Antibiotic Resistance of Bacteria: A Global Challenge

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Bacterial resistance to antibiotics poses a serious challenge to the prospect of chemotherapy. Rational use of antibiotics is most desirable but it cannot provide a permanent solution to the problem. In this article the biochemical and genetic basis of antibiotic resistance in bacteria is discussed with examples. The non-clinical aspects of antibiotic-resistance are also dealt with in brief.

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1. The Wonder Drugs

Antibiotics constitute one of the most significant contributions of modern science. The discovery of these life-saving drugs transformed the health-care scene during the last century. A significant decline in the fatality rate of many diseases was noticed after the introduction of antibiotics into clinical practice. For example, 20 to 85% death rate due to pneumonia in the US during the 1930s came down to about 5% in the 1960s, 100% death due to chronic infection of a heart valve was reduced to 5%, 20 to 90% fatality due to epidemic of spinal meningitis infection caused by the bacterium *Neisseria meningitides*, was decreased to almost 2%. Antibiotics help not only in curing the diseases but also in their prevention. Penicillin, for instance, prevents throat infection caused by *Streptococcus* and thereby prevents recurrences of rheumatic fever in susceptible individuals. Onset of a sexually transmitted disease may be prevented by the timely use of the proper antibiotic. By eliminating the causative organism of diphtheria from carriers (people harboring the organism without falling sick) using an antibiotic, the chances of dissemination of infection could be effectively reduced.

2. What They Are

In 1942, Selman Waksman defined antibiotics as low molecular

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weight chemical substances produced by microorganisms, having growth-inhibitory effects on other microorganisms in high dilution. However in the present context, any chemical substance either of microbial, synthetic or semi-synthetic origin, used in the clinical treatment of infections, is called antibiotic. Thus streptomycin produced by fermentation is an antibiotic. The sulpha drugs and quinolones, produced by chemical synthesis, are antibiotics. Ampicillin (semisynthetic penicillin), produced partly by fermentation and partly by chemical synthesis, is also an antibiotic. The discussion in this article will be confined to the antibacterial antibiotics.

The significance of antibiotics in nature remains a riddle. They are secondary metabolites and therefore are not indispensable for sustenance of life of the producer organism. The widely- believed notion about their role in preventing the growth of other organisms which thrive on the same source of food in natural environments, is essentially anthropocentric and yet to be validated. The role of antibiotics as signaling molecules in natural ecosystems has been suggested by some scientists.

3. Historical Background

Though the use of microorganisms for the control of infections was in vogue even in ancient civilizations, the modern era of antibiotics was ushered only at the beginning of the twentieth century. During the screening of hundreds of synthesized organic arsenical compounds for efficacy in the treatment of syphilis, salvarsan was discovered by Sahachiro Hata in 1909 in the laboratory of Paul Ehrlich, the German Nobel-laureate. Prontosil, the first sulfonamide drug, was developed by Gerhard Domagk in 1932 for commercial use. The serendipitous discovery of penicillin by Alexander Fleming at St Mary's Hospital (UK) in 1928 and the subsequent isolation and purification of the compound by Florey and Chain made a giant stride in the field of chemotherapy. Availability of such a compound with activities against a wide range of infectious organisms and (unlike sulfonamides) no loss of activity in the presence of biological materials such as pus,

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provided a distinct advantage to the physicians in controlling bacterial infections. Discovery of streptomycin, the antitubercular antibiotic, by Albert Schatz, a graduate student in the laboratory of Selman Waksman (associated with the discovery of a series of antibiotics in the 1940s and 1950s) at Rutgers University (USA) in 1943, led to the idea that all the infections could be controlled whenever required. Major pharmaceutical companies started diverting substantial portion of their funds, earmarked for research on anti-infectives, to the development of other drugs.

