BACTERIAL RESISTANCE TO ANTIMICROBIALS*

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The development of bacterial resistance to antimicrobials is a matter of great importance; if sensitive organisms are supplanted by resistant ones, then a previously potent drug may become useless. Over the last 3 decades there has been a continuing race between the discovery of new antibiotics and the growing resistance of microorganisms to existing antimicrobial agents.

The development of resistance of bacteria during therapy is essentially due to a remarkable, and at times elaborate, capacity of these microbes to circumvent the action of the antibiotics, rather than to any clearly defined *in vivo* changes involving susceptibility of the host. Furthermore, this enlarging frontier of resistance of pathogens to antimicrobials has its origins in, and is largely explained by, a study of microbial genetics. Drug resistance is due to mutation and is therefore entirely pre-adaptive. Moreover, such drug-resistant organisms may be either drug tolerant, when there exist physiologic pathways unaffected by the antibiotic, or drug destroying (e.g. penicillinase-producing staphylococci). The drug acts as a selecting agent, destroying or suppressing the sensitive forms, and the resistant forms then proliferate. This process may occur in 3 main ways:

(*a*) Drug resistance may arise in a single clone or strain of organism.

(*b*) The original sensitive organism and the resistant replacing organism are different strains of the same species.

(*c*) The original drug-sensitive population is replaced by a different species, which is drug resistant.

The origin and incidence of resistance of microbes is in a large measure due to the prevalence of the drug in their environment (e.g. widespread use and misuse of antibiotics) but is also due to selection by natural exposure to the antibiotic-producing fungi in the ground or by selective pressures bearing only a fortuitous relation to antibiotics.[1]

Not only is this problem of antibiotic resistance of great heuristic and clinical importance, but the field itself has grown and mushroomed in a startling fashion during the last 10 years. A complete review of the entire subject is impracticable in an article of this nature. Our attention, therefore, will be focused primarily on 3 major aspects: the nature and mechanism of evolution of drug resistance, the extent and changing pattern of this resistance, and a brief glimpse at possible therapeutic measures.

THE NATURE AND INHERITANCE OF RESISTANCE TO ANTIBIOTICS

In the past 20 years, bacteria have become one of the groups of organisms most intensively studied genetically and biochemically. Cytologic and genetic studies have demonstrated that the genetic material of bacterial cells consists of single molecules of deoxyribonucleic acid (DNA). The genetic material of all other free-living organisms is also DNA, but the bacteria and blue-green algae differ from higher plants and animals in that the nucleus consists of only a single DNA molecule without the nuclear proteins characteristic of the chromosomes of higher organisms and is not separated from the cytoplasm by a nuclear membrane. Bacterial cells on the whole lack internal membranes and membrane-bound organelles, and most of the chemical reactions such as respiration and photosynthesis, which are confined to these organelles in higher organisms, take place on the inner surface of the cell membrane in bacteria. The differences in membranes, together with other differences such as the lack of the usual type of mitotic apparatus in bacteria, have prompted cytologists to class these types of cells as 'prokaryotes', as distinct from 'eukaryotes' which include all higher plants and animals.[2]

The usefulness of antibiotics in treating disease resides in their differential action on the cells of bacteria and those of the host organism, which in turn depends on the differences in metabolism and structure between the two types of cells. For example, one large group of antibiotics affects the bacterial cell membrane (e.g. the bacteriocidal drugs such as the penicillins, cephalosporins, bacitracin, ristocetin, the polymixins, and perhaps the aminoglycosides, streptomycin, kanamycin and neomycin).

