PLASMID-MEDIATED TRANSFERABLE CHLORAMPHENICOL AND TETRACYCLINE RESISTANCE IN *SALMONELLA TYPHI* (Vi PHAGE TYPE 25) ISOLATED IN PENINSULAR MALAYSIA

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SUMMARY

A clinical isolate of Salmonella typhi *(Vi phage type 25), resistant to chloramphenicol, streptomycin and tetracycline, was examined for the presence of R plasmids. Results from conjugation, agarose gel electrophoresis and transformation experiments indicated that it harboured a single large self-transmissible R plasmid which coded for both the chloramphenicol and tetracycline resistance traits.*

INTRODUCTION

Bacterial plasmids are extrachromosomal genetic elements which are capable of stable autonomous replication in their host cells. [1] R plasmids confer upon bacteria resistance to many different antibiotics, and their role in bacterial antibiotic resistance has been well documented. [2-7]

Salmonella typhi is the causative agent of typhoid fever, and it is normally controlled by drugs, especially chloramphenicol which is the drug of choice in the treatment of typhoid fever. However, in recent years, chloramphenicol-resistant strains of *S. typhi* have been isolated in many parts of the world with increasing frequency. [8-21] Some of these chloramphenicol-resistant strains caused outbreaks of typhoid fever, and almost all of them were known to be associated with R plasmids. [9,10,11,13,15,21]

In Malaysia, Jegathesan and Khor [20] have reported that four out of 693 strains of *S. typhi* were resistant to chloramphenicol and other antibiotics. Two of the four strains have been shown to transfer their resistance traits. However, no attempt has been made to demonstrate physically the presence of R plasmids in these strains. In view of this, we chose to study the basis of antibiotic resistance of a multiple drug resistant clinical isolate of *S. typhi* (Vi phage type 25).

In this paper we report the genetic evidence provided by conjugation, agarose gel electrophoresis and transformation experiments that a large conjugative R plasmid was responsible for two of the antibiotic resistance traits in the multiresistant *S. typhi* isolate.

MATERIALS AND METHODS

Bacterial strains

The *S. typhi* strain was isolated from blood culture and stool of a patient and it was identified by the methods described in Edwards and Ewing [22] and Cowan [23] in the Bacteriology Laboratory of the Kuala Lumpur General Hospital. It was then forwarded to the Division of Bacteriology, Institute for Medical Research, Kuala Lumpur, for Vi-

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phage typing. The patient initially did not respond clinically to chloramphenicol administered in standard dosage. However, he recovered after cotrimoxazole (Bactrim) was given.

Escherichia coli CSH56, [24] a plasmidless strain which is sensitive to all antibiotics tested except nalidixic acid, was used as the recipient in the mating experiment. *E. coli* JA221, [25] a plasmidless antibiotic-sensitive strain, was used in the transformation experiment. *E. coli* V517 [26] was used to provide size reference plasmids of known molecular weight.

Media

LB broth [24] was used as the liquid rich medium. Agar plates were prepared by solidifying LB broth with 1.5% Difco Bacto-agar.

Antibiotic sensitivity test

Antibiotic sensitivity of the *S. typhi* strain was determined on Isosensitest agar (Oxoid) by the disc diffusion technique [27] with *E. coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 as sensitive controls. The antibiotic-discs used were: ampicillin (Ac) 10 μg, carbenicillin (Cb) 100 μg, cefuroxime (Cf) 30 μg, chloramphenicol (Gm) 10 μg, cotrimoxazole (Ct) 25 μg, gentamicin (Gm) 10 μg, kanamycin (Km) 30 μg, nalidixic acid (Nx) 30 μg, streptomycin (Sm) 10 μg and tetracycline (Tc) 10 μg.

Mating procedure and analysis of transconjugants

Bacterial matings were performed on the surface of LB agar plates by mixing 0.1 ml of donor culture and 0.1 ml of recipient culture in the late-exponential phase of growth, followed by incubation at 37°C for two hours. After mating, the surface of the agar was flooded with 1 ml of LB broth, and the cells resuspended. Ten-fold serial dilutions of the mating mixture were then prepared in 0.85% saline. 0.1 ml aliquots of various dilutions were plated on LB selective agar plates containing Nx (100 μg/ml) and Cm (25 μg/ml). The controls containing the donor and recipient alone were treated in exactly the same way as the mixed matings. Neither the donor *Salmonella* strain nor the recipient *E. coli* grew on the selective medium.

To examine the acquisition of unselected resistance traits, single transconjugant colonies growing on the selective medium were purified and toothpicked onto LB agar plates containing either Sm (100 μg/ml) or Tc (20 μg/ml). The donor and recipient cells were also inoculated on the same antibiotic plates as the transconjugants to act as controls.

Transfer frequency is expressed as the number of transconjugants per ml of the mating mixture divided by the number of donor cells per ml of the same mating mixture.

Isolation of plasmid DNA and agarose gel electrophoresis

The rapid extraction method of Kado and Liu [28] was used to isolate plasmid DNA from the *Salmonella* donor and the *E. coli* transconjugants. Horizontal agarose gel electrophoresis for the detection of plasmid DNA was performed according to Meyers *et al.* [29]

Transformation and analysis of transformants

Plasmid DNA extracted from one of the *E. coli* tranconjugants by the method of Kado and Liu [28] was first dialysed against TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 7.5). It was then used to transform *E. coli* JA221 to Cm resistance and Tc resistance, respectively, following the method described by Dagert and Ehrlich. [30]

Transformant colonies growing on the Cm and Tc plates were purified and toothpicked onto different LB plates, each containing Cm (25 μg/ml), Sm (100 μg/ml) or Tc (20 μg/ml). *E. coli* JA221 cells were also inoculated on the same antibiotic plates as the transformants to act as control.

