THE INCIDENCE OF TRANSMISSIBLE DRUG RESISTANCE FACTORS AMONG STRAINS OF *ESCHERICHIA COLI* IN THE PRETORIA AREA

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Antibiotic-resistant members of the family Enterobacteriaceae, which can transmit their resistance to other members of this family of Gram-negative bacteria, have been isolated in many countries.[1-4] This infectious resistance is controlled by a number of resistance determinants (R-determinants) which are linked on a piece of extrachromosomal genetic material. The transfer of this material to another cell is controlled by a transfer factor (TF). This factor is responsible for the appearance of a conjugation tube between the resistant and sensitive bacteria and also for the transmission of the R-determinants through this tube. The complex of transfer factor attached to resistance determinants is called an R-factor. Characteristics of this system are that the sensitive recipient organism becomes resistant to a number of antibacterial agents simultaneously and that the degree of resistance to some of the antibiotics is remarkably high. Strains of *Salmonella typhimurium* and Citrobacter carrying R-factors have been isolated in Pretoria.[4] In order to gain an impression of the prevalence of R-factors in the Pretoria area, it was decided to investigate locally isolated human strains of *Escherichia coli*.

METHODS AND MATERIALS

Two hundred and seven strains of *Escherichia coli* were isolated from an equal number of patients. One hundred and seventy were from faeces and 37 from urine specimens investigated by the routine section of this laboratory. Gram-negative bacteria which formed non-mucoid red colonies on MacConkey agar were regarded as *E. coli* for purposes of this experiment. A fully sensitive derivative of the multiple drug-resistant *Salmonella typhimurium* and *E. coli strain* E27, previously used,[4] both of which are sensitive to 25 μg./ml. of sulphadiazine, streptomycin, ampicillin, chloramphenicol and tetracycline, were employed as recipients for R-factors in conjugation experiments. The R-factor was eliminated from the *S. typhimurium* strain by means of acriflavine,[4] and this sensitive organism is now called RD42. The liquid medium was Difco Penassay broth and the plating medium was Difco MacConkey agar. Sensitivity to antibiotics was determined by streaking loopfuls of overnight broth cultures onto Penassay base agar, into which appropriate concentrations of the agents had been incorporated. Sulphonamide sensitivity tests were done on a minimal medium[5] in order to overcome interference from sulphonamide inhibitors present in nutrient media.[3] Transfer experiments were done by mixing 1 ml. of overnight broth cultures of resistant donor *E. coli* and sensitive recipient *S. typhimurium* RD42 in 5 ml. of warm broth. The mixture was incubated at 37°C. One ml. of the overnight mixture was added to 10 ml. of warm selenite broth to inhibit the donor *E. coli*, and again incubated. Dilutions of this culture to yield about 200 colonies/plate were then spread on 5 drug-containing MacConkey agar plates. Each plate contained a different drug. After overnight incubation, pale resistant Salmonella colonies were picked off into broth and subsequently tested for their complete spectrum of resistance. Broth cultures of donor and recipient organisms were also plated on drug-free and drug-containing media as controls. Experiments were also done to determine whether the Salmonella strain RD42, which had newly-acquired R-factors from different resistant *E. coli*, was infectious for this property. This was done by mixing the newly-resistant Salmonella with the sensitive *E. coli* E27. These experiments were done as above with the exception that the selenite step was excluded. The resistance-spectrum was also determined for any newly-resistant *E. coli* E27 so produced.

RESULTS

Forty-six of the 207 *E. coli* strains examined were sensitive to 25 μg./ml. of sulphadiazine, streptomycin, ampicillin, chloramphenicol and tetracycline, respectively, and were discarded. The remaining 161 strains were resistant to at least 25 μg./ml. of one or more of the 5 drugs and could be divided into 16 groups according to their pattern of resistance (Table I). The resistance of these strains to sulphadiazine was 100 μg./ml. and resistance to ampicillin and chloramphenicol was 250 μg./ml. Strains resistant to tetracycline grew on agar containing 100 μg./ml., but resistance to streptomycin never exceeded 25 μg./ml.

