Multiple Drug Resistance and the Presence of R Factors in Enteric Pathogens

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THE TRANSFER OF drug resistance between **Shigella** and **Escherichia coli** was first demonstrated in Japan (Ochiai et al, 1959; Akiba et al, 1960). The term R factor has been used to denote the genetic determinant responsible for such transferable drug resistance.

It is now known that such factors may be present in many genera of gram negative bacilli (Mitsuhashi et al, 1967). Transfer can take place between different taxonomic groups, such as the **Enterobacteriaceae, Pseodomonas, Vibrio** and **Pasteurella** (Chabbert et al, 1969).

The use of antibiotics has resulted in the selection of bacteria carrying R factors, and drug-resistant gram-negative enteric bacilli is on the increase in many countries. This paper reports the incidence of multiple drug resistance and presence of R factors in enteric pathogens in Singapore.

Materials and Methods

The sensitivity patterns of 168 **salmonellae** and 44 **shigellae** isolated from clinical specimens have already been described (Sng and Lam, 1970; Sng and Lam, in press). Those resistant to ampicillin, streptomycin, tetracycline, chloramphenicol, kanamycin, neomycin and sulphonamide were selected for the present study. The R factor recipient strain used was **E.coli K12,** which was provided by Dr. H. Fukumi from Japan.

Media: Brain Heart Infusion (BHI, Difco) was used as propagating media for R factor transfer. For selection of resistant strains, DST (Oxoid) agar plates containing ampicillin (A; 25μg/ml), streptomycin (S; 12.5μg/ml), tetracycline (T; 25μg/ml) and sulphadiazine (Su; 100μg/ml) were prepared. To differentiate **E.coli** from non-lactose fermenters, eosin methylene blue (EMB) agar with 0.5% lactose was used.

Transfer of R factor from **salmonellae** and **shigellae:** 0.1 ml. of an overnight broth culture of each donor **(salmonellae** and **shigellae)** and recipient **(E.coli K12)** were mixed in 5 ml. of BHI broth. After incubation at 37°C overnight, serial ten-fold dilutions of the cultures were prepared in sterile normal saline. 0.1 ml. from each dilution was spread over the surface of DST agar plates containing the drugs. After overnight incubation at 37°C, resistant colonies were cultured on EMB plates for one day. Lactose fermenting colonies were then subcultured twice, after which drug resistance was determined on DST agar incorporated with the anti-microbial agents. A strain of **E.coli K12,** which had not been used as a recipient, was used as a control to check on the effectiveness of the anti-microbial drugs.

Transfer of R factor to S.typhi: The donor was **E.coli K12** which had previously been made resistant to ampicillin, tetracycline, chloramphenicol and sulphadiazine after conjugation with a strain of **Sh.flexneri** carrying the R factor. Six sensitive S.typhi strains were used as recipients. The procedure was the same as that mentioned above, except that non-lactose fermenting colonies were selected from EMB agar plates, subcultured and tested for drug resistance.

TABLE I

Distribution of Drug-Resistant Strains

Strains	Number	A	S	T	C	K	N	Su	No. resistant	% resistant
S.typhi	104	–	–	5	–	1	1	2	10	9.6
other salmonellae	64	1	9	6	–	1	1	14	23	35.7
Sh.flexneri	26	1	2	3	1	1	1	10	10	38.5
Sh.sonnei	18	–	1	1	–	–	–	6	6	33.3

TABLE II

Distribution of Multiple Resistant Strains

Species	No.	Resistance patterns	% resistant strains with multiple resistance
S.typhi	1	S K N	10
S.abony	1	K N	
S.derby	5	S Su	
S.paratyphi B	1	A S Su	34.8
S.weltevreden	1	S Su	
Sh.flexneri	1	A S T C Su	
	1	T Su	30
	1	S T K N Su	
Sh.sonnei	1	S Su	
	1	S T	33.3

Results

Among the strains studied, 9.6% of S.typhi, and about one-third of the other **salmonellae** and **shigellae** were resistant to either ampicillin, streptomycin, tetracycline, chloramphenicol, kanamycin, neomycin or sulphadiazine **(Table I).**

Multiple resistance was shown by one (10%) of the insensitive **S.typhi**, eight (34.8%) of the other resistant **salmonellae**, and three (30%) of the resistant **Sh.flexneri (Table II).** Two (33; 3%) of the resistant **Sh.sonnei** were multiple resistant. Resistance to kanamycin and neomycin always occurred together, and there was close association between resistance to streptomycin and sulphadiazine. Five **S.derby** strains had the same multiple resistance pattern.

