THE ISOLATION OF *ENTEROBACTERIACEAE* POSSESSING THE PROPERTY OF TRANSMISSIBLE MULTIPLE-DRUG RESISTANCE

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Bacteria may become resistant to chemotherapeutic agents and antibiotics as a result of discrete mutations.[1] The chromosomal sites responsible for this change in phenotype may be disseminated in suitable bacterial populations by chromosomal transfer following conjugation, by transduction in which a bacteriophage conveys the genetic material to a recipient, and by transformation in which deoxyribonucleic acid liberated from the resistant donor penetrates the sensitive recipient.[1] These are the 3 'classical' *in vitro* methods of genetic exchange among bacteria, but their importance in Nature is difficult to assess.[2] In 1957 some *Shigella* strains isolated in Japan were resistant to sulphonamide, tetracycline, chloramphenicol and streptomycin, and by 1959 about 10% of *Shigella* isolated in that country possessed this quadruple resistance.[3,4] In 1960 Japanese workers[4] reported that these multiple-resistant strains could transmit their resistance-pattern *en bloc* to other sensitive *Enterobacteriaceae*, both *in vitro* and in the intestinal tract of experimental animals and human volunteers. They also showed that this multi-drug resistance is controlled by a genetic element which is distinct from the bacterial chromosome, is self-replicating, and transmissible at high rates to other bacteria by direct contact. This contagious transmission is the fourth method by which bacteria may become resistant to antibiotics and applies *in vivo*.[4] The portion of the genetic element responsible for transmission is called the resistance transfer factor (RTF). To it are attached the determinants of resistance (R-determinants) and the entire complex is called the R-factor.[5] In 1962 strains of *Salmonella typhimurium* with this property of transmissible multi-drug resistance were reported from England,[6] and in 1963 strains with similar properties were encountered in West Germany.[7] In 1965 Anderson and Datta[8] reported multiple-resistant strains of *S. typhimurium* also capable of transmitting ampicillin resistance. This paper reports the isolation of multiple drug-resistant strains of *S. typhimurium* able to transmit the property to other genera of the family *Enterobacteriaceae*.

METHODS

The patient was a White girl aged 1 year who was admitted to the Pretoria General Hospital on 14 July 1965 with bronchopneumonia. One month before admission she had an attack of gastroenteritis which subsided without specific treatment. From a rectal swab submitted to the routine section of this Department, a strain of *S. typhimurium* was isolated which was resistant to sulphonamide, tetracycline, chloramphenicol, streptomycin and ampicillin as determined by disc diffusion techniques. The patient had no gastro-intestinal symptoms and, since her respiratory condition had responded to tetracycline, she was discharged after 7 days. The strain of *S. typhimurium* was then investigated for the property of transmissible drug resistance. The techniques used were those of Datta[6] and Anderson and Datta[8] and acriflavine treatment of resistant strains was done according to Watanabe and Fukasawa.[9] Two weeks after her discharge from hospital a further stool specimen was obtained from the patient. From this specimen a strain of *S. typhimurium* was again isolated and 10 colonies of *Escherichia coli* and 4 non-lactose fermenting colonies, which were eventually allocated to the *Citrobacter* group, were also picked off plates for further investigation.

