Editorial

Antibiotics Resistance and Stewardship

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Since Alexander Fleming has discovered antibiotics (penicillin) in 1928 a new era of treatment of many diseases has started. Treatment of lots of diseases takes a new step, which in turn improves morbidity as well as mortality. In old ages, a human had to face many epidemics and pandemics of infectious diseases like typhus, cholera, HIV/AIDS, smallpox, measles, Spanish flu, tuberculosis, etc. In all the above-mentioned epidemics and pandemics millions of people died. We are still facing one of the biggest pandemics of the human race (COVID 19). A plethora of work is being done on treatment and prevention by vaccination but the average number of patients of COVID 19 is still increasing due to the virus's ability to mutate.[1]

Nowadays different types of antibiotics are being used from the range of infections like pneumonia, UTI, tuberculosis to the treatment of carcinomas and organ transplantation. Unnecessary usage of these is making a bacterium resistant to it.

Antibiotic is the chemical agents which are used against the bacteria by killing it (bactericidal) or by inhibiting its growth (bacteriostatic). Examples of Bacteriostatic antibiotics are chloramphenicol, ethambutol, macrolides, etc. and bactericidal antibiotics are penicillin, cephalosporin, vancomycin, etc.

Just like the other medications, antibiotics also have side effects like clostridium difficile infection caused by unnecessary intake of antibiotics like cephalosporin, clindamycin, etc.

Due to unnecessary usage of antibiotics and use not as prescribed, individually as well as hospital-based, bacteria are becoming resistant to these drugs. Antibiotics resistance is the most serious medical issue being faced nowadays globally, MRSA, VRSA, and MDR are some of the examples of resistance against antibiotics. A lot of work is being done nowadays to make a better healthcare system that can supervise and improve antibiotics usage. It may be difficult because every physician or surgeon has his variable way of prescribing antibiotics according to diseases and areas even with the presence of gold standard rules.

According to CDC, almost 23000 people die per year in the USA with the infection of multi-drug resistant bacteria. As the use of antibiotics increases, it becomes essential to do the check and balance of it by doing supervision. CDC in 2006 provides guidelines regarding antibiotics use in the Management of Multi-drug resistant organisms in a hospital. In 2009 CDC gave a (Get smart to healthcare campaign) to guide regarding antibiotics use in emergency care setups.[2]

The antibiotics stewardship program (ASP) was given by CDC in 2007 and further modified in 2016 helps a lot in managing the use of the antibiotic in many hospitals. Following mechanisms are used by the antibiotics to kill the bacteria.

The cell wall is an essential part of many bacteria. This class of antibiotics disrupts the cell wall by acting on the peptidoglycan (an essential part of the bacterial cell wall), which in turn destroys the bacterial cell as cell wall is the imperative part of only bacteria not of human, so the drugs which are used against the cell wall of bacteria do not affect the human

cell, making it safe for a human to a certain level. Examples of cell wall antibiotics are B-lactams, bacitracin, vancomycin, etc.

The cell membrane is the important part of bacterial structure this antibiotics class disrupts the cell membrane by acting on the phospholipid of the membrane, causing the leakage of cytoplasmic content, as a result, bacteria die. As cell membrane is also part of human cells so these antibiotics can cause harm to human normal cells. That's why these antibiotics cannot be used systemically, and are given at the local wound side. For example polymyxin.

Antibiotics act on the 50S or 30s subunits of ribosomes causing decreased protein synthesis by hindering the process of translation (a process in which proteins are formed by translating the mRNA). These proteins not only give strength to the structure of bacteria but also important for the survival of bacteria. After the attack of antibiotics on bacterial protein synthesis, the growth of the bacteria stops, making the antibiotics bacteriostatic. For example tetracycline, macrolides, etc.,

Some of the Antibiotics of this class act on nucleic acid synthesis. It exactly acts on the topoisomerase 2 (an enzyme that relaxes the bacterial DNA before its replication) for example quinolones. Antibiotic of this class also affect the RNA polymerase and causes stoppage of bacterial growth for examples rifampin. Some of the group of antibiotics which acts on the DNA and causes cell destruction are also being used in the cancer treatment because these antibiotics also affect the human's rapidly dividing cell (cancerous cell). For example bleomycin, doxorubicin, etc.

