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The *mef*(A) Gene Predominates among Seven Macrolide Resistance Genes Identified in Gram-Negative Strains Representing 13 Genera, Isolated from Healthy Portuguese Children

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Of the 176 randomly selected, commensal, gram-negative bacteria isolated from healthy children with low exposure to antibiotics, 138 (78%) carried one or more of the seven macrolide resistance genes tested in this study. These isolates included 79 (91%) isolates from the oral cavity and 59 (66%) isolates from urine samples. The *mef*(A) gene, coding for an efflux protein, was found in 73 isolates (41%) and was the most frequently carried gene. The *mef*(A) gene could be transferred from the donors into a gram-positive *E. faecalis* recipient and a gram-negative *Escherichia coli* recipient. The *erm*(B) gene transferred and was maintained in the *E. coli* transconjugants but was found in 0 to 100% of the *E. faecalis* transconjugants tested, while the other five genes could be transferred only into the *E. coli* recipient. The individual macrolide resistance genes were identified in 3 to 12 new genera. Eight (10%) of the oral isolates and 30 (34%) of the urine isolates for which the MICs were 2 to >500 μg of erythromycin per ml did not hybridize with any of the seven genes and may carry novel macrolide resistance genes.

The use of macrolide and related antibiotics (ketolides, oxazolidinones, streptogramins, and lincosamide) has increased dramatically over the last 15 years. A number of different mechanisms of macrolide resistance have been reported for gram-negative bacteria. These mechanisms include two esterase genes [*ere*(A) and *ere*(B)] found in *Escherichia coli* and *Enterobacter*, *Klebsiella*, *Citrobacter*, and *Proteus* species (1) and more recently, in *Providencia stuartii*, *Pseudomonas* species, and *Vibrio cholerae* (5, 17, 22). These mechanisms also include three phosphorylase genes [*mph(*A), *mph*(B), and *mph*(D)] found in *E. coli* (14, 15) and *Pseudomonas* (12) and one rRNA methylase gene [*erm*(B)] previously found in *E. coli* and *Actinobacillus*, *Klebsiella*, *Neisseria*, and *Wolinella* species (1, 4, 18–21). The strains described above were principally clinical isolates from hospital settings and/or from patients with clinical disease (17, 18, 20, 21), with members of the family *Enterobacteriaceae* and *Pseudomonas* species primarily isolated from France and Japan (1, 5, 11, 12, 14, 15). In contrast, relatively little is known about the presence of these six macrolide resistance genes in gram-negative bacteria from healthy individuals.

Recently, Cousin et al. found the efflux gene *mef*(A) in *Neisseria gonorrhoeae* and *Acinetobacter junii* (3, 7). This gene has been transferred, by conjugation in the laboratory, into a variety of gram-negative species, including *Eikenella corrodens*, *Haemophilus influenzae*, *Kingella denitrificans*, *Moraxella catarrhalis*, commensal *Neisseria*, and *Neisseria meningitidis* recipients using both gram-negative and gram-positive donors (7). These results suggested that the *mef*(A) gene may also be widespread among gram-negative species, so the *mef*(A) gene was included in the study of a group of randomly selected, commensal, gram-negative bacterial strains from oral and urine samples, collected from healthy children with low exposure to antibiotics from Lisbon, Portugal, for the presence of these seven acquired genes coding for macrolide resistance.

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MATERIALS AND METHODS

Population and bacterial strains. A total of 176 randomly chosen, commensal, gram-negative bacterial strains were isolated from oral and urine samples collected from healthy children in Lisbon, Portugal, who were participating in a randomized study designed to assess the safety of low-level mercury exposure from dental amalgam restorations. Children were 8 to 11 years of age during the recruitment period of February 1997 through April 1998, while isolates were from cultures obtained between December 1997 and March 1999 (Table 1). From the records, we found that during the collection period, five or six children per year received some type of medication from the doctors and included both antibiotics and nonantibiotic drugs. The bacteria represented 13 different genera (Table 1) and included 87 isolates from the oral cavity and 89 isolates from urine. The isolates were identified using CHROMagar orientation medium (DRG International, Inc., Mountainside, N.J.), standard biochemicals (10), and API kits according to the manufacturer's instructions (Biomerieux, Hazelwood, Mo.).

