

Short Communication

R-Factor in *Salmonella enterica* Serovar Typhi: Transfer to and Acquisition from *Escherichia coli*

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SUMMARY: Blood culture isolates of *Salmonella enterica* serovar Typhi showing high degrees of resistance to ampicillin, chloramphenicol, cotrimoxazole and tetracycline (ACCoT-resistance) transferred their full resistance phenotype to antibiotic-sensitive *S. enterica* serovar Typhi strains through the primary recipient *Escherichia coli* C600. Transfer frequencies were 0.80×10^{-5} and 0.80×10^{-6} , respectively, in the primary and secondary transfer experiments. The *Escherichia coli* isolates from urinary tract infection cases showing high minimum inhibitory concentration values ($\mu\text{g/ml}$) to A (2,000-5,000), C (2,000-5,000), Co (250-1,200), and T (500-2,000) also transferred ACCoT-resistance to the antibiotic-sensitive *S. enterica* serovar Typhi and then to *E. coli* C600 with transfer frequencies 0.61×10^{-6} and 0.98×10^{-5} , respectively. Curing experiments revealed the loss of ACCoT-resistance from the original and the transconjugant *S. enterica* serovar Typhi strains. Results suggest that R-factor from other enteric bacteria is acquired by *S. enterica* serovar Typhi, and that it (R-factor) is unstable in nature.

Enteric fever due to infection with multidrug resistant (MDR) *Salmonella enterica* serovar Typhi is a cause of great concern. We reported on the emergence and reemergence of MDR *S. enterica* serovar Typhi isolates in our previous publication (1). The emergence of MDR *S. enterica* serovar Typhi is due to indiscriminate use of antibiotics in the treatment of enteric fever. Under such selective pressure of antibiotics, the antibiotic-sensitive bacteria may acquire resistance traits from antibiotic-resistant strains belonging to the same or different genera, and thus become resistant to one or more antibiotics. The R-plasmid has clinical importance in the acquisition and spread of antibiotic resistance among bacteria. In *S. enterica* serovar Typhi, R-plasmid-encoded resistance to ampicillin (A), chloramphenicol (C), cotrimoxazole (Co), and tetracycline (T) has been reported to be transferable (2). Plasmid-mediated transferable drug resistance has also been reported among *E. coli* isolates (3). Therefore, the surveillance of R-factor among bacterial isolates is essential to trace the source of infection. Herein we report on a study of 70 *E. coli* isolates (1995 - 2001) from urinary tract infection (UTI) cases that included antibiogram, minimum inhibitory concentration (MIC) value determination, and transferability of R-factor by in vitro conjugation to evaluate the acquisition of drug resistance by *S. enterica* serovar Typhi isolates. Transferability of drug resistance in *S. enterica* serovar Typhi has also been studied.

Drug-resistant (resistance pattern ACCoT) and drug-sensitive blood culture isolates of *S. enterica* serovar Typhi (representative isolates) obtained from 1991 to 2001, and a total of 70 *E. coli* isolates obtained from UTI cases from 1995 to 2001 (10 per year) at the Calcutta School of Tropical Medicine, Kolkata, India, were used in the present study. The antibiotic resistant *S. enterica* serovar Typhi strains ($n = 10$) selected for the present study were highly resistant in terms of MIC

values ($\mu\text{g/ml}$) to A and C (>2,000), Co (>1,200), and T (>1,500).

Nutrient broth was used for inoculum preparation and for mating experiments. Mueller Hinton agar (MHA) was used for antibiotic susceptibility test and MIC value determination. For the selection of transconjugant strains, MacConkey agar was used.

The agar dilution method (4) was used to screen for antibiotic resistance of the *E. coli* isolates against six antibiotics [$\mu\text{g/ml}$]: A [10], C [30], Co [25], T [10], nalidixic acid (Nx) [30], and ciprofloxacin (Ci) [5], and for MIC value determination using the agents at different concentrations ranging from 50 to 2,500 $\mu\text{g/ml}$ for Co and T, and 50 to 5,000 $\mu\text{g/ml}$ for A and C. The antibiotic mixed agar plates were spot-inoculated with the broth cultures of *E. coli* isolates using 10^4 CFU/spot, and then incubated at 37°C for 18 h. The *E. coli* ATCC 25922 strain was used as a control.