These antibiotics inhibit folic acid synthesis. Folic acid is essential for the production of certain nucleotides which are used to make the DNA. Bacteria have to form their folic acid intrinsically, on the contrary to humans as a human can take it from food and can store it in the body. These antibiotics competetively inhibit the attachment of para-amino benzoic acid (PABA) with folic acid and prevent its synthesis. The above-mentioned mechanism is shown by sulphonamide. While secondly, an anti-biotic named trimethoprim reversibly attaches with the dihydrofolate reductase and prevents its function (reduction of dihydrofolic acid to tetrahydrofolic acid).

Resistance of bacteria from antibiotics is a continuous ongoing process. It takes time and multiple factors. It can be multifactorial because bacteria also advantage of their environment. Or it can be entirely due to intrinsic characteristics.

There are important ways that are used by bacteria to make themselves resistant.

1. Mutation
2. DNA (gene) incorporation

Mutation occurs naturally and specific to some sort of antibiotics. In it, some members of the bacterial population developed such a mutation in its genome which helps that specific bacterial population to survive even in the presence of antibiotics. By that mutation, they evolve special ways to tackle the antibiotic and survive. These special ways are, change in the active target site of bacteria where antibiotic is to bind, inactivation of an antibiotic molecule, efflux of antibiotics by special channels, etc. So this special bacterial population that becomes mutant remains safe and the rest of the bacteria are killed by antibiotics (showing the survival of the fittest considering the dwarven theory). Now mutant population proliferates and the whole new population is mutant and resistant to antibiotics. Here important point to note is, if an individual is taking broad-spectrum antibiotics for a disease in which broad-spectrum antibiotic is not recommended or not taking it as prescribed by the physician for some infection, he is making infectious agent causing disease more powerful by giving it favorable environment to become mutant to a broad-spectrum antibiotic.

Gene transfer is the most important mechanism to generate resistance in bacteria. It an evolutionary process, every bacterium has its special ability to do the transfer genetic material. Antibiotics which we form are mostly a special organic source, the bacteria also somewhere are the part of the same source showing the source of antibiotics and bacteria share a common niche.

This helps bacteria to exchange some of the genetic material from the source of the antibiotic or other bacteria. Bacteria develop exceptional power to trade the gene from its environment as well as from other bacteria.

It happens commonly by following three ways

1. Ancorporation of DNA
2. Transduction
3. Conjugation

Only some of the special types of bacteria can do the natural transformation of the gene for the development of resistance. In a hospital setup, where there can be a lot of bacteria due to the presentation of various patients with different infections, the most common mode of gene attachment to get resistance is conjugation instead of naked DNA transfer which is comparatively a simple way of genetic material relocation. In humans, GIT tract, when some bacteria are naturally present and others are there to cause disease, in the presence of antibiotics bacteria achieve suitable environment to make the gene pass on by conjugation process, which occurs by the cell to cell union. Mobile genetic substances are the substances that are used as a conveyance in the conjugational process. These are plasmids and transposons. These mobile genetic elements play an important part in the progress and spread of bacterial resistance. Integration is also an efficient way to transport genes in the form of the genetic cassette to attach a new gene to chromosomes along with the requirements necessary for its impression.

Bacteria evolve a lot of different methods to obtain resistance against antibiotics. a single bacterium can have a single method as well as more than one method in it, more than one methods are there to support the resistance of the single way or enhance it. For example, fluoroquinolones possess three methods to deal with the antibiotic resistance 1: change of the attachment site of the fluoroquinolones (DNA gyrase and topoisomerase 4) 2: increases the number of pumps which are there to throw out the substances (antibiotic) 3: making a protein which can be present on the target site as a guard to prevent the attachment of antibiotic. Sometimes bacteria prefer to stay stick on a single way to get resistance, even in the presence of more than one method in it. For example in B-lactam bacteria, penicillin enters the bacterial cell and attaches with the penicillin-binding protein, and continues further to do the cell destruction. In gram-negative bacteria B-lactamase enzymes are produced to destroy antibiotics, bacteria control the antibiotic concentration coming inside the cell by different porin molecules present on the outer membrane and get enough time to produce a sufficient amount of B-lactamase enzymes inside. But in gram-positive bacteria, penicillin-binding site, a target site for an antibiotic is changed to achieve the goal of resistance, but some gram-positive bacteria also generate the B-lactamase enzymes. Bacterial resistance mechanisms are divided into four various sections to explain how bacteria get resistant. These are 1: change in antibiotic structure by doing an addition of some new molecule in it and destroying it 2: stopping the antibiotic to reach the active site 3: target site modification 4: cell modification as a whole.

