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Erythromycin-Resistant *Neisseria gonorrhoeae* and Oral Commensal *Neisseria* spp. Carry Known rRNA Methylase Genes

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Two *Neisseria gonorrhoeae* isolates from Seattle and two isolates from Uruguay were resistant to erythromycin (MIC, 4 to 16 μg/ml) and had reduced susceptibility to azithromycin (MIC, 1 to 4 μg/ml) due to the presence of the self-mobile rRNA methylase gene(s) *ermF* or *ermB* and *ermF*. The two Seattle isolates and one isolate from Uruguay were multiresistant, carrying either the 25.2-MDa *tetM*-containing plasmid (Seattle) or a β-lactamase plasmid (Uruguay). Sixteen commensal *Neisseria* isolates (10 *Neisseria perflava-N. sicca*, 2 *N. flava*, and 4 *N. mucosa*) for which erythromycin MICs were 4 to 16 μg/ml were shown to carry one or more known rRNA methylase genes, including *ermB*, *ermC*, and/or *ermF*. Many of these isolates also were multiresistant and carried the *tetM* gene. This is the first time that a complete transposon or a complete conjugative transposon carrying an antibiotic resistance gene has been described for the genus *Neisseria*.

Neisseria gonorrhoeae isolates obtained in Denver, Colo., and Edinburgh, United Kingdom, and having high-level resistance to erythromycin (MIC, >8 μg/ml) and reduced susceptibility to azithromycin (MIC, 2 to 4 μg/ml) have been described (4, 33). The MICs for these isolates were higher than those normally associated with chromosomal *mtr* mutations (3, 8, 13, 15, 30). Unfortunately, these isolates were not available for examination of the mechanisms of resistance. During a gonorrhea outbreak in 1994 to 95 in Seattle, Wash., caused by strains containing the 25.2-MDa plasmid encoding tetracycline resistance of the Pro$^-$/IA-1,2 class, two isolates resistant to both tetracycline and erythromycin (MICs, ≥16 μg/ml) were identified. Two additional gonococci, isolated in 1991 and 1995 in Uruguay and for which an erythromycin MIC (4 μg/ml) higher than that previously found in this setting was determined, were available for study. In addition to the identification of these isolates with high-level erythromycin resistance (4, 33), plasmids carrying an *ermC* gene (34) and conferring erythromycin resistance to both *N. gonorrhoeae* and *N. meningitidis* have been created. These findings prompted us to evaluate whether these four *N. gonorrhoeae* isolates had acquired one or more of the *erm* genes known for other urogenital species (2). Investigations further sought to define the location of these methylase genes (plasmid versus chromosome), to determine whether their location was on conjugative units, as have been found in many other species (2, 18, 19, 26, 29), and to examine the transfer of such genes to other isolates and species. The methylase gene composition of oral commensal *Neisseria* spp. for which the erythromycin MICs were 4 to 16 μg/ml was compared to that of the gonococcal isolates.

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MATERIALS AND METHODS

Bacterial isolates. Erythromycin-resistant (Em^r) Pro$^-$/IA-1,2 *N. gonorrhoeae* isolates were isolated in Seattle during the 1994-1995 gonococcal outbreak (Table 1). Both Seattle isolates (94-965 and 95-1) were also tetracycline resistant. The 1995 Uruguay isolate, 581, was Pro$^-$/IB-3, and the 1991 Uruguay isolate, 1101, was nonrequiring Proto/IB-3. Strain 1101 carried the 3.2-MDa β-lactamase plasmid and was resistant to penicillin in addition to erythromycin, while all the other *N. gonorrhoeae* isolates did not carry a β-lactamase plasmid. Six other Tc^r Pro$^-$/IA-1,2 *N. gonorrhoeae* isolates that were from the Seattle outbreak but that were not resistant to erythromycin were available for comparison with the two Em^r Tc^r *N. gonorrhoeae* isolates. The NRL (*Neisseria* Reference Laboratory, University of Washington, Seattle) strains were isolated prior to 1986. The *N. gonorrhoeae* isolates were confirmed by biochemical methods (11). Auxotypes, protein I serovars, and plasmid contents of the gonococcal isolates were determined by established methods (5, 6, 9, 32).