The genes of prokaryotes appear to function in a manner similar to those of eukaryotes, that is, in controlling the cellular production of proteins by specifying the synthesis of messenger, transfer and ribosomal RNA molecules. However, minor differences must occur, as another large group of antibiotics, which may be classified as bacteriostatic or cytostatic, work by interfering with some step in the chain of events leading from the gene to the synthesis of the completed protein. Some (e.g. chloramphenicol, echinomycin, daunomycin, nogalamycin, mithramycin, actinomycin, mitomycin and puromycin)[3-6] interfere with the duplication of DNA, the transcription of DNA into RNA or the stability or correct functioning of RNA molecules. Others, such as streptomycin and the bacteriostatics, chloramphenicol, oleandomycin, erythromycin and tetracycline, affect the ribosomes where protein formation occurs, causing errors in the incorporation of amino acids.[7]

Mutations of various types can be envisaged which would confer resistance to antibiotics. Any mutation which altered the nature of the bacterial membrane in such a way as to prevent the entrance of an antibiotic into the cell or prevent its reaction with the membrane would confer resistance. Other mutations cause the cellular production of enzymes which are capable of inactivating antibiotics. Mutations are also known which change the structure of organelles in such a way that they are no longer sensitive to the action of a particular antibiotic.

It must be emphasized that mutation in bacteria, as in higher organisms, is a random, non-directed process. Mutation to resistance to antibiotics has been shown to be pre-adaptive and not postadaptive, as was widely believed earlier.[8] This means that a mutation to resistance may

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occur by chance at any time in any cell, irrespective of whether or not that cell or any of its ancestors has ever been in contact with the particular antibiotic. A mutation to resistance may occur before exposure to the drug, and does not arise as a result of an adaptation of metabolic pathways occurring due to exposure. It has been shown that some bacteria isolated before the use of a particular antibiotic may carry genetic resistance to that antibiotic.

Once a mutation to antibiotic resistance has occurred in a bacterial cell, it may be transferred to the progeny of that cell during ordinary cell division, or to other cells by a variety of different means by which some bacteria are able to transfer genetic material. The inheritance of a particular form of resistance depends on the number of separate genes necessary to confer resistance and on the type of gene involved (chromosomal or extrachromosomal).

Monogenic and Polygenic Resistance

It has been shown that resistance to high concentrations of streptomycin or isonicotinic acid hydrazide (isoniazid) may be acquired by bacteria by the mutation of a single gene.[8] On the other hand, high-level resistance to penicillin and most other antibiotics cannot be acquired in one mutational step, but requires the simultaneous presence of mutations in a number of different genes. If a large population of sensitive cells is treated with a high concentration of streptomycin, we should expect that one in a million of these cells (taking 10^{-6} as an average mutation rate) might have undergone a mutation to resistance during one generation. Note that this figure is independent of drug treatment, i.e. just as many mutations may be expected in the absence of the drug as in its presence. The sensitive cells will be killed by the drug, but the few resistant cells will soon multiply and form a new, highly-resistant population. On the other hand, if resistance to high concentrations of penicillin requires the presence in any cell of, say, 5 different mutant genes, each of which mutates at a rate of 10^{-6}, then the chance of these 5 mutations occurring in the same cell during the same generation is only 10^{-30}.

Each individual gene causing resistance to penicillin confers a slight degree of resistance, and these act additively. If a low concentration of penicillin is used on an initially sensitive population, we should expect 5×10^{-6} cells to have undergone a single resistance mutation in any generation. The penicillin will kill the completely sensitive cells and a new population of low-level resistants will establish itself. If the concentration of drug is now doubled, 4×10^{-6} of the low-level resistant cells (all of which now carry one gene for resistance) will have undergone mutation of a second gene. By stepwise increase of drug concentration with sufficient time for cell proliferation between increases, a population of high-level resistants can be selected. The therapeutic implications of the monogenic and polygenic forms of inheritance will be discussed later.

Inheritance and Transfer of Resistance Genes

A. Chromosomal genes. Genes for resistance to antibiotics located on the bacterial chromosome may be transferred to other cells in the following way (see Hayes for review):[9]

1. By normal cell division. Each resistant cell, when it divides, passes its resistance genes to each of its 2 daughter cells.

