A Multi-resistance Plasmid Isolated from Commensal *Neisseria* Species is Closely Related to the Enterobacterial Plasmid RSF1010

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pFM739, an R plasmid from *Neisseria sicca* that encodes penicillin, streptomycin and sulphonamide resistance, and the enterobacterial IncQ(P-4) plasmid RSF1010, which encodes streptomycin and sulphonamide resistance, were incompatible, and were mobilized by the same conjugative plasmids. Restriction mapping confirmed a high degree of similarity between both R plasmids; pFM739 carried DNA fragments corresponding to the known replication and resistance regions of RSF1010. pFM739 also carried an extra segment with the same restriction map as that described for the β -lactamase-coding region of transposon Tn3. It is suggested that the R plasmids isolated from commensal *Neisseria* sp. could have resulted from transposition of a Tn3-like genetic element to an RSF1010-like plasmid, and that they contain deletion derivatives of transposon Tn3.

INTRODUCTION

The recent isolation, in our laboratory, of commensal *Neisseria* spp. strains carrying multi-resistance plasmids (Rotger *et al.*, 1981; Pintado *et al.*, 1985) raised questions about the origin, transfer mechanisms and possible spread of these plasmids to pathogenic bacteria colonizing the same mucosal surfaces. The problem of the introduction of R plasmids into the genus *Neisseria* is of increasing concern in view of the possibility of commensal strains becoming a reservoir of R plasmids (Genco *et al.*, 1984) and the fact that R plasmids seem to have reached strains of *Neisseria meningitidis* (Dillon *et al.*, 1983). The plasmids we have isolated from strains of *Neisseria sicca*, *Neisseria subflava* and *Neisseria mucosa* had the same molecular size (9.45 kb) and restriction patterns, and coded for a TEM-type β -lactamase, a streptomycin phosphotransferase and a sulphonamide-resistant dihydropteroate synthetase (Pintado *et al.*, 1985). They were also stably maintained in *Escherichia coli* and *Pseudomonas putida* (Rotger *et al.*, 1981). These properties suggested the possibility of such R plasmids from *Neisseria* spp. being related to R plasmids of the IncQ(P-4) group. Such plasmids are of similar size and host range and encode the same mechanisms of streptomycin and sulphonamide resistance (Heffron *et al.*, 1977; Barth *et al.*, 1981). In this communication we show that a close relationship exists between pFM739, from *N. sicca*, and RSF1010, a representative IncQ(P-4) plasmid encoding streptomycin and sulphonamide resistance.

METHODS

Bacterial strains, plasmids and culture media. Strains and plasmids used in this investigation are listed in Table 1. *E. coli* was grown in Luria Bertani (LB) broth containing 0.5% (w/v) yeast extract, 1% (w/v) tryptone (Difco) and 1% (w/v) NaCl, or in the same medium solidified with 2% (w/v) agar (Difco). This medium was also used for selection in all genetic transfer experiments, except when sulphamethoxazole was the selective agent. In this case Mueller-Hinton agar (BBL) supplemented with 5% (v/v) lysed horse blood was used. Incubations were always done at 37 °C.

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Table 1. *Bacterial strains and plasmids used*

Strain	Relevant characteristics*	Origin†
<i>E. coli</i> K12 HB101	<i>ara-14 galK2 hsdS20 lacY1 leu mtl-1 proA2 recA13 rpsL20 supE44 thi xyl-5</i>	V. Rubio
<i>E. coli</i> K12 NEM259	<i>hsdR_k hsdM_k⁺ met supE supF</i>	M. Vicente
<i>E. coli</i> K12 185	Nal ^R	J. M. Ortiz
Plasmid		
pFM739	Ap ^R Sm ^R Su ^R	This laboratory; Pintado <i>et al.</i> (1985)
pFM202	Ap ^R	This laboratory; Rotger & Nombela (1983)
RSF1010	Sm ^R Su ^R IncQ(P-4)	J. Jofre
R124	Tra ⁺ Tc ^R IncFIV	J. Jofre
pIP113	Tra ⁺ Tc ^R IncN	J. Jofre
RP4	Tra ⁺ Ap ^R Km ^R Nm ^R Tc ^R IncP(P-1)	J. Jofre
Rts1	Tra ⁺ Km ^R IncT	J. Jofre

* Ap, ampicillin; Sm, streptomycin; Su, sulphonamides; Tc, tetracycline; Km, kanamycin; Nm, neomycin.

