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SHORT COMMUNICATION

# A TRANSFERABLE RESISTANCE PLASMID IN VIBRIO CHOLERAE AND ESCHERICHIA COLI ISOLATED FROM THE SAME PATIENT

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**Abstract**

This study attempted to determine whether or not resistance plasmids present in *Vibrio cholerae* and *Escherichia coli* isolated from the same patient with diarrhoea were identical. Both species were resistant to ampicillin, kanamycin, tetracycline and gentamicin and both species harboured conjugative plasmids of approximately 112 megadaltons belonging to incompatibility group C. The results showed that the resistance plasmids of both organisms appeared to be identical, which confirms that genetic material can be transferred *in vivo* between organisms of different families. The implications of such a transfer are discussed.

*Key words*: *Escherichia coli*; *Vibrio cholerae*; Drug resistance, Microbial, Plasmids; Diarrhoea.

## Introduction

The transfer of resistance to several drugs between species of *Shigella* and *Escherichia coli* in mixed cultivation was demonstrated in 1959 (1). It was subsequently observed that plasmid-mediated drug resistance could be transferred between species of the family Enterobacteriaceae (2) and Baron and Falkow reported the exchange of genetic material between members of the families Enterobacteriaceae and Vibrionaceae (3). Since then, Huq *et al.* have reported isolating from the same patient *V. cholerae* (MARVc) and *E. coli* with similar antibiotic resistance patterns (4).

We report here the presence and identification of two large plasmids from two bacterial species of different families (*V. cholerae* and *E. .coli*) isolated from the stools of a patient with severe watery diarrhoea.

## Materials and methods

In September, 1981 both *V. cholerae* and *E. coli* were isolated from stool samples of a patient admitted to the hospital of the International Centre for Diarrhoeal Disease Research, Bangladesh. These strains were preserved in blood agar base slants in paraffin oil, and were later tested for their susceptibility to antibiotics by the method of Bauer *et al.* (5), using a standard *E. coli* (ATCC 25922) resistance control. The ability of the two strains to transfer drug resistance to *E. coli* K-12 ($Lac^+ F^- Nx^r$) was tested by conjugation experiments (6). Using the method of Birnboim and Doly (7), deoxyribonucleic acid (DNA) was extracted from the two strains and from bacteria with plasmids of known molecular weights. Plasmids were separated by agarose gel electrophoresis following the method of Meyers *et al.* (8). The incompatibility group of the plasmids was determined by standard methods (9). Plasmids were cured of *V. cholerae* by acridine orange treatment (10). Enterotoxin testing was performed, using the Chinese Hamster Ovary cell assay (11).

## Results

The plasmid profiles and drug resistance patterns of the *V. cholerae* and *E. coli* isolated from the

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patient were found to be the same (Table). A plasmid of approximately 112 Mdal was present in both the species and was self-transmissible to an *E. coli* K-12 strain (Fig.). Both plasmids were found to belong to incompatibility group C. This large plasmid was eliminated from the *V. cholerae* isolate by acridine orange treatment. The strain then became susceptible to all tested antibiotics, thus confirming the plasmid-mediated nature of antibiotic resistance.

## Discussion

The findings of this study suggest that the presence of a plasmid in strains of both *V. cholerae* and *E. coli* with a high molecular weight and showing multiple drug resistance characteristics is the outcome of a transfer *in vivo* of plasmid DNA among these members of the Enterobacteriaceae and the Vibrionaceae. The identical resistance patterns of the *E. coli* and *V. cholerae* isolates were completely transferred *in vitro*, as a single unit, to an *E. coli* K-12 strain. The conjugative 112–Mdal plasmid was responsible for these resistances (Fig.). Further investigation revealed that both plasmids belonged to incompatibility group C. The similarity of molecular weight, antibiotic resistance pattern and incompatibility group strongly suggests that the *V. cholerae* and *E. coli* strains from this patient contained identical plasmids of common origin.

These findings confirm the occurrence of a transfer *in vivo* of genetic material between organisms of the same and of different families. Resistance plasmids which are shared between an intestinal pathogen and species of the normal intestinal flora can be potentially dangerous (12, 13). Although the pathogen may be eliminated, the normal gut flora may contain a persistent reservoir of drug resistance. This may be transferred to a new pathogen and thus complicate antibiotic treatment.

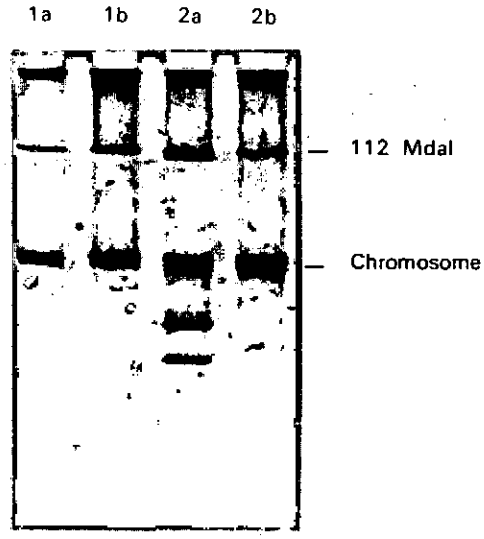


*Fig. — Electrophoretic patterns of plasmid DNA of parent bacterial strains and their respective transconjugates. Lanes 1a and 1b: V. cholerae parent and transconjugate. Lanes 2a and 2b: E. coli parent and transconjugate.*

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CHARACTERISTICS OF DRUG RESISTANCE PLASMIDS OF *V. CHOLERAE* AND *E. COLI*

| Pathogens | Resistance pattern | Plasmid profile(s) | Resistance pattern of the transconjugants | Plasmid profile of the transconjugants | Incompatibility grouping |
|---|---|---|---|---|---|
| *V. cholerae* | AKTG | MW = 112 Mdal | AKTG | MW = 112 Mdal | C |
| *E. coli* | AKTG | MW = 112 Mdal<br>100 "<br>7.8 "<br>5.8 " | AKTG | MW = 112 Mdal | C |

Mdal = megadalton; A = ampicillin; K = kanamycin; T = tetracycline; G = gentamicin; MW = molecular weight.

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