2. By conjugation. Episomic fertility factors exist in some species of bacteria. These extrachromosomal pieces of DNA may replicate independently of the chromosome in the cytoplasm, or may become incorporated into the chromosome and behave as if they were an integral part of it. Alternation between the chromosomal and cytoplasmic states occurs. When present in cells, these fertility factors such as K12, the well-studied F factor of *E. coli*, alter the host cell in such a way that it is able to form a conjugation tube with another cell lacking an F factor and transfer either the F factor itself (when it is in the cytoplasmic state) or a part of the bacterial chromosome (when F is in the chromosomal state). Recombination occurs between the DNA of the donor and recipient cells and any gene of the donor may be incorporated into the chromosome of the recipient.

3. By transformation. Free DNA isolated from bacteria of some species can enter competent cells and recombine with the recipient cell's DNA. In this way small blocks of genes may be transferred.

4. By transduction. Some bacteriophages are capable of including host bacterial DNA molecules in their protein coats in the course of intracellular multiplication. When these phages attack other bacteria, recombination for small blocks of genes can occur between the DNA obtained from the previous host and that of the present host. Since a transducing phage has usually substituted bacterial DNA for a part of its own viral DNA, the remaining viral genome is unable to complete the course of infection, and the infected cell survives. Resistance genes, like any other bacterial genes, may be transmitted from one cell to another by transducing phages.

Transformation and transduction studies provide a convenient method for studying the mode of inheritance of resistance to a particular antibiotic. Since only a small piece of the DNA of the donor cell is transferred at a time to the recipient cell, resistance due to a single gene may be transferred in one step of transformation or transduction, while a resistance based on polygenes cannot be transformed or transduced in one step.

B. Resistance controlled by genes carried by plasmids and episomes. In addition to their normal complement of genes carried on the chromosome, bacteria may also carry extra genetic material in cytoplasmic plasmids or episomes, such as the F factor mentioned above. These classes of genetic elements which are not necessary for the normal existence of bacteria have only recently been discovered to be of great importance in the problem of drug resistance. Watanabe and Fukasawa in Japan first isolated shigella strains that were resistant to high concentrations of several antibiotics.[10] It was subsequently shown that this resistance was not of the usual chromosomal type but was carried by cytoplasmic resistance factors. These R factors are DNA, and usually carry genes which act as fertility factors, allowing the cells which contain them to conjugate with other cells lacking them. They also carry genes which permit their independent replication, genes

for resistance for various numbers of antibiotics and sometimes genes which confer an abnormally high resistance to certain metal ions.[11] Judging from their size, they probably also carry additional genes whose functions are as yet unknown. The R factors are readily passed from one cell to another of the same species, or, in some cases, to different species during conjugation. In this way resistance to a number of antibiotics can be transferred simultaneously from harmless enteric bacteria to pathogenic species.

The origin of R factors is not known and the resistance genes they carry appear to be different from the known chromosomal genes conferring resistance to the same antibiotics. Resistance to each of the antibiotics carried by the R factors seems to be of a single gene type rather than the polygenic type carried by the chromosomes. Different R factors may also carry different genes for resistance to the same antibiotic.[12] The similarity of R factors to episomes such as F and to prophages, together with the established fact that some episomes and phages can exchange genes with the chromosome, makes it seem likely that R factors are F factors which have at some time in the past picked up resistance genes from the chromosome of some unknown organism.[13]

Possible Host Factors

The aforegoing has placed prime emphasis on bacterial genetics in the development of microorganismal resistance to antibiotics during therapy. Very little is known about possible host factors in this regard. One may consider poor absorption of antibiotics in sick patients, walled-off lesions, inadequate protein binding and transfer of the drug to the site of infection, development of antibodies to the antibiotic being used and changes in local tissue pH during infection. The only well-documented host factor important in altering the sensitivity of urinary bacteria to antibiotics is the pH level of the urine. Thus benzylpenicillin, ampicillin, oxytetracycline and sulphathiazole have an increased antibacterial effect in acid conditions and vice versa. On the other hand, streptomycin and kanamycin are most active in alkaline media and kanamycin is almost without effect in acid conditions.[14]

