Review Article

Mechanisms of Development of Antibiotic Resistance in Bacteria Among Clinical Specimens

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ANTIBIOTICS.[1]:

An antibiotic is a Chemical substance formed as a metabolic by-product in bacteria or fungi that kills or inhibits the growth of bacteria. Antibiotics belong to the group of antimicrobial compounds used to treat infections caused by microorganisms, including fungi and protozoa. Antibiotics can be produced either naturally, using microorganisms, or synthetically.

ANTIBIOTIC RESISTANCE.[2-5]:

Antibiotic resistance is a specific type of drug resistance when a microorganism has the ability of withstanding the biological effects of antibiotics. In human medicine the major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics in clinical medicine. There has probably been a gene pool in nature for resistance to antibiotic as long as there has been for antibiotic production, for most microbes that are antibiotic producers are resistant to their own antibiotic.

A. HISTORY OF ANTIBIOTIC RESISTANCE:

Alexander Fleming first discovered antibiotics through a providential experiment. By 1928, Fleming was investigating the properties of *Staphylococci*. He was already well known from his earlier work, and had developed a reputation as a brilliant researcher, but his laboratory was often untidy. On 3 September 1928, Fleming returned to his laboratory having spent August on holiday with his family. Before leaving he had stacked all his cultures of *Staphylococci* on a bench in a corner of his laboratory. On returning, Fleming noticed that one culture was contaminated with a fungus, and that the colonies of *Staphylococci* that had immediately surrounded it had been destroyed, whereas other colonies further away were normal. Fleming identified the mould that had contaminated his culture plates as being from the *Penicillium* Genus, His work eventually led to the large-scale production of penicillin from the mold *Penicillium notatum* in the 1940s. In 1946, penicillin became generally available for treatment of bacterial infections; especially those caused by *Staphylococci* species and *Streptococci* species. Initially, the antibiotic was effective against all sorts of infections caused by Gram-positive bacteria. Penicillin had unbelievable ability to kill these bacterial pathogens without harming the host that harbored them. As early as the late 1940s resistant strains of bacteria began to appear. Currently, it is estimated that more than 70% of the bacteria that cause hospital-acquired infections are resistant to at least one of the antibiotics used to treat them.

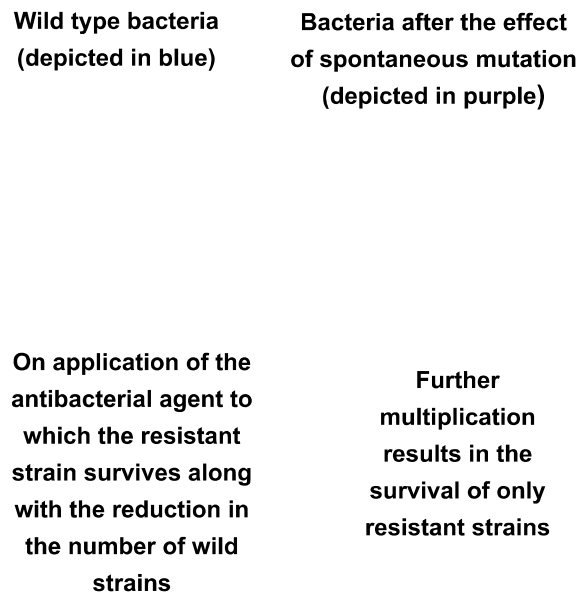
B. MODE OF GAINING RESISTANCE:

Bacteria can gain resistance through two primary ways:

1. By mutation, and
2. By using a built-in design feature to swap DNA (called horizontal gene transfer) Bacteria share resistance genes.

(1) Mutation

Microbes reproduce by dividing every few hours, allowing them to evolve rapidly and adapt quickly to new environmental conditions. With each replication, spontaneous mutations arise, and some of these mutations may help an individual microbe survive exposure to an antimicrobial.



(2) Gene Transfer:

Microbes may also acquire genes from each other, including genes that make the microbe drug resistant.

Hospital Use:

Critically ill patients are more susceptible to infections and, thus, often require the aid of antimicrobials for treatment. However, the heavier use of antimicrobials in these patients can worsen the problem by selecting for antimicrobial-resistant microorganisms. The extensive use of antimicrobials and close contact among sick patients creates a fertile environment for the spread of antimicrobial-resistant germs.

C. MECHANISMS OF RESISTANCE:

The four main mechanisms by which microorganisms exhibit resistance to antimicrobials are:

1. **Drug inactivation or modification:** e.g. enzymatic deactivation of Penicillin G in some penicillin-resistant bacteria through the production of beta-lactamases.

