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CONJUGAL TRANSFERABILITY AND SPONTANEOUS LOSS OF R-PLASMIDS IN STRAINS OF *S. TYPHI* AND *S. PARATYPHI A*: COMPARISON OF FREQUENCIES AT 30° C AND 37° C

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HIGH incidence of multiple drug resistance in gram-negative enterobacteria has posed a serious therapeutic problem in the last two decades. The drug resistance in the majority of the multidrug resistant organisms is mediated by extra-chromosomal genetic elements called R-plasmids, which are transferable to other enterobacteria[1]. In the earlier decade, several investigators expressed the fear that these plasmids might be transferred to potential enteric pathogens like *Salmonella typhi* leading to great health hazards. Indeed, in 1972 outbreaks of typhoid due to *S. typhi* strains carrying plasmid-mediated chloramphenicol resistance did occur, causing high mortality[2]. Interestingly, all the strains from these outbreaks harboured incompatibility group H1 R-plasmids[2]. In view of this, we had examined the transfer frequency of various plasmid groups in crosses between *Escherichia coli* K12/R^+ and *S. typhi* under ecological factors that prevail in the human gut[3]. It was observed that at 37° C, IncH1 and IncH2 plasmids are transferred at extremely low frequencies (in the order of 10^{-6} or less) compared to other incompatibility group plasmids. Of the gut associated factors, temperature seems to be the most detrimental for the conjugal transfer of IncH1 and IncH2 plasmids, because they possess a thermosensitive transfer system[2]. In crosses at 22°–28° C, plasmids of groups H1 and H2 are transferred at 10,000 fold higher frequencies[2]. It has, therefore, been suggested that these plasmids are likely to be more extensively spread in extra-intestinal situations like sewage[2]. The transfer frequency of other R-plasmid groups at 22°–28° C has not been investigated so far to enable speculations on their relative transfer in intra-intestinal and extra-

TABLE 1

Frequencies† of conjugal transfer and spontaneous loss of R-plasmids of different incompatibility groups

R-Plasmid group	*S. typhi*				*S. paratyphi A**			
	Transfer frequency		Per cent loss after ten sub-cultures		Transfer frequency		Per cent loss after ten sub-cultures	
	30° C	37° C	30° C	37° C	30° C	37° C	30° C	37° C
IncFI	4.1×10^{-5}	5.1×10^{-3}	100	90	7.7×10^{-5}	6.9×10^{-4}	100	96
IncFII	5.6×10^{-5}	4.2×10^{-4}	100	100	6.6×10^{-6}	3.6×10^{-5}	100	97
IncFIV	2.9×10^{-3}	2.3×10^{-3}	100	98	1.5×10^{-4}	5.1×10^{-4}	100	90
IncI1	6.1×10^{-4}	6.5×10^{-4}	28	11	6.6×10^{-5}	6.7×10^{-5}	42	15
IncN	3.0×10^{-5}	9.3×10^{-5}	43	10	6.5×10^{-4}	3.4×10^{-4}	40	12
IncC	$1.7 \quad 10^{-4}$	5.4×10^{-4}	47	16	3.9×10^{-6}	3.1×10^{-6}	35	14
IncA	5.9×10^{-4}	6.3×10^{-4}	42	17	4.1×10^{-4}	3.3×10^{-4}	40	7
IncH1	2.6×10^{-2}	1.1×10^{-6}	25	8	3.0×10^{-3}	1.4×10^{-6}	20	9
IncH2	9.5×10^{-3}	3.6×10^{-6}	21	11	1.4×10^{-3}	2.2×10^{-6}	26	12

† Frequencies presented are mean values for three experiments.

