomonas aeruginosa.[7] Aminoglycoside molecules have a net positive charge and most Gram negative bacilli have a net negative charge. The Mg++ and Ca++ ions found in the bacterial environment compete with aminoglycoside molecules for the negatively charged receptor sites on bacterial surface, so that less drug will be taken up and antimicrobial activity diminishes. Information regarding environmentally mediated drug resistance is used to standardize testing methods that minimize the impact of environmental factors so that microorganism mediated resistance is more accurately determined.[5,8]

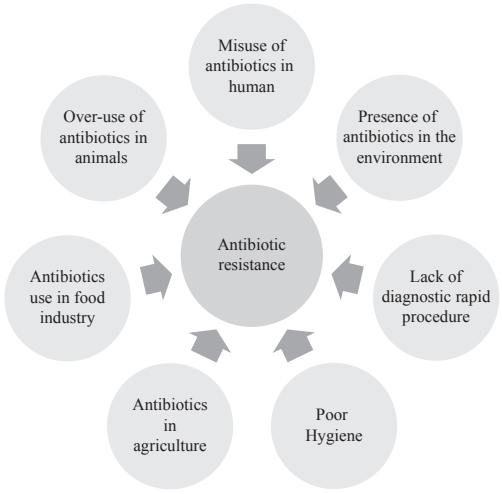

Figure 1. Reasons behind emergence and spread of antibiotic resistance

Development of antibiotic resistance

The development of drug resistance is a natural process among bacteria.[9] However, several factors augment the development of drug resistance. This involve antibiotic overuse and abuse, inexact diagnosis and improper antibiotic prescribing, patient sensitivity loss and self-medication, bad healthcare environments, poor personal hygiene, and widespread agricultural use.[10-12] The development of antibiotic resistance to an antibiotic by a microorganism can be of two types: intrinsic and acquired.[5,13]

Intrinsic resistance

Drug resistance resulting from the normal genetic, structural, or physiologic state of microorganism is referred to as intrinsic (or natural) resistance. It is natural, inherited characteristic that is usually associated with a species or genus or a group of strains (Table 1). E.g. Gram negative bacilli are resistant to vancomycin. Therefore, this is a predictable resistance of a particular microorganism. Intrinsic resistance profiles are useful as markers in the identification of certain bacteria, and to determine which drug should be tested against a particular microorganism.[5,13,14]

Table 1. Examples of intrinsic resistance

Intrinsic resistance	Mechanism
Anaerobic bacteria versus aminoglycosides	Lack of oxidative metabolism to drive uptake of aminoglycosides
Gram positive bacteria versus aztreonam (ß-lactam)	Lack of penicillin binding proteins (PBPs)
Gram negative bacteria versus vancomycin	Lack of uptake due to inability of vancomycin to penetrate outer membrane
Pseudomonas aeruginosa versus sulphonamides, trimethoprim, tetracycline, chloramphenicols	Lack of uptake due to inability of antibiotics to achieveeffective intracellular concentrations
Klebsiella spp. versus ampicillin (ß-lactam)	Production of ß-lactamases that destroy the drug
Aerobic bacteria versus metronidazole	Inability to anaerobically reduce drug to its active form

Acquired resistance

Drug resistance that results from altered cellular physiology and structure caused by changes in a microorganism's genetic makeup by acquiring genes coding for resistance is known as acquired resistance. In other words, it is an emergence of resistance in bacteria that are ordinarily susceptible antibiotics, by acquiring the genes coding for resistance. It is a character associated with only some strains and unpredictable and that's why laboratory methods to detect resistance are necessary. Most of the antibiotic resistance shown by bacteria belong to this category. Overuse and misuse of antibiotics is the single most important cause of development of acquired resistance.[1,5,13,14] The evolution of resistant strains is a natural phenomenon, which can occur among bacteria especially when an antibiotic is overused.

Use of a particular antibiotic poses selective pressure in a population of bacteria which in turn promotes resistant

bacteria to thrive and the susceptible bacteria to die off. Thus the resistant bacterial populations flourish in areas of high antimicrobial use, where they enjoy a selective advantage over susceptible populations. The resistant strains then spread in the environment and transfer the genes coding for resistance to other unrelated bacteria. The other factors favoring the spread of antibiotic resistance include poor infection control practices, poor hand hygiene practices, inadequate sanitary conditions, inappropriate food-handling, irrational use of antibiotics by doctors, without following antimicrobial susceptibility report, and uncontrolled sale of antibiotics over the counters without prescription.[15,16] In the presence of selective antibiotic pressure, bacteria acquire new genes by either of two mechanisms. i. mutational (chromosome mediated) drug resistance can develop due to mutation in the genes. It is typically seen in Mycobacterium tuberculosis, developing resistance to anti-tubercular drugs (Table 2).[5,13,14] ii. extrachromosomal (plasmid mediated) resistance or transferable drug resistance or infectious drug resistance: It is plasmid coded and usually transferred by mechanisms of gene transfer such as conjugation, transduction or transformation. The resistance coded plasmid, called R plasmid can carry multiple genes, each coding for resistance to one class of antibiotic (Table 2).[5,13,14,17,18]