Media. Luria-Bertani (LB) agar (Difco Laboratories, Division of Becton Dickinson & Co., Sparks, Md.) unsupplemented or supplemented with ≥25 μg of erythromycin per ml was used to grow the clinical isolates. The *E. coli* strain HB101 and *Enterococcus faecalis* JH2-2 recipients were grown without antibiotic. All isolates were incubated at 36.5°C.

Agar dilution susceptibility testing. Erythromycin MICs were determined by the agar dilution method as described by the National Committee of Clinical Laboratory Standards (13) for all isolates. MIC breakpoints are not available for macrolides for most gram-negative species. We did not attempt to distinguish resistance from susceptibility and used MICs.

Detection of acquired genes. Isolates were initially screened by DNA-DNA hybridization of whole-cell dot blots and/or DNA dot blots as previously described (2–4, 6–9, 18). PCR was performed on selected isolates to confirm the presence of the various macrolide genes (3, 4, 8). Oligonucleotide probes for PCR and DNA-DNA hybridization are listed in Table 2. Radiolabeled probes were used as previously described (8).

TABLE 1. Distribution of macrolide resistance genes found in gram-negative isolates from the oral cavity and urine samples

Genus	Oral isolates		Urine isolates	
	No. of isolates	Gene(s) carried[a]	No. of isolates	Gene(s) carried
Acinetobacter	3	1 *mef*(A) 1 *ere*(A) 1 *ere*(B), *erm*(B)		
Citrobacter	4	1 *ere*(A) 1 *mef*(A), *erm*(B) 1 *mef*(A), *mph*(A) 1 *mef*(A), *ere*(A), *ere*(B), *mph*(A)	1	1 *mef*(A)
Enterobacter	6	2 *mef*(A) 1 *erm*(B) 1 *ere*(B) 1 *mef*(A), *ere*(B), *mph*(A) 1 *mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(B)		
Escherichia	7	3 *mef*(A) 2 *erm*(B) 1 *ere*(a) 1 *mph*(A), *mph*(B), *mph* (D)	19	2 *mef*(A) 12 *mph*(A) 1 *mph*(B) 1 *mph*(A), *mph*(B) 1 *mef*(A), *mph*(B) 1 *mef*(A), *mph*(D) 1 *mef*(A), *ere*(B), *mph*(A), *mph*(B) *mph*(D)
Klebsiella	19	5 *mef*(A) 4 *ere*(A) 2 *erm*(B) 2 *mef*(A), *erm*(B) 1 *mef*(A), *mph*(A) 1 *ere*(A), *ere*(B), *mph*(D) 1 *mef*(A), *ere*(A), *mph*(A), *mph*(B) 1 *mef*(A), *ere*(A), *mph*(A) 1 *mef*(A), *ere*(B), *mph*(D) 1 *mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(D), *erm*(B)	6	1 *mef*(A) 2 *erm*(B) 1 *mph*(A) 2 *mph*(A), *mph*(B), *mph*(D)
Morganella			1	1 *mef*(A)
Pantoeae	1	1 *mef*(A), *ere*(A), *mph*(A), *mph*(D), *erm*(B)		
Providencia	1	1 *mef*(A)		
Pseudomonas	31	14 *mef*(A) 3 *ere*(A) 2 *mef*(A), *ere*(A) 1 *mef*(A), *erm*(B) 1 *mef*(A), *mph*(B) 1 *mph*(A), *mph*(B), *mph*(D) 2 *erm*(B), *ere*(A), *ere*(B) 1 *mef*(A), *ere*(A), *mph*(D) 1 *mef*(A), *ere*(A), *mph*(D), *erm*(B) 1 *mef*(A), *ere*(B), *mph*(A), *mph*(D) 2 *ere*(A), *ere*(B), *mph*(B), *mph*(D) 1 *ere*(A), *mph*(A), *mph*(B), *mph*(D) 1 *ere*(A), *mph*(A), *mph*(B), *erm*(B)	19	3 *erm*(B) 4 *mef*(A) 1 *mph*(D) 2 *mph*(A) 1 *mef*(A), *mph*(B) 3 *mef*(A), *erm*(B) 1 *mph*(A), *mph*(B) 1 *mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(B), *mph*(D) 2 *mef*(A), *ere*(B), *mph*(A), *mph*(B), *mph*(D) 1 *mef*(A), *ere*(B), *mph*(A), *mph*(B), *mph*(D), *erm*(B)
Proteus			13	1 *mef*(A) 1 *erm*(B) 7 *mph*(D) 1 *mph*(A), *mph*(D) 2 *mph*(A), *mph*(B) 1 *mef*(A), *erm*(B)
Ralstonia	1	1 *mef*(A), *mph*(A)		
Serratia	3	1 *mef*(A), *erm*(B) 1 *mef*(A), 1 *mef*(A), *ere*(A), *ere*(B)		
Stenotrophomonas	3	1 *mph*(A) 1 *mef*(A), *ere*(A), *mph*(A) 1 *mef*(A), *mph*(A), *mph*(D)		
Total	79		59	

