TRANSFERABLE DRUG RESISTANCE AND THE ECOLOGIC EFFECTS OF ANTIBIOTICS

by

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Antibiotics were first introduced as chemotherapeutic agents in the early 1940s with the advent of penicillin for the treatment of human infections. They have since grown in number to some 50 different types. Approximately 20 of these antibiotics have common usage in man and animal.

Antibiotics are used to control infectious diseases of man; to treat and control diseases in animals and food crops; to stimulate the growth of animals; and to preserve foods. They are the "wonder drugs."

Large as the benefits may be from the use of antibiotics, certain risks threatened their effectiveness from the very beginning. The greatest of these risks was the emergence of resistant bacteria. As resistance developed, an antibiotic would become less effective at initially established doses, therefore, dosages had to be greatly increased.

It has long been known that resistance to drugs may be produced by bacteria. The commonly known methods by which resistance develops are by mutation, by the replacement of drug-sensitive bac-

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teria strains by resistant strains, or by transduction in which genes are carried from one bacterial cell to another by infecting phages or bacterial viruses. Normally, resistance by these means develop at a relatively low frequency.

In 1959, the Japanese scientists, Ochiai and Akiba and their co-workers were able to show that a more efficient and more disturbing mechanism for drug resistance was at work in gram negative enterobacteria--the mechanism of transferable drug resistance.[1,37,55]

By this mechanism, bacteria suddenly become resistant to several antibacterial drugs at once and are able to transfer this resistance to other susceptible bacteria of the same specie or different species simply by cell-to-cell contact. In vitro, it has been shown that this process of transferring resistance continues until nearly all of the organisms coming into contact with the infectious bacteria become resistant.

Since the discovery of transferable drug resistance in Japan, it has been detected in many countries. Other names given transferable drug resistance are infectious drug resistance, multiple drug resistance, and multiply drug resistance.

Transferable drug resistance

Transferable drug resistance is brought about by conjugation--

a transfer of genetic information from one bacteria cell to another through direct cell contact.[16]

Exactly how this genetic information or drug resistance moves from the donor cell to the recipient cell by conjugation is not definitely known. Investigators agree, however, that resistance to antibiotics transmitted by conjugation does occur, both in vitro[1,23,52,53] and in the intestinal tract of man and animal.[22,25,50,55]

In theory, transferable drug resistance concerns an element called the R-factor (for "resistance").[55] It is postulated that the R-factor has two components: one component is the Resistance Transfer Factor (RTF) which may be extrachromasomal[52,54,55] and contains DNA[16,17]; the genetic material that specifies the design and construction of future generations. The other component is called the Resistance Determinant (R-d); the genetic fragment that determines the specific drug's resistance.[5]

The RTF component or episome units receive and transmit the drug resistance.[52] However, the RTF is not activated for drug resistance until it comes in contact with the R-determinant. Both are needed for the transfer of drug resistance.[5]

The RTF may itself transfer the R-determinants or the R-determinants may be present in the cell in the absence of the RTF.[5,6] How the R-d actually gets into a bacterial cell is academic when we consider that one such donor cell will activate

the RTF in any recipient cell it contacts and the recipient cell then becomes a donor cell thus continuing a chain reaction. This process is thought to be maintained as long as there is constant antibiotic pressure.

When one adds a small number of bacteria with R-factor to a culture of drug-sensitive cells, there is a rapid increase in the relative number of drug-resistant cells. Within 20 minutes, a transfer of resistance can be completed between cells. In 24 hours or so, the culture may become almost completely resistant. This rapid spread of R-factors or resistance to the once sensitive bacteria occurs at a much faster rate than the overall growth of the culture.[27,52,53]

The defense system set up by bacteria carrying the R-factor is very sophisticated. They can ward off attack not only to one but to several antibiotics simultaneously.[27] In turn, the R-factor can pass this resistance to bacteria of other strains--nonpathogenic bacteria can transfer their resistance to bacteria that are pathogenic.[23,53]

Transferable drug resistance is known only to occur among gram-negative bacteria and primarily of the enterobacteria. No gram-positive bacteria have been shown to carry this resistance.

Resistance factors can infect all genera of the Enterobacteriaceae.[23] The more important pathogenic members of the Enterobacteriaceae are Salmonella, Shigella and Escherichia coli. These bacteria are found in the lower intestinal tract of man and animal. The first two can cause salmonellosis including typhoid fever and bacillary dysentery, to name a few diseases. Pathogenic E. coli may cause infections of both the intestinal and urinary tracts.

