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. 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. These are the three classical in vitro methods of genetic exchange among bacteria, but their importance in Nature is difficult to assess. 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. In 1960 Japanese workers 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. 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. In 1962 strains of Salmonella typhimurium with this property of transmissible multi-drug resistance were reported from England, and in 1963 strains with similar properties were encountered in West Germany. In 1965 Anderson and Datta 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 and Anderson and Datta and acriflavine treatment of resistant strains was done according to Watanabe and Fukasawa. 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 10⁻³ 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 10⁻¹ 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 10⁻¹ 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.
DISCUSSION

The origin of RTF and R-determinants is obscure. 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, and that RTF transmitted resistance comes to expression immediately in a new recipient whereas chromosomal resistance is usually recessive. Also the experience of the Japanese workers was that quadruple transmissible resistance was present from the start of their investigations. These facts are difficult to reconcile with chromosomal gene pick-up theories and a de novo origin of R-factors has been suggested. Anderson and Datta presented evidence that treatment of calves with ampicillin resulted in an increase in resistant organisms in the intestinal flora of the patient. The strains are resistant to sulphonamide, tetracycline, chloramphenicol, streptomycin and ampicillin and are capable of contagiously transmitting this pattern of resistance in vivo to sensitive E. coli and Providencia strains. The public health importance of the phenomenon is mentioned.

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REFERENCES


Case Report

ATYPICAL SPHEROCYTOSIS IN AN AFRICAN GIRL

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Hereditary spherocytosis is best known as a disorder affecting people of European origin, although it is by no means confined to this group. The disease has been reported in Egyptians by Salah, in Filipinos by Stransky and Dausis-Lawas, and Kline and Holman in a comprehensive search of the literature reported that 42 bona fide cases had been described in Negroes. In the African this condition is considered to be a distinctly rare entity. Gelfand has not seen it in an African in the Rhodesias, and Foy and Kondi recorded, without mentioning any details, one typical case of hereditary spherocytosis in Kenya. In the South African Bantu, Merskey and Baskind and Gon each reported a case of chronic haemolytic anaemia resembling acholuric jaundice. In neither case was a family study carried out and in the former, comprehensive techniques for the exclusion of antibodies had not been evolved at the time of recording. Metz was the first to report a Bantu family where the diagnosis of hereditary spherocytosis could be established and he mentioned a further case in a Bantu male. Recently, Spector and Metz recorded another Bantu family with hereditary spherocytosis. The true incidence of this disease in races other than European is not known. Whether the paucity of reports in Africans indicates that the disorder is rare in this race, or results from failure in diagnosis, or in reporting of known cases, is not possible to assess. In view of the apparent rarity of this disorder in Africans, this paper presents a case in a Bantu girl which, for reasons to be mentioned later in the report, is considered to be a case of atypical spherocytosis or 'type-B' of Young, Izzo, Altman and Swisher.

METHODS

Routine haematological studies were performed by standard methods. Autohaemolysis studies were done by the method described by Cartwright; glucose utilization studies by the...