[a] Gene(s) carried on the isolates. The number is the number of isolates carrying the gene(s) shown.

TABLE 2. Primers used in this study

Gene	Primer	Sequence (5′→3′)	Reference(s)
mef(A)	mefF	TGT GCA TAT TTC TAT TAC G	This study
	mefR	CCA ATT GGC ATA GCA AG	This study
	mefI	GCT GTG CAA TAA TGG GGC	This study
ere(A)	ereAF	GCC GGT GCT CAT GAA CTT GAG	This study
	ereAR	CGA CTC TAT TCG ATC AGAGGC	This study
	ereAI	TCA CTG GCT AGA GCT AGT CTT	This study
ere(B)	ereBF	GCC TTG AAG CTA TGG CTC C	This study
	ereBR	GGC CCA TTG GTA GGC AAC	This study
	ereBI	TTG GAG ATA CCC GAG TTG TAG	This study
erm(B)	ermBF	GAA AAG GTA CTC AAC CAA ATA	7, 8
	ermBR	AGT AAC GGT ACT TAA ATT GTT TAC	7, 8
	ermBI	AGC CAT GCG TCT GAC ATC TAT	7, 8
mph(A)	mphAF	GTG AGG AGG AGC TTC GCG AG	This study
	mphAR	TGC CGC AGG ACT CGG AGG TC	This study
	mphAI	GAT ACC TCC CAA CTG TAC GCA	This study
mph(B)	mphBF	TTA AAC AAG TAA TCG AGA TAG C	This study
	mphBR	CCT TGT ACT TCC AAT GCT T G	This study
	mphBI	GCG TAT GGA TGC AGT AAG AGC	This study
mph(D)	mphDF	GTG TTC TTG CTT GGC TCG TAA	This study
	mphDR	ATC TGG TCG GGG TTG ATA A	This study
	mphDI	GCG GAT CTC CTC CCA GAG TG	This study

Mating. Fifteen donors and one transconjugant donor were selected to be mated with erythromycin-susceptible *E. faecalis* JH2-2 and/or erythromycin-susceptible *E. coli* HB101. The erythromycin MIC for *E. faecalis* JH2-2 was <0.5 μg/ml, and the erythromycin MIC for *E. coli* HB101 was 16 μg/ml. Matings were performed on agar plates, and transconjugants were identified as previously described because they expressed erythromycin resistance (6–8, 18). The transconjugants were selected with 5 μg of erythromycin per ml for *E. faecalis* JH2-2 and 25 to 50 μg of erythromycin per ml for *E. coli* HB101. The presence of acquired macrolide resistance genes in 5 to 10 of the transconjugants from each mating pair was determined by DNA-DNA hybridization and PCR for each of the macrolide resistance genes as previously described (2–4, 7, 8, 18). Positive controls were used in each assay.

DNA-DNA hybridization. DNA-DNA hybridization of Southern blots, whole-cell bacterial dot blots, whole-cell DNA dot blots, and/or PCR dot blots was performed as previously described. DNA was hybridized with the appropriate ^{32}P-labeled probe as previously described (2).