Other gram-negative bacteria against which multiple drug resistance can be transferred include: Serratia,[15,49] Vibrio,[10,28] Pasteurella,[21] and Pseudomonas.[23,42] Transferable drug resistance can occur between species of genera in Enterobacteriaceae, Brucellaceae, Pseudomonadceae, and Spirillaceae.

The drugs most frequently reported against which transferable drug resistance develops are: streptomycin, chloramphenicol, tetracyclines, and the sulfonamides. Resistance has also been reported against penicillin,[2] ampicillin,[2,13] furazolidone,[46] kanamycin, neomycin, paromomycin,[57] celphaloridine, and gentamicin.

The frequency with which antibiotic resistance is transferred from one bacteria cell to another in nature is not definitely known. It is known that any contact with antibiotics will bring about an increase in resistance. In general, the more frequently a drug is used the higher the incidence of the corresponding resistance.

It is not known how long resistance remains after antibiotics are withdrawn. One study with pigs showed bacteria resistant to one of the antibiotics as long as seven months after the pigs no longer had access to the drug, although the resistance was decreasing.[45]

Investigators believe that resistance will continue only under constant antibiotic exposure.[46] The use of antibiotics in feed to promote growth in animals can result in the emergence and the continuance of transferable drug resistance in intestinal bacteria.[9] Also, an antibiotic may perpetuate antibiotic resistance to another unrelated antibiotic.[29] That is, an organism may become resistant to one antibiotic and the resistance perpetuated for an indefinite period of time by the presence of another antibiotic.

Bacteria may spontaneously lose the R-factor[11,12,29,43,54] or lose the capacity to transfer the R-factor yet retain the resistance itself.[55] This failure to transfer resistance has been observed among R-factors associated with Salmonella[9,46] and other enteric bacteria.[42,55] In vitro, the resistance factors of multiple drug-resistance can be eliminated by treatment of the resistant cells with acridines.[54]

The phage type by which bacterial strains are identified may be changed by transfer factors. This change has been observed in Salmonella. When infected, the phage type of one strain of bacteria can be converted and resemble the phage type of another.[4,7] No studies have been reported on serotype changes accompanying R-factor resistance. Such studies are needed since serotypes in Salmonella are germane to epidemiological investigations.

Occurrence and distribution

Scientists do not know the origin of the R-factors, nor how far back in unrecorded history they go. Neither do they know the mechanism of developing new types of R-factors.[38,58]

In Japan, the R-factor can be traced back to 1955 but no further. This, despite the extensive use in that country of sulfanilamide since 1945 and of dihydrostreptomycin, tetracycline and chloramphenicol since 1950.[38]

In the United States, R-factors have been demonstrated in bacteria isolated and stored over a number of years. In fact, an E. coli isolated in the 1930's and lyophilized in 1946 was found to contain an R-factor mediating resistance to tetracycline and streptomycin.[44]

It has been suggested that R-factors may have come about from gene pickup by the resistance-transfer factor. That is, the transfer factor picked up resistance genes from the chromosome of some unknown bacteria. First picking up one resistance gene, then another, and another until bacteria were resistant to a number of drugs.[55,58]

On the other hand, epidemiological surveys suggest that multiple drug resistance has not developed step by step but existed from the beginning or developed all at once.[37] The first isolation of R-factor in Japan was resistant to four drugs.

Distributions of R-factors are world wide. Multi-resistant strains of bacteria have been found with increasing frequency in many different parts of the world.

Japan:

Transferable resistance was first brought to light in Japan in 1959. Strains of Shigella resistant to streptomycin, chloramphenicol, tetracycline, and sulfanilamide were found in 1955. Subsequently, Japanese workers isolated Escherichia coli strains resistant to the same four drugs during an epidemic caused by Salmonella flexneri in 1957 and in a patient afflicted with S. flexneri in 1958. In still another patient, S. flexneri and E. freundii resistant strains were isolated.[37]

To account for the increasing number of antibiotic-resistant bacteria they were finding, Japanese scientists advanced the R-factor theory. The antibiotic-resistant Shigella prevalent in Japan were investigated.[36] Their studies showed that the dysentery-causing bacteria Shigella, which were resistant, could pass this resistance to E. coli. In turn, they found that E. coli could pass the resistance to sensitive shigellae.[1,37,55]

Great Britain:

In Great Britain, transferable drug resistance was discovered in 1962; the first isolation of multiple resistance reported outside of Japan. Strains of Salmonella typhimurium that had been isolated during an outbreak of gastro-enteritis in London in 1959 were found to be resistant to streptomycin, tetracycline, and sulfathiazole. This triple resistance could be transferred to a strain of Shigella sonnei and back again to S. typhimurium.[11]

In 1965 resistance to ampicillin and penicillin were reported in strains of S. typhimurium isolated in 1962.[2] Also, outbreaks of enteric infections due to strains of E. coli,[8] and Shigella[31] have been found to be carrying transferable drug resistance.

