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# Molecular Basis of Resistance to Macrolides and Other Antibiotics in Commensal Viridans Group Streptococci and *Gemella* spp. and Transfer of Resistance Genes to *Streptococcus pneumoniae*

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**We assessed the mechanisms of resistance to macrolide-lincosamide-streptogramin B ($MLS_B$) antibiotics and related antibiotics in erythromycin-resistant viridans group streptococci (*n* = 164) and *Gemella* spp. (*n* = 28). The macrolide resistance phenotype was predominant (59.38%); all isolates with this phenotype carried the *mef*(A) or *mef*(E) gene, with *mef*(E) being predominant (95.36%). The *erm*(B) gene was always detected in strains with constitutive and inducible $MLS_B$ resistance and was combined with the *mef*(A/E) gene in 47.44% of isolates. None of the isolates carried the *erm*(A) subclass *erm*(TR), *erm*(A), or *erm*(C) genes. The *mel* gene was detected in all but four strains carrying the *mef*(A/E) gene. The *tet*(M) gene was found in 86.90% of tetracycline-resistant isolates and was strongly associated with the presence of the *erm*(B) gene. The $cat_{pC194}$ gene was detected in seven chloramphenicol-resistant *Streptococcus mitis* isolates, and the *aph(3′)-III* gene was detected in four viridans group streptococcal isolates with high-level kanamycin resistance. The *intTn* gene was found in all isolates with the *erm*(B), *tet*(M), *aph(3′)-III*, and $cat_{pC194}$ gene. The *mef*(E) and *mel* genes were successfully transferred from both groups of bacteria to *Streptococcus pneumoniae* R6 by transformation. Viridans group streptococci and *Gemella* spp. seem to be important reservoirs of resistance genes.**

Viridans group streptococci (VGS) and *Gemella* spp. are commensal bacteria of the human upper respiratory tract, although they also cause systemic diseases, including bacterial endocarditis, bacteremia (especially in neutropenic patients), and pneumonia (12, 15, 23, 24). These bacteria can exchange genetic material with other bacteria sharing their habitat, making their resistance profiles good markers for the risk of the emergence of resistance to some antibiotics in *Streptococcus pneumoniae* and *Streptococcus pyogenes* (4).

Resistance to macrolides and related antibiotics has spread among VGS (10, 17, 27, 36, 48, 54). Two major mechanisms account for resistance to macrolide, lincosamide, and streptogramin B ($MLS_B$) antibiotics in streptococci: the first is mediated by methylation of the ribosomal target of these antibiotics ($MLS_B$ resistance). The methylase responsible for this activity is encoded by the *erm* genes (erythromycin ribosome methylase) (26). Expression of $MLS_B$ resistance can be constitutive ($cMLS_B$) or inducible ($iMLS_B$) (25). The streptococcal *erm*(B) gene is associated with conjugative transposons of the Tn*916*-Tn*1545* family that also confer resistance to tetracycline [by the *tet*(M) gene] and/or kanamycin [by the *aph*(*3′*)-*III* gene]. These elements also contribute to the dissemination of multidrug resistance by integration into larger conjugative transposons, like Tn*5253* and Tn*3872*, that encode additional antimicrobial resistance determinants (i.e., the $cat_{pC194}$ gene). A core element in the biology of these transposons is the integrase, encoded by the *intTn* gene, which is absolutely required for their transposition movements (11, 30, 39, 40).

The second major mechanism of resistance to $MLS_B$ antibiotics is mediated by an active efflux pump, encoded by the *mef*(A/E) gene. The expression of the *mef*(A/E) gene produces resistance to 14- and 15-membered macrolide compounds, and the resulting phenotype is designated M. There are two subclasses of the *mef*(A/E) gene: *mef*(A), originally found in *S. pyogenes*, and *mef*(E), originally found in *S. pneumoniae* (5, 51, 53). *mef*(A) and *mef*(E) are 90% identical at the nucleotide level but are characterized by major genetic differences (9). Genetic elements carrying the *mef*(A) and *mef*(E) genes were recently characterized in *S. pneumoniae*. The *mef*(A)-carrying element is a 7.2-kb defective transposon (Tn*1207.1*) that contains eight open reading frames (ORFs) (46) and that appears to be part of a longer conjugative transposon, named Tn*1207.3* (45). The element that contains the *mef*(E) gene, designated MEGA (macrolide efflux genetic assembly), is approximately 5.5 kb and contains five ORFs (16). Both elements contain an ORF adjacent to *mef*, designated ORF5 in Tn*1207.1* and *mel* in MEGA, which shares approximately 35% identity with the *msr*(A) gene of *Staphylococcus aureus*.