2. **Alteration of target site:** e.g. alteration of Penicillin Binding Protein (PBP) the binding target site of penicillins in Methycillin Resistant Staphylococcus Aureus (MRSA) and other penicillin-resistant bacteria.

3.**Alteration of metabolic pathway:** e.g. some sulphonamide-resistant bacteria do not require para-aminobenzoic acid (PABA), an important precursor for the synthesis of folic acid and nucleic acids in bacteria inhibited by sulphonamides.

4. **Reduced drug accumulation**: by decreasing drug permeability and/or increasing active efflux (pumping out) of the drugs across the cell surface.

D. MODE OF ANTIBIOTIC RESISTANCE SPREAD. [2-5]:

The acquisition and spread of antibiotic resistance in bacteria:

Antibiotic resistance in bacteria may be an inherent trait of the organism (e.g. a particular type of cell wall structure) that renders it **naturally resistant**, or it may be **acquired** by means of mutation in its own DNA or acquisition of resistance-conferring DNA from another source.

1. Inherent (natural) resistance:

Bacteria may be inherently resistant to an antibiotic. For example, an organism lacks a transport system for an antibiotic; or an organism lacks the target of the antibiotic molecule; or, as in the case of Gram-negative bacteria, the cell wall is covered with an outer membrane that establishes a permeability barrier against the antibiotic.

2. Acquired resistance:

Several mechanisms are developed by bacteria in order to acquire resistance to antibiotics. All require either modification of existing genetic material or acquisition of new genetic material from another source. Acquisition takes place by following mechanisms.

a. Vertical Gene Transfer:

Spontaneous mutation frequency for antibiotic resistance is about 10^{-8}- 10^{-9}. This means that one in every 10^{8}- 10^{9} bacteria in an infection will develop resistance through process of mutation. Once resistance genes have developed, they are transferred directly to all the bacteria's progeny during DNA replication. This is known as **vertical gene transfer** or **vertical evolution**. In the selective environment of the antibiotic, the wild types (non mutants) are killed and the resistant mutant is allowed to grow and flourish.

b. Horizontal Gene Transfer:

Another mechanism beyond spontaneous mutation is responsible for the acquisition of antibiotic resistance. Lateral or **horizontal gene transfer** (HGT) is a process whereby genetic material contained in small packets of DNA can be transferred between individual bacteria of the same species or even between different species. There are at least three possible mechanisms of HGT, equivalent to the three processes of genetic exchange in bacteria. These are **Transduction, Transformation and Conjugation.**

COMMONLY OCCURRING DRUG RESISTANCE:

1. Methicillin resistant *Staphylococci*
2. Vancomycin resistant *Enterococci*
3. Multidrug resistant *Pseudomonas aeruginosa*
4. Extended Spectrum of Beta-Lactamases (ESBLs)

Methicillin-resistant *Staphylococcus aureus* (MRSA). [7-12]**:**

Methicillin Resistance Staphylococci (MRS) is a major pathogen causing nosocomial and community acquired infection throughout the world. All the species of Staphylococci, *S. aureus* and Coagulase Negative Staphylococci (CNS) has been found to be the common bacterial agent recovered from blood stream

infections, skin and soft tissue infections, post-operative wound infections. MRSA has emerged as an important pathogen cause of community-associated infections in both pediatric and general population. MRSA is also known as oxacillin-resistant *S. aureus* (ORSA) and multiple-resistant *Staphylococcus aureus*. If a bacterium carries several resistance genes, it is called multi drug resistant or, informally, a super bug. It was first discovered in the UK in 1961. MRSA has ability to grow in the presence of beta-lactams and its derivatives, including cephalosporin and penicillin. This resistance is intrinsic and can be transferred to susceptible strains through horizontal transfer of this *mecA* gene. The *mecA* gene, structural determinant encoding Pencillin binding protein 2a (PBP2a), is found in all MRS strains and therefore considered a useful molecular marker of putative methicillin resistance in *S. aureus*.

Methicillin resistance is mediated by penicillin-binding protein (PBP) 2a. PBP 2a and gene encoding it have been found in all MRS. PBP 2' (or PBP 2a), which, unlike intrinsic set of PBPs (PBP 1 to 4) of *S. aureus*, has remarkably reduced binding affinities to beta-lactam antibiotics. Despite presence of inhibitory concentrations of beta-lactam antibiotics, MRSA can continue cell wall synthesis solely depending upon uninhibited activity of PBP 2a resulting in a loss of target affinity.