We also examined 16 isolates of commensal *Neisseria* spp., including 10 *N. perflava-N. sicca*, 2 *N. flava*, and 4 *N. mucosa* (Table 1). These isolates were confirmed by biochemical methods. The commensal *Neisseria* spp. were clinical isolates collected from the periodontal pockets of seven periodontitis patients seen at the Graduate Periodontics Clinic at the University of Washington, Seattle, between 1991 and 1995, isolates collected from oropharyngeal specimens from six patients attending the DeKalb County Sexually Transmitted Disease Clinic in 1986 (designated CTM before the number [7]), and type strains (NRL strains) obtained from Joan Knapp (22, 23).

Media. GC base or GCP broth (Difco Laboratories, Detroit, Mich.) supplemented as previously described (9, 20) was used for routine culturing of *N. gonorrhoeae*, *Neisseria* spp., and *Enterococcus faecalis*.

Antimicrobial susceptibilities. Mueller-Hinton medium (Difco) was used to determine the MICs for the commensal *Neisseria* spp., *N. meningiditis*, and *E. faecalis* transconjugants, and supplemented GC medium base (Difco) was used for *N. gonorrhoeae*, as recommended by the National Committee on Clinical Laboratory Standards for aerobic bacteria (12). The antibiotic concentrations tested were as follows: erythromycin, 0.06 to 32 μg/ml; azithromycin, 0.03 to 8 μg/ml; and tetracycline, 0.06 to 32 μg/ml. MIC plates were incubated at 36.5°C for 24 h with CO_2 for *N. gonorrhoeae* and without CO_2 for commensal *Neisseria* spp., *N. meningitidis*, and *E. faecalis* transconjugants.

Proteinase K treatment. Isolates and transconjugants were treated with proteinase K as previously described (2) and used as templates for the PCR assays. Each proteinase K-treated sample was not used more than three times, since repeated freezing and thawing has been shown to degrade DNA samples (2).

PCR of the *ermF* gene. The PCR primers used in the study were F_1 (5′ CGGGTCAGCACTTTACTATTG 3′, starting at bp 1235) and F_2 (5′ GGACC TACCTCATAGACAAG 3′, antisense sequence ending at bp 1700). The expected size of the PCR fragment was 466 bp (2, 14, 28). Each 100-μl reaction mixture contained 2 U of *Taq* polymerase (Boehringer Mannheim Indianapolis, Ind.), 200 mM deoxynucleoside triphosphate, 1× PCR buffer I (1.5 mM $MgCl_2$), and 100 ng of each primer. Ten to 40 ng of DNA or 1 to 2 μl of proteinase K-treated bacteria were used as the DNA template. The PCR conditions were as follows: denaturing at 94°C for 30 s, annealing at 50°C for 30 s, and elongation

TABLE 1. MICs for and antibiotic resistance determinants of *N. gonorrhoeae* and commensal *Neisseria* spp.

Isolate	Date	Location[a]	MIC (μg/ml) of[b]:			Resistant gene carried[c]	
			ERY	AZM	TET	*erm*	*tet*
N. gonorrhoeae[d]							
94-965	1994	Seattle	16	4	16	F	M
95-1	1995	Seattle	16	4	16	B, F	M
1101	1991	Montevideo	4	1	1	F	None
581	1995	Montevideo	4	1	4	F	None
N. perflava-N. sicca							
CTM 1.2	1986	DeKalb	4	ND	1	B, C	None
CTM 4.3	1986	DeKalb	8	ND	16	B	M[e]
CTM 7.2	1986	DeKalb	8	ND	16	B	M[e]
10004	1991	Seattle	16	ND	16	C	M[e]
10915	1991	Seattle	16	ND	16	B, C, F	M[e]
30423	1991	Seattle	16	ND	8	B, C	M[e]
31212	1991	Seattle	16	ND	8	C	M[e]
3006	1995	Seattle	4	ND	8	B, C, F	None
33107	1995	Seattle	8	ND	1	B	None
NRL 45	Before 1986	NRL	4	ND	2	B	None
N. flava							
CTM 5.4	1986	DeKalb	16	ND	1	C	None
NRL 69	Before 1986	NRL	8	ND	1	B	None
N. mucosa							
CTM 2.2	1986	DeKalb	8	ND	16	B, C	M[e]
CTM 8.1	1986	DeKalb	8	ND	16	B, C	M[e]
10502	1991	Seattle	8	ND	>16	C	None
NRL 76	Before 1986	NRL	8	ND	1	B	None

[a] Seattle, Wash.; Montevideo, Uruguay; DeKalb County, Ga.
[b] ERM, erythromycin; AZM, azithromycin; TET, tetracycline. ND, not determined.
[c] F, *ermF*; B, *ermB*; C, *ermC*; M, *tetM*.
[d] Both Seattle *N. gonorrhoeae* isolates carried the *tetM* gene on the 25.2-MDa plasmid; strain 1101 carries the 3.2-MDa β-lactamase plasmid and is penicillin resistant.
[e] The commensal species carried the *tetM* gene on the chromosome, where it was not mobile.