West Germany:

In West Germany, in 1963, resistance of Salmonella strains against four antibiotics was reported in the course of a Salmonella

infection in an infant treated with chloramphenicol. The resistance pattern bore no relation to the drug therapy used since chloramphenicol alone had been administered in treatment. This gave rise to the assumption that there had been a transfer of multiple resistance to the Salmonella. On investigation, an R-factor was found present in an enteropathogenic strain of Escherichia coli that was responsible for the resistance.[30]

These findings, like the observations made by the Japanese and British, showed that in pathogenic intestinal bacteria, resistance can arise in an indirect manner, namely by transfer. Isolates of drug resistance in pathogenic E. coli have become prevalent in Germany.[33]

Hungary:

In Hungary, in 1965, the episomal nature of drug resistant strains of Shigella was studied and verified. This study was prompted by the remarkably high incidence of multiple resistant strains. A majority of the Shigella strains isolated were found to be carrying episomal resistance thereby helping to explain the rapid increase in the incidence of multiple resistance in that country.[26]

Israel:

In Israel, multiple drug-resistant strains of Shigella rapidly appeared in 1956. It has been postulated that the marked increase in resistance in that country may be due to the spread of R-factors since they have been found in strains of Shigellae.[12,19,56] Transferable resistance to chloramphenicol in a strain of S. typhi has also been observed in a typhoid carrier treated with the drug.[9]

United States:

In the United States, bacteria carrying transferable antibiotic drug resistance were first reported in 1966.[24] Resistance was found in isolates of Salmonella, E. coli, and Shigella bacteria taken from humans in the Chicago area between September 1964 and February 1966. Also, reports of transferable resistance were published in 1966 on isolations of E. coli, Proteus, Pseudomonas, and Klebsiella bacteria from humans in the New England area.[42,43] and on clinical isolations of Salmonella furnished by laboratories in New York, Massachusetts, and Pennsylvania.[20] Transferable resistance to multiple antibiotics have since been associated with outbreaks of Shigellosis in Georgia,[18] and New York.[40]

Evidence indicates that resistance transfer factors are widespread in the United States. They have been found not only in the large metropolitan areas of the United States but also in the less populated regions.[48]

Other Countries:

Other countries and areas reporting incidence of infectious or transferable drug resistance include: The Netherlands,[34,35] Czechoslovakia,[41] Canada,[12,14] Brazil,[59] Greece,[13,27] Switzerland,[13] and South Africa.[59] No country appears to be exempt. Wherever R-factors have been looked for they have been found.

Relevance to developing nations

The immediate importance of transferable resistance to the world is that it poses a serious threat to effective treatment and control of enterobacterial diseases of both animal and man. Many of the organisms affected are notorious pathogens. Infections of the genito-urinary tract caused by enteric bacteria resistant drugs is a serious therapeutic problem.[42]

Enteric pathogens resistant to antibiotics emerge during treatment. The administration of a single antibiotic, to a patient with Shigellosis, has been followed by the appearance of multiple drug resistance.[55] The administration of low-level antibiotics in livestock feeds can cause and perpetuate drug resistance.[45]

In diseases of the intestinal tract or the excretory system where the commonest pathogen is E. coli, antibiotic-resistant

strains may multiply rapidly during antibiotic therapy. Resistant strains can become the dominant microorganisms and transfer RTF to all available Enterobacteriaceae.[24]

One of the most striking pictures from the onset was the early period of susceptibility of bacteria to antibiotics.[19] This was the period of early clinical use. Since then there has been a marked increase in the number of resistant enterobacterial strains.[12,55]

Because of resistance, infections are becoming more and more difficult to treat. Resistance by R-factors may be so high that the infections caused by the bacteria infected with the R-factors are not amenable to antibiotic therapy. Epidemic outbreaks due to the emergence of enteric pathogens resistant to antibiotics have occurred with increasing incidence with Shigella,[18,31,40] Salmonella,[43] and E. coli.[8]