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4. Emergence of Antibiotic-Resistant Bacteria

The euphoria did not last long. Within four years following the introduction of penicillin during the Second World War, occurrence of resistant strains was reported. According to an estimate by the The Centers for Disease Control and Prevention (USA), 13,300 patients died of antibiotic-resistant bacterial infection in the US during 1992. An incredible 150% increase in the occurrence of drug-resistant *Pneumococci* was noted between 1987 and 1994. A 20-fold increase in the frequency of hospital-acquired *Enterococci*, resistant to vancomycin, was seen between 1989 and 1993. The frequency of methicillin-resistant *Staphylococcus* rose from 2% in 1975 to 32% in 1992. By this time, resistance to virtually all the therapeutically useful antibiotics had been evidenced. Emergence of methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE) have raised serious concern all over the world since these two antibiotics were believed to be invincible when they were released in the market. A couple of years back, some Gram-negative bacteria (*Escherichia coli, Klebsiella pneumoniae)* were found to produce an enzyme, which confers resistance to virtually all the commonly used antibiotics including carbapenems, one of the last resorts in the clinical management of infections caused by multidrug-resistant organisms. The organisms were believed to be originated from India. That is why the enzyme was named New Delhi Metallo β-lactamase (NDM-1). Some of the other most important types of multiple drug-resistant organisms include extended-spectrum beta-lactamase producers (which are resis-

tant to cephalosporins and monobactams) and penicillin-resistant *Streptococcus pneumoniae*. Studies on resistance in soil bacteria have been shown that these organisms serve as a reservoir for multiple antibiotic resistances. A recent analysis of 264 soil isolates obtained from different natural habitats in and around Hyderabad has identified 5 isolates resistant to as many as 10 antibiotics. During the recent past, soil metagenomics also revealed several aminoglycoside resistances in nonculturable bacteria. Notwithstanding the availability of so many antimicrobial agents, infectious diseases still remain the second leading cause of death worldwide. Eventually, the widespread occurrence of antibiotic-resistant bacteria has added a new dimension to the emerging threat of bioterrorism.

5. Genetic and Biochemical Basis of Antibiotic Resistance

5.1 *Genetic Basis*:

5.1.1 ***Spontaneous Mutation and Gene Transfer***: Antibiotic-tolerance in bacteria emerges as a result of error in DNA replication, a phenomenon known as spontaneous mutation having a frequency of one in 10^7 cells. During the epidemic of *Shigella* infections in Japan during 1950s, it was observed that bacteria could transfer copies of antibiotic resistance genes to susceptible bacteria thus making the latter antibiotic-tolerant. The mechanism predominantly used for this purpose is conjugation[1], a powerful gene-mobilizing mechanism. The other known mechanisms of gene transfer among bacteria, e.g., transformation[2] and transduction[3] also play some role in dissemination of antibiotic resistance. Resistance thus acquired is transferred to the progeny cells by a process called vertical gene transfer. Another process, called horizontal gene transfer[4], enables bacteria to transfer copies of antibiotic resistant genes even to the distantly related species in their neighborhood and thus contributes significantly in dissemination of resistance. Different types of such resistant genes, when accumulated in a single cell, result in multi-resistant phenotype.

[1] A process of gene transfer in bacteria facilitated by direct uptake of exogenous genetic material (such as plasmid DNA) from a related strain in the surroundings. Transformation takes place naturally in some species and can also be attained by artificial means. This technique is being used very frequently in the field of biotechnology for the purpose of cloning.

[2] Another process of bacterial gene transfer mediated by bacteriophages which carry DNA from one cell to another. This process is known to occur in several bacterial species such as *Salmonella, Escherichia, Micrococcus* etc.

[3] The process which involves the transfer of genetic material from one bacterial cell to another by direct cell-to-cell contact through a tube-like structure known as pilus.

[4] The movement of genetic material between unrelated species of bacteria. This can happen through direct incorporation of free DNA by bacterial cells. This direct form of gene transfer, for instance in the soil or in the digestive tract of animals, is the most common mode for the transfer of genetic material. It fosters rapid dissemination of antibiotic resistance in the bacterial community. Horizontal gene transfer can happen either through transformation, transduction or conjugation.

