FIRST ISOLATES OF CHLORAMPHENICOL RESISTANT *S. TYPHI* IN MALAYSIA

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INTRODUCTION

Interest in the antibiotic sensitivity of *S. typhi* was stimulated by the report of a massive outbreak of chloramphenicol resistant typhoid in Mexico in 1972 (Anderson and Smith, 1972). The strain implicated in this outbreak had two important features: it was highly resistant to chloramphenicol and this resistance resulted from the carriage of a transferable resistance factor (R factor) which coded resistance to streptomycin, sulphonamides, and tetracycline. Since then transferable chloramphenicol resistance has been reported in the typhoid bacillus in countries such as India (Paniker and Vimala, 1972), Vietnam (Butler *et. al.*, 1973, Brown *et. al.*, 1975), Thailand (Lampe *et. al.*, 1974) and Indonesia (Sanborn and Lesmana, 1975). The incidence of such strains seems to be on the increase and they already constitute a substantial proportion of cultures isolated from typhoid patients in countries like Vietnam and Thailand (WHO, 1974).

The bacteriology division of the Institute for Medical Research in Kuala Lumpur, Malaysia has been watching out for the emergence of such strains in this country for some time but until late 1978 no such strains were encountered (Jegathesan, 1978). Subsequently 4 strains have been isolated. This paper discusses the bacteriological features of these first isolates of chloramphenicol resistant *S. typhi* in Malaysia.

MATERIALS AND METHOD

S. typhi strains

693 *S. typhi* strains forwarded from all over Malaysia to the Institute for Medical Research for Viphage typing between January 1978 and December 1979 were monitored for their sensitivity to antibiotics.

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Antibiotic Sensitivity Tests

All the strains were routinely screened for resistance by a comparative disc diffusion method (Stokes amd Waterworth, 1972) against the following antibiotics: ampicillin 10ug, chloramphenicol 10ug, cotrimoxazole 25ug, tetracycline 10ug, gentamycin 10ug, streptomycin 10ug and kanamycin 30ug.

Resistant strains were retested for their antibiograms by "spotting" with a replicating apparatus onto antibiotic incorporated agar plates and examining for growth after 24 hours. Strains not inhibited by 30 ug/ml of chloramphenicol, ampicillin, tetracycline, neomycin, kanamycin, streptomycin or 50 ug/ml of nalidixic acid or 300 ug/ml of sulphadiazine were regarded as resistant.

Transfer Experiments

The recipient organisms used were *E. coli* W3110, *E. coli* W1802 and *Salmonella saint paul,* all resistant to nalidixic acid.

Conjugation mixtures consisted of log phase cultures of the donor and recipient strains in a ratio of approximately 1:100. Controls of the donor and recipient strains were also set up. After overnight incubation at 37°C, appropriate dilutions of the mixtures and controls were plated on selection plates. The selection plates consisted of MacConkey Agar containing 50 ug/ml of nalidixic acid and 30 ug/ml of either chloramphenicol or ampicillin or tetracycline or kanamycin. Transconjugants growing on the selection plates were then tested by multipoint replication to ascertain the patterns of antibiotic resistances transferred.

RESULTS

Of the 693 strains of *S. typhi* tested, 4 strains were shown to be resistant to chloramphenicol and other antibiotics using the disc diffusion and agar dilution methods.

The characteristics of these 4 strains are summarised in Table I. The strains came from a 20 year old Chinese female from Kuala Lumpur, a 6 year old Malay female from Mentakab, a 30 year old Chinese female from Seremban and a 54 year old Malay male from Kota Baru.

Phage typing was carried out on these strains and one was found to be phage type E_1, another a degraded type A and two were untypeable.

The resistance shown by two of the four strains was found to be transferable to the sensitive recipient *E. coli* strains. Transfer of resistance was not shown by the remaining two strains. The first strain showed resistance to chloramphenicol and ampicillin which was seen to be transferred together in all 18 transconjugant *E. coli* colonies picked for study. The second strain showed resistance to ampicillin, chloramphenicol, kanamycin, neomycin, streptomycin and sulphadiazine. 24 transconjugant E. *coli* colonies were picked for study and three patterns of resistance transfer were observed. 18 colonies had picked up resistance to all the 6 antibiotics, 1 colony showed resistance to ampicillin, chloramphenicol, streptomycin and sulphadiazine while 5 colonies exhibited resistance to chloramphenicol, kanamycin and neomycin only.