PCR. Seven different PCRs were performed to detect each of the seven genes separately. PCR assays for the *erm*(B) and *mef*(A) genes were conducted as previously described (2–4, 7, 8, 17). For the other five genes, new assays were developed using 2 U of *Taq* polymerase (Perkin-Elmer Cetus, Norwalk, Conn.), 200 μM (each) deoxynucleotide triphosphates, 1× PCR buffer (1.5 mM $MgCl_2$), 100 ng of each primer, and ≥200 ng of whole DNA as the template. To detect the *ere*(A) and *ere*(B) genes, PCR was performed as follows: an initial denaturation step (96°C for 3 min); followed by 35 cycles of PCR, with 1 cycle consisting of denaturation (96°C for 30 s), annealing (56°C for 1 min), and elongation (72°C for 2 min). For the *mph*(A), *mph*(B), and *mph*(D) genes, the initial denaturation, denaturation, and elongation times and temperatures were the same, but annealing was done at 57°C for 1 min. For all assays, the final step was 72°C for 10 min, followed by incubation at 4°C. Positive and negative controls were included in each run. The PCR products were visualized on a 1.5% agarose gel as previously described. Southern blots and/or dot blots of the PCR products were hybridized using an internal ^{32}P-labeled probe to verify the PCR products as previously described (2–4, 7, 8, 18).

RESULTS

Macrolide susceptibility and detection of the seven macrolide resistance genes. Of the 13 genera, four were found in both the oral and urine samples (Table 1). The erythromycin MICs for the isolates ranged from 2 to >500 μg/ml; the erythromycin MICs for most of the isolates were ≥64 μg/ml. The MICs for isolates within a species varied, with *Pseudomonas* (MICs, 4 to >500 μg/ml) and *Klebsiella* (MICs, 16 to >500 μg/ml) having the widest range (data not shown). Of the 176 isolates, 138 (78%) isolates, including 79 (91%) isolates from the oral cavity and 59 (66%) isolates from urine samples, hybridized with one or more of the seven gene probes used (Table 1). Forty-four (56%) oral and 40 (68%) urine isolates carried one of the seven macrolide genes, while 35 (44%) oral and 19 (32%) urine isolates carried two or more of the seven macrolide genes examined. The *mef*(A) gene was found in 74 (54%) of the isolates, including 51 (65%) of the oral isolates and 22 (37%) of the urine isolates and was the most common gene found in this population. The *mph*(A) gene was the second most commonly found gene; it was found in 45 (33%) isolates, which included 18 (23%) oral isolates and 27 (46%) urine isolates.

The other five genes, *erm*(B), *ere*(A), *ere*(B), *mph*(B), and *mph*(D), were found in 14 to 22% of the total isolates (Table 1). The distribution of the *erm*(B) gene was similar in the isolates from the oral cavity (22%) and urine samples (19%), while the *ere*(A) gene was found more commonly in the oral isolates (38%) than in the urine isolates (1%). In contrast, the *mph*(B), *mph*(D), and/or *ere*(B) genes were more common in the urine isolates than in the oral isolates (Table 1).

The *mef*(A) gene has previously been found in *A. junii* (7), but this is the first report of this gene in the other 12 genera examined (Table 3). The *mph*(A) gene was identified in eight new genera, the *mph*(D) gene was identified in seven new genera, the *erm*(B) gene was found in six new genera, the *ere*(B) gene was found in five new genera, and the *ere*(A) gene

TABLE 3. Macrolide resistance genes found in new genera

Type of gene	Gene	No. of new genera	New genera
rRNA methylase	*erm*(B)	6	*Acinetobacter*, *Enterobacter*, *Pantoeae*, *Proteus*, *Pseudomonas*, *Serratia*
Efflux (major facilitator)	*mef*(A)	12	*Citrobacter*, *Enterobacter*, *Escherichia*, *Klebsiella*, *Morganella*, *Pantoeae*, *Proteus*, *Providencia*, *Pseudomonas*, *Ralstonia*, *Serratia*, *Stenotrophomonas*
Esterase	*ere*(A)	3	*Pantoeae*, *Pseudomonas*, *Stenotrophomonas*
	ere(B)	5	*Acinetobacter*, *Citrobacter*, *Enterobacter*, *Pseudomonas*, *Stenotrophomonas*
Phosphorylase	*mph*(A)	8	*Citrobacter*, *Enterobacter*, *Klebsiella*, *Pantoeae*, *Pseudomonas*, *Proteus*, *Serratia*, *Stenotrophomonas*
	mph(B)	3	*Enterobacter*, *Pseudomonas*, *Proteus*
	mph(D)	7	*Escherichia*, *Klebsiella*, *Pantoeae*, *Proteus*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*

and the *mph*(B) genes were found in three new genera (Table 3).