Mobile genetic elements, viz., transposons[5] and integrons[6] can also transfer antibiotic resistance genes *en block* to susceptible bacteria. Resistance genes mostly reside on bacterial plasmids. However, in some cases they are also found to be located on chromosomes and in a few cases organized in operons. It was believed earlier that antibiotic-resistant strains were less competent to survive in the environment and are slow-growers compared to their antibiotic-sensitive counterparts, but subsequent studies have established that such disadvantages might be counterbalanced by additional compensatory mutations.

5.2 *Biochemical Basis*

5.2.1 ***Drug Inactivation by Microbial Enzymes***: Among several mechanisms involved in the development of antibiotic resistance, drug modification plays a significant role in rendering many therapeutically useful drugs useless. For example β-lactamase, an enzyme elaborated by many Gram-positive and some Gram-negative bacteria, converts penicillin into penicilloic acid, which is therapeutically inactive. The production of the enzyme is inducible in Gram-positive bacteria whereas it is constitutive in Gram-negative bacteria. This reaction also forms the basis of chemical assay of the antibiotic since penicilloic acid can be titrated with iodine. The enzyme also provides a therapeutic target in the management of infections caused by penicillin-resistant bacteria. Likewise, chloramphenicol is converted to the therapeutically inactive compound 1, 3-Diacetoxychloramphenicol by chloramphenicol acetyl transferase (CAT), produced by some resistant bacteria. N-acetylation of kanamycin by a bacterial enzyme leads to tolerance of the organism to the antibiotic.

5.2.2 ***Modification of Target Site***: The other mechanism of antibiotic-resistance e.g., modification of the target is best exemplified by streptomycin and erythromycin resistance of bacteria. Both of these antibiotics act by ribosomal binding thereby inhibiting bacterial protein synthesis. Modification of the S12 protein of the 30S subunit of the ribosome makes the ribosome insensitive to streptomycin. Mutations affecting protein L4 or L12 of the

[5] **S**mall segments of DNA which can hop from one DNA location to another. It is found as part of a bacterium's nucleoid (conjugative transposons) or in plasmids. It contains a number of genes, coding for antibiotic resistance or other traits, flanked at both ends by insertion sequences called transposase, which catalyzes the cutting and resealing of the DNA during transposition. Thus it is able to cut itself out of a bacterial nucleoid or a plasmid and insert into another location leading to the transmission of antibiotic resistance among a population of bacteria.

[6] A mobile genetic element, which contains sitespecific recombination system capable of capturing and mobilising gene cassettes. It is composed of an *intI* gene encoding an integrase, a recombination site *attI*, and a promoter (5' to the cassette) that mediates expression of the resistant determinant. The 3' end of the cassette has a variable region termed as 59bp element, which contains the recognition sequence of the integrase. The integrase is able to integrate or excise genes-cassettes (usually antibiotic-resistance genes), by site-specific recombination. For dissemination, the integron must be able to insert itself into a plasmid or transposon. To date integrons are found in Gram-negative bacteria.

50S ribosomal subunit render it resistant to erythromycin. Another example is the modification of the β-subunit of RNA-polymerase of *Mycobacterium* leading to the failure of the anti-tubercular drug rifampicin to bind the subunit and inactivate the enzyme.

5.2.3 ***Reduction in Permeability to Antibiotics***: In some cases, emergence of mutants with reduced permeability of the cell membrane to antibiotics compared to that of the wild-type strain, leads to tolerance to the antibiotic. For example *Neisseria gonorrhoea*, the causative organism of gonorrhea (one of the most prevalent sexually transmitted disease), can gain antibiotic resistance by acquiring a mutation in the gene encoding the membrane protein 'porin', thus inhibiting the transport of the antibiotics penicillin and tetracycline into the cell and rendering the cells immune to the effect of drugs.