DISCUSSION

Transferable chloramphenicol resistance in *S. typhi* which has not been reported in Malaysia till late 1978 has now been shown to occur. Two isolates have been shown to possess transferable resistance not only to chloramphenicol but to ampicillin and other antibiotics as well. Such transferable resistance is dependant on extrachromosomal genetic material (plasmids) which have been called 'R' factors.

The fear is that such resistant strains will increasingly be isolated in the future posing a serious public health and therapeutic problem as far as the treatment, management and control of typhoid are concerned. Typhoid fever is still a serious problem in this country and chloramphenicol is widely used in its treatment. Ampicillin has often been used as an alternate drug. The two isolates described in this paper have been shown to possess transferable resistance not only to chloramphenicol but to ampicillin as well. In view of this, continued vigilance is absolutely essential to monitor the emergence of resistant strains so that rational therapeutic approaches can be planned.

The authors are not really surprised that they have been able to encounter such strains of *S. typhi.* 'R' factor mediated resistance to chloramphenicol and other antibiotics have been shown to be present in other Salmonella serotypes isolated in this country for many years (Khor and Jegathesan – unpublished data) and it was only a matter of time before some *S. typhi* strains were similarly able to pick up these 'R' factors. Furthermore *S. typhi* strains possessing transferable resistance to chloramphenicol and other antibiotics are rampant in some of the neighbouring countries. For instance in Thailand, chloramphenicol resistant *S. typhi* strains constitute a substantial proportion of cultures isolated from typhoid patients (Lexomboon and Unkurapiana, 1978).

The resistant strains isolated in Malaysia have come from patients from divergent geographical locations and are also of different phage types. This points to a multifocal emergence of 'R' factor acquisition. This probably indicates that the 'R' factors coding for resistance were initially present in non pathogenic commensal organisms such as E. coli. The indiscriminate use of antibiotics has resulted in selection pressure which ensured, first the prevalence of such 'R' factors in these bacteria and subsequently their transfer to the typhoid bacillus on separate occasions.

Further spread of such transferable drug resistance can only be controlled by more prudent use of antibiotics not only in humans but in animals as well.

It is the purpose of this paper to point out the potential problem that will ensue if the emergence and spread of chloramphenical resistance in *S. typhi* is to increase in the future and the need for vigilance and the necessity for steps to be taken to minimise this danger.

SUMMARY

Four strains of *S. typhi* isolated in Malaysia were found to show resistance to chloramphenicol and other antibiotics. In two of these strains it was possible to show that this resistance was transferable. This problem which is widespread in neighbouring countries and undetected in Malaysia till recently has now been shown to exist in this country. Fears that the incidence of such strains will increase in the future are expressed and the need for vigilance is emphasised.

Table I

Characteristics of chloramphenicol resistant *S. typhi* strains isolated in Malaysia

Strain	Agar Dilution Tests	Results of Transfer Experiments			Patterns transferred	Frequency (Proportion of total no. of colonies tested)	Phage type
		Recipient strains					
	Resistant to:	E. coli W1802	E. coli W3110	Salmonella saint paul			
1. Isolated from the stool of a 20 year old Chinese Female, Kuala Lumpur.	A (100 ug/ml) C (100 ug/ml)	Transfer seen	Transfer seen	Not done	AC	18/18	E_1
2. Isolated from a stool of a 6 year old Malay Female, Mentakab.	A (100 ug/ml) C (100 ug/ml) K (30 ug/ml) N (30 ug/ml) S (20 ug/ml) SU (300 ug/ml)	Transfer seen	Transfer seen	Not done	ACKNSSu ACSSu CKN	18/24 1/24 5/24	A degraded
3. Isolated from the stool of a 30 year old Chinese Female, Seremban.	A (100 ug/ml) C (100 ug/ml) S (20 ug/ml) Su (300 ug/ml) T (30 ug/ml)	Not transferred	Not transferred	Not transferred	–	–	untypeable
4. Isolated from blood of a 54 year old Malay Male, Kota Baru.	A (100 ug/ml) C (50 ug/ml) S (20 ug/ml) Su (300 ug/ml) T (30 ug/ml)	Not transferred	Not transferred	Not transferred	–	–	untypeable

Key to antibiotics:
A – Ampicillin
C – Chloramphenicol
K – Kanamycin
N – Neomycin
S – Streptomycin
Su – Sulphadiazine
T – Tetracycline

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