Transfer of antibiotic resistance was carried out by in vitro conjugation experiments following the protocol of Jevanand et al. (2), in two sets:

Set I: MDR *S. enterica* serovar Typhi isolates were used as donors. The recipient strains were *E. coli* C600 (Nx^R, F⁻) and drug-sensitive *S. enterica* serovar Typhi, which were used in the primary and secondary transfer experiments, respectively. Transconjugants were selected on MacConkey agar media containing Nx and C in the primary, and C alone in the secondary, conjugation experiments.

Set II: MDR *E. coli* isolates showing a resistance pattern of ACCoTNxCi were used as donors. The recipient strains used were the drug-sensitive *S. enterica* serovar Typhi in the primary, and *E. coli* C600 (Nx^R, F⁻) in the secondary, transfer studies. The selection plates contained C, and C plus Nx, respectively, in the primary and secondary conjugation experiments.

Identity of the transconjugant and acquisition of R-factor by the transconjugant were confirmed by performing the indole test and the antibiotic susceptibility test, respectively. Transfer frequencies were calculated in terms of the final total count of the recipient strain in the mating mixture (5).

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The original MDR *S. enterica* serovar Typhi, and the transconjugant *S. enterica* serovar Typhi that acquired the ACCoT-resistance from MDR *E. coli*, were selected for curing experiments. The experiments were carried out with ethidium bromide following the protocol of Anjanappa et al. (6) with slight modification. The strains were grown in nutrient broth containing 0.3 mg/ml of ethidium bromide for 7 days at 37°C then a loop full of the culture was streaked on nutrient agar plates. One hundred colonies were transferred to different antibiotic plates, incubated for 18 h at 37°C, and the percentage of total curing was determined on the basis of antibiotic sensitivity of the colonies.

The *E. coli* isolates were MDR showing various resistance patterns: ACCoTNxCi (34%), ACCoT (60%), and CCoNxCi (6%) (Fig. 1). MIC values ($\mu\text{g/ml}$) of the isolates were 2,000-5,000 for A and C, 250-1,200 for Co, and 500-2,000 for T (Fig. 2).

The resistance patterns transferred from the donors to the recipients and the transfer frequency are shown in Table 1. The MDR *E. coli* isolates (ACCoTNxCi) transferred the ACCoT-resistance to the antibiotic-sensitive strains of *S. enterica* serovar Typhi with a frequency of 0.61×10^{-6} , which was then transferred to *E. coli* C600 from the *S. enterica* serovar Typhi transconjugant with a frequency of 0.98×10^{-5} ; NxCi-resistance was not transferred. MDR *S. enterica* serovar Typhi showed *en bloc* transfer of antibiotic resistance (ACCoT) first to *E. coli* C600 with a frequency of 0.80×10^{-5} and then to drug-sensitive strains of *S. enterica* serovar Typhi (transfer frequency: 0.80×10^{-6}). MIC values of the transconjugants for A, C, Co, and T were similar to those of their corresponding donor strains.

Both the original MDR *S. enterica* serovar Typhi and the transconjugant *S. enterica* serovar Typhi strains showed 100% sensitivity to A, C, Co, and T after incubation for 7 days in nutrient broth containing 0.3 mg/ml ethidium bromide, indicating the loss of ACCoT-resistance.

The emergence of *S. enterica* serovar Typhi isolates showing resistance to single to multiple antibiotics during 1991 - 1995 and again during 1999 - 2001, along with the emergence of isolates (1996 - 1998) showing sensitivity to the antibiotics tested for, has been reported (1). In *S. enterica* serovar Typhi, plasmid-mediated resistance to A, C, Co, and T has also been reported from India (2) and other parts of the world (7-9). The selection exerted by antibiotic treatment of enteric fever