The purpose of this study was to study the prevalence and genetic basis of resistance to $MLS_B$, tetracycline, chloramphenicol, and kanamycin among VGS and *Gemella* spp. from the human microbiota. We also investigated the elements that carry erythromycin resistance genes and their possible transfer mechanisms.

## MATERIALS AND METHODS

**Bacterial strains.** Between October 2001 and March 2003, 164 isolates of VGS and 28 *Gemella* sp. isolates resistant to erythromycin were isolated from 178 patients in the Microbiology Department of the "Lozano Blesa" Clinical University Hospital (Zaragoza, Spain). These isolates originated from pharyngeal exudates (*n* = 88), sputa (*n* = 54), bronchial aspirates (*n* = 17), nasal swab

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TABLE 1. Distributions of VGS and *Gemella* sp. strains according to macrolide resistance phenotypes and genotypes

| Bacterium | Total no. of isolates | No. (%) of M-phenotype isolates[a] | cMLS$_B$-phenotype isolates[b] | | iMLS$_B$-phenotype isolates[b] | |
|---|---|---|---|---|---|---|
| | | | No. (%) of isolates | No. with *mef*(A/E) | No. (%) of isolates | No. with *mef*(A/E) |
| VGS | 164 | 97 (59.15) | 50 (30.49) | 24 | 17 (10.37) | 10 |
| *S. mitis* | 125 | 76 (60.80) | 35 (28.00) | 15 | 14 (11.20) | 8 |
| *S. oralis* | 28 | 28 (46.43) | 13 (46.43) | 8 | 2 (7.14) | 1 |
| *S. sanguinis* | 7 | 7 (100.00) | | | | |
| *S. salivarius* | 3 | 1 (33.33) | 1 (33.33) | 1 | 1 (33.33) | 1 |
| *S. anginosus* | 1 | | 1 (100.00) | | | |
| *Gemella* spp. | 28 | 17 (60.71) | 9 (32.14) | 3 | 2 (7.14) | |
| *G. haemolysans* | 12 | 9 (75.00) | 3 (25.00) | 2 | | |
| *G. morbillorum* | 16 | 8 (50.00) | 6 (37.50) | 1 | 2 (12.50) | |

[a] All strains with the M phenotype harbored the *mef*(A/E) gene.
[b] All strains with the cMLS$_B$ or iMLS$_B$ phenotype harbored the *erm*(B) gene.

samples ($n = 13$), oral swab samples ($n = 5$), and other samples ($n = 15$). Strains were identified on the basis of colony morphology, α-hemolysis, optochin susceptibility, and Gram staining. Isolates were identified to the species level by using the API 20 Strep system (bioMerieux, Marcy-l'Etoile, France). The isolates were assigned to a group or species according to the criteria described by Facklam (13). Different isolates from the same patient were differentiated by colony morphology, species identification, antimicrobial resistance phenotype and genotype, and/or pulsed-field gel electrophoresis pattern (data not shown).

**Detection of MLS$_B$ resistance phenotypes.** Macrolide resistance phenotypes were classified as described by Seppälä et al. (47). The M phenotype was confirmed by the induction test described by Malke (29).

**Antimicrobial agents.** The following antibiotics were tested: erythromycin, clindamycin, tetracycline, minocycline, and chloramphenicol (all from Sigma, St. Louis, Mo.); azithromycin, (Pfizer, Madrid, Spain); miocamycin (Menarini, S.A., Badalona, Spain); and kanamycin (Amersham Life Science, [manufactured for USB in China]).