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Genetic transfer. *E. coli* cells were transformed with isopropanol-precipitated plasmid DNA, as described by Cohen *et al.* (1972). Mating experiments were done by mixing exponential phase cultures of donor and recipient strains (1:1), and incubating for 90 min. Alternatively, the mating mixture was retained on nitrocellulose filters as described previously (Rotger *et al.*, 1981). The antibiotics used for selection and their concentrations were: carbenicillin, 500 µg ml⁻¹; kanamycin, 50 µg ml⁻¹; nalidixic acid, 50 µg ml⁻¹; streptomycin, 25 µg ml⁻¹ or 300 µg ml⁻¹ for selection of plasmid or chromosomal resistance markers respectively; sulphamethoxazole, 1 mg ml⁻¹; and tetracycline, 10 µg ml⁻¹. The results obtained were consistently reproduced after several determinations.

Preparations and characterization of plasmid DNA. The rapid method of Holmes & Quigley (1981) was used for plasmid detection. For restriction endonuclease analysis, plasmid DNA was purified by centrifugation of cleared lysates, and prepared according to Grinstead *et al.* (1978), in caesium chloride ethidium bromide equilibrium density gradients. Covalently closed circular DNA preparations were exhaustively dialysed and ethanol-precipitated. Restriction endonucleases were purchased from BRL and used according to the manufacturer's instructions. Electrophoresis was done in polyacrylamide (4%, w/v) or agarose (0.7 to 1%) gels, essentially as described by Meyers *et al.* (1976). λ phage DNA, digested with *Hind*III and *Eco*RI, was used as a molecular size marker (Southern, 1979). Analyses with each endonuclease or pair of endonucleases were done at least twice, with reproducible results.

Plasmid incompatibility tests. To study incompatibility between plasmids pFM739 and RSF1010, the former was transformed into *E. coli* K12 HB101(RSF1010) by selecting for carbenicillin resistance. Several of the corresponding transformant clones were analysed by agarose gel electrophoresis in order to examine the resulting plasmid complement. The experiment was repeated three times with the same results.

RESULTS

Incompatibility of plasmids pFM739 and RSF1010

Plasmid pFM739, from *N. sicca* 739, has been described as an extrachromosomal element encoding a triple resistance phenotype. In order to examine compatibility relationships between pFM739 and the enterobacterial plasmid RSF1010, the former was transformed into *E. coli* K12 HB101(RSF1010), and the cells were plated on to medium containing carbenicillin. Of 12 transformant clones analysed, 11 carried only one plasmid, which was the same size as pFM739, whereas the remaining clone carried both plasmids (data not shown). This clone was subcultured overnight and plated on to non-selective medium. Another 12 clones were examined and all of them carried only one plasmid, which was the same size as pFM739. Similar experiments were done with pFM202, a 7 kb gonococcal plasmid (Rotger & Nombela, 1983); however, pFM202

Table 2. Mobilization of plasmids pFM739 and RSF1010 by pIP113 and RP4

After mating, cells were plated on media containing: nalidixic acid, to determine the number of recipients; nalidixic acid with tetracycline (conjugative plasmid marker), to determine the number of transconjugants; or streptomycin (mobilized plasmid marker), to measure mobilization of the non-conjugative plasmid.

