DISCOVERY AND MECHANISM OF ACTION OF ANTIBIOTICS PRODUCED FROM SOIL BACTERIA: A REVIEW

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ABSTRACT: Antibiotics are chemicals that kill or inhibit the growth of bacteria and are used to treat bacterial infections. Antibiotics are produced in nature by soil bacteria and fungi. Antibiotics are also known as antibacterial and antimicrobial. Antibiotics prevent the bacterial cells from multiplying so that the bacterial population remains the same, thus allowing the host’s defence mechanism to fight the infection or kill the bacteria. The first true antibiotic ‘Penicillin’ was discovered by Alexander Fleming in 1928. After the discovery of Penicillin many researchers using the similar discovery and production techniques, discovered many other antibiotics in 1940s and 1950s i.e., streptomycin, chloramphenicol, erythromycin, vancomycin, etc. This review is focused on the classification, discovery and mechanism of action of antibiotics so that it will help the future researchers to find the better antibiotics to overcome the antibiotic resistance problem.

Keywords: Antibiotics resistant, soil bacteria, origin, mechanism of action.

INTRODUCTION

Antibiotics are one of the most important commercially exploited secondary metabolites produced by the bacteria that kill or inhibit the growth of other bacteria and are used to treat bacterial infections. Antibiotics are also known as antibacterial and antimicrobials (Kourkouta et al., 2018; Abbas et al., 2014) and are grouped together on the basis of their action. Each antibiotics works only against certain types of bacteria or parasites which is why different antibiotics are used to treat different types of infection. Like antibiotics there also exist antifungal and antivirals for fungus and virus infection as antibiotics doesn't work on fungal and viral infections.

Soil is a dynamic and excellent habitat for a huge variety of life forms. It is also proven by many theories and explanation that vast majority of novel antibiotics have been detected by screening of "wild isolates". These isolated microbes make many bioactive, which is why many antibiotic bacteria are produced from the soil and other natural habitats (Hutchings et al., 2019; Sethi et al., 2013). Microorganisms such as bacteria, fungi, Actinomycetes, algae and protozoa etc, are very small forms of life that can either be as single cells or as colonies of cells that functions in soil. Various factors such as temperature, salt concentration, pH, carbon sources etc. enhance the microbial population in soil (Begum et al., 2017; Mandal et al., 2019;). Also, many microorganisms exist in topsoil, where there is plenty of food sources rather than subsoil (Abbas et al., 2014).
Soil microbes have many essential roles in the ecosystem. These microorganisms are primary decomposer of organic matter. Beside this they also provide nitrogen through nitrogen fixation process to help growing plants, which also detoxify the harmful chemicals such as toxins, and also produce products that might stimulate plant growth (Salim et al., 2017).

Apart from all these importance, soil microorganisms have had another importance in human life and that is that they are the source of most of the antibiotic medicines which are used to fight several bacterial diseases. Natural soil contains over 109 microorganisms per gram or an acre of soil might contain approximately 130 pounds each of algae and protozoa, 890 pounds insects, nearly 900 pounds of earthworms and about 2000 pounds each of bacteria and fungi and thus soil is an ideal reservoir for bioactive micro biota, which is why soil bacteria plays a significant role in antibiotic discovery (Demain and Fang,2000; Abbas et al., 2014).

Today nearly 500 antibiotics are found each year and over 75-80% of the commercially and medically useful antibiotics have been obtained from soil isolates especially from the genus Streptomyces, Actinomycetes, Bacillus (MVS Sandhya et al.,2014; Abbas et al.,2014).

Studies have also suggested that bacteria are present in diverse ecological habitats and are easy to isolate, culture, maintain and to improve their strains (Babasaki et al., 1985; Amrita Saha et al., 2014). Because of the resistant endospore formation and production of vital antibiotics like Bacitracin etc., Bacillus species are the most predominant soil bacteria that inhibit the growth of other organisms (Abbas et al.,2014). In addition, almost 90% of Actinomycetes genera have been isolated from soil and are useful in industrial as well as pharmaceutical sections (Sapkota et al.,2020; Bawazir and Shantaram,2018). Therefore, there are many species such as Penicillin, Streptomyces and Bacillus that have been studied continuously to understand their ability to produce antibiotics (Brock TD and Madogan MT, 1991).

HISTORY OF ANTIBIOTICS

Origin and discovery

Selman Waksman known as ‘Father of antibiotic’ also used the word ‘antibiotic’ in 1941 for the first time which means any small molecule made by a microbe that antagonizes the growth of other microbes (Rafiq et al., 2018). An earlier trace of tetracyclines have been detected in human skeletons dredge in Nubia and during the Roman occupation of Egypt but origin of the tetracycline still remains a mystery (Basset EJ et al.,1980; Kate Gould, 2016). Probably Pyocyanase was the first antibiotic to be used to treat human infections. Rudolf Emmerich (1856–1914) and Oscar Löw (1844–1941) discovered that the green bacteria isolated from injured patients' bandages inhibited the growth of other microbes (Levy SB, 2002; Kate Gould, 2016).