at 72°C for 2 min. The cycle was repeated 35 times. Plasmid pBF4 (2), containing the cloned *ermF* gene, and water were used as positive and negative controls, respectively. The PCR products were dried on a lyophilizer, resuspended in 10 μl of sterile H_2O, run on 1.5% agarose gels, and stained with ethidium bromide for visualization. Southern blots of these gels were hybridized with labeled *ermF*-containing plasmid probes for confirmation of PCR products as previously described (2).

PCR primers and conditions for the *ermA*, *ermB*, and *ermC* genes. A_F (5′ CTTCGATAGTTTATTAATATTAGT 3′) and A_R (5′ TCTAAAAAGCATGT AAAAGAA 3′), B_F (5′ AGTAACGGTACTTAAATTGTTTAC 3′) and B_R (5′ GAAAAGGTACTCAACCAAATA 3′), and C_F (5′ GCTAATATTGTTTAAA TCGTCAAT 3′) and C_R (5′ TCAAAACATAATATAGATAAA 3′) have been described previously (2). The PCR conditions for the *ermB* reaction were the same as those for the *ermF* reaction. The PCR assay used for *ermA* consisted of denaturing at 94°C for 30 s, annealing at 48°C for 1 min, and elongation at 72°C for 2 min; that used for *ermC* consisted of denaturing at 94°C for 30 s, annealing at 43°C for 1 min, and elongation at 72°C for 2 min.

DNA hybridization. DNA was extracted from *N. gonorrhoeae*, commensal *Neisseria* spp., *N. meningitidis*, and selected transconjugants as previously described (9, 20). Uncut whole-cell DNA was visualized on a 0.7% agarose gel stained with ethidium bromide, and Southern blots were prepared. Fragment probes prepared from rRNA methylase genes from the cloned plasmids pEM9592, pJIR229, pBR328:33RV, pBF4, and pJI3, which carried the genes *ermA*, *ermB*, *ermC*, *ermF*, and *tetM*, respectively, or oligonucleotide probes for the appropriate genes were used (2, 16). The DNA probes were labeled with the appropriate Genius 3 chemiluminescence kit as recommended by the manufacturer (Boehringer). Hybridization under stringent conditions and detection were done according to the manufacturer's instructions as previously described (2, 19). Positive and negative controls were included in each Southern blot.

Hybridization of PCR products. Plasmids pEM9592, pJIR229, pBR328:33RV, and pBF4 or oligonucleotide probes for *ermA*, *ermB*, *ermC*, and *ermF* were labeled with nonradioactive Genius kits as recommended by the manufacturer (Boehringer). The labeled plasmids were used for hybridization with Southern blots of the appropriate PCR product or purified whole-cell DNA. The hybridization and wash steps were performed at stringent temperatures according to the manufacturer's instructions. Detection was done with a CDP-Star detection kit at a reagent concentration of 1:1,000 as described by the manufacturer (Boehringer).

Sequencing. The *ermF* PCR products from *N. gonorrhoeae* and commensal *Neisseria* spp. were sequenced separately with primers $ermF_1$ and $ermF_2$. A *Taq* Dye Deoxy terminator cycle sequencing kit (Applied Biosystems, Foster City, Calif.) was used for PCR amplification, and the filtered PCR products (Nuclean D50 filters; Kodak, Rochester, N.Y.) were examined on a model 373A sequencer (Applied Biosystems) (2, 19). The two sequences for each isolate were overlapped, aligned and compared with the known GenBank sequence of *ermF* (accession no. M14730) by use of GCG software (Genetics Computer Group, Madison, Wis.). The putative amino acid sequences were determined from the DNA sequences and also compared (with the known GenBank sequence of ErmF 19).

