Molecular cloning and transposition of a kanamycin resistance determinant from *Campylobacter jejuni* between replicons in *Escherichia coli*

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This paper reports a restriction map of a fragment of DNA encoding kanamycin resistance cloned from plasmid DNA of *Campylobacter jejuni* ABA94 in the recombinant plasmid pRS9421-1. In transposition experiments, kanamycin-resistant R751:km9421 transconjugants appeared at frequencies of $10^{-7}$ per donor cell. These transconjugants harboured a plasmid 4 kb larger than the parental 49 kb plasmid R751. Restriction enzyme analysis and Southern blot hybridization of these transconjugants showed that the kanamycin resistant determinant had transposed from recombinant plasmid pRS9421-1 to plasmid R751.

Key words: *Campylobacter jejuni*, cloning, kanamycin resistance, transposition.

*Campylobacter jejuni* is recognized as a common cause of diarrhoeal diseases in humans (Blaser & Reller 1981). *Campylobacter jejuni* can harbour a variety of resistance determinants and plasmid-mediated resistance (Taylor & Courvalin 1988), in particular resistance to tetracycline is common (Tenover *et al.* 1985; Sagara *et al.* 1987). Resistance to kanamycin (Km') in *C. jejuni* on the other hand appears to be uncommon. However, when present, it is often mediated by a plasmid which also encodes tetracycline resistance. In conjugation experiments performed by Kotarski & coworkers (1986), kanamycin-susceptible, tetracycline-resistance segregants carrying plasmid DNA 4 kb smaller than the 59 kb parental plasmids were detected. In contrast, they found that the kanamycin-resistant, tetracycline-susceptible segregants contained no detectable plasmid DNA. This suggests that the Km' may be located on a transposable element. Similar findings were also obtained in our laboratory where we have observed that the Km' in *C. jejuni* ABA94 was transferred to the *C. fetus* C607 recipient without concomitant transfer of plasmid DNA (Ansary & Son 1992). Thus Km' in *C. jejuni* ABA94 appears also to be located on a translocatable element.

It was therefore decided to construct the physical map of the chimeric plasmid pRS9421-1 containing the cloned Km' determinant from *C. jejuni* ABA94, and to conduct a series of experiments to determine if the cloned Km' determinant was capable of transposition from the recombinant plasmid pRS9421-1 to a number of replicons in *Escherichia coli*.

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Materials and Methods

**Bacterial Strains and Plasmids**

The recombinant plasmid pRS9421-1 harbouring the cloned Km' determinant from *C. jejuni* ABA94 plasmid was from the DNA library constructed in our laboratory. The kanamycin resistant *C. jejuni* ABA94 strain was isolated from stool of a patient with gastroenteritis at the University Hospital, Kuala Lumpur, in which plasmid DNA was characterized, and has been previously described (Ansary & Son 1992). The shotgun cloning experiment was carried out with total pure plasmid DNA of *C. jejuni* ABA94 and the multicopy-number plasmid pUC18 vector (Vieira & Messing 1982) prepared by CsCl-ethidium bromide density gradient centrifugation as described by Maniatis *et al.* (1982). The chimeric plasmid pRS9421-1 was constructed by digesting purified *C. jejuni* ABA94 plasmid DNA and pUC18 vector DNA with EcoRI at 37°C for 3 h and then ligating the digests with T4 ligase.
Plasmid DNA Isolation and Restriction Endonuclease Mapping
Organisms were screened for plasmid DNA by the procedure of Bimboim & Doly (1979), followed by caesium chloride-ethidium bromide density gradient centrifugation to obtain pure plasmid DNA. Digestions by restriction endonucleases 

\[ \text{AvaI, BamHI, BglI, BglII, Clal, EcoRI, EcoRV, HindIII, HincII, PstI, ScaI, Sall, Scal, SmaI and XhoI} \]

(Bioehringer Mannheim, Amersham International plc, UK or Bethesda Research Laboratory, USA) were performed according to the manufacturer's directions. DNA fragments were analyzed by agarose gel electrophoresis according to standard procedures (Maniatis et al. 1982). The approximate molecular mass of the plasmids was determined by comparison with plasmids of E. coli V517 (Macrina et al. 1978).

Transposition Studies
Plasmids pBR322 or pACYC184 were introduced by transformation (Hanahan 1983) and plasmid R388 or R751 were introduced by conjugation into E. coli HB101 (Sm') carrying the test recombinant plasmid pRS9421-1. Subsequent steps in the detection of constructs containing transposon-carrying derivatives of plasmids pACYC184, pBR322, R388 or R751 were carried out as described by Bennett et al. (1988).

Results and Discussion
A recombinant plasmid designated pRS9421-1 was found to confer Km' to the recipient E. coli HB101 Sm' strain. The information from the restriction endonuclease analysis of pRS9421-1 plasmid was sufficient to construct a preliminary partial restriction map of the recombinant plasmid pRS9421-1 containing a 4.15 kb insert fragment cloned from C. jejuni ABA94 plasmid (Figure 1) conferring resistance to kanamycin.

Based on the results obtained in our earlier studies involving C. jejuni ABA94 (Ansary & Son 1992), we decided to test the nature of the cloned Km' determinant in recombinant plasmid pRS9421-1. From the results obtained, all attempts to detect transposition of the Km' determinant from recombinant plasmid pRS9421-1 into plasmids pACYC184, pBR322 and R388 were unsuccessful, despite repeating the experiments several times. The transposition frequencies observed were less than \(10^{-9}\) per donor (limit of detection, \(10^{-9}\) per donor). Therefore, although in many cases the approach used in this study can be successful, in this particular instance it may be inappropriate; an approach which works for one transposon cannot be guaranteed to work for an entirely different element (Tu & Cohen 1980; Coleman & Foster 1981; Tsai et al. 1987; Jennifer & Berg 1990).

On a more promising note, plasmid R751 can mobilize the kanamycin resistance determinant from recombinant plasmid pRS9421-1. In the transposition studies, E. coli HB101 Sm' carrying the test plasmid pRS9421-1 (Km') and the R751 (Tp') plasmid was mated with E. coli K12 Nal'. Transconjugants were selected on iso-sensitest agar containing the appropriate concentrations of trimethoprim, kanamycin and the counter-selecting nalidixic acid. Transconjugants which inherited resistance to both kanamycin and trimethoprim were recovered at frequencies of \(10^{-7}\) transconjugants per donor cell and were screened for the presence of plasmid R751 altered in size by the alkaline lysis procedure (Maniatis et al. 1982). As far as is known,