Table 2. Differences between mutational and transferable drug resistance

Mutational drug resistance	Transferable drug resistance
Mutations confer resistance usually to one drug at a time	Mediated by plasmids, may confer resistance to multiple drugs or all drugs used in treatment, simultaneously
Degree of resistance is low	Degree of resistance is usually high
It can be overcome by administering high dose of drug	It cannot be overcome by high dose of drug
It can be prevented by treatment with a combination of drugs e.g. multidrug therapy is used in tuberculosis using a combination of four to five different classes of drugs such as isoniazid, rifampicin, pyrazinamide, ethambutol, and streptomycin	It cannot be prevented by treatment with a combination of drugs
It does not spread from cell to cell	It spreads to other cells of same or different species by various methods of gene transfer such as conjugation, transduction, and transformation
Resistant mutants are usually metabolically defective	Resistant mutants are metabolically normal
The virulence of resistant mutants may be decreased	Virulence is not decreased

Mechanisms of antibiotic resistance

Bacteria develop drug resistance by several mechanisms.

1. Decreased permeability across the cell wall limiting uptake of drug: Some bacteria alter their cell membrane porin channels, thereby preventing antibiotics from entering into the cell (limiting drug uptake). E.g. *Pseudomonas, Enterobacter,* and *Klebsiella* species against drugs such as imipenem, aminoglycosides, and quinolones.[7,17,19] The biofilm matrix formed by some bacteria makes antibiotics difficult to enter the bacteria.[20]

2. Efflux pumps: Some bacteria possess efflux pumps which mediate active trasnportation of the drug from the cell, soon after their entry, thereby preventing the intracellular accumulation of the drug. e.g. *E.coli* and other *Enterobacteriaceae* members against tetracyclines, chloramphenicol; staphylococci against macrolides and streptogramins; *Staphylococcus aureus* and *Streptococcus pneumoniae* against fluoroquinolones.[21,22]

3. By enzymatic inactivation: Some bacteria can inactivate the antibiotics by producing various enzymes. e.g. Beta-lactamase enzyme produced by both Gram positive and Gram negative bacteria, breaks down the beta-lactam ring, thereby inactivating the beta-lactam antibiotics. Chloramphenicol acetyl transferase produced by Gram negative bacilli destroys the structure of chloramphenicol. Similarly, both Gram positive and Gram negative bacteria can produce acetyl transferases, adenyl transferases, and phosphotransferases that destroy aminoglycosides.[18,21,23,24]

4. By modifying the target sites: Methicillin resistance in *S.aureus* is mediated by a chromosomally mediated gene called mec A gene, which alters penicillin-binding protein (PBP) present on *S.aureus* cell membrane to PBP-2a. PBP is an essential protein needed for cell wall synthesis of bacteria. Beta lactam drugs bind and inhibit this protein, thereby inhibit the cell wall synthesis. The altered PBP-2a of methicillin resistant *Staphylococcus aureus* (MRSA) strains has less affinity for beta lactam antibiotics; Hence, MRSA strains are resistant to all beta lactam antibiotics.[13,25-27]

Similarly, penicillin resistance in pneumococci has been reported increasingly nowadays. This resistance is due to alteration of penicillin-binding protein (PBP) to PBP-2b. This altered PBP-2b has low affinity for ß-lactam antibiotics. The gene coding for altered PBP is acquired by transformation and horizontal transfer of DNA from related *streptococcal* species. Quinolone resistance seen in many Gram positive bacteria, particularly *Staphylococcus aureus* and *Streptococcus pneumoniae* is due to mutations in DNA gyrase enzyme. Vancomycin resistance in enterococci (VRE) is due to an alteration in the target site of vancomycin, mediated by van gene. [13,25,26]

Beta- lactamase enzymes

Beta lactamase enzymes produced by both Gram positive and Gram negative bacteria, can hydrolyze the ß-lactam ring (the active site) of ß-lactam antibiotics and inactivate them. They are plasmid coded, and transferred from one bacterium to other mostly by conjugation, except in *Staphylococcus aureus* where they are transferred by transduction. Beta-lactamases are of various types.[16,18,22]

i. Extended spectrum ß-lactamases (ESBL): Bacteria producing ESBL enzymes are resistant to all penicillins, first, second, and third generation cephalosporins and monobactams; however, remain susceptible to carbapenems and cephamycins. The resistance can be overcome by use of ß-lactam along with ß-lactamase inhibitor (e.g. sulbactum or clavulanic acid).[16,18,22]

ii. AmpC ß-lactamases: AmpC ß-lactamase producing bacteria are resistant to cephamycins (e.g. cefoxitin and cefotetan), in addition to the antibiotics to which ESBL producing bacteria are resistant. But they are susceptible to carbapenems. The resistance cannot be overcome by a combination of ß-lactam plus ß-lactamase inhibitor.[16,18,22]

ii. Carbapenamases: Carbapenamase producing bacteria are resistant to all those antibiotics to which AmpC ß-lactamase producing bacteria are resistant. In addition, they are also resistant to carbapenems. The resistance cannot be overcome by a combination of ß-lactam plus ß-lactamase inhibitor. The most important carbapenemase enzymes are *Klebsiella pneumoniae* carbapenemase, New Delhi metallo-beta-lactamase.[19,20]