Mating experiments. Recipients included *N. gonorrhoeae* F62, with chromosomally mediated resistance to rifampin (25 μg/ml), streptomycin (250 μg/ml), and nalidixic acid (25 μg/ml) (21, 22, 27); *N. gonorrhoeae* CDC36N, with chromosomally mediated resistance to nalidixic acid (25 μg/ml) and carrying the 4.4-MDa β-lactamase plasmid (20); *E. faecalis* JH2-2, resistant to rifampin (25 μg/ml) and fusidic acid (25 μg/ml) (2, 19, 24, 26); *N. meningitidis* NRL9205 (serogroup A), resistant to streptomycin (250 μg/ml) and rifampin (20 μg/ml) (23); and *N. mucosa* CTM 1.1, with chromosomally mediated resistance to streptomycin (250 μg/ml) and rifampin (20 μg/ml). Donors included Em^r *N. gonorrhoeae* isolates and selected isolates from each of the commensal *Neisseria* species. Donors and recipients were grown separately for 24 h on agar plates. The donor and recipient isolates were each resuspended in 0.5 ml of GCP broth to form turbid suspensions ($>10^8$/ml), mixed together, and plated on a GC agar (Difco) plate without antibiotics as previously described (22, 23, 27). The mixture was incubated at 36.5°C in 5% CO_2 for 24 h. *N. meningitidis*, *N. mucosa*, and *N. gonorrhoeae* F62 transconjugants were selected on medium containing streptomycin (150 μg/ml) and erythromycin (10 μg/ml). The transconjugants were confirmed by growth on rifampin (25 μg/ml) (21–23). *N. gonorrhoeae* CDC36N transconjugants were selected on medium containing penicillin (10 μg/ml) and erythromycin (10 μg/ml). The transconjugants were verified by growth on nalidixic acid (25 μg/ml) and the presence of the 4.4-MDa β-lactamase plasmid (21–23, 27). JH2-2 transconjugants were selected on medium containing rifampin (10 μg/ml) and erythromycin (10 μg/ml). The *E. faecalis* transconjugants were confirmed by growth on medium supplemented with streptomycin (150 μg/ml) and by use of chromosomal DNA probe specific for *E. faecalis* (2). *N. meningitidis* transconjugants were confirmed by growth on medium supplemented with rifampin (20 μg/ml).

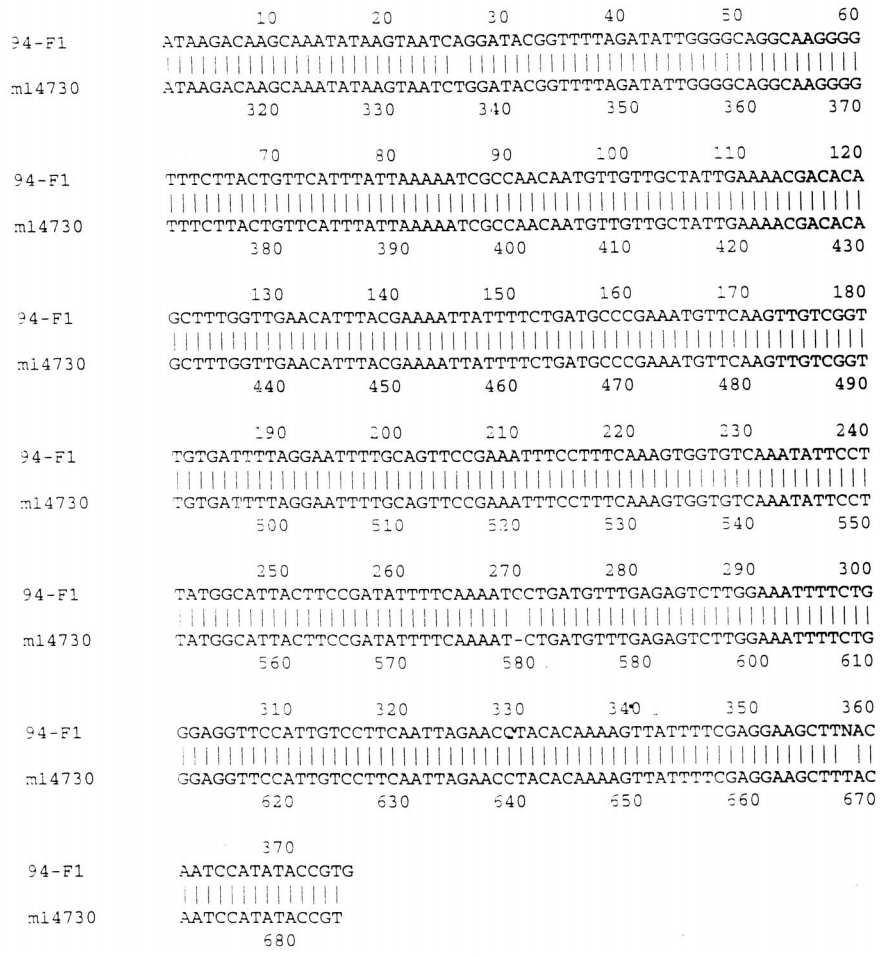

FIG. 1. DNA sequence homology between the GenBank *ermF* sequence (listed as m14730) and the PCR product from *N. gonorrhoeae* 94-965 (listed as 94-F1) (99% identity over 374 bp).