5.2.4 ***Exclusion of Antibiotics from the Cell***: Among the mechanisms involved in the resistance of bacteria to tetracycline, energy mediated efflux is a powerful strategy, which does not allow the drug to accumulate in sufficient concentration to exert its inhibitory effect. It is mediated by a trans-membrane export protein that functions as an elctroneutral antiport system. The protein catalyzes the exchange of a tetracycline-divalent metal cation complex for a proton.

5.2.5 ***Overproduction of a Target Metabolite***: In some cases, the molecule, which is competitively antagonized by the antibiotic, is overproduced. For example, sulphonamides act by competitively inhibiting the enzyme dihydropteroate synthetase, which plays a crucial role in the synthesis of folate. Sulfonamides (or sulfa drugs) are structural analogs of p-aminobenzoic acid (PABA), the substrate of this enzyme. The indispensable role of folate in the synthesis of nucleic acids viz., DNA and RNA is well-known. In some PABA-overproducing mutants of *Staphylococcus aureus*, sulfonamide molecules are outnumbered by the substrate and therefore the activity of the enzyme is not inhibited even in the presence of the drug.

5.2.6 ***Gene Deletion***: Gene deletion is also known to contribute to the mechanism of antibiotic resistance. Deletion of the *katG* gene in some strains of *Mycobaterium tuberculosis* is associated with the tolerance of the organism to the anti-tubercular drug isonicotinic acid hydrazide (INH). It is postulated that INH is actually a prodrug [15], which is converted into the active form by catalase/peroxidase, encoded *by katG*. Thus, absence of the *katG* gene makes the organism immune to the effect of the drug.

6. Adaptive Resistance

In the laboratory, a bacterial population can adapt to tolerate an antibiotic following exposure to gradually increasing concentration of the drug. While resistance due to mutation occurs in low frequency, resistance due to adaptation imparts tolerance to the whole population at a time. It is usually caused by a change in the lipid composition of the cell wall ultimately leading to reduced permeability to the antibiotic. The maintenance of the resistance phenotype requires the presence of an antibiotic in the growth medium unlike mutational resistance, which does not require the presence of the selection pressure. However, with the evidence available so far, adaptive resistance does not appear to play any significant role in therapeutics.

A special type of non-heritable resistance to chloramphenicol and ampicillin was also found to be induced in *Escherichia coli* when it was incubated in the presence of repellants like sodium acetate, acetyl salicylate, salicylate and sodium benzoate. The inducers in turn made the cells tolerant to ampicillin and nalidixic acid. Induction of tolerance to structuarally unrelated antibiotics by a variety of substances including some pharmaceuticals (acetyl salicylate) is commonly known as phenotypic antibiotic resistance and it appears to have some significance in therapeutics.

7. Non-Specific Resistance

Apart from harboring antibiotic resistance genes in mobile genetic elements such as plasmids and transposons, many bacterial species possess an intrinsic mechanism for resistance to multiple

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structurally unrelated compounds. The chromosomal multiple antibiotic resistance (*mar*) locus of some members of the *Enterobacteriaceae* confers resistance to several antibiotics, household disinfectants, organic solvents and other toxic chemicals. In *E coli*, multiple antibiotic resistance is known to be conferred by different sets of genes. The mar phenotype is induced following exposure to a variety of chemicals with aromatic rings such as salicylates, non-antibiotic antimicrobials (triclosan) and also superoxide radicals.

Bacteria can also survive antibiotics by the formation of biofilms, a multilayer conglomeration of diverse-species of bacteria, embedded in a self-produced exopolysaccharide matrix and specially attached to surfaces including objects such as prostheses or catheters. Antibiotic-resistance of bacteria in many infections (e.g., gingivitis, cystic fibrosis) is attributed to the biofilms formed by them. The precise mechanism of this resistance is still not very clear. Bacteria detached from the biofilms are found to be antibiotic-sensitive. Slow diffusion of antibiotics and a slow rate of growth and metabolism are believed to contribute significantly to antibiotic-tolerance in biofilms. Further, biofilms could be an ideal niche for horizontal gene mobilization, allowing resistance genes to be transferred and expressed rapidly. Conjugation promotes formation of biofilms in *E. coli*. The ability of bacteria to respond to quorum sensing [16] is a pre-requisite for the formation of biofilm. It is not surprising therefore that some quorum sensing inhibitors (baicalin hydrate, cinnamaldehyde, hamamelitannin) have recently been found to enhance the efficacy of some antibiotics against pathogens.