THE CHANGING SPECTRUM OF BACTERIAL RESISTANCE

With the genetic mutational model in mind, it is propitious to pass to a consideration of the changed spectrum of bacterial resistance to antibiotics over the last few decades. The recognition of the development of bacterial resistance to an antibiotic may be made on clinical and bacteriological grounds. Clinically, there will be initial improvement in the patient's condition, and then, as the mutant resistant strain emerges and proliferates, so will there be a recrudescence of the patient's symptoms and signs. From the bacteriological viewpoint, a fall-and-rise phenomenon will be observed. For example, in the case of tuberculosis treated with isoniazid or streptomycin alone, the viable population of a culture or a lesion exposed to the drug initially shows a fall associated with killing of the sensitive organisms, followed by a rise as the resistant mutants multiply and replace them.[15] Further, resistant organisms can be isolated in culture and then tested for resistance to varying concentrations of the antibiotic concerned. The serologic and other characteristics of the resistant mutant may also be established.

A study cannot be made of all the organisms pathogenic to man. One method of approach is to consider serially the more common microorganisms and their sensitivity or resistance to various antibiotics.[16]

Staphylococci

The mutation potential of these cocci is high.[17] The emergence and dissemination, especially within the hospital milieu, of resistant staphylococci have caused serious concern in recent years. Penicillinase-producing staphylococci have exhibited resistance to penicillin G, tetracyclines, streptomycin, chloramphenicol, erythromycin, novobiocin and neomycin as each drug came into use. At present nearly two-thirds of staphylococci isolated from patients are resistant to penicillin G, streptomycin, tetracycline or erythromycin, and a smaller proportion are resistant to chloramphenicol and novobiocin.[18] Practically all strains are still sensitive to vancomycin and bacitracin.[19,20] The introduction of methicillin in 1959 seemed to be an answer to this problem. However, methicillin-resistant strains of *Staph. aureus* (coagulase positive) were first recognized in England in 1960.[21] Such strains have subsequently been recognized elsewhere. They are pathogenic in man and infection may have fatal consequences.[22] These methicillin-resistant strains of *Staph. aureus* have rather consistent characteristics. They are all resistant to penicillin, streptomycin, tetracycline, oxacillin, cloxacillin, nafcillin and certain of the cephalosporins, and many are also resistant to chloramphenicol and erythromycin.[23] These strains existed before the introduction of methicillin and have spread alarmingly in hospitals in the last few years,[23] although there is little evidence that such spread is directly related to the use of methicillin in hospitals.[24] The fact that methicillin-resistant strains are also resistant to many other antibiotics, as outlined above, probably explains their selection and spread in hospitals.[25] Despite the fact that their incidence is low at present, these strains are widespread, virulent and communicable, and it is likely that they will continue to survive and spread.

Group A Streptococci

Penicillin remains the drug of choice in the prophylaxis and therapy of group A streptococcal infections. There is no evidence of naturally-occurring penicillin-resistant strains. Clinically, group A streptococci may fail to be eliminated from an infected area by the concomitant presence of penicillinase-producing *Staph. aureus*. The latter destroys the penicillin and therefore the streptococci survive.[25] Penicillin-resistant streptococci have, however, been successfully selected for *in vitro* studies.[26]

Sulphonamides. A programme of mass sulphadiazine prophylaxis carried out in the armed forces during World War II produced an initial dramatic effect, but later the emergence of sulphonamide-resistant strains occurred. These strains have not led to any serious problem in civilian life.[27]

Erythromycin. This is the best antibiotic for streptococcal eradication in patients allergic to penicillin. Only one instance of naturally-occurring resistant strains has been reported, and this in a centre where erythromycin

was extensively used in the control of infection.[28]

Tetracyclines. Group A streptococci, resistant to tetracyclines, were first isolated from man in 1952.[28] Since this original observation, resistant strains have been found in various areas. These strains are resistant to tetracycline, chlortetracycline, oxytetracycline and dimethylchlortetracycline. The resistant strains are as virulent as the sensitive ones and tetracyclines should not be used as effective substitutes for penicillin or sulphonamides for streptococcal prophylaxis. Moreover, these drugs are unsatisfactory therapeutic agents for established streptococcal infection, and even if the organism is sensitive, they are inferior to penicillin or erythromycin.