Vancomycin-resistant Enterococcus. [13-17]**:**

Enterococci are known to be major causes of infections. Commonly, they infect the urinary tract, abdomen, and bile tract. The two common pathogens are *Enterococcus faecalis* and *Enterococcus faecium*. *Enterococcus faecalis* strains cause 80-90% of the infections and *E. faecium* the remaining 10-20% of the infections. The *Enterococci* are the fourth cause of hospital infections and hence it is important to know the adequate treatment. **Vancomycin-resistant *Enterococcus*** (VRE), are bacterial strains of the genus *Enterococcus* that are resistant to the glycopeptide antibiotic vancomycin. Many species in the genus *Enterococcus* possess intrinsic resistance to commonly used antibiotics. Intrinsic resistance represents naturally encoded chromosomal characteristics. These resistance mechanisms affect primarily the aminoglycosides and beta lactam antibiotics, and create therapeutic challenges for the treatment of serious infections such as endocarditis or septicemia. To become VRE, vancomycin-sensitive *Enterococci* typically obtain new DNA in the form of plasmids or transposons which encode genes that confer vancomycin resistance. In addition to intrinsic resistance, Enterococci can acquire genetic determinants that confer resistance to other antibiotics. There are six different types of vancomycin resistance shown by *Enterococcus*: *Van-A, Van-B, Van-C, Van-D, Van-E and Van-F*. The emergence and increasing frequency of VRE has presented both therapeutic and infection control challenges.

Multidrug Resistant *Pseudomonas.* [18 22]**:**

The human opportunistic pathogen, *Pseudomonas aeruginosa*, is a major cause of infectious-related mortality among the critically ill patients, and carriers the highest case fatality

rate of all gram-negative infections. It is a leading cause of nosocomial infections and is responsible for 10% of all hospital-acquired infections. Infections caused by *P. aeruginosa* are often severe and life threatening and are difficult to treat because of the limited susceptibility to antimicrobial agents and the high frequency of an emergence of antibiotic resistance during therapy, thus resulting in severe adverse outcomes. The problem of antibiotic resistance in *P. aeruginosa* is on the increase. The heightened level of drug resistance is a result of the de novo emergence of resistance in a specific organism after exposure to antimicrobials as well as of patient-to-patient spread of resistant organisms. Accumulation of resistance after exposure to various antibiotics and cross-resistance between agents may result in multidrug-resistant (MDR) *P. aeruginosa*. This condition was found primarily in patients with cystic fibrosis, where persistent infection with *P. aeruginosa* leads to the sequential emergence of resistance to multiple antibiotic agents. These MDR P. aeruginosa strains may be transmitted from patient to patient and sometimes lead to outbreaks among cystic fibrosis patients attending the same clinic. Now recently it is shown that it poses serious infection of all the organ system and thus treatment is the crucial part.

EXTENDED-SPECTRUM OF BETA LACTAMASES (ESBLs).[23-25]:

ESBL stands for **Extended Spectrum Beta-Lactamase**, which are enzymes that mediate resistance to third generation cephalosporins e.g., ceftazidime, cefotaxime, and ceftriaxone and monobactams e.g., aztreonam but do not affect cephamycins (e.g., cefoxitin and cefotetan) or carbapenems (e.g., meropenem or imipenem). *Escherichia coli, Klebsiella pneuminiae, Klebsiella oxytoca* are the most ESBL-producing pathogens. ***Extended***-spectrum Beta-lactamase producing *E. coli* is highly resistant to an array of antibiotics and infections by these strains are difficult to treat. In many instances, only two oral antibiotics and a very limited group of intravenous antibiotics remain effective. According to the amino acid sequences extended-spectrum beta-lactamases are typed as **TEM (**found in a single strain of *E. coli* isolated from a blood culture from a patient named Temoniera in Greece, hence the designation TEM)' **SHV (**sulphydryl variable), **CTX-M (**cefotaxime), **OXA (**oxacillin), **AmpC (**ampicillin chromosomal).

All extended spectrum of beta-lactamases have serine at their active sites. They share several highly conserved amino acid sequences with penicillin binding proteins (PBPs). Beta-lactamases attack the amide bond in the beta-lactam ring of penicillins and cephalosporins, with subsequent production of pencillinoic acid and cephalosporic acid, respectively, ultimately rendering the compounds antibacterially inactive.

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