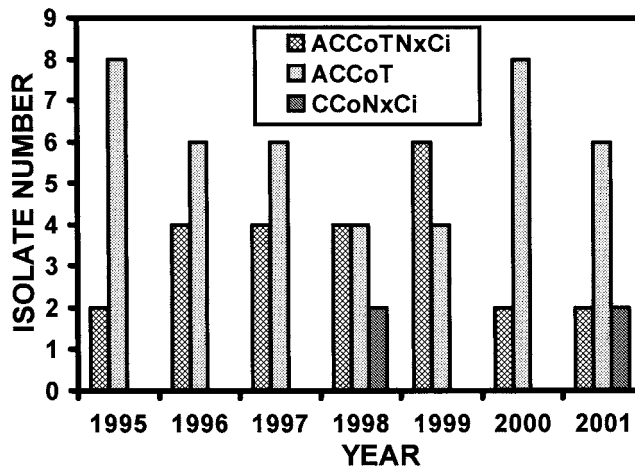


Fig 1. Antibiotic susceptibility pattern of *E. coli* isolates from urinary tract infection cases, 1995-2001 ($n = 70$). Abbreviations are in Table 1.

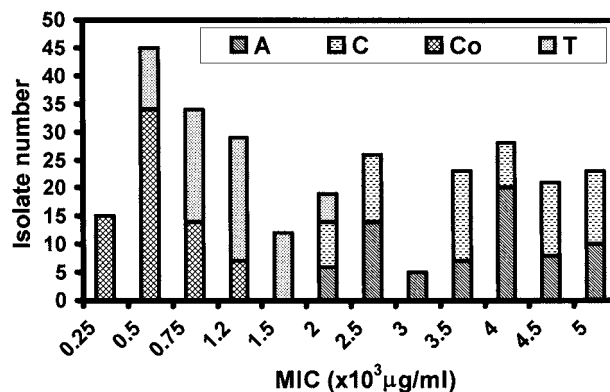


Fig 2. Minimum inhibitory concentration values of antibiotics to inhibit *E. coli* isolates ($n = 70$). Abbreviations are in Table 1.

may be the cause of acquisition of R-plasmid (10). In *S. enterica* serovar Typhi the R-plasmid is an unstable plasmid that may appear or disappear at any time resulting in the emergence of drug-resistant or drug-sensitive isolates. Results of conjugation and curing experiments supported this phenomenon.

Table 1. Transfer of antibiotic resistance from donor to recipient strains and transfer frequencies

Set No.	Donor (Resistance pattern)	Recipient (Resistance pattern)	Transconjugant (Resistance pattern)	Conjugation frequency
I	<i>S. enterica</i> serovar Typhi (ACCoT)	<i>E. coli</i> C600 (Nx)	<i>E. coli</i> C600 (ACCoTNx)	0.80×10^{-5}
	Transconjugant <i>E. coli</i> C600 (ACCoTNx)	<i>S. enterica</i> serovar Typhi (Sensitive)	<i>S. enterica</i> serovar Typhi (ACCoT)	0.80×10^{-6}
II	<i>E. coli</i> (ACCoTNxCi)	<i>S. enterica</i> serovar Typhi (Sensitive)	<i>S. enterica</i> serovar Typhi (ACCoT)	0.61×10^{-6}
	Transconjugant <i>S. enterica</i> serovar Typhi (ACCoT)	<i>E. coli</i> C600 (Nx)	<i>E. coli</i> C600 (ACCoT Nx)	0.98×10^{-5}

A, ampicillin; C, chloramphenicol; Co, cotrimoxazole; T, tetracycline; Nx, nalidixic acid; Ci, ciprofloxacin.

We were also interested in searching for the probable origin of the R-plasmid. In this study, it was considered that *E. coli*, a commensal bacterium, might be the source of dissemination of the plasmid conferring resistance to A, C, Co, and T in *S. enterica* serovar Typhi, based on the fact that most of the *E. coli* isolates (1995 - 2001) showed a common resistance pattern (ACCoT), which was transferred to the drug-sensitive *S. enterica* serovar Typhi with the same degree of resistance. Datta et al. (10) reported the acquisition of R-plasmid by *S. enterica* serovar Typhi from an other bacterial strain. Thus, the present findings suggest that the MDR *S. enterica* serovar Typhi most likely arise from sensitive isolates by acquisition of R-factor from antibiotic-resistant enteric bacteria.

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