Bacteria have a special ability to produces such enzymes, which can help to create resistance. These enzymes cause additions of some specific molecules in the antibiotic which make it nonfunctional. The actions which bacteria carry to cause the structural change in antibiotic are acetylation, acetylation, and phosphorylation. Acetylation is mostly related to the following antibiotics, these are aminoglycosides, chloramphenicol streptogramins. Acetylation is done in following antibiotics to make them incapable to work, are lincosamides and aminoglycosides. Phosphorylation of chloramphenicol and aminoglycosides are done to make them powerless. All the above-mentioned ways are used to prevent the reach of antibiotics to reach the action site. One of the most important examples of enzymes that causes structural changes in antibiotics is aminoglycosides modifying enzymes. These are acetyltransferases, adenyltransferases, and phosphotransferases. The gene for these enzymes are obtained by mobiles genetic substances and are also present in the chromosomes of some bacteria like enterococcus faecium. These aminoglycosides modifying enzymes causes the changes in hydroxyl and amino groups of aminoglycosides making it impotent for bacteria. These enzymes are somehow also related to different bacterial classes, e.g. phosphoryl transferases are mainly present in both of the gram-stained species, and it affects streptomycin and kanamycin but does not affect the tobramycin and gentamycin. And similarly, aceytranasferases are mainly present in gram-negative bacteria especially in Enterobacteriaceae, and pseudomonas affects amikacin and gentamycin. Showing the diversity of resistance, bacterial gene coding is also different in various bacteria even for the same enzyme. Tn400I like transposon, a genomic area on which a special type of gene is present. These genes are encoded for

the formation of an enzyme which two functions at the same time, this enzyme can cause acetylation and phosphorylation at the same time making bacteria more powerful to show resistance against antibiotics. This enzyme is present in famous resistant bacteria like MRSA and VRSA, it makes the bacteria resistant to almost all the aminoglycosides except streptomycin. Similarly, an antibiotic is known as chloramphenicol acts through inhibiting the protein formation in bacteria by acting on the peptidyl transfer of 50S subunit of the ribosome. An enzyme known as chloramphenicol acetyltransferase is produced by both gram-positive and gram-negative bacteria make them resistant to it. The genome coding for these enzymes are mostly acquired by bacteria by mobile gene element or already incorporated in chromosomes of some bacteria.

Resistance against antibiotics is commonly generated by destroying the amide group of the B-lactam of antibiotics, this, in turn, destroys the antibiotic molecules. This demolition of the amide group is done mainly by an enzyme produced by certain bacteria called, B lactamase. It was discovered even before the proper launching of penicillin in the market in 1940. Similarly, when penicillin was being used widely, bacteria evolve and they start gene-rating the B-lactamase against it and was mainly being done by the Staphylococcus aureus. Genetically its was seen that plasmid was the route that was being used by staph aureus to create resistance in many other bacteria. Then scientists generated another antibiotic which was having other types of B lactam. That antibiotic was ampicillin. But in 1960, another B lactamase was obtained which was having the capacity to destroy ampicillin, and it was obtained from a patient named temoneiria so the plasmid which was carrying it was named on the patient's name by TEM-1. So from 1960 scientists started to make new B-lactams and bacteria kept on creating B-lactamase against it by mobile gene elements. The gene which was generating B-lactamases was named the BLA gene, it was the part of the extra chromosome of bacteria and it was also getting spread by integrations.

Almost 1000 B-lactams have been discovered till now, these are divided by two ways. Ambler classification was based on the sequence of amino acids, while the second classification is named Bush-Jacoby. It was based on the functionality and substrate used. Amblers classification divides the bacteria into 4 different groups. These are classes A, B, C, D. Most of the B-lactamases which was are active against extensive broad-spectrum antibiotics will fall in ambler class A. Now bacteria evolve enzymes from the enzymes like an enzyme generated from the TEM-3, it evolves from the TEM-1 penicillin's and had specific power to hydrolyze the 3rd generation cephalosporin and penicillin and monobactum but does not affect cephomycin and carbapenem. ambler class A is inhibited by the tazobacum and clavulanic acid. This characteristic helps to separate from amber class C, which works against 3rd generation cephalosporin but is not inhibited by clavulanic acid and tazobactum. There is another large group of enzymes that are against carbapenem is called carbapenemase. There are divided into serine carbapenemase and metallo carbapenemase. Serine includes ambler class A and D, while Metallo carbapenemase inclu-des ambler class B.