The identity of *erm* genes in the transconjugants was confirmed by PCR and hybridization of the PCR products as described above (2).

PFGE. Pulsed-field gel electrophoresis (PFGE) was used to compare the Em^r *N. gonorrhoeae* isolates to six Tc^r Pro^-/IA-1,2 Seattle *N. gonorrhoeae* isolates which were part of the outbreak. The isolates were digested with *Nhe*I or *Spe*I (Promega, Madison, Wis.) as previously described (31, 32). The PFGE patterns were compared and assumed to be genetically related if they were identical or had three or fewer band differences.

PFGE was also used to compare the *N. meningitidis* transconjugants with the donor and recipient *N. meningitidis* isolates by use of the *N. gonorrhoeae* protocol and one enzyme (31, 32). This procedure allowed us to verify that the *N. meningitidis* transconjugants were related to the recipient rather than the donor *N. meningitidis*.

RESULTS

Characterization of macrolide-resistant *N. gonorrhoeae*. The two Seattle Pro^-/IA-1,2 isolates were identified because of the high MICs of erythromycin (16 μg/ml) and azithromycin (4 μg/ml) for them (Table 1). Both Seattle isolates carried 25.2-MDa plasmids (tetracycline MIC, 16 μg/ml) which hybridized with the *tetM* probe (data not shown). For the two Uruguay isolates, the erythromycin MIC was 4 μg/ml and the azithromycin MIC was 1 μg/ml (Table 1). Uruguay isolate 1101 was resistant to penicillin and carried a 3.2-MDa β-lactamase plasmid (data not shown). All four *N. gonorrhoeae* isolates carried an *ermF* gene, which encodes a known rRNA methylase, and one isolate (95-1) also carried *ermB* (Table 1). The other isolates did not hybridize with *ermA*, *ermB*, or *ermC* gene probes, while 95-1 did not hybridize with *ermA* or *ermC* gene probes (data not shown). The *ermF* probe hybridized with the chromosomal fraction of the gel when whole-cell DNA was used for the Southern blots, suggesting a chromosomal location for the *ermF* gene. In addition, the 2.6- and 3.2-MDa β-lactamase plasmids or the 25.2-MDa *tetM*-containing plasmid common to *N. gonorrhoeae* was found, but no other plasmids were found in any of the four isolates.

PCR fragments of the *ermF* genes from two strains of *N. gonorrhoeae* (94-965 and 1101) were sequenced. The DNA sequence and amino acid homologies between the PCR fragment from 94-965 and the *ermF* gene originally identified in colonic *Bacteroides* spp. were both 99% identical over 374 bp (Fig. 1 and 2). Results were similar (95% identity) for *N. gonorrhoeae* 1101 from Uruguay (data not shown). The G+C content of the PCR fragment was approximately 35%, which differs from the 50% G+C content found in the *N. gonorrhoeae* chromosome, suggesting a non-*Neisseria* origin for these genes. PCR fragments of the *ermB* gene from strain 95-1 were also sequenced. The DNA sequence and amino acid homologies between the PCR fragment and the *ermB* gene previously characterized from *Clostridium perfringens* (GenBank accession no. X58285) were over 99% identical over 342 bp.

The six Tc^r Pro^-/IA-1,2 *N. gonorrhoeae* isolates obtained

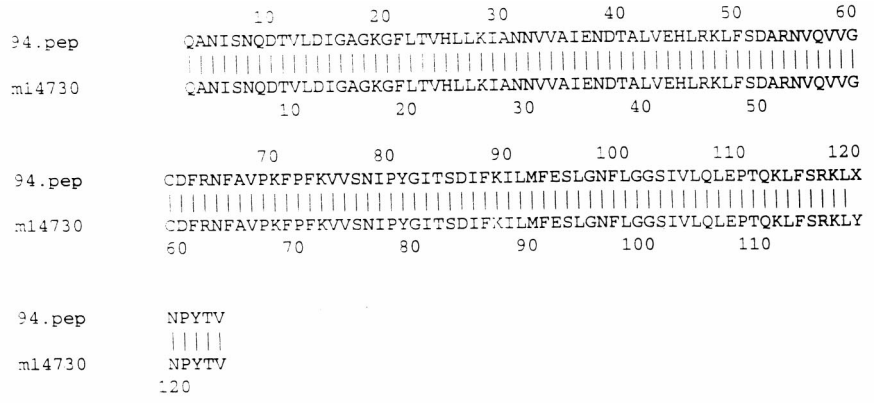

FIG. 2. Amino acid homology between the GenBank *ermF* sequence (listed as m14730) and the PCR product from *N. gonorrhoeae* 94-965 (listed as 94.pep) (99% identity over 123 amino acids).