Conjugative plasmid	Conjugation per recipient	Mobilized plasmid	Mobilization frequency	
			Per transconjugant	Per recipient
pIP113	0.4	RSF1010	1.0×10^{-6}	4.2×10^{-7}
pIP113	0.3	pFM739	4.1×10^{-6}	1.1×10^{-6}
RP4	0.4	RSF1010	1.0	0.4
RP4	0.8	pFM739	0.9	0.7

and RSF1010 stably coexisted in the same cells. These results clearly showed that pFM739 displaced RSF1010 upon appropriate selection. We concluded that pFM739, a plasmid isolated from a commensal strain of *N. sicca*, belongs in the IncQ(P-4) incompatibility group, which has been classically regarded as a group of *Pseudomonas* and enterobacterial plasmids (Guerry *et al.*, 1974).

Genetic mobilization

N. sicca 739 can transfer pFM739 to *E. coli* in an apparently conjugal process, although no plasmid DNA large enough to encode sex factor activity has been detected in the donor strain (Pintado *et al.*, 1985). In order to compare plasmid pFM739 and RSF1010 further with regard to their mobilization by conjugative plasmids between *E. coli* strains, we transferred plasmids R124 (IncFIV), pIP113 (IncN), RP4 (IncP) and Rts1 (IncT) to *E. coli* K12 HB101 carrying either pFM739 or RSF1010. Plasmids were transferred by conjugation, and transconjugants were selected and shown to maintain both plasmids stably. Mobilization of pFM739 and RSF1010 was studied by mating with *E. coli* K12 185 (nalidixic acid resistant) and by selecting for either the mobilized or the conjugative plasmid independently. It is known that RSF1010 can be efficiently mobilized by IncP plasmid but very poorly or not at all by others (Barth *et al.*, 1981). Our results confirmed this, since RSF1010 was mobilized at a high frequency by RP4 (IncP) and much less efficiently by pIP113 (IncN). Other conjugative plasmids gave no detectable mobilization (Table 2). The behaviour of pFM739 was again identical to that of RSF1010 in this respect (Table 2), suggesting a high degree of similarity.

Restriction endonuclease analysis

The comparison between the *N. sicca* plasmid pFM739 and the enterobacterial plasmid RSF1010 was extended by analysis with several restriction endonucleases. This analysis enabled us to estimate a size of 9.45 kb for pFM739, by addition of the sizes of its fragments. This value is more accurate than the approximate value of 6.0 MDa that was estimated by agarose gel electrophoresis of the plasmid (Pintado *et al.*, 1985). Both plasmids had unique restriction sites for *EcoRI* (data not shown) and pFM739 had an additional unique site for *BamHI*.

Several bands were common to both plasmids after digestion with restriction endonucleases and electrophoresis of the fragments: four after digestion with *HinfI*, three with *AvaI*, two with *HincII* and one with *PstI* (Fig. 1). Partial and double digestions were also done and all these data enabled us to prepare a restriction map of pFM739 (Fig. 2); this was compared with the published restriction map of RSF1010 (Bagdasarian *et al.*, 1981). The restriction patterns of sulphonamide and streptomycin resistance determinants as well as the replication region of RSF1010 appeared to be shared by pFM739. Notable differences were observed between the 2.44 kb *AvaI* fragment of pFM739 and the corresponding fragment of RSF1010; the former was

Fig. 1. Agarose (lanes *a* to *h*) or polyacrylamide (lanes *i* to *l*) gel electrophoresis of fragments produced by treatment of plasmids pFM739 and RSF1010 with various restriction endonucleases. (*a*) pFM739 treated with *Eco*RI plus *Bam*HI; (*b*) pFM739 treated with *Ava*I; (*c*) RSF1010 treated with *Ava*I; (*d*) λ phage DNA treated *Hind*III [sizes of fragments in kb are 23.7, 9.46, 6.61, 4.26, 2.26, 1.98]; (*e*) pFM739 treated with *Pst*I plus *Bam*HI; (*f*) pFM739 treated with *Pst*I; (*g*) RSF1010 treated with *Pst*I; (*h*) λ phage DNA treated with *Eco*RI plus *Hind*III [sizes of fragments in kb are 21.8, 5.24 and 5.05 (appearing in one band in this gel), 4.21, 3.41, 1.98, 1.9, 1.57, 1.32, 0.83, 0.84]; (*i*) pFM739 treated with *Hinf*I; (*j*) RSF1010 treated with *Hinf*I; (*k*) pFM739 treated with *Hinc*II; (*l*) RSF1010 treated with *Hinc*II.

