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H-K. Young<sup>\*</sup>, M.V. Jesudason, <sup>\*</sup>Department of Biological Sciences, University of Dundee, DD1 4HN, UK and CMC Hospital, Vellore, S. India.

Increasing resistance to many antimicrobial drugs in developing countries is now of great concern. In a previous study carried out in Vellore, South India, we revealed that 64% of enterobacterial urinary pathogens were resistant to 10 mg/Ltrimethoprim (Tp). The majority (58.2%) of Tp resistance was located on transferable resistance plasmids (R-plasmids) (Young et al 1986). The source and reservoirs of the resistance genes are still unknown. In the present study the role of drinking water as a source/reservoir of antibiotic resistance genes in and around Vellore has been investigated. Samples were collected from drinking water sources in both urban and rural areas. Each sample was processed immediately. 10 mlof water was filtered through a sterile  $0.45 \mu \text{m}$  membrane filter which was then placed on the surface of a modified MacConkeys medium containing either ampicillin (Ap) or Tp at a concentration of 10 mg/L. These two drugs were chosen because they are still the most commonly used antimicrobials in India. In all cases, after overnight incubation at  $37^{\circ}$ C, confluent growth was observed so no quantitative assessment of the resistant organisms could be made.

The principal aim of the study was, however, to determine the prevalence of plasmid borne resistance genes within bacteria from water sources. A loopful of resistant bacteria was therefore streaked for single colonies on a fresh selective plate. A total of 44 morphologically distinct colonies, resistant to either or both Ap and Tp, were purified for further study. Of the 24 isolates recovered on Ap containing media, only 5 (20.8%) were resistant to lOmg/L Tp. In contrast 70% (14/20) of the bacteria isolated on Tp containing media were found to be resistant to 10 mg/L Ap. The minimum inhibitory concentration (MIC) of Ap and Tp was determined for each organism. Fifty percent of strains were resistant to at least 100 mg/L Ap although only 6.8% of the total population had MICs of Ap >1000mg/L. A much higher proportion (15.9%) were found to be resistant to high levels of Tp (MIC >1000mg/L).

Trimethoprim resistant strains were tested for the presence of transferable R-plasmids by conjugation with the rifampicin resistant <u>Escherichia coli</u> strain J53-2. A total of 66.7% of the highly resistant strains were shown to possess transferable Tp R-plasmids. Analysis of the transconjugants revealed that only 40% of the plamsids conferred linked resistance to streptomycin (Sm), indicative of the presence of the promiscuous Tp resistance transposon Tn7. This low incidence of linked Tp/Sm resistance on transferable Tp R-plasmids is similar to previous findings in clinical plasmids isolated in the Vellore area (Young & Storey 1989). Restriction analysis of the plasmids revealed several different plasmids were responsible for Tp resistance in these strains. However, one plasmid type, conferring resistance to Tp, Sm, sulphamethoxazole and chloramphenicol, was identified in bacteria isolated from four geographically distinct locations.

The results of this survey confirm that drinking water in Southern India harbours large numbers of antibiotic resistant organisms. Furthermore a significant proportion of these bacteria were shown to transfer their resistance to standard <u>E. coli</u> strains. The storing of water and the failure of the local population to boil water prior to its consumption, mean that commensal bacteria, and subsequently pathogenic bacteria, will have transient exposure to large numbers of antibiotic resistant organisms which have the potential to pass on their resistance genes.

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Young, H-K. et al (1986) J. Antimicrob. Chemother. 17: 615-621 Young, H-K., Storey, D. (1989) J. Pharm. Pharmacol. 41: 38P