One **S.paratyphi** B and three **Sh.flexneri** were found to carry R factors **(Table III).** All the strains were multiple resistant, but the resistance patterns which were transferred were different. All the multiple resistant **Sh.flexneri** carried R factors. In the case of **S.paratyphi B.** only resistance to ampicillin, but not streptomycin and sulphadiazine, was transferable.

Of the six **S.typhi** strains tested, one was found capable of receiving R factor.

TABLE III

Resistance Patterns of R Factors Transferred

Species	No.	Resistance patterns	% resistant strains carrying R factors
S.Paratyphi B	1	A	1.6 (non-S.typhi salmonellae)
Sh.flexneri	1	A S T C Su	30
	1	S T K N	
	1	T Su	

Discussion

R factors are extrachromosomal particles of DNA which confer on their cell hosts drug resistance, often multiple in nature, and may be transferred from cell to cell either by conjugation or transduction (Watanabe, 1969). As such transfer can occur between different genera of bacteria, it is possible for drug resistance to spread from non-pathogens to pathogens, thus giving rise to therapeutic problems (Chabbert et al, 1969). In Japan, the increased isolation frequency of multiple resistant **shigellae** strains, which reached 95% in 1965, has been ascribed to the appearance and distribution of strains carrying R factors (Mitsuhashi et al, 1967).

At the moment, drug resistance in enteric pathogens is not so common in Singapore (Sng and Lam, 1970; Sng and Lam, in press), but the percentage of multiple resistance amongst resistant strains is fairly high. Amongst resistant strains, 10% **S.typhi**, 34.8% of the other **salmonellae**, 30% **Sh.flexneri** and 33.3% **Sh.sonnei** showed multiple resistance.

From these strains, four (28.6%) were found to carry R factors. All the three multiple resistant **Sh.flexneri** carried R. factors. This constitutes 30% of resistant strains, and is less than what was found by Mitsuhashi et al (1967) in Japan, where 81% were found to carry R factors.

One **S.paratyphi B**, resistant to ampicillin, streptomycin and sulphadiazine, could only transfer resistance to ampicillin. Resistance transfer in **S.paratyphi B** is not so common, but has been reported before (Datta, 1968).

The R factor in **S.typhi** appears to be rare fortunately, Mare (1967) studied 506 strains of which 4 were found resistant. None of them transferred R factors, though they could receive them. However, Sompolinsky and Aboud (1967) have reported isolation of **S.typhi** carrying two R factors from the faeces of a patient. In our series, none of the **S.typhi** carried R factors, but one of the six strains tested received R factor from a donor. Thus the **S.typhi** are potential carriers of R factors.

R factors are present in enteric pathogens in Singapore, and could further increase through the frequent administration of antibiotics. The danger is that **S.typhi** strains might acquire the R factors. To prevent this, it is perhaps desirable that more thought should be given to the role of antibiotics in the treatment of uncomplicated enteritis of bacterial origin. Aserkoff and Bennett (1969) found that patients with **S.typhimurium** infection, when given antibiotics, took longer to clear the organism from their intestines than those not given the drugs. Furthermore, 97% of those given antibiotics excreted strains which had acquired antibiotic resistance, whereas none of those untreated excreted resistant strains.

Summary

The resistance patterns of 168 **salmonellae** and 44 **shigellae** were studied. Drug resistance was not common, but amongst the resistant strains, multiple resistance was found in 10% **S.typhi**, 34.8% of the non-**S.typhi salmonellae**, 30% **Sh.flexneri** and 33.3% **Sh.sonnei.** In four (28.6%) of these multiple resistant strains, the R factor was found. All three **Sh.flexneri** with multiple resistance carried R factors. The resistance patterns transferred were all different. One

S.paratyphi B also carried the R factor. Though **S.typhi** did not carry the R factor, one of six strains tested could receive it. In the discussion, attention is drawn to the potential danger that exists should **S.typhi** strains acquire R factors. It is suggested that there should be greater discrimination in the use of antibiotics for enteric infections.

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