The incidence of enterobacteria with transferable drug resistance has increased in direct proportion to the use of antibacterial drugs. In Japan, where antibiotics are widely used, the incidence of antibiotic-resistant strains of Shigella has increased enormously.[37] Whereas, almost no cases of Shigella resistance were reported in 1956, hospitals in three major cities in 1964 reported some 50 percent of the Shigella isolates were resistant to streptomycin, chloramphenicol, the tetracyclines, and the sulfonamides.[58]

In England, through RTF, resistance is rapidly increasing.[12] Antibiotic resistance in S. typhimurium increased from 2.9 percent during 1961-62, to 21 percent in 1963-64, to 61 percent in 1964-65.[9] Chloramphenicol and furazolidone resistance appeared in 1964.[9]

In 1966, high incidence of transferable drug resistance was found in England among strains of E. coli isolated from human beings and domestic animals suffering from diseases of the alimentary tract. The incidence appeared to be directly related to the extent that the drug had been used and was found to be increasing. In pigs, the incidence of resistance was twice as great in those isolated in 1965 as in those isolated in 1960-62. Also, neomycin and furazolidone resistance was found among the 1965 strains but not among the 1960-62 strains.[47]

Extensive and sometimes indiscriminate use of antibiotics in agriculture creates a dual problem. Strains of bacteria emerge which are resistant to antibiotics or which harbor R-factors.[39,51] It is reported that these bacteria are not only a hindrance to the effective treatment or control of the animal infection but are also a potential source of human infection or drug resistance.[2,9] E. coli strains from animals which received therapeutic drugs or low levels of antibiotics in their feed may possibly serve as vectors of drug resistance to humans.[2,9,46]

In 1965, the Enteric Reference Laboratory in Britain reported that of 2544 human S. typhimurium cultures examined, drug resistant type 29 comprised 22 percent. There were six deaths. Many times a connection could be demonstrated between the bovine and human infection caused by type 29. Where a connection was not demonstrated it could usually be deduced because of resistance to furazolidone, a drug used only in calves. Of all the 2544 S. typhimurium cultures, 63 percent or more represented types (including type 29) predominantly of bovine origin.[9]

Increases in drug resistance have been noted in the general population. In Great Britain, Escherichia coli strains isolated at random from healthy people show a high incidence of multiple-drug resistance.[46] Where antibiotics are used extensively, the incidence of strains with transferable drug resistance in the population at large is of the order of 1 in 10.[32]

Extrapolating from data available, the incidence of resistance to antibiotics can be expected to increase in any country as the use of antibiotics increases. This could become serious, particularly in countries where there is a sanitation problem.

In many countries, Salmonellosis has increased in the last two decades and may be considered a public health threat. In the underdeveloped countries, Shigellosis, or bacillary dysentery, presents a most serious threat to the health of mankind. Also,

epidemics occur most frequently in over-crowded populations with inadequate sanitation. Resistance to antibiotics in these countries could have serious consequences.

The World Health Organization Expert Committee on Antibiotics in its second report (1961) made the following observations: "Bacterial resistance to antibiotics is the principal obstacle to their successful therapeutic use. When resistance develops during the course of treatment, it may deprive an antibiotic of its proper therapeutic effect in the patient being treated. More important in the long run is the effect on the general community, since the elimination of sensitive strains and the dissemination of resistant ones lead to a situation in which many infections are resistant ab initio and alternate treatment must be adopted. For this reason, the estimation of bacterial sensitivity or resistance to antibiotics has assumed great importance. Such estimations are an essential prerequisite for the rational use of antibiotics and for preserving the efficacy of this important group of therapeutic substances."[60]

SUMMARY

Transferable drug resistance, discovered by Japanese workers in 1959, is a most important type of drug resistance of the enterobacteria. It can transfer multiple resistance quickly and effectively

from one bacterial cell to another of the same or different species simply by cell-to-cell contact; a feature distinguishing it from all other known forms of resistance.

Transferable drug resistance is confined principally to the Enterobacteraceae and can be transferred to some of the other gram-negative bacteria. Some that inhabit the alimentary tract also invade the genito-urinary tract. R-factors are prevalent in strains of Salmonella, Shigella, and E. coli and can be found throughout the world. There have been reports of transferable drug resistance in Europe, the Middle East, Southeast Asia, North and South America, and Africa.

Each year, reports of multi-resistant strains of bacteria appear with increased frequency. Also, resistant factors are being found to confer resistance to more and more antibacterial drugs. As a result, infections once considered susceptible to effective treatment by antibiotics are becoming all the more troublesome to treat. In countries where there are medical or sanitation problems, this development of transfer resistance unless controlled can be particularly serious.

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