**Antimicrobial susceptibility testing.** Antimicrobial susceptibility testing was performed by a standard agar diffusion test with commercially available disks (Bio-Rad, La Coquette, France) and a standard agar dilution method according to the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS) (32, 33). *S. pneumoniae* ATCC 49619 was used as a control strain. The cutoff points for resistance to each antibiotic were those recommended by the NCCLS (34). The MIC breakpoint for miocamycin resistance was as defined by the Comité de l'Antibiogramme de la Societé Française de Microbiologie (Antibiogram Committee of the French Microbiology Society) (7). As there are no defined MIC breakpoints for *Gemella* spp., we used those for VGS, given the similarities between these two bacterial genera.

**Detection of antibiotic resistance genes.** Antibiotic resistance genes were detected by PCR with oligonucleotide primers specific for each gene. DNA samples were prepared as described by Ausubel and Frederick (3). The *erm*(A), *erm*(B), *erm*(C), and *mef*(A/E) genes were amplified as reported by Sutcliffe et al. (52). The *erm*(A) subclass *erm*(TR) gene was detected as described by Seppälä et al. (49). The *mel* gene was detected with primers designed on the basis of the sequence of MEGA and *mel* in *S. pneumoniae* (GenBank accession no. AF274302). The forward and reverse primers were 5′-CAT GAG CGG TGG TGA AGA-3′ and 5′-TAG GGA TTT AGC GGC ATT AT-3′, respectively. Cycling conditions were 1 cycle at 94°C for 4 min; 35 cycles of 94°C for 1 min, 56°C for 1 min, and 72°C for 1 min; and 1 cycle at 72°C for 10 min. The PCR mixture contained 4 mM $MgCl_2$, 0.2 mM deoxynucleoside triphosphates, 0.25 mM each primer, and 0.5 U of *Taq* polymerase in a final volume of 20 μl. The tetracycline resistance gene *tet*(M) and the *intTn* gene were examined by established protocols (11). The $cat_{pC194}$ gene was detected with oligonucleotides catD (5′-GAA ACA TAA AAC AAG AAG GA-3′) and catR (5′-ATA GAA AGA GAA AAA GCA TT-3′), designed from the sequence of staphylococcal plasmid pC194 (GenBank accession no. NC_002013). The PCR conditions were as follows: 35 cycles of 94°C for 30 s, 46°C for 45 s, and 72°C for 2 min with 2.5 mM $MgCl_2$, 0.5 mM deoxynucleoside triphosphates, 1 mM each primer, and 1.75 U of *Taq* polymerase. The *aph*(3′)-*III* gene, which encodes the aminoglycoside-modifying enzyme, was amplified as described by van de Kludert and Wiegenthart (56).

Amplifications were performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, Conn.). The PCR products were resolved by electrophoresis on 1.5% agarose gels. To discriminate between *mef*(A) and *mef*(E), the *mef*(A/E) amplicon was digested with BamHI. The *mef*(A) amplicon contains one BamHI site, so restriction generates two fragments of 282 and 64 bp, respectively, whereas the *mef*(E) amplicon contains no BamHI restriction sites. PCR reagents and BamHI enzyme were purchased from Promega (Madison, Wis.).

**Transformation assays.** Precompetent *S. pneumoniae* R6 cells were prepared as described previously (18). Total genomic DNA (1 μg/ml) from strains carrying either the *mef*(A/E) or the *erm*(B) gene and competence-stimulating peptide (1 μg/ml) were added to precompetent *S. pneumoniae* R6 cells. Erythromycin-resistant transformants were isolated on Mueller-Hinton agar (Bio-Rad), supplemented with 5% blood (MHB) containing 2 or 8 μg of erythromycin per ml. The transformation frequency is expressed as the number of CFU of the transformants divided by the number of CFU of the recipients. The stabilities of the transformant strains were assessed by successive plating on MHB. Erythromycin resistance was determined by the agar diffusion test.

**Statistical analysis.** The $\chi^2$ test was used to determine whether differences in resistance rates among the isolates with different macrolide resistance phenotypes and between groups of isolates were significant.