during the 1994-1995 Seattle outbreak had *Nhe*I PFGE patterns (Fig. 3, lanes 1 to 6) that were indistinguishable from the *Nhe*I PFGE patterns of the two Em^r Tc^r *N. gonorrhoeae* isolates (Fig. 3, lanes 7 and 8), but the Seattle Em^r isolates differed from the two Em^r *N. gonorrhoeae* isolates from Uruguay (Fig. 3, lanes 9 and 10). The two Uruguay isolates appeared unrelated to the Seattle isolates or to each other (the PFGE patterns differed by more than three fragments) (Fig. 3). Similar results were found when *Spe*I was used for PFGE analysis (data not shown). Both enzymes gave identical patterns for the eight Seattle isolates, strongly suggesting a very close relationship between the two Em^r and the six Em^s *N. gonorrhoeae* isolates from the outbreak.

Characterization of macrolide resistance in commensal *Neisseria* spp. The erythromycin MICs for the other *Neisseria* spp. ranged from 4 to 16 μg/ml (Table 1). Compared to the *N. gonorrhoeae* isolates studied, these commensal species contained a more heterologous group of known *erm* genes (*ermB*, *ermC*, and *ermF*). Among the 10 *N. perflava-N. sicca* isolates, 4 carried the *ermB* gene and had erythromycin MICs ranging from 4 to 8 μg/ml; 2 carried *ermC* and had an erythromycin MIC of 16 μg/ml; 2 carried both *ermB* and *ermC* and had erythromycin MICs of 4 to 16 μg/ml; and 2 carried *ermB*, *ermC*, and *ermF* and had erythromycin MICs of 4 to 16 μg/ml (Table 1). Among the four *N. perflava-N. sicca* strains isolated before 1990, three carried one *erm* gene, while three of six strains isolated after 1990 carried multiple *erm* genes. One *N. flava* strain carried *ermC*, and the other strain carried *ermB* (erythromycin MICs, 8 to 16 μg/ml). Among the four *N. mucosa* strains, two carried both *ermB* and *ermC*, one carried *ermB*, and one carried *ermC*; the erythromycin MIC for all four strains was 8 μg/ml (Table 1).

To confirm the presence of the *erm* genes, we used PCR sequencing. The PCR fragment of the *ermF* gene from *N. perflava-N. sicca* 10915 was sequenced; the DNA sequence homology between the PCR fragment and the *ermF* gene from *Bacteroides* spp. showed 97% identity over 374 bp, and the amino acid homology was 94% (data not shown).

Transfer of erythromycin resistance. All four of the *N. gonorrhoeae* isolates and seven of the commensal *Neisseria* sp. isolates were examined for their ability to transfer the Em^r phenotype to *Neisseria* and *E. faecalis* recipients (Table 2). *N. gonorrhoeae* donors transferred the *ermF* gene at frequencies of 10^{-6}/recipient with the two different *N. gonorrhoeae* recipients, 10^{-7}/recipient with *N. meningitidis* as the recipient, and 10^{-7} to 10^{-8}/recipient with *E. faecalis* as the recipient.

The commensal species carried a variety of *erm* genes and were able to transfer *ermF*, *ermC* and *ermF*, *ermB* and *ermC*, all three *ermC*, or *ermB* (Table 2) at frequencies ranging from 10^{-5} to 10^{-9} for *E. faecalis* and *N. meningitidis*. Matings were done at least twice, and only a portion of the transconjugants were characterized and described in Table 2. The donor *N. mucosa* CTM 2.2 could move the *ermB* gene but not the *ermC* gene to the recipient *N. mucosa* CTM 1.1 (Table 2) at a frequency of 10^{-8}/recipient. The other *N. mucosa* donor (CTM 8.1) and the various *N. perflava-N. sicca* and *N. flava* donors used in the matings, which transferred *erm* genes to *E. faecalis* and/or *N. meningitidis* recipients, could not transfer *erm* genes at measurable frequencies ($>10^{-9}$/recipient) to the recipient *N. mucosa* CTM 1.1 (Table 2). Both *E. faecalis* and *N. meningitidis* recipients were able to acquire one or more *erm* genes. No *N. meningitidis* with *ermF* was isolated from the transconjugants with the commensal donors, but *ermF* was found in *N. meningitidis* transconjugants when *N. gonorrhoeae* carrying the *ermF* gene was used as the donor (Table 2).

