

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<sup>3</sup>, it was seen that fertility inhibition positive (*fi*<sup>+</sup>) type of R-plasmids are highly unstable in *S. typhi*. This led us to speculate that *fi*<sup>+</sup> R-plasmids are less likely to be involved in R<sup>+</sup> *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<sup>4</sup>. Donor strains were *Escherichia coli* K12 J53 F<sup>-</sup> Lac<sup>+</sup> bearing R-plasmids of various incompatibility groups<sup>5</sup> listed in table 1. These were kindly provided by Dr. E. M. Lederberg, Plasmid Reference Centre, Stanford. Recipient 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<sup>6</sup>.

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, HI, 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)<sup>7</sup>. Further, loss of certain plasmids is accelerated by bile salt<sup>3</sup>. 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<sup>+</sup> *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<sup>1-4</sup>. The infection may occur in any organ such as liver, spleen, kidney, lung, brain, adrenal, pancreas and mammary gland<sup>3,4</sup>. 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<sup>5</sup>.

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

intra-peritoneal route. The post-mortem of the Guinea pig was done after 9 days of post inoculation when the animal succumbed with infection. The pathogen was also recovered on SDA from the visceral organs of the laboratory animal and also demonstrated in the impression smear by Gram's method. The swabs were also cultured from the eyes of ten healthy bullocks.

The microscopic examination of the corneal scrapings by potassium hydroxide technique revealed thin, branched filaments compatible with *Nocardia*. The smear showed gram positive, branched, thin filaments. Inoculated slant of Sabouraud's medium showed raised, irregular, orange coloured colonies on 6th day of inoculation at 37° C (figure 1). The remaining one slant was badly contaminated with easily growing moulds. The growth was further subcultured on the above medium for biochemical and animal pathogenicity tests. The identification of the organism was confirmed by its typical microscopic morphology, gram positive, partial acid-fast staining character besides its inability to hydrolyse xanthine, tyrosine and casein. The isolate proved pathogenic to Guinea pig as evidenced by the death with 9 days of inoculation of culture by intraperitoneally. The pathogen was reisolated from the liver, spleen and diaphragm of the animal; and also demonstrated in the impression

smears prepared from these internal organs. It is interesting to note that swabs cultured from the eyes of ten apparently healthy bullocks failed to yield *N. asteroides*.

The isolation of *N. asteroides* and its direct demonstration in corneal scrapings of the diseased eye is quite suggestive that the animal in question suffered from Nocardial ocular infection. However, on the contrary, the pathogen could not be recovered from the eye material of ten apparently healthy bullocks. This indicates that *N. asteroides* is not a normal flora of the eye. This observation is consistent with an earlier report<sup>6</sup> that *Nocardia* sp. are inhabitants of soil rather than commensals in animals.

Although no epidemiological investigation was carried out to establish the source of infection, the owner of the bullock reported that a trauma was inflicted into the eye by a sharp wooden splinter while ploughing the field. This speculation is further supported by the finding that *N. asteroides* is widely prevalent as saprophyte in the soil of many parts of the world including India<sup>7,8</sup>. Moreover, reference can be made here to the recent work<sup>9</sup> where the trauma to the eye is one of the predisposing factors in the causation of mycotic keratitis and conjunctivitis in animals.

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**Figure 1.** *Nocardia asteroides* isolated from a corneal ulcer case in a 7-year old bullock on Sabouraud's dextrose agar after 6 days of incubation at 37° C.

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