## RESULTS

Of the 164 isolates of VGS examined in this study, 125 were identified as *Streptococcus mitis*, 28 were identified as *Streptococcus oralis*, 7 were identified as *Streptococcus sanguinis*, 3 were identified as *Streptococcus salivarius*, and 1 was identified as *Streptococcus anginosus*. Among the *Gemella* spp. identified, 12 were identified as *Gemella haemolysans* and 16 were identified as *Gemella morbillorum*.

**MLS$_B$ resistance phenotypes and macrolide resistance genes.** Table 1 shows the distributions of the MLS$_B$ phenotypes and the macrolide resistance genes among the species tested. The M phenotype was the most prevalent among the VGS (59.15%) and *Gemella* spp. (60.71%); all the isolates with this phenotype gave the expected 346-bp PCR product corresponding to the *mef*(A/E) gene. This was the only phenotype found among the *S. sanguinis* isolates. All M-phenotype strains were negative by the induction test. The cMLS$_B$ phenotype was the second most prevalent. *S. oralis* displayed the highest percentage of strains with this phenotype (46.43%). Finally, the iMLS$_B$ phenotype was the rarest phenotype in both VGS and *Gemella* spp. The *erm*(B) gene was detected in all strains with the cMLS$_B$ or the iMLS$_B$ phenotype. This gene was found in combination with the *mef*(A/E) gene in 45.76% of the cMLS$_B$ isolates and 52.63% of the iMLS$_B$ isolates. None of the isolates contained the *erm*(A) subclass *erm*(TR), *erm*(A), or *erm*(C) gene. The amplicons of *mef*(A/E) were subjected to restriction

TABLE 2. Resistance to tetracycline correlated with the presence of the *tet*(M) gene in VGS and *Gemella* spp. with different macrolide resistance phenotypes

| Bacterium | M-phenotype isolates | | $cMLS_B$-phenotype isolates | | $iMLS_B$-phenotype isolates | |
|---|---|---|---|---|---|---|
| | No. (%) $Tet^{r}$ [a] | No. with *tet*(M) | No. (%) $Tet^{r}$ | No. with *tet*(M) | No. (%) $Tet^{r}$ | No. with *tet*(M) |
| VGS | 13 (13.40) | 7 | 40 (80.00) | 35 | 15 (88.24) | 15 |
| *S. mitis* | 10 (13.16) | 7 | 30 (85.71) | 26 | 12 (85.75) | 12 |
| *S. oralis* | 1 (7.69) | | 9 (69.23) | 8 | 2 (100.00) | 2 |
| *S. sanguinis* | 1 (14.29) | | | | | |
| *S. salivarius* | 1 (100.00) | | | | 1 (100.00) | 1 |
| *S. anginosus* | | | 1 (100.00) | 1 | | |
| *Gemella* spp. | 4 (14.29) | 4 | 8 (88.89) | 8 | 2 (100.00) | 2 |
| *G. haemolysans* | 1 (8.33) | 1 | 2 (66.67) | 2 | | |
| *G. morbillorum* | 3 (18.75) | 3 | 8 (75.00) | 8 | 2 (100.00) | 2 |

[a] $Tet^{r}$, tetracycline resistant.

analysis to differentiate between *mef*(A) and *mef*(E). The *mef*(E) gene was predominant in VGS (95.42%) and gemellae (95.00%). Only six VGS isolates (three *S. mitis* isolates, two *S. oralis* isolates, and one *S. sanguinis* isolate) and one *G. haemolysans* isolate carried the *mef*(A) subclass. The *mel* gene, ORF2 of MEGA, was found in all *mef*(A/E)-containing *Gemella* sp. isolates and 96.95% of the VGS strains containing *mef*(A/E). The four *mef*(A/E)-containing VGS that did not carry this ORF included one *S. mitis* isolate and three *S. oralis* isolates.

**Non-$MLS_B$ resistance genes *tet*(M), $cat_{pC194}$, and *aph*(3′)-ITIII**. Tetracycline resistance (including intermediate resistance) was the most common non-$MLS_B$ resistance found in VGS (41.46%) as well as in *Gemella* spp. (57.14%); it was mainly found in isolates with the $cMLS_B$ and $iMLS_B$ phenotypes ($P < 0.0001$) (Table 2). In all cases, resistance to tetracycline was associated with resistance to minocycline. Among the species tested, *S. sanguinis*, the isolates of which displayed only the M phenotype, showed the lowest rates of resistance to these antibiotics. The *tet*(M) gene was detected in a large proportion (86.90%) of tetracycline-resistant strains, particularly in those with the *erm*(B) gene ($cMLS_B$ and $iMLS_B$ phenotypes) ($P < 0.005$) (Table 2).