CTX-M is another enzyme that is present in Klebsiella pneumonia, E-coli and other enter bacteria, and this enzyme is the best example of taking a gene from the environment and use it against antibiotics. It takes the genome from an environmental bacterium klayuvera spp. which has no pathologic effect on a human. it works by horizontal gene transfer, and it is the cause of the most widely spread resistance against cephalosporin.

Class B enzymes are Metallo-lactamases, they have the quality to work against carbapenem as well. These enzymes used the metallic ion (zinc) to destroy the lactum ring, and are inhibited by EDTA.

Class C lactamases are the enzymes that make bacteria resistant against penicillin, cephalosporin with cephamycin, and it is not inhibited clavulanic acid.

Class D lactamases can hydrolyze the oxacillin and are poorly inhibited by clavulanic acid. it has many variants, OXA-11 for pseudomonas ariginosa. OXA 48, OXA 23, etc. are prevalent in many clinical species, OXA-23 and OXA 58 are now spreading worldwide.

Many antibiotics have their target site inside the cell. The antibiotic has to go inside the cell by crossing the membrane to express the function. So bacteria develop different methods to decrease the intake of antibiotics and if molecules of antibiotics get inside,

bacteria try to throw it out. it is specifically shown by gram-negative bacteria. Hydrophilic antibiotics will be unable to directly cross the outer membrane and will need the channel to go inside bacteria do some changes in the porin to prevent the entry of antibiotics. For example, special proteins are produced by E coli to get them attached to porin to make the outer membrane-impermeable or less permeable for antibiotics, these proteins are OmpF, OmpC, and PhoE. Pseudomonas shows OprD.

Bacteria have to generate a piece of machinery to throw an antibiotic out of the cell. Special pumps are formed which efflux of the antibiotics. It was first seen in 1980 when, the pump was discovered to throw the tetracycline out of the cell by E´coli by the action of special gene tet gene, as well as the mef gene shown by pneumococci

There are five major families of efflux pump. 1: major facilitator superfamily 2: small multidrug family 3: resistance nodulation cell division family 4: ATP binding cassette family 5:

Multi drugs and toxic compounds exclusion family.

Many antibiotics have to act by going inside the cell. As it goes inside attaches to its target side, but here bacteria form some protein and send them to the target cell, blocking the site. For example for tetracycline, antibiotics went inside the cell trying to act on the target site but bacteria by genes Tet-M produces such a protein which compete for the site for antibiotic and block the target site and antibiotic expression cannot be done showing resistance. Similarly, Tet-O changes the structure of the target site so the antibiotic cannot attaches to it. Similarly, fluoroquinolones Qnr acts on DNA gyrase and topoisomerase inside the cell. The Qur faces resistance after the gene expression, these genomes are gyrA-GyrB and parC, parE for DNA gyrase and topoisomerase IV respectively. Similarly, linezolid is a grampositive antibiotic and mutation occurs and causes domain Vof rRNA changes to get resistance. Another way to alter the target site is enzymatic. The most common enzymatic change is the methylation of the ribosomes by an enzyme that is produced after erm (erythrocyte ribosomal methylation) gene expression. As the site of action of macrolides, linc-osamide and streptomycin B is almost at the same point in ribosomal RNA, erm gene expression, make a bacteria-resistant from all three of these group. Once the erm gene is expressed sometimes bacteria from an untranscribed mRNA which is almost inactive in the absence of antibiotic, it becomes ready for translation whenever antibiotic comes, and start the translation and make proteins, so bacteria get resistant rapidly due to the presence of already made mRNA. This kind of erm expression is shown in well -known MRSA and other gram-positive organisms. Cfr gene-mediated resistance against linezolid is another example of enzymatic change in the target area. The presence of Cfr mediated enzymes makes a bacterium resistant to not only linezolid but also to phenols, streptogramin, and lincosamide. Sometimes bacteria create an alternative to the target area, which is inactive and antibiotics cannot work on it so became useless. Bacteria containing the mecA gene, mostly seen in MRSA, generate PBP2 a fake target site in place of PBP (normal target site on cell wall) and make bacterium resistant to B-lactams, penicillin. The presence of the Van a gene in the bacteria makes many bacteria resistant to vancomycin. Vancomycin acts on the cell wall and kills the bacterial cell after destroying its cell wall. So bacteria transport a special type of gene through HGT or plasmid from other bacteria and studies show, it comes from an environmental bacteria which is not pathogenic for humans through plasmids. In this way bacteria bypass the site of action making themselves resistant. Many variants are there now of van gene, these are van X, van B, etc.