* Both phage types of *S. paratyphi A* (*i.e.*, 1 and 2) showed similar frequencies.

intestinal situations. The present study was therefore undertaken and data on conjugal transfer frequencies in laboratory strains of *Salmonella typhi* and *Salmonella paratyphi A* phage types 1 and 2 are presented here. In our earlier studies[3], it was seen that fertility inhibition positive (*fi*$^+$) type of R-plasmids are highly unstable in *S. typhi*. This led us to speculate that *fi*$^+$ R-plasmids are less likely to be involved in R$^+$ *S. typhi* outbreaks. Currently, the spontaneous loss of various R-plasmids was also investigated in *S. typhi* and *S. paratyphi A* at 30° and at 37° C.

Conjugation was conducted in Penassay broth at 30° C and 37° C by the method described previously[4]. Donor strains were *Escherichia coli* K12 J53 F$^-$ Lac+ bearing R-plasmids of various incompatibility groups[5] listed in table 1. These were kindly provided by Dr. E. M. Lederberg, Plasmid Reference Centre, Stanford. Recipent cultures were nalidixic acid (Nal) resistant mutants of the above mentioned *Salmonella* strains. Transfer frequencies were estimated as ratio of transconjugant to donor cells in mating mixture after 18 hours of mating. The frequency of loss of plasmid from *Salmonella* strains was determined by the technique of Watanabe and Ogata[6].

Results on transfer frequency and genetic stability of plasmids at 30°C and 37° C are shown in table 1. Transfer frequencies were least for IncH1 and IncH2 plasmids at 37° C, both in *S. typhi* and *S. paratyphi A*. However, at 30° C both IncH1 and IncH2 showed about 3 log increase in transfer frequency. On the other hand, most other incompatibility group plasmids showed lower transfer at 30° C. Plasmids of groups FI, FII and FIV were extremely unstable both at 37° C and 30° C, whereas, those of groups N, H1, H2 C and A were remarkably unstable at 30° C but less so at 37° C.

The human intestine and extra-intestinal situations like sewage are the most probable sites where *Salmonella* might acquire R-plasmids. It should be realized, however, that transfer of plasmids in the human gut is restricted by factors such as bile, pH, anaerobiosis, fatty acids, colicins, bacteriophages and temperature (in case of IncH1 and IncH2 plasmids)[7]. Further, loss of certain plasmids is accelerated by bile salt[3]. Under extra-intestinal situations at temperature around 30° C on the other hand, only IncH1 and IncH2 plasmids seem to have a greater likelihood of being transferred to *S. typhi*. At 37° C and 30° C, IncFIV plasmids show transfer frequencies comparable with those of IncH1 and IncH2 at 30° C, but like IncFI and IncFII plasmids, these plasmids too are highly unstable in *Salmonella*. It is likely that by virtue of the higher transferability of IncH1 plasmids at 30° C, the R$^+$ *S. typhi* responsible for the drug resistant typhoid outbreaks might have emerged in extra-intestinal situations like sewage.

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ETIOLOGICAL SIGNIFICANCE OF *NOCARDIA ASTEROIDES* IN CORNEAL ULCER OF CATTLE

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NOCARDIA asteroides is one of the well recognized aerobic pathogens for man, canine and very rarely other species of animals[1-4]. The infection may occur in any organ such as liver, spleen, kidney, lung, brain, adrenal, pancreas and mammary gland[3,4]. However, available literature reveals no information on nocardial ocular infection in cattle. The present paper records the isolation and identification of *N. asteroides* from the diseased eye of an Indian bullock.

A seven-year old Haryana bullock brought to the Veterinary Hospital constituted the animal for this study. The right eye of the animal showed ulcer of the cornea with oedema. A portion of the corneal scrapings was examined microscopically in 10% KOH; smears were also examined by Gram's technique. The remaining material was inoculated on tubes of Sabouraud's dextrose agar (SDA) and Sabouraud's medium with chloramphenicol (0.05 mg/ml). These were incubated at 37° C and examined daily upto 21 days before discarding them negative. The details of the isolate were studied according to recommended procedures[5].

The pathogenicity test was conducted in a Guinea pig using the addition of 5% gastric mucin in equal parts with a heavy suspension of a 72-hr culture by