Eight isolates (10%) from the oral cavity (two *Acinetobacter* isolates, three *Enterobacter* isolates, two *Klebsiella* isolates, and one *Pseudomonas* isolate) were negative for all seven macrolide resistance genes. Thirty (34%) isolates from urine samples (one *Acinetobacter* isolate, eight *Escherichia* isolates, five *Klebsiella* isolates, four *Morganella* isolates, four *Proteus* isolates, and eight *Pseudomonas* isolates) were negative for all seven macrolide resistance genes. These isolates, representing seven genera, did not hybridize with any of the gene probes used [*ere*(A), *ere*(B), *mph*(A), *mph*(B), *mph*(D), *mef*(A), or *erm*(B)]. The erythromycin MICs for oral isolates ranged from 2 to 256 μg/ml; the erythromycin MICs for five isolates were ≥64 μg/ml. The erythromycin MICs for urine isolates ranged from 2 to >500 μg/ml; the erythromycin MICs for 24 isolates were ≥64 μg/ml.

Characterization of specific isolates. Fifteen isolates from 10 genera were chosen for further characterization (Table 4). There was no apparent correlation between MIC and the number or type of macrolide resistance gene(s) carried (Table 4). Mating experiments were done using the 15 isolates as donors and *E. faecalis* and/or *E. coli* as the recipient(s) (Table 4). *A. junii* 329 has been included in Table 4 for comparison (7). The presence of acquired macrolide resistance genes were determined in 5 to 10 transconjugants, from each mating pair. The *mef*(A) gene was transferred to the *E. faecalis* recipient at frequencies from 10^{-5} to 10^{-9}/recipient. The *mef*(A) gene also transferred to the *E. coli* recipient at frequencies from 10^{-5} to 10^{-9}/recipient. One transconjugant, *E. coli* 11 donor (HB101 transconjugant) carrying the *mef*(A) gene, was used as a donor and mated with the *E. faecalis* recipient. Transconjugants from this mating were detected at low frequencies, indicating that the HB101 transconjugant maintained its ability to retransfer the *mef*(A) gene (Table 4).

In six of the gram-negative donors, both the *erm*(B) and *mef*(A) genes were present, and the overall transfer of macrolide resistance genes varied from 10^{-5} to 10^{-9}/recipient (Table 4). In five of the matings, the *mef*(A) gene was transferred and detected in the *E. faecalis* transconjugants, while the *erm*(B) gene was detected in 25 to 100% of the *E. faecalis* transconjugants. With the *Klebsiella oxytoca* 561 donor, the *erm*(B) gene was not detected in the *E. faecalis* transconjugants even after multiple mating experiments (Table 4). The esterase and phosphorylase genes were not detected in the *E. faecalis* transconjugants from any of the matings.

Seven of the donors carried macrolide resistance genes in addition to the *erm*(B) and/or *mef*(A) genes, with two carrying a total of six different macrolide resistance genes and one carrying a total of five different macrolide resistance genes. These donors were mated with the *E. coli* recipient, and the frequency of transfer ranged from 10^{-5} to 10^{-9} per recipient (Table 4). All the macrolide resistance genes carried in the donors were identified in the *E. coli* transconjugants tested.

DISCUSSION

In this study, 78% of the randomly selected, commensal, gram-negative bacteria from a healthy population with low exposure to antibiotics were found to carry one or more of the seven macrolide resistance genes tested. Unfortunately, there is little previously published data from other countries available for comparison of data, because most studies have focused on macrolide resistance in pathogenic bacteria from clinical settings and/or diseased hosts (1, 3, 4, 6, 7, 9, 11, 12, 14–18, 22). However, we can compare these results with those of an earlier study by Luna et al. (8) on 615 randomly selected, commensal, gram-positive isolates from the same group of Lisbon children as the current study over the same time period. In that study, 222 (36%) isolates carried one or more of four different rRNA methylases and *mef*(A) efflux gene, which is less than 50% of the rate we found in the current gram-negative study, indicating that in this Lisbon population, acquisition of macrolide resistance genes is more prevalent in commensal, gram-negative isolates than in commensal, gram-positive isolates. The *mef*(A) and *erm*(B) genes were examined in both groups of bacteria, and the percentage in each population was compared. For the *mef*(A) and *erm*(B) genes, 9 and 60% were found in the gram-positive bacteria, respectively, while 54 and 19% were found in the gram-negative isolates, respectively.