Only 4.27% of VGS isolates were resistant (including intermediately resistant) to chloramphenicol. There were no significant differences between the three macrolide resistance phenotypes ($P > 0.5$). The seven chloramphenicol-resistant strains were all *S. mitis*, and all of them carried the $cat_{pC194}$ gene. None of the *Gemella* sp. isolates were resistant to chloramphenicol.

High-level kanamycin resistance was found in two *S. mitis* isolates and two *S. oralis* isolates. The *aph*(3′)-*III* gene was detected only in the isolates that also harbored the *erm*(B) gene (one *S. mitis* isolate and two *S. oralis* isolates).

In one strain with the *mef*(A/E) gene (M phenotype), the *tet*(M) gene was associated with the $cat_{pC194}$ gene, whereas three isolates harboring the *erm*(B) gene showed this combination. The *erm*(B), *tet*(M), and *aph*(3′)-*III* genes were found together in one *S. mitis* isolate with the $iMLS_B$ phenotype.

***intTn* gene.** The *intTn* integrase gene, from conjugative transposons of the Tn*916*-Tn*1545* type and from Tn*5253* and Tn*3872*, was detected in all the isolates with one or more of the following genes: *erm*(B), *tet*(M), *aph*(3′)-*III*, and $cat_{pC194}$.

**Transformation of macrolide resistance genes.** Transformation experiments were performed with *S. pneumoniae* R6 as the recipient. The *mef*(E) gene could be transferred from different species of VGS and from *Gemella* spp., always in association with the *mel* gene. Although 38 *erm*(B)-carrying strains were used as DNA donors, only strain 208 could transfer it to *S. pneumoniae* R6, and this occurred at a frequency of $3.3 \times 10^{-9}$ (Table 3). All the transformants were stable, as erythromycin resistance remained after 10 consecutive platings and after 1 year of storage at −80°C.

## DISCUSSION

The M phenotype was predominant (59.38%) among the erythromycin-resistant VGS and *Gemella* spp. analyzed here, whereas the $iMLS_B$ phenotype was the rarest (9.90%). Other investigators have also shown that the M phenotype is predominant in VGS from the oropharynx (1, 21, 48). However, the $cMLS_B$ phenotype is the one the most commonly encountered among VGS isolated from the bloodstream (2, 8, 43, 58). In this study, only *S. sanguinis* displayed the M phenotype, in agreement with the results of Rodriguez-Avial et al. (42), who found a statistically significant ($P < 0.01$) prevalence of the M phenotype among blood isolates of this bacterial group. PCR amplification of macrolide resistance genes was performed to clarify the mechanisms of resistance. Among our strains, the *erm*(B) gene was always detected in isolates with the $cMLS_B$ or the $iMLS_B$ phenotype, as reported by other investigators (20, 38, 43, 48). However, the *erm*(A) subclass *erm*(TR), *erm*(A), and *erm*(C) genes were not detected. The only description of the *erm*(A) subclass *erm*(TR) gene in VGS was in clinical isolates of *S. anginosus* by Jacobs et al. (22). As far as we know, the *erm*(A) and *erm*(C) genes have never been detected in VGS or *Gemella* spp. The *mef*(A/E) gene was found in all the strains with the M phenotype and 45.76 and 52.63% of strains with the $cMLS_B$ and $iMLS_B$ phenotypes, respectively, with *mef*(E) being the predominant subclass (95.36%). Although the *mef*(A/E) gene has been described in erythromycin-resistant VGS with the three macrolide resistance phenotypes (20, 22, 27, 38, 43, 48), most investigators did not differentiate between the two subclasses of the *mef*(A/E) gene. Only Arpin et al. (2) reported on the prevalence of *mef*(E) in clinical isolates of VGS, and they obtained results similar to ours. It is