Fig. 2. Restriction maps of plasmids pFM739 and RSF1010. The location of resistance genes and the replication origin in RSF1010 has been described by Badgasarian *et al.* (1981), Barth *et al.* (1981) and Sakaguchi (1982). The dotted line below pFM739 indicates the region which does not appear in RSF1010. The 0.2 kb *Ava*I fragment of RSF1010 and pFM739 was not detected in our electrophoretic system, but could be deduced from the difference with other endonuclease restriction treatments and from the previously published analysis of RSF1010. A, *Ava*I; B, *Bam*HI; E, *Eco*RI; H, *Hinc*II; P, *Pst*I.

0.85 kb longer, and carried the single *Bam*HI site of pFM749 as well as *Pst*I and *Hinc*II restriction sites, all of which are absent in RSF1010. The restriction map of this extra region is very similar to that of the β -lactamase-encoding region of Tn3 (Heffron *et al.*, 1981). This observation is in agreement with the ability of pFM739 to code for a TEM-type β -lactamase (Rotger *et al.*, 1981; Cenamor *et al.*, 1986).

DISCUSSION

The discovery of multi-resistance plasmids in commensal *Neisseria* spp. (Pintado *et al.*, 1985) represents a clear demonstration that these bacteria can harbour and maintain resistance plasmids that have arisen in nature. The results presented above clearly show that a high degree

of similarity exists between pFM739, a plasmid isolated from *N. sicca*, and RSF1010, a typical IncQ(P-4) R plasmid that was originally isolated from enteric bacteria (Guerry *et al.*, 1974), and that codes for streptomycin and sulphonamide resistance. Both plasmids were incompatible and were mobilized by the same conjugative plasmids. Their restriction maps showed that pFM739 could have resulted from the transposition of a fragment of a Tn3-like genetic element to a plasmid of the RSF1010 type. Consistent with this interpretation, pFM739 DNA, labelled with [α - 32 P]GTP by nick-translation, hybridized with the DNA fragments obtained by *Ava*I digestion of RSF1010 (data not shown). In fact, the transposition of Tn3-like genetic elements to RSF1010-related plasmids, which acquire the capacity to code for a TEM-type β -lactamase in this way, has been shown to occur in nature as well as in the laboratory (Heffron *et al.*, 1977; Yamada *et al.*, 1979). Plasmids that arose in this way, were very similar to pFM739, but carried the whole transposon. On the other hand, gonococcal β -lactamase plasmids have been shown to carry a Tn3 fragment, with a restriction map very similar to that of the Tn3 fragment that seems to be part of pFM739, and these are considered to be deletion derivatives of plasmid bearing the entire Tn3 transposon. The possession of this incomplete and therefore non-transposable Tn3-like segment seems to be the only similarity between pFM739 and the gonococcal penicillinase plasmids, which so far do not encode any other resistances and which have restriction patterns and incompatibility groups very different from those of the plasmid described here (Fayat *et al.*, 1982).

The spread of IncQ plasmids to other, possibly pathogenic bacterial species was first reported by Albritton *et al.* (1982), who identified sulphonamide-resistance plasmids in *Haemophilus ducreyi*. The presence of pFM739-like plasmids in several strains of commensal *Neisseria* spp. points to the possibility of further spread of such nonconjugative multiple resistance plasmids to other pathogenic or opportunistic bacteria.

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