Pneumococci

Penicillin remains the antibiotic of choice for the treatment of pneumococcal infections. Despite extensive and long usage there are no reports of penicillin-resistant pneumococci and there is no trend towards decreasing susceptibility.[29]

Erythromycin likewise remains efficacious in pneumococcal infections[29] and is the best alternative agent for pneumococcal therapy in patients who cannot be given penicillin.

Tetracycline-resistant pneumococci were reported in Australia in 1962[30] and subsequently in other countries. With this resistance, there is a marked correlation with previous tetracycline therapy. The hospital may be an important site for the transmission of resistant strains and the term 'hospital pneumococcus' has been coined.[31] Tetracycline-resistant strains appear late and the reasons for this are unknown.

Meningococci

Until 1963 the sulphonamides were regarded as the agents of choice for both the prophylaxis and therapy of meningococcal infections. At that time an outbreak of meningococcal disease occurred at 2 naval installations in California. Adequate proof was obtained for the existence of sulphonamide-resistant meningococci—both in prophylaxis and therapy.[32] Later evidence was forthcoming that sulphonamide-resistant meningococci are distributed throughout the civilian population of the USA. It is of interest to note that most of the recent cases of meningococcal infection were due to groups B and C, while group A was the main offender during World War II.[33] Almost all the sulphonamide-resistant meningococci have been of group B, with a few resistant strains from group C. It is now clear that sulphonamides can no longer be relied upon for chemoprophylaxis or therapy of meningococcal infections. Penicillin G, however, is the therapy of choice and is most efficacious in the management of meningococcal meningitis or infections where sulphonamide-resistant strains exist. Chloramphenicol is a valuable substitute in patients allergic to penicillin.

Gonococci

Sulphonamides were initially used with high success-rates in gonococcal infections.[34] While almost all strains of gonococci were initially susceptible, with cure rates of up to 90%, resistant forms soon emerged and shortly before World War II therapeutic failures of near 85% were experienced. At this time penicillin was introduced and was highly active against all strains and effective treatment was once more obtained. However, strains of gonococci with decreased sensitivity to penicillin then made their appearance.[34] Whereas previously, cure in 90 - 100% of cases of gonorrhoea was obtained with a single injection of 300,000 units of penicillin, the practice now, in the light of decreased sensitivity, is to use a minimal dosage of nearly 2·5 million units of penicillin G.

Tetracyclines are also of value and no resistance has been found. Rapid emergence of resistance followed use of streptomycin. Erythromycin and chloramphenicol provide alternate modes of attack.

The Enterobacteriaceae

While the enterobacteriaceae have generally been considered as relatively harmless constituents of the normal bowel flora, the bacterial disease spectrum has shown remarkable changes inasmuch as many of these bacteria are now causative of serious infections and pathology in man. They mutate readily. In a hospital population under study, enteric organisms such as *Escherichia coli,* proteus species, *A. aerogenes, Ps. aeruginosa* and enteric cocci accounted for only 12% of the infections and 9% of the deaths in 1935 ; but in 1957 they accounted for more than one-third of bacterial infections and about half of the deaths from bacteraemia.[35]

The increased prevalence of serious infections caused by enteric bacteria is due to:

(*a*) certain strains of enterobacteriaceae naturally resistant to antimicrobials increasing in number as other sensitive strains are eliminated,

(*b*) resistant mutants from originally sensitive strains emerging under the selective influence of antibiotics, and

(*c*) resistance factors, as described above, which may confer resistance on other enterobacteriaceae or on other Gram-negative organisms.