8.1 *The Antibiotic Paradox*

In 1992, Professor Stuart B Levy, Director of the Center for Adaptation Genetics and Drug Resistance at Tufts University School of Medicine (USA), proposed the theory of Antibiotic Paradox. It states that the widespread use of antibiotics itself is promoting the emergence of resistant strains. The basis of this theory lies in the fact that our body (as well as our surroundings)

is populated by millions of bacteria most of which are susceptible to antibiotics. The frequency of occurrence of antibiotic-resistant bacteria is usually negligible in these naturally occurring populations. Their growth is restrained by the susceptible bacteria, which outnumber them. Nowadays we resort to indiscriminate use of antibiotics on trifle illness (*a pill for every ill*) and even in cases like common cold for which the antibacterial antibiotics are of no use. These antimicrobials annihilate or suppress the friendly bacteria and thus provide opportunity to the antibiotic-resistant bacteria to grow and predominate in the population. We also often dump unused antibiotic formulations on the soil. Thus antibiotics help to select resistant bacteria in the soil by killing or suppressing the susceptible bacteria.

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Evidences available in the literature speak volumes in support of the theory. Following the Second World War, outbreaks of dysentery caused by sulfonamide-resistant *Shigella* sp. was a major concern in Japan. The situation was improved by the introduction of a number of newly discovered antibiotics viz., streptomycin, and chloramphenicol, into the clinical practice in the year 1950. However by 1956, again a strain of *Shigella flexneri*, resistant to sulfonamides and all these three new antibiotics, was detected. In the year 1969, the frequency of occurrence of the multidrug-resistant strains was found to be 69% among the 10462 strains tested. Fluroquinolones were used only in 1.4% of the cases during 1983–85. The fluoroquilone-resistance was not detected in *E coli* tested during the period, 1983 to 1990. With a sharp rise in the use of this drug in clinical practice during 1991 to 1993, 28% of the *E. coli* strains tested were found to be fluoroquinolone-resistant. A couple of years back, a team of investigators led by Ranadhir Chakraborty, at the Department of Biotechnology in the North Bengal University, demonstrated a correlation between the prevalence of resistance to specific antibiotics in some river isolates, obtained from north Bengal, and over the counter sale of the same antibiotics in and around the town of Shiliguri.

8.2 *Other Examples*

The use of antibiotics in poultries and animal farms for clinical management of the infections of the livestock and also as a growth promoter is well-documented. Substantial portion of the antibiotics applied to the animals is excreted into the soil, leading to the selection of antibiotic-resistant strains. Washed by rain and carried to the ponds and rivers, these antibiotics enter the body of fishes, which serve as a reservoir of resistant bacteria. Emergence of resistant strains in fishes is also favored by the antibiotics, used for the control of infections in fish farming. Isolation of antibiotic-resistant bacteria from fishes and other marine organisms has been reported from several laboratories across the globe.

9. Prudent Use Not a Permanent Solution

It may appear from Prof Levy's theory that the problem of antibiotic-resistance could be bypassed by avoiding the use of antibiotics. In the UK a joint committee on the use of antibiotics in Animal Husbandry and Veterinary Medicine was set up in 1968 under the Chairmanship of Prof. M M Swan. Antibiotics were routinely used during that time in the country for the purpose of promoting growth of animals. The committee recommended a stricture on the use of therapeutically useful antibiotics for this purpose. Use of tetracycline as a growth promoter was prohibited in March 1971. However, during the 16 months of prohibition the incidence of pigs carrying tetracycline-resistant organisms was not found to decrease. Following the fall of socialism in Eastern Europe, and also because of commercial blockade inflicted on the country, Cubans had less access to antibiotics during the 1990s. After 10 years, it was found that the degree of antibiotic-resistance in the harmless bacteria isolated from the mouth of Cubans was almost equal to that of their counterparts from Mexico, where antibiotics is available without prescription and also used in agriculture. Widespread resistance to a number of therapeutically useful antibiotics was observed by the investigators at the Centre for Cellular and Molecular Biology