TABLE 3. Transfer by transformation of erythromycin resistance determinants from VGS to *S. pneumoniae* R6

| Donor | Donor phenotype | Donor gene(s) | Frequency of transformation | Transformant phenotype | Transformant gene(s) |
|---|---|---|---|---|---|
| *S. mitis* 130 | M | *mef*(E), *mel* | $1.5 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. mitis* 143 | M | *mef*(E), *mel* | $3.0 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. mitis* 147 | M | *mef*(E), *mel* | $7.8 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. mitis* 183 | M | *mef*(E), *mel* | $5.3 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. mitis* 220 | M | *mef*(E), *mel* | $1.1 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. mitis* 208 | $cMLS_B$ | *erm*(B) | $3.3 \times 10^{-9}$ | $cMLS_B$ | *erm*(B) |
| *S. oralis* 47 | M | *mef*(E), *mel* | $6.7 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. oralis* 288 | M | *mef*(E), *mel* | $1.7 \times 10^{-6}$ | M | *mef*(E), *mel* |
| *S. oralis* 296 | M | *mef*(E), *mel* | $2.1 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. salivarius* 137 | $iMLS_B$ | *erm*(B), *mef*(E), *mel* | $1.1 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *S. salivarius* 172 | $cMLS_B$ | *erm*(B), *mef*(E), *mel* | $2.0 \times 10^{-6}$ | M | *mef*(E), *mel* |
| *G. haemolysans* 70 | M | *mef*(E), *mel* | $1.7 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *G. haemolysans* 78 | M | *mef*(E), *mel* | $1.9 \times 10^{-6}$ | M | *mef*(E), *mel* |
| *G. haemolysans* 79 | M | *mef*(E), *mel* | $6.3 \times 10^{-6}$ | M | *mef*(E), *mel* |
| *G. morbillorum* 95 | M | *mef*(E), *mel* | $1.7 \times 10^{-7}$ | M | *mef*(E), *mel* |
| *G. morbillorum* 256 | M | *mef*(E), *mel* | $8.9 \times 10^{-8}$ | M | *mef*(E), *mel* |

important to differentiate between the two subclasses of *mef*(A/E) because of the genetic differences between them (9) and the information that it gives about the possible origins of these genes and gene transfer with other bacteria. Recently, Gay and Stephens (16) and Santagati et al. (46) described two genetic elements, MEGA and Tn*1207.1*, carrying *mef*(E) and *mef*(A), respectively. Both elements possess an ORF (*mel* in MEGA) adjacent to the *mef* gene that shares approximately 35% identity with the *msr*(A) gene from staphylococci. The *mel* gene was amplified by PCR to localize the *mef*(A/E) gene in our strains. The *mel* gene was detected in all but four isolates, suggesting that these strains have elements similar to MEGA or Tn*1207.1*. The absence of this ORF from the four VGS could be due to the lack of *mel* or the complete elements or to the presence of another *mel*-like gene with major nucleotide sequence differences. We are performing experiments to determine which of these hypotheses is true. Only a few studies have looked at the carriage of *mef*(A) and *mef*(E) elements. The first description of such elements was in *S. pneumoniae*, which remains the most studied bacterium (16, 46). However, Luna et al. (28) performed a broad study with gram-positive and gram-negative bacteria and detected the eight ORFs of Tn*1207.1* in seven *Streptococcus* spp. and two *Enterococcus* spp. That group detected only ORF5 to ORF8 in four *Staphylococcus intermedius* isolates.