TABLE 4. Transfer of macrolide resistance genes

Donor	ERY[a] MIC (μg/ml)	Gene(s) carried	Recipient	Frequency[b]	Gene(s) transferred
Oral isolates					
Acinetobacter junii 329[c]	2	*mef*(A)	*E. faecalis*	9.5×10^{-6}	*mef*(A)
Citrobacter freundii 16	256	*mef*(A), *erm*(B)	*E. faecalis*	1.6×10^{-8}	*mef*(A), *erm*(B)[d]
Enterobacter cloacae 240	256	*mef*(A)	*E. faecalis*	5.3×10^{-9}	*mef*(A)
Escherichia coli 11	64	*mef*(A)	*E. faecalis*	6.3×10^{-7}	*mef*(A)
		mef(A)	*E. coli*	3.9×10^{-9}	*mef*(A)
E. coli HB101 transconjugant[e]		*mef*(A)	*E. faecalis*	2.0×10^{-10}	*mef*(A)
Klebsiella sp. 7	128	*mef*(A)	*E. faecalis*	3.0×10^{-7}	*mef*(A)
Klebsiella sp. 8	256	*mef*(A), *erm*(B)	*E. faecalis*	2.9×10^{-8}	*mef*(A), *erm*(B)[f]
		mef(A), *erm*(B)	*E. coli*	4.5×10^{-8}	*mef*(A), *erm*(B)
Klebsiella sp. 9	256	*mef*(A), *ere*(B), *mph*(D)	*E. faecalis*	5.1×10^{-6}	*mef*(A)
Klebsiella sp. 106	128	*mef*(A)	*E. faecalis*	2.4×10^{-8}	*mef*(A)
K. oxytoca 561	128	*mef*(A), *erm*(B), *ere*(A), *ere*(B), *mph*(A), *mph*(B)	*E. faecalis*	2.0×10^{-7}	*mef*(A), *erm*(B)[g]
			E. coli	3.8×10^{-5}	*mef*(A), *erm*(B), *ere*(A), *ere*(B), *mph*(A), *mph*(B)
Pantoeae agglomerans 323	256	*mef*(A), *ere*(A), *mph*(A), *mph*(D), *erm*(B)	*E. faecalis*	1.3×10^{-7}	*mef*(A), *erm*(B)[h]
			E. coli	4.7×10^{-9}	*mef*(A), *erm*(B), *ere*(A), *mph*(A), *mph*(D)
Pseudomonas sp. 203	>500	*mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(D), *erm*(B)	*E. faecalis*	4.0×10^{-8}	*mef*(A), *erm*(B)[i]
			E. coli	2.2×10^{-7}	*mef*(A), *ere*(A), *ere*(B), *mph*(A), *mph*(D), *erm*(B)
Pseudomonas sp. 333	128	*mef*(A), *erm*(B)	*E. faecalis*	1.0×10^{-5}	*mef*(A), *erm*(B)[j]
		mef(A), *erm*(B)	*E. coli*	4.0×10^{-9}	*mef*(A), *erm*(B)
Pseudomonas putida 366	>500	*ere*(A), *mph*(A), *mph*(D), *mef*(A)	*E. faecalis*	2.5×10^{-9}	*mef*(A)
Serratia liquefaciens 136	64	*mef*(A), *ere*(A), *ere*(B)	*E. faecalis*	7.2×10^{-8}	*mef*(A)
Urine isolates					
Morganella morganii 236	500	*mef*(A)	*E. faecalis*	6.7×10^{-8}	*mef*(A)
Proteus sp. 21	>500	*mef*(A), *mph*(A)	*E. faecalis*	1.0×10^{-9}	*mef*(A)