Alexander Fleming (1881–1955) discovered penicillin in 1928, which is used as a treatment for bacterial infection and was possible through the work of Florey and Chain who managed to efficiently purify the antibiotic produced but according to various article published many researchers have also observed the mechanism of antibiotics (Gaynes, 2017). Like in 1870, Sir John Scott Burdon-Sanderson (1828–1905) described how culture fluid covered in mould inhibited the growth of bacteria. After a year, Joseph Lister (1827–1912) experimented with ‘Penicillium glaucum’ (sic) and demonstrated that it had an antibacterial effect on human tissues, and in 1875, Dr John Tyndall (1820–1893) presented his experiments with Penicillium notatum to the Royal Society. Finally, in 1897, Ernest Duchesne (1874–1912) observed Arab stable boys treating saddle sores with mould propagated on their saddles. He took this mould, confirmed as Penicillium notatum, and used it to successfully treat induced typhoid in guinea pigs (Fleming A, 1929; Porter R, 1999).

In 1910, the first antibiotic, Salvarsan was deployed. The introduction of penicillin marked the beginning of so-called ‘golden era’ of antibiotics. The discovery of antibiotics in just over 100 years have notoriously changed the existence of modern medicine and extended the average human lifespan by approximately 23 years (Hutchings et al., 2019). After streptomycin was isolated in 1944 from an organism found in soil i.e., Streptomyces griseus because of which a worldwide search began. Eli Lilly had the bright idea of asking...
Christian missionaries to send back a soil sample from every exotic place that they visited. A sample from Borneo sent in 1952 grew *Streptomyces orientalis*, from which vancomycin was eventually extracted; then in 1958 vancomycin became available for patients (Saga and Yamaguchi, 2009; Levine DP, 2006). Daptomycin was first evaluated in the late 1980s and was derived from a soil organism, *Streptomyces roseosporus*, which was obtained from Mount Ararat in Turkey (Eisenstein et al., 2010).

Figure 1. Timeline showing the decade new classes of antibiotic reached the clinic. The antibiotics are coloured per their source: green = actinomycetes, blue = other bacteria, purple = fungi and orange = synthetic. At the bottom of the timeline are key dates relating to antibiotic discovery and antimicrobial resistance, including the first reports of drug resistant strains: methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant enterococci (VRE), vancomycin-resistant *S. aureus* (VRSA) and plasmid-borne colistin resistance in Enterobacteriaceae (Adopted by Hutchings et al., 2019).

MODE OF ACTION

The mode of action of antimicrobial agents depends on the basis of their function. Antibiotics disrupt essential processes or structures in the bacterial cell. And depending upon these effects the antibiotics works in two ways that is either as (a) bacteriostatic - they will inhibit or stop the growth of bacteria and will keep them in a stationary phase of growth, for example tigecycline, linezolid, macrolides, sulphonamides, tetracyclines and streptogramins; or (b) bactericidal which means it kills the bacteria, for example β-lactam antibiotics, glycopeptide antibiotics, fluoroquinolones and aminoglycosides.

Antibiotics disrupt the essential processes or structures in the bacterial cell. The antibiotics that affect a wide range of bacteria are known as broad-spectrum antibiotics and which affect only a few types of bacteria known as narrow-spectrum antibiotics.
The mode of action of antibiotics are as follows:

(i) inhibition of the cell wall synthesis;
(ii) inhibition of nucleic acid synthesis;
(iii) inhibition of folate metabolism;
(iv) inhibition of protein synthesis (Etebu and Arikekpar, 2016).

The major target sites of bacterial cell are shown in Figure 2.

**Figure 2** showing targets of bacterial cell (Adopted from Etebu and Arikekpar, 2016)

**Inhibition of cell wall synthesis**

Cell walls of microorganisms are constructed by peptidoglycan. Being the outermost and primary component of the wall, peptidoglycan layer is most important for cell wall structural integrity. B-Lactam antibiotics are bactericidal and act by inhibiting the synthesis of the peptidoglycan layer of bacterial cell walls. Glycan polysaccharide strands are linked by crosslink that bind polypeptides bound to N-acetyl muramic acid (NAM) of each polysaccharide strands. The D-alanyl-alanine portion of peptide chain is cross linked by glycine residues in the presence of penicillin binding proteins (PBPs). This cross-linking strengthens the cell wall. β-lactams and the glycopeptides inhibit cell wall synthesis (Garima Kapoor et al., 2017, David C. Hooper, 2001). β-Lactam antimicrobials form covalent linkages and are known to interact with transpeptidases directly because of which their actions can be studied by use of labelled β-lactam molecules to tag the enzymes, which are also designated penicillin binding proteins because of this property. β-Lactams as transpeptidase inhibitors thus block the conversion of immature to mature peptidoglycan. Because many bacteria have several distinct but essential transpeptidases, β-lactam resistance by target alteration requires alteration of several targets, making development of high-level resistance by mutation unlikely (Goffin and Ghuysen, 1998; Hakenbeck et al., 1999).