Conjugative transposons from the Tn*916* family and large composite structures like Tn*5253* and Tn*3872* are found in different species of the genus *Streptococcus* (11, 40, 44). Tn*916* encodes resistance to tetracycline [*tet*(M)] alone, but some of the other elements mentioned carry other resistance determinants, such as the $cat_{pC194}$, *aph*(*3′*)-*III*, and *erm*(B) genes (40). To detect these conjugative transposons, we amplified the $cat_{pC194}$, *aph*(*3′*)-*III*, and *erm*(B) resistance genes and the *intTn* gene, which encodes a protein responsible for transposition of Tn*916*-like elements and composite transposons. Tetracycline was the drug to which resistance was the most frequently encountered among our isolates, and tetracycline resistance had a strong association ($P < 0.0001$) with the *erm*(B) gene. The same was true for the *tet*(M) gene ($P < 0.005$), which was found in 79.49% of the *erm*(B) isolates. Rodriguez-Avial et al. (43) also described this association. They found that 75.80% of VGS isolated from blood carried both genes. Clermont and Horaud (6) detected the *tet*(M) gene in clinical *S. anginosus* isolates that also carried chromosomal elements similar to Tn*916*. Olsvik et al. (35) reported on two tetracycline-resistant *G. morbillorum* strains carrying the *tet*(M) gene. The *tet*(M) gene appears to be widespread in erythromycin-resistant VGS and *Gemella* spp., as is the case in pneumococci (39, 50). The $cat_{pC194}$ gene is believed to be the main gene responsible for chloramphenicol resistance in pneumococci, whereas other classes of *cat* genes are more prevalent in streptococci of groups A, B, and G (55, 57). We detected the $cat_{pC194}$ gene in all chloramphenicol-resistant VGS, but we found no significant association with the *erm*(B) gene, as reported in pneumococci by Seral et al. (50). To our knowledge, this is the first description of the $cat_{pC194}$ gene in *S. mitis*. The *aph*(*3′*)-*III* gene was detected in two high-level kanamycin-resistant isolates of VGS (two *S. oralis* isolates and one *S. mitis* isolate) harboring the *erm*(B) gene. Although some studies have investigated high-level aminoglycoside resistance in VGS (6, 14, 19), the *aph*(*3′*)-*III* gene has been described in only six kanamycin-resistant *S. anginosus* isolates (6).

The association between the *tet*(M), *erm*(B), *aph*(*3′*)-*III*, and/or $cat_{pC194}$ genes with the *intTn* gene suggests the presence of elements similar to the Tn*916* family of conjugative transposons and composite elements like Tn*3872* and Tn*5253*. It also demonstrates that resistance to tetracycline, erythromycin, kanamycin, and chloramphenicol in VGS and *Gemella* spp. could be due to the acquisition of these highly mobile elements. In this sense, filter mating experiments have demonstrated the conjugation of *tet*(M) and *erm*(B) determinants from oral streptococci to *Enterococcus faecalis* JH2-2 and also the transfer of a Tn*916* native conjugative transposon from the same bacteria to *Streptococcus parasanguinis* in an oral biofilm model (6, 41).

The transfer of the *mef*(E) determinant by transformation clearly indicates that gene transfer can occur between VGS or *Gemella* spp. and *S. pneumoniae*, as is the case for genes encoding penicillin-binding proteins (37). In pneumococci, the *mef*(E) gene is part of a genetic insertion element that lacks the

genes necessary for transposition and is inserted at various chromosomal sites (16). As mentioned above, this element could be found in our *mef*(E)-positive isolates; therefore, transformation probably plays an important role in the transfer of this resistance determinant. The fact that *mef*(E) and *mel* were always transferred together suggests that *mel* could be involved in the efflux mechanism of erythromycin resistance, as suggested by Gay and Stephens (16). However, our results suggest that transformation does not play an important role in the transfer of the *erm*(B) gene, because only 1 of the 38 *erm*(B)-positive isolates used as donors could transfer this gene to *S. pneumoniae* R6, and transfer occurred at a very low frequency. Furthermore, both *S. salivarius* isolates that possessed the *erm*(B) and *mef*(E) genes were able to transfer only the latter gene. The *erm*(B) gene is located in conjugative transposons from the Tn*916* family, meaning that conjugation is the major means by which it is transferred (6, 40, 44). However, the main reason why *mef*(E) gene transfer was more efficient than *erm*(B) gene transfer could be differences in the percent identities with the host DNA. The critical step of transformation is the integration of the transforming DNA into the bacterial chromosome. Homologous recombination can occur only if the introduced DNA and the host DNA share a minimum of 70% nucleotide sequence identity. Larger allelic differences can be incorporated if substantial amounts of flanking identity are present on both sides of a heterologous marker (31). The efficiency of transformation varies with the length of identity available for recombination; therefore, it seems probable that the *mef*(E)-*mef*(