RESULTS

The 2 isolates of *S. typhimurium* and all 4 *Citrobacter* colonies had identical patterns of resistance. They were resistant to 25 μg./ml. of streptomycin, 100 μg./ml. of sulphadiazine and 750 μg./ml. of chloramphenicol, tetracycline and ampicillin. The 10 colonies of *E. coli* were sensitive to 20 μg./ml. of the above drugs. In mixed culture with a sensitive laboratory strain of *E. coli* (E 27) the *S. typhimurium* strains transmitted their full resistance pattern to it at a rate of about 10^{-2}/donor cell. The *E. coli* strain E 27 could be distinguished from the donors by sugar-fermentation reactions and susceptibility to a particular bacteriophage. The degree of resistance of the *E. coli* recipient was the same as that of the donor *S. typhimurium*. The *Citrobacter* strains also transmitted the 5 R-determinants to *E. coli* strain E 27 at a rate of about 10^{-4}/donor cell. The *S. typhimurium* also transmitted the full resistance-pattern to a *Providence* strain NCTC 9295 which could be distinguished from the *Salmonella* by biochemical reactions and phage susceptibility. The rate of transmission was low (about 10^{-4}/donor cell), but the degree of resistance acquired by the *Providence* strain was the same as that of the donor *S. typhimurium*. Two of the patient's sensitive *E. coli* isolates were grown in mixed culture with the *S. typhimurium* strain. Both these *E. coli* strains were converted to the same pattern and degree of resistance of the *S. typhimurium* donor at a rate of about 10^{-4}/donor cell. In all transmission experiments appropriate controls ruled out the possibility of bacteriophage-mediated transduction, and in no instance was segregated transfer of R-determinants encountered. Multiple-resistant recipient *E. coli* or *Providence* strains still retained the biochemical reactions and phage susceptibility of the corresponding drug-sensitive cultures. When grown in the presence of 1·8 μg./ml. of acriflavine, 10% of colonies of *S. typhimurium* lost their entire resistance pattern, 3·5% of the *Citrobacter* did the same and 6% of colonies of the newly-resistant *E. coli* strain E 27 also lost their resistance and became sensitive to 20 μg./ml. of the drugs. Control cultures had a corresponding figure of less than 1%. No segregated elimination was observed. The low figures for elimination of RTF episomes by acriflavine is characteristic of these episomes.[3,6]

DISCUSSION

The origin of RTF and R-determinants is obscure.[4,5,9] One view is that different RTF picked up single R-determinants by recombination with bacterial chromosomes and then acquired multiple resistance by combining with one another. Another explanation is that a single RTF serially picked up the different chromosomal resistance sites. Against these arguments are the facts that the biochemical mechanisms of multiple drug resistance may differ from those of non-RTF mediated resistance,[4] and that RTF transmitted resistance comes to expression immediately in a new recipient whereas chromosomal resistance is usually recessive.[10] Also the experience of the Japanese workers was that quadruple transmissible resistance was present from the start of their investigations.[4] These facts are difficult to reconcile with chromosomal gene pick-up theories and a *de novo* origin of R-factors has been suggested.[5] Anderson and Datta[8] presented evidence that treatment of calves with ampicillin resulted in an increase of ampicillin-resistant strains of *S. typhimurium* and that this resistance was contagious. Whatever their ultimate origin, the R-factors appear to be selected by drugs[4,5] and this fact may necessitate a review of the use of these agents in human and veterinary medicine.

It is not known whether both the *S. typhimurium* and the *Citrobacter* originally possessed the R-factor or (more likely) if one strain infected the other in the intestine of the patient. *Salmonella typhimurium* is a common animal parasite which often infects man. If it was the primary resistant organism in this case it could mean that a reservoir of R-factors already exists in the local animal population.

It is surprising that no multiple-resistant *E. coli* were isolated from the patient, particularly as the *S. typhimurium* strain could transmit its resistance to the patient's *E. coli in vitro*. The rate of transmission was low, however, and resistant clones may have been missed. The rate of transmission of multi-drug resistance factors differs among various recipients and is also influenced by the presence of other episomes.[10] However, the existence of strains which harbour R-factors have now been demonstrated in South Africa, and *in vivo* transmission of their drug-resistance to other *Enterobacteriaceae* may interfere with future therapeutic efficacy.[11]

SUMMARY

This paper reports the isolation of strains of *Enterobacteriaceae* with the property of transmissible multiple drug-resistance in South Africa. The strains are a *Salmonella typhimurium* and an organism belonging to the *Citrobacter* group, both of which were isolated from the stools of a White child. The strains are resistant to sulphonamide, tetracycline, chloramphenicol, streptomycin and ampicillin and are capable of contagiously transmitting this pattern of resistance *en bloc* to sensitive *E. coli* and *Providence* strains. The public health importance of the phenomenon is mentioned.

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