(Hyderabad, India) among bacterial isolates, obtained from different types of samples (soil, ice, cyanobacterial mat), collected from Antarctica, where exposure of the organisms to antibiotics is highly improbable. During an exchange program conducted at the Leibniz Institute of Freshwater Ecology and Inland Fisheries (Stechlin, Germany), it was found that incidence of chloramphenicol-resistance (19.69%) was not substantially lower than that of erythromycin-resistance (24.24%) in various types of lake-isolates, sampled from some least populated areas of north Germany. It is well-known that use of chloramphenicol for therapeutic purpose was banned in the Western countries quite some time back while erythromycin is still being used. Many other reports of this sort reveal that prudent use of antibiotics might help slow down the emergence of resistant strains but the strategy cannot ensure complete reversal and disappearance of resistance.

10. Co-occurrence of Antibiotic Resistance with Tolerance to Other Antimicrobials

Antibiotic resistance genes are often located on plasmids, which also carry genes conferring resistance to many other chemicals including heavy metals and disinfectants. Substances like heavy metals and disinfectants are persistently present in the environment since they are part and parcel of the industrial civilization. Consequently, they select resistant strains among the naturally occurring population of bacteria and in the process, cross-select antibiotic-resistant strains. In some cases, bacteria are found to harbor plasmids conferring resistance to a number of antibiotics and heavy metals. It is also known that in the absence of antibiotics, resistance plasmids and their bacterial hosts co-evolve in such a way that, after several generations, they grow better than a strain that lacks the plasmid. The metabolic burden needed to maintain such a big plasmid does not seem to pose any challenge to the organisms and they are found to carry it through generations without any selection pressure. That is why it is said that antibiotic resistance genes are easy to get but difficult to lose.

11. Search for New Antibiotics

An antibiotic research reported by the London School of Economics and Political Science (LSE) showed that bacterial and parasitic diseases are the second leading cause of death worldwide. Because of the emergence of drug-resistant 'superbugs', (like MRSA and VRE), traditional antibiotics and its derivatives are becoming nonfunctional. The whole world is thus confronted with a looming drug crisis because of frequent emergence of resistant strain and development of new antibiotics is desperately needed. The search for new antibiotics during the past few decades was mostly based on the chemical modification of the existing drugs but in the post genomic era, molecular genetics is being used to identify essential new targets with the help of high throughput screening and combinatorial chemistry. Structural biology is being applied to rapidly explore and optimize the interactions between lead compounds and their biological targets. Genomic tools are helping us to select for antibacterial targets and understand bacterial resistance. On the other hand, the availability of a plethora of natural products and combinatorial synthetic small molecule libraries, provides ample opportunities to dig into these resources in search of new chemotherapeutic agents. A number of approaches to the discovery of new antibacterials are on the anvil. Some of them are based on the forward chemical genetic approach while the others are based on the reverse chemical genetic strategy (*Box* 1).

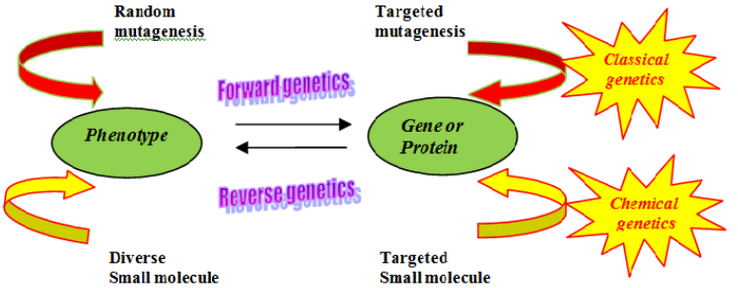
12. Doomsday Ahead?