TABLE I. RESISTANCE PATTERNS AND INCIDENCE OF R-FACTORS IN 207 STRAINS OF *E. coli*

Resistance pattern	*Faeces*	*Urine*
Sensitive	41	5
SuSmACmT	56 (16)	13 (5)
SmACmT	3 (0)	0 (0)
SuSmA T	8 (1)	1 (0)
SuSm	6 (1)	2 (1)
SuSm T	5 (0)	4 (0)
SuSm CmT	2 (1)	2 (0)
Su ACmT	18 (2)	3 (1)
Su Cm	2 (0)	0 (0)
Su	11 (0)	1 (0)
Su ACm	1 (0)	0 (0)
Su CmT	3 (1)	1 (0)
T	4 (0)	1 (0)
Su A T	1 (0)	1 (0)
Su T	4 (1)	0 (0)
A T	2 (0)	1 (0)
SuSmA	3 (0)	2 (0)

Su=sulphonamide, Sm=streptomycin, A=ampicillin, Cm=chloramphenicol and T=tetracycline. Figures in brackets indicate number of strains which possess R-factors.

While donor organisms always grew readily on the drug-containing plates, the recipient controls, plated in pure culture, never showed growth. Twenty-one of the 129

resistant *E. coli* from faeces and 7 of the 32 resistant *E. coli* from urine transmitted their full pattern and degree of resistance to the sensitive *S. typhimurium* strain RD42 when grown with the latter organism. An additional 2 faecal strains of *E. coli*, the one resistant to all 5 drugs and the other resistant to sulphonamide, ampicillin, chloramphenicol and tetracycline, only transferred portions of their resistance pattern. The former transmitted streptomycin resistance while the latter transmitted resistance to sulphonamide and streptomycin. All the newly resistant Salmonella transmitted antibiotic resistance to the sensitive *E. coli* E27. With 5 exceptions the entire resistance pattern was again transmitted. The degree of newly-acquired resistance in all experiments was exactly equivalent to that pertaining in the donor organism.

DISCUSSION

Thirty of the 161 resistant strains of *Escherichia coli* were capable of transmitting drug resistance to a sensitive *Salmonella typhimurium*. This incidence (19%) is low compared with results of a recent investigation in England[6] where 19 of 20 resistant *E. coli* from human sources harboured R-factors. The use of other and possibly more competent sensitive recipients might have increased the present incidence. It is well known[7] that sensitive strains vary in their ability to accept R-factors. The use of sodium selenite favoured survival of Salmonella in the mating mixtures, but even this selection may not have been severe enough to detect low transmission rates. The level of streptomycin resistance of all the donor and newly-resistant recipient strains never exceeded 25 μg./ml. This is low in comparison with the high levels of resistance attainable by chromosomal mutation in bacteria. Resistance to ampicillin and chloramphenicol was of the order of 250 μg./ml., while that to tetracycline and sulphonamide was 100 μg./ml. These values for transmissible drug resistance accord well with our previous findings[4] and also those of Japanese[1] and English[2] workers and are characteristic for contagious acquired resistance. The 130 resistant *E. coli* strains which failed to transfer their resistance were resistant to the same levels of drugs as the 30 infectious strains. This is an anomaly. It seems unlikely that these strains could have accumulated sufficient step-wise individual chromosomal mutations to achieve the level and spectrum of antibiotic resistance. R-determinants require transfer factors to render them infectious. Resistant strains have been described[8] which have lost the transfer factor but still harbour resistance determinants. The non-infectious resistant strains described here may be of this variety. Segregation of resistance determinants occurred in 2 transfers from *E. coli* to *S. typhimurium* and in 5 transfers from *S. typhimurium* to *E. coli*. The higher rate of segregation of R-determinants in *S. typhimurium* agrees with results obtained by other workers.[5] Despite the uncertainty about the origin of R-determinants and transfer factors, the selection of strains carrying these factors is favoured by the use of antibacterial agents.[9] The existence of organisms possessing infectious drug resistance poses a therapeutic problem of as yet undefined dimensions and may eventually necessitate stricter control over antibiotic administration to man and animals. This survey has attempted to partially define the problem in this area.

SUMMARY

Infectious drug resistance was encountered in 19% of 161 drug-resistant *Escherichia coli* from human sources. These *E. coli* transferred their resistance spectrum to a sensitive Salmonella indicator strain during conjugation. This transfer often involved resistance to 5 different drugs. The resistant *E. coli*, which failed to transmit their resistance pattern contagiously, often had similar degrees and spectra of resistance as the infectious varieties, and it is postulated that the former may have lost a factor responsible for the transfer of resistance determinants. The public health importance of these findings is mentioned.

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