[a] ERY, erythromycin.
[b] Number of transconjugants per recipient.
[c] Data from reference 7.
[d] 33% of *E. faecalis* transconjugants carried both the *mef*(A) and *erm*(B) genes, all carried *mef*(A).
[e] Transconjugant from *E. coli* 11 mated with *E. faecalis* JH2-2 and selected on erythromycin.
[f] 50% of *E. faecalis* transconjugants carried both the *mef*(A) and *erm*(B) genes; all carried *mef*(A).
[g] No *erm*(B) gene was detected in the *E. faecalis* transconjugants with the donor *K. oxytoca* 561.
[h] 100% of the *E. faecalis* transconjugants carried both the *mef*(A) and *erm*(B) genes.
[i] 50% of the *E. faecalis* transconjugants carried both the *mef*(A) and *erm*(B) genes; all carried *mef*(A).
[j] 25% of the *E. faecalis* transconjugants carried both the *mef*(A) and *erm*(B) genes; all carried *mef*(A).

The distribution of the seven macrolide resistance genes in the gram-negative bacteria varied by genus and location. However, with only four genera found in both the oral cavity and urine samples, it was not surprising that these seven different genes were found in different percentages of the oral and urine isolates. The *mef*(A) gene was identified in at least one isolate from each of the 13 genera examined, while the *mph*(A) gene was the second most commonly found gene overall (Table 1). Each of the seven macrolide resistance genes was identified in a number of new genera, suggesting that the host range of these genes is larger than the literature has suggested (Table 3).

We had 8 isolates from the oral cavity and 30 isolates from urine samples that did not carry any of the known genes. The erythromycin MICs for the majority of the isolates were ≥64 μg/ml. Susceptibility to clindamycin, determined by agar dilution, was examined for these strains. The clindamycin MICs for most of the isolates was the same or plus or minus up to eightfold of their erythromycin MICs (data not shown). Other *erm* genes have been identified in gram-negative *Actinobacillus* spp. (21), *Neisseria* spp. (18), and a variety of anaerobic gram-negative genera (2, 19–21). Thus, it is possible that some of the other known rRNA methylase genes are present in the 38 gram-negative strains found in this study. We are currently testing these isolates for the presence of the *erm*(A), *erm*(C), *erm*(F), *erm*(G), and *erm*(Q) genes, which are the most widespread genes found other than the *erm*(B) gene. Three interesting isolates were identified. For one *Acinetobacter* isolate, the erythromycin MIC was 16 μg/ml, and the clindamycin MIC was >500 μg/ml. For one *E. coli* isolate, the erythromycin MIC was 64 μg/ml, and the clindamycin MIC was >500 μg/ml. For one *Pseudomonas* isolate, the erythromycin MIC was 8 μg/ml, and the clindamycin MIC was 500 μg/ml. These three isolates may carry a lincosamide resistance gene. These isolates and all of the nonreactive isolates will be examined for the

presence of the *lnu*(A) and *lnu*(B) transferase genes, which have previously been found only in *Staphylococcus* and *Enterococcus*, respectively (19).

The *mef*(A) gene transferred to both the *E. coli* and *E. faecalis* recipients, with all transconjugants receiving and maintaining this gene. In contrast, the *erm*(B) gene, a gene of gram-positive bacterial origin, transferred and was maintained in some but not all *E. faecalis* transconjugants examined (Table 4). The variability in transfer and maintenance appears to be donor specific, since the same recipient was used with each mating. The reason for this variability is currently being examined but could be due to the location of the *erm*(B) gene (plasmid versus conjugative transposon). However, all seven genes were associated with mobile elements in most of the donors examined and were able to conjugally transfer these genes to unrelated recipients in the laboratory. It is also likely that these strains would be able to conjugally transfer these genes to unrelated bacteria in their human hosts as well.

Few studies have examined erythromycin MICs for members of the family *Enterobacteriaceae* or for *Pseudomonas* species. Both groups have been thought to be innately nonsusceptible to erythromycin due to innate multidrug-resistant transporters which confer resistance to 14-membered macrolides (16). Clearly, more work needs to be done to determine whether the data from this group of bacteria can be generalized to bacteria from other geographic locations, isolates collected during different time periods, and populations of bacteria from humans, animals, and the environment.

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