The proportion of antibiotic-resistant enteric bacteria isolated from infected patients has increased since the introduction of antibiotics.[36] Enteropathogenic *E. coli* resistant to neomycin, kanamycin, tetracycline or chloramphenicol are documented. R factors mediate resistance to sulphonamides, chloramphenicol, streptomycin and tetracycline,[37] and also to neomycin and kanamycin,[38] ampicillin and cephalothin,[39] and furazolidine.[40] More recently, resistance of the same type to several new aminoglycosides such as bluensomycin, spectinomycin and gentamicin has been reported.[1]

While most strains of *E. coli* isolated from clinical sources remain sensitive to ampicillin, colistimethate, tetracycline or chloramphenicol, the 'hospital strains' are much more resistant to tetracycline or chloramphenicol.[36]

Shigellae resistant to sulphonamides, streptomycin, chloramphenicol and tetracyclines have been described. Although *Salmonella typhosa* can receive multiple resistance factors *in vitro* from organisms with such R factors, wild strains of *S. typhosa* possessing R factors have not yet been isolated.[41] In India, about 9% of 687 *S. typhosa* strains were resistant to at least 16 μg./ml. of chloramphenicol.[42] However, the incidence in South Africa of

strains resistant to more than 10 μg./ml. of chloramphenicol is only 0·8%.[43] The absence of R factors among wild strains of *S. typhosa* is difficult to explain.

THERAPEUTIC MEASURES

From the aforegoing discussion on the changing spectrum of drug resistance and its explanation in terms of bacterial genetics, a few therapeutic pointers arise:

The correct antibiotic for the particular organism should be selected whenever possible. This obviates overgrowth of the organism (at the expense of other flora), where it is entirely non-sensitive, and where it is partly sensitive, the eventual emergence by serial mutation of full-blown resistance is avoided.

The drug should be administered for a sufficient length of time. By lengthy dosage, the phenomenon of microbial persistence is avoided. Several microbes may not divide for short periods during exposure to antibiotics and may thus survive brief dosage schedules.

The drug should be given in adequate dosage. Small doses are likely to lead to the emergence of drug resistance because sensitive or partially sensitive microbes are 'given the chance or time' to mutate further and resistance is consequently selected.

Antibiotics should, in certain cases, be used in combination. This is particularly true for therapy of tuberculosis where a one-step mutational resistance to streptomycin is common and is prevented by use of streptomycin in combination with INH or PAS.

There seems to be little which can be achieved in the case of resistance determinants and their transfer factors. R-factor-mediated resistance in all enterobacteriaceae and several other genera of Gram-negative bacteria remains one of the most serious challenges and dangers of the present antibiotic era. *In vitro* studies show that bacteria can be 'cured' of R factors as well as other cytoplasmic episomes by treatment with acridine dyes.[44] Therefore, deeper understanding of this phenomenon at the intracellular level will hopefully lead to a solution *in vivo*.

SUMMARY

The development of bacterial resistance to antibiotics during therapy poses challenging biological and clinical problems. Over the last few decades the susceptibility of various microorganisms to antibiotics has changed. The changed pattern is outlined. A major factor in its development has been the widespread misuse of antibiotics. Their extensive and often indiscriminate use in man and animals has served as a form of natural selection for the emergence of resistant mutants.

The genetic basis for this resistance is by single or multiple mutation of the DNA of the bacterial cell. The mutation is passed on by cell division and is transmitted to other bacteria by 3 means: conjugation, transformation and transduction. The special case of resistance factors and their transfer elements is outlined.

Therapeutic aims comprise a deeper understanding of events at the microbial genetic level. Clinically, the formula comprises employing the correct antibiotic, in the adequate dosage for a sufficient time. In several instances combined antibiotic therapy is called for, especially where one-step mutation is common, as in the case of mycobacteria with use of streptomycin or isoniazid alone.

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