The glycopeptides bind to D-alanyl D-alanine portion of peptide side chain of the precursor peptidoglycan can subunit. Vancomycin produces a block at the same step as the β-lactams by producing steric hindrance to
transpeptidase action, thereby preventing conversion of immature to mature peptidoglycan. Thus, this large drug molecule vancomycin prevents binding of this D-alanyl subunit with the Penicillin binding protein, and hence inhibits cell wall synthesis (Garima Kapoor et al., 2017).

Inhibition of nucleic acid synthesis
Several bacteria use enzymes that do not exist in human cells for replication. Fluoroquinolones inhibit DNA gyrase, which is needed for bacterial DNA replication. Fluoroquinolones nicks the double-stranded DNA then introduces negative supercoils and reseals the nicked ends. This is necessary to prevent excessive positive supercoiling of the strands when they separate to permit replication or transcription. In Gram-positive bacteria, the major target of action is topoisomerase IV which nicks and separate’s daughter DNA strand after DNA replication. (Garima Kapoor et al., 2017; Liwa and Jaka, 2015).

Also, nucleic acid synthesis is dependent on folate i.e., folic acid but sulphonamides inhibit bacterial folate synthesis by acting as an antimetabolite which inhibits metabolic pathways. Sulfonamides competitively inhibit the conversion of pteridine and p-aminobenzoic acid (PABA) to dihydrofolic acid by the enzyme pteridine synthetase. Sulfonamides have a greater affinity than PABA for pteridine synthetase (Liwa and Jaka, 2015).

Agents such as trimethoprim act at a later stage of folic acid synthesis and inhibit the enzyme dihydrofolate reductase (Garima Kapoor et al., 2017).

Inhibition of protein synthesis
The process of mRNA translation occurs over three sequential phases i.e., initiation, elongation and termination which involves the ribosome and a host of cytoplasmic accessory factors (Garrett, 2000). The ribosome organelle is composed of two ribonucleoprotein subunits, the 50S and 30S. Drugs that inhibit protein synthesis are among the broadest classes of antibiotics and can be divided into two subclasses: the 50S inhibitors and 30S inhibitors.

Inhibitors that bind to the 30S ribosomal subunit interfere primarily with initiation process, although some also interferes with pairing of the mRNA codon with the AA-tRNA anticodon, and therefore results in impairing elongation. Inhibitors that bind to the 50S ribosomal subunit i.e., to the elongation factors, which are transiently linked to ribosome at certain steps of the cycle, which interferes with steps involved in the elongation process (Cocito et al., 1997).

Through analysing it’s clear that 30S binders such as Aminoglycosides like Gentamicin, Amikacin, Tobramycin etc bind irreversibly to 30S subunit of ribosomes, whereas 50S binders can bind to 50S subunit in following ways:

a. **Binding to peptidyl transferase**
   Some antibiotics bind to the peptidyl transferase component of 50S ribosome thereby blocking peptide elongation.
   Ex: Chloramphenicol

b. **Inhibitors of amino acid-acyl-tRNA Complex binding**
   It includes those antibiotics which bind to 50S subunit in such a way that they block the binding of amino acid-acyl-tRNA complex and hence inhibit peptidyl transferase action and hence peptide elongation. Ex: Clindamycin (Ahamed Basha, ICAR).

c. **Reversible Binders**
   These are also known as bacteriostatic as they bind to 50S subunit in a reversible manner to temporarily block peptide elongation. Example: Macrolides like Azithromycin, Erythromycin, Roxithromycin, Clarithromycin etc.
d. t-RNA binding blockers

This class of antibiotics block the binding of tRNA to 30S ribosome-mRNA complex. Tetracyclines like doxycycline, minocycline, plain tetracycline etc (Ahamed Basha, ICAR).

ANTIBIOTIC RESISTANCE

Alteration in the target sites of antibiotics is a common mechanism of resistance. Target site changes often result from spontaneous mutation of a bacterial gene on the chromosome and selection in the presence of the antibiotic. Antimicrobials are one of the most successful forms of therapy in medicine, however the efficiency of antimicrobials is compromised by a growing number of antibiotic resistant pathogens (Lambert PA, 2005). Resistance can be described in two ways:

Intrinsic resistance is when a bacterial species is naturally resistant to a certain antibiotic or family of antibiotics, without the need for mutation or gain of further genes.

Acquired resistance is said to occur when a particular microorganism obtains the ability to resist the activity of a particular antimicrobial agent to which it was previously susceptible.

Mechanisms of acquired resistance include the presence of an enzyme that inactivates the antimicrobial agent, post- transcriptional or post-translation modification of the antimicrobial agent’s target, reduced uptake to the antimicrobial agent and active efflux of the antimicrobial agent (Dowling et al., 2017).

CONCLUSION

The discovery of antibiotics was a must needed dose for the harmful bacteria or microbes. Today we are in a peace knowing that several infections can be controlled or treated through these antibiotics. But as time is passing a problem of antibiotic resistance is also arising. Therefore, discovering the new and novel antibiotics is need of an hour. Understanding the concept of antibiotic mechanism will help further upcoming researchers and also those who are in the medicinal field.

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