In the past few decades, because of the emergence of resistant strains, therapeutic benefit of some of the potent antibiotics was completely lost. Over nearly 20 years, from the early 1980s to the late 1990s, not a single truly new antibiotic was introduced into clinical use. Even now, barely a trickle has reached the market since 1999. Only five new antibiotics were approved from 2003 to 2007, compared to the 16 approved during 1983 to 1987. The situation for the treatment of Gram-negative infections is even bleaker. The impermeable nature of the Gram-negative envelope

Box 1. Chemical Genetics

Chemical genetics is a chemistry-based approach, which involves diverse small-molecule compounds to elucidate biological pathways in a manner analogous to the mutagenesis strategies used in classical genetics. Screening small-molecule libraries to induce a phenotype of interest represents the forward chemical genetic approach, whereas the reverse approach involves small molecules targeting a single protein. The prerequisite for this analysis is a collection of potential compounds (natural products, combinatorial libraries, inventories and chemi-informatics) and high throughput functional assays (in *vitro* binding assays, cellular assays, yeast, bacteria, animal models, bioinformatics).

In forward genetics, small molecules are used to attain a specific phenotype and try to find the gene that controls it; in reverse genetics, small molecules are used to manipulate genes or proteins to characterize their role by identifying the resulting phenotype.



and presence of multiple efflux pumps in combination with other resistance mechanisms contribute to the difficulty of this task. If the emergence of antibiotic resistance continues to follow the present trend, it is reasonable to assume that resistance to the new antibiotics will emerge sooner or later. In fact, reduced susceptibility to linezolid and daptomycin has already been encountered in the clinical setting. Despite prudent usage, the life span of a new antibiotic is cut short by the emerging resistance. So it is evident that a continuous supply of structurally novel antibacterial agents with multiple mode of action is needed to combat the problem of drug resistance. The optimism associated with antimicrobial peptides because of their broad-spectrum activity, absence of cross-resistance with the existing antibiotics and low probability of developing resistance is hindered by their poor bioavailability and high cost involved in their manufacture. Physicians are often taking resort to multiple antibiotic therapies, which are likely to be more effective than using a single

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antibiotic. Scientists are also looking for alternative strategies e.g., enhancing the susceptibility of bacteria to antibiotics by using plant-derived antimicrobials (viz., thymol, carvacrol, cinnamaldehyde, allyl isothiocyanate). Let us hope for a better future while being aware of the precarious situation that is prevailing at present.

13. Conclusion

The foregoing discussion on antibiotic-resistance in bacteria is focused on the clinical impact of the phenomenon. But there are many other aspects which are overshadowed by its devastating effects on the prospect of chemotherapy. The history of antibiotics as therapeutic agents is less than 100 years old while antibiotic biosynthetic pathways and antibiotic-resistance genes are believed to have evolved thousands of years ago. So in nature both antibiotic biosynthetic pathways and mechanisms involved in antibiotic resistance must have some other significance, which warrants extensive investigations. The multiplicity and non-specificity of efflux pumps and occurrence of resistance-conferring genes in non-pathogenic bacteria hint at some other role of antibiotics in evolution. It is also believed that bacteria sense antibiotics as an environmental stress. Hence there might be some correlation between antibiotic-resistance and stress tolerance of bacteria. Antibiotic-resistance has been detected in many bacteria isolated from extreme environments. Enhanced rate of horizontal gene transfer was observed by some investigators in some food-borne bacteria treated with sublethal levels of stress factors. Therefore, it is obvious that the clinical aspect of antibiotic-resistance is only the tip of the iceberg and most of the aspects in the study in antibiotic-resistance of bacteria remain unexplored till now. We hope that thorough investigations will provide more insights in the years to come.

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