The 25.2-MDa plasmid, conferring tetracycline resistance, was transferred from a Seattle *N. gonorrhoeae* donor to *N. gonorrhoeae* and *N. meningitidis* recipients but not to *E. faecalis*

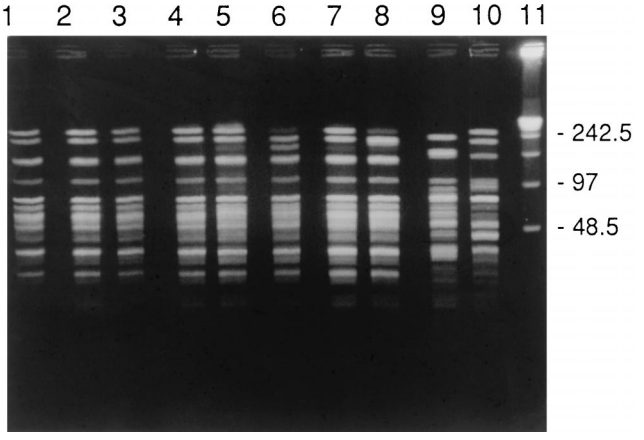

FIG. 3. PFGE with *Nhe*I. Lanes 1 to 6, Tc^r *N. gonorrhoeae*, with isolates in lanes 1 to 3 being isolated before and isolates in lanes 4 to 6 being isolated after the Em^r Tc^r *N. gonorrhoeae* isolates from Seattle; lanes 7 and 8, Em^r Tc^r *N. gonorrhoeae* isolates from Seattle; lanes 9 and 10, Em^r isolates from Uruguay; lane 11, λ standard. Numbers at right are molecular weight standards.

TABLE 2. Mobility of *erm* genes in representative transconjugants

Donor	*erm* gene(s) carried[a]	Recipient	Transconjugants	
			No. tested	*erm* genes transferred[a]
N. gonorrhoeae				
94-965[b]	F	*N. meningitidis* 9205	1	F
94-965[b]	F	*N. gonorrhoeae* CDC36N	1	F
94-965[b]	F	*N. gonorrhoeae* F62	1	F
94-965	F	*E. faecalis* JH2-2	1	F
95-1	F	*E. faecalis* JH2-2	1	F
1101	F	*N. gonorrhoeae* CDC36N	1	F
		E. faecalis JH2-2	1	F
581	F	*E. faecalis* JH2-2	1	F
N. perflava-N. sicca				
10915	B, C, F	*E. faecalis* JH2-2	2	F
		E. faecalis JH2-2	5	C, F
10915	B, C, F	*N. meningitidis* 9205	1	B, C
33006	B, C, F	*E. faecalis* JH2-2	2	C
33006	B, C, F	*N. meningitidis* 9205	6	C
33006	B, C, F	*E. faecalis* JH2-2	1	B, C, F
30423	B, C	*N. meningitidis* 9205	8	B
31212	C	*N. meningitidis* 9205	2	C
N. flava NRL 69	B	*N. meningitidis* 9205	2	B
N. mucosa				
CTM 8.1	B, C	*E. faecalis* JH2-2	1	B
CTM 2.2	B, C	*E. faecalis* JH2-2	1	C
		E. faecalis JH2-2	2	B, C
		N. mucosa CTM 1.1	5	B

[a] See Table 1, footnote *c*, for *erm* gene designations.
[b] The 25.2-MDa plasmid did transfer in these matings.

recipients (data not shown). No plasmids carrying the *tetM* gene were found in the commensal species, and we were unable to transfer tetracycline resistance from these species to either *N. meningitidis* or *E. faecalis*. However, this result was anticipated, since we have previously shown that commensal *Neisseria* sp. isolates carry an incomplete *tetM* transposon in the chromosome and were unable to transfer *tetM* by conjugation (16, 17, 25).