Antibiotics

Chloramphenicol, nalidixic acid, streptomycin sulphate and tetracycline HCl, added to agar or broth, were purchased from Sigma Chemical Co., U.S.A.

RESULTS

Antibiotic resistance phenotype

The *S. typhi* strain was shown by disc diffusion tests to be resistant to Cm, Sm and Tc, and sensitive to Ac, Cb, Cf, Ct, Gm, Km and Nx.

Transfer of Cm resistance by conjugation to *E. coli*

Cm resistance was transferred from the *S. typhi* donor to the *E. coli* CSH 56 recipient at a frequency

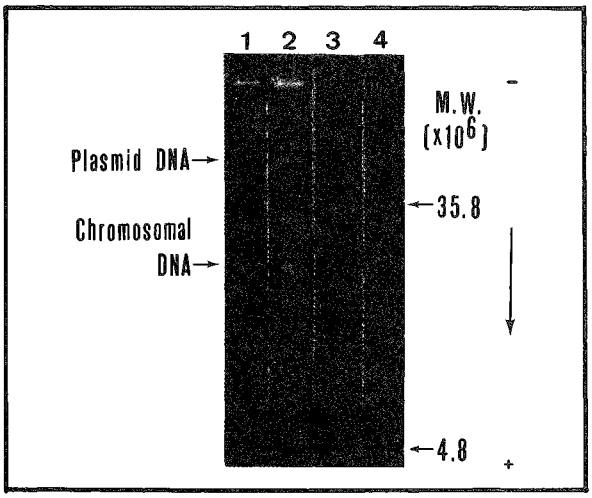

Fig. 1 Agarose gel (0.5%) electrophoresis of DNA extracted from *S. typhi* donor (1), *E. coli* CSH56 transconjugant (2), *E. coli* CSH56 recipient (3) and *E. coli* V517 (4).

of 1.3×10^{-6}.

Coinheritance of unselected resistance traits

Fifty *E. coli* transconjugant colonies growing on the selective agar plates were purified and toothpicked onto media containing antibiotics to determine the nature of other antibiotic resistance traits that had been cotransferred by conjugation. All the transconjugants were found to have acquired resistance to Tc. They were, however, sensitive to Sm.

Isolation of plasmid DNA

As shown in Fig. 1, after electrophoresis a slower-migrating DNA band was detected behind the chromosomal DNA band in both the *Salmonella* donor and the *E. coli* transconjugant, but was absent in the *E. coli* CHS56 recipient. This band indicates the presence of a high molecular weight (M.W. greater than 35.8×10^6) plasmid DNA in both the donor and the transconjugant.

Transformation

Dialysis against TE buffer, 25 μl of the plasmid DNA extracted from one of the *E. coli* transconjugateds was used to transform *E. coli* JA221. Both Cm resistant and Tc resistant transformants were obtained, although at low frequencies (Table I).

Coinheritance of unselected resistance traits

All the transformant colonies were purified and toothpicked onto media containing antibiotics to determine the nature of other antibiotic resistance traits that had been cotransferred by transformation. As shown in Table I, all the transformants were resistant to Cm and Tc but sensitive to Sm, irrespective of their origins.

DISCUSSION

A strain of *S. typhi* (Vi phage type 25) resistant to Cm, Sm and Tc was isolated from a patient in Peninsular Malaysia. From the intergeneric mating experiment, *in vitro* transfer of Cm resistance was demonstrated from the multiresistant *S. typhi* strain to a susceptible *E. coli* strain at a frequency of 1.3×10^{-6} at 37°C. The other resistance trait transferred simultaneously by conjugation was Tc.

The cotransfer of Cm and Tc resistance traits suggests that they are linked on a conjugative R plasmid. The physical evidence of a plasmid was provided by agarose gel electrophoresis, and confirmation that the two resistance traits were encoded by this plasmid was provided by the transformation experiment. Plasmid DNA extracted from one of the *E. coli* transconjugants transformed competent antibiotic-sensitive *E. coli* cells to Cm and Tc resistance.

Combining the results from both conjugation and transformation experiments, it can be concluded that a single large (M.W. greater than 35.8×10^6) self-transmissible R plasmid conferring

TABLE I
TRANSFORMATION OF *E. COLI* JA221 WITH PLASMID DNA EXTRACTED FROM ONE OF THE *E. COLI* TRANSCONJUGANTS

Selective antibiotic	Number of transformant colonies	Number of transformants growing when toothpicked onto plates with Cm	Sm	Tc	Resistance pattern transferred
Cm	5	5	0	5	CmTc
Tc	7	7	0	7	CmTc

resistance to Cm and Tc is present in the multidrug resistant *S. typhi* isolate. The Sm resistance trait is most probably determined by a chromosomal gene.

The antibiotic resistance pattern of this plasmid is different from that reported during the Mexico outbreak in 1972. [9] It is possible that this resistance plasmid was acquired by the *S. typhi* strain from other resistant commensal bacteria in the bowel of the patient as reported by Datta, Richards and Datta [31] and Threlfall, Ward, Rowe and Robins-Browne. [32] Such resistant strain would be very dangerous if it initiated an epidemic outbreak.

The isolation of a multiresistant *S. typhi* strain, and the findings that its Cm and Tc resistance traits are plasmid-mediated and can be transferred simultaneously by conjugation are of concern to public health and therapeutic treatment. In view of this, it is important that surveillance be maintained to monitor and suppress the emergence of antibiotic resistant *S. typhi* strains in Malaysia.

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