Drug resistance in tuberculosis

In clinical practice, mutational drug resistance is a major problem in the treatment of tuberculosis. The mechanism of drug resistance in tubercle bacilli is due to point mutation in the genome of *Mycobacterium tuberculosis* which occurs at an approximate rate of 1 in 108 cell divisions (Table 3). This can be effectively checked by multi-drug therapy. Incidence of resistance to one drug is independent of that to another. Hence, the probability of a strain to be resistant to two drugs is much lower, than when these drugs are used independently. Unfortunately, this was not implemented properly and failure to adhere to the multi-drug regimen is the most important reason for development of resistance, which may be due to a combination of lapses in prescribing practices, prolonged duration of regimen, poor patient compliance, development of toxicity to the drugs, and improper supervision and follow-up. As a result, the resistance has built up in tubercle bacilli over the years, reducing the efficacy of treatment.[27-33]

Table 3. Drug resistant genes present in *Mycobacterium tuberculosis*

Drugs	Drug resistant genes
Isoniazid	Enoyl ACP reductase (inhA) Catalase-peroxidase (katG) Alkyl hydroperoxide reductase (AhpC)
Rifampicin	RNA polymerase subunit B
Pyrazinamide	Pyrazinamidase (pncA)
Ethambutol	Ribosomal protein subunit 12 (rpsL)
Streptomycin	Ribosomal protein subunit 12 (rpsL) 16S ribosomal RNA (rrs) Aminoglycoside phosphotransferase gene (strA)
Fluoroquinolones	DNA gyrase (gyrA and gyrB)

A very serious consequence of unchecked drug resistance has been the emergence and spread of multi-drug resistant tuberculosis (MDR-TB). Though the term multi-drug resistance means only resistance to two or more drugs, in the context of tuberculosis, it specifically refers to resistance to rifampicin and isoniazid, two first-line drugs, with or without resistance to one or more other first-line anti-tubercualr drugs. This is because rifampicin and isoniazid form the sheet anchor of short-term chemotherapy and any strain resistant to both these drugs is unlikely to respond to treatment. MDR-TB is a global problem, and its presence in those with concomitant HIV infection makes it more dangerous.[16,27,33] Extensively drug-resistant tuberculosis (XDR-TB) is defined as MDR plus resistance to fluoroquinolones (levofloxacin, maoxifloxacin, gatifloxacin), and one of the second-line injectables (amikacin, capreomycin, kanamycin). The treatment of XDR-TB is very difficult, and has a very rapidly progressing clinical course with high mortality. [16,27]

Prevention of drug resistance

- Antibiotic resistance is accelerated by the misuse and overuse of antibiotics, as well as poor infection prevention and control. Steps can be taken at all levels of society to reduce the impact and limit the spread of resistance.[1,26,33] The development of antibiotic resistance can be prevented or minimized by:
- Avoiding the indiscriminate use of antibiotics where they are of no clinical value.
- Refraining from the use of antibiotics commonly employed for generalized infections for topical applications.
- Using correct dosage and schedule (complete course) of the proper antibiotic to overcome an infection quickly.
- Using combinations of antibiotics (multi-drug therapy) of proved effectiveness, that have different modes of action so that the organism cannot quickly undergo adaptation.
- Using a different antibiotic when an organism gives evidence of becoming resistant to the one used initially.
- Maintaining sufficiently high levels of the drug in the tissues to inhibit both the original population and first step mutants.
- Using antibiotics in situations where a bacterial infection is either proven or strongly suspected.
- Choosing a drug that targets the specific organism to be eradicated rather than opting for a more broad-spectrum drug.
- Preventing infections by regularly washing hands, preparing food hygienically, avoiding close contact with sick people, practicing safer sex, and keeping vaccinations up to date.

ANTIBIOTICS IN VETERINARY PRACTICE

Antibiotics used in veterinary practice can lead to emergence of drug resistant strains that may be transferred to humans. Incorporation of antibiotics in animal feeds may cause animals to grow more rapidly, but associated with increase of drug resistant strains in farm workers. Hence use of antibiotics in animal feeds must be controlled.[4,30-36]

CONCLUSION

Antibiotic resistance is a global health and development threat. Antibiotic resistance is emerging and spreading to dangerously high levels all over the world, threatening our ability to treat common infectious diseases. The emergence and spread of resistance is worsening, where antibiotics can be bought without a prescription over the pharmacy counters. Similarly, antibiotics are often over-prescribed by health workers and veterinarians and over-used by the public. Without urgent action, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill. Surveillance of antibiotic usage and resistance must be improved by implementing antibiotic stewardship programs. Although the pace at which resistance is spreading continues to increase, the health care community has limited data on the magnitude of the effect of this problem on health and economic outcomes. Further study in this area is essential.

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