DISCUSSION

This is the first description of a known *erm* gene(s) in the genus *Neisseria*, since both the TEM β-lactamase and the *tetM* genes have incomplete transposons in *N. gonorrhoeae*, *N. meningitidis*, and the commensal *Neisseria* spp. (5, 16, 17). The data indicates that the *ermF* genes have been in *N. gonorrhoeae* since at least 1991, the *ermB* genes have been in *N. gonorrhoeae* since 1995, and various *erm* genes have been in three commensal species (*N. perflava-N. sicca*, *N. flava*, and *N. mucosa*) since at least the 1980s (Table 1). Whether *erm* genes are relatively new (last 20 years) in *Neisseria* spp. or whether they predate the identification of the β-lactamase plasmids in *N. gonorrhoeae* (5) is currently under investigation. Donors carrying the 25.2-MDa plasmid with the *tetM* gene transferred this gene into *N. gonorrhoeae* and *N. meningitidis* recipients but not into *E. faecalis* (data not shown), indicating that the *ermF* gene had a wider host range than the gonococcal 25.2-MDa plasmid (17, 22) or the gonococcal 24.5-MDa and β-lactamase plasmids (17, 23). Although 10 (63%) of the commensal *Neisseria* isolates carried the *tetM* gene (Table 1), none could move this gene, as has previously been described (16, 17).

N. gonorrhoeae with reduced susceptibility to erythromycin (MICs, 2 to 4 μg/ml) has been reported since the 1960s (1, 15). Some studies have shown a positive association between reduced susceptibility to penicillin, erythromycin, chloramphenicol, and tetracycline and *mtr* mutations (3). It was hypothesized that resistant *N. gonorrhoeae* isolates for which erythromycin MICs were 2 to 4 μg/ml were due to the presence of *mtr* mutations (3, 8). However, the maximum azithromycin MICs for these isolates generally were 0.25 to 0.5 μg/ml (unpublished observations). Based upon our finding with the two Uruguay isolates, for which the erythromycin MIC was 4 μg/ml and the azithromycin MIC was 1 μg/ml (Table 1), it is tempting to speculate that other *N. gonorrhoeae* isolates for which erythromycin MICs are 2 to 4 μg/ml also may carry *erm* genes with or without *mtr* mutations. We are currently examining isolates obtained during different decades and for which erythromycin MICs range from 0.5 to 8 μg/ml. It will be of interest to determine whether the characteristics attributed to the *mtr* mutations are due to the combination of *mtr* mutations and *erm* genes or whether reduced susceptibility to penicillin and tetracycline is associated with *mtr* mutations but reduced susceptibility to erythromycin is associated not with *mtr* mutations but with the presence of *erm* genes (3). Clinically, this information will be of interest because many infections in homosexual and bisexual men in Seattle-King County (10) are due to *N. gonorrhoeae* isolates with a phenotypic pattern (reduced susceptibility to erythromycin, penicillin, and tetracycline) suggesting *mtr* mutations. Some of these isolates have been shown to carry an *mtr* mutation by sequencing of PCR products. One can speculate that one or more of the four Em^r *N. gonorrhoeae* isolates in this study may carry both *erm* genes and *mtr* mutations. However, *mtr* mutations cannot be transferred by conjugation, nor do they influence the transfer of coresident β-lactamase plasmids. Since the *ermF-tetQ* transposons in colonic *Bacteroides* spp. are able to transfer mobilizable plasmids between *Bacteroides* spp., the ability of mobile *ermF* to conjugally transfer gonococcal β-lactamase plasmids is under investigation (2).

Three of the *N. gonorrhoeae* isolates and over 60% of the commensal species isolates were multiresistant (Table 1). The *ermF* gene in *N. gonorrhoeae* and the *ermB*, *ermC*, and *ermF* genes in the commensal species (Table 2) were able to move themselves by conjugation to other *Neisseria* spp. and to *E. faecalis* recipients. This finding implies that these *erm* genes are associated with complete conjugative elements, and this is the first description of complete transposable elements in *Neisseria*. Previously described TEM β-lactamase genes and the *tetM* gene are both on incomplete elements (5, 9, 17).

There was greater diversity among the *erm* genes carried by the 16 commensal *Neisseria* isolates than by the 4 *N. gonorrhoeae* isolates. Within this study, there was no association of erythromycin MIC with the number of *erm* genes found or with a particular gene among the commensal *Neisseria* spp. Further studies are needed to determine whether clinical isolates of *N. gonorrhoeae* carrying the *ermC* gene can be found or whether carriage of this gene is unique to the commensal species. Studies are needed to determine the influence of these *erm* genes on the treatment of gonococcal disease with the newer macrolides. It also remains to be determined if the commensal *Neisseria* spp. are reservoirs for these *erm* genes, how long these genes have actually been in the genus, whether most *erm* genes are on mobile conjugative elements, and whether isolates carrying *erm* genes are more likely to be multiresistant than the general *Neisseria* population.

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