In this type of resistance generation, bacteria do special changes in many areas of the cell to get survival from the antibiotics as it is always too susceptible to get targeted by the host immune system and antibiotics. For example daptomycin, an antibiotic acts in three ways .1 chelation with the calcium 2 oligomerization of daptomycin-calcium complex on outer and then inner surface of the cell membrane through cardiolipin .3 in the cell membrane, it acts as a pore to make a channel between the inner and outer layer of bacterium which changes the electrochemical gradient of cell and causes destruction of the cell. To get resistance from it, bacteria develop a system which is known as LiaFSR. It is a system of three proteins LiaF, LiaS, and LiaR. This system works collectively and negatively charges the cell, and increases the minimal inhibitory concentration of the antibiotic, and dispersion of cardiolipin over the cell membrane, which cannot work with the DAP and make the bacterium resistant to it.

Supervision of antibiotic usage has become very imperative because it's really necessary to check the proper dosage and to see whether it is recommended or not. In hospital setup as well as in outdoor, a patient can present with many different infectious diseases. Unnecessary and over usage of antibiotics causes the bacteria resistant to it. Antibiotic resistance is one of the most crucial issues of public health nowadays. The reason behind this is the prescription of antibiotics even if it's not essential which causes resistance and less discovery of new antibiotics as compared to resistance.

In many countries usage of antibiotics can only be done after the prescription of expert doctors. But in some other countries, antibiotics can be taken over the counter.

Not knowing about side effects and easy availability makes a person susceptible to do self-medication. In self-medication for any infection, the patient tries to take a broad-spectrum antibiotic. In this way, the patient got better in his / her symptoms but it is helping a bacteria to get resistant. Similarly, in tertiary care hospitals, many different antibiotics are being used for many diseases, so hospitals became the habitat of many organisms, in turn helping bacteria to get resistance. It is essential to prevent resistance of bacterium by full energy.

Some of the important elements were presented by CDC in struggle, to cover the issue of antibiotic resistance. These are

Leadership for stewardship is necessary to start different programs at a smaller level and should have leadership quality to use all kinds of resources in the given time. He/she may be a clinician

Accountability is essential regarding the management of resources including financial matters.

The pharmacy expert is also a co-leader and provides an expert opinion regarding the drugs used in programs.

Action should be taken to implement an intervention, take the feedback and preauthorization for preferring antibiotic use.

Tracking the prescription of antibiotics, help to do the intervention, and consider the outcome of the prescription is also an important part of the stewardship of antibiotics.

Responses of the use of the antibiotic and its outcomes such as resistance. These reports should be sent to the pharmacist, nurses, etc.

Educate the people around you like the prescriber, pharmacist, and nurses regarding the adverse effect, and proper usage.

Prospective audit, feedback, and preauthorization are the most important factors which can be used to start the evidence-based treatment. For example, a prospective audit is done on clostridium defficle causing pseudomembranous colitis is done showing the cause is the consistent use of antibiotics. Preauthorization will be done to start a new drug. Then action will be done to implement. Then feedback should be taken to see the effects of the new drug after usage. The graph shows the development of new antibiotics production between2013-2019.

Conclusively in developed countries stewardship works differently as compared to developing countries.

In developing countries, common people should be educated regarding the use, merits, demerits, and adverse effects of antibiotics. They should be educated that antibiotic is the medicines which should not be self-medicated.

Another issue is the availability of the drug. It should not be easily available. The sale of over-the-counter antibiotics should be stopped.

New possible ways should be considered regarding the production of a new antibiotic. Because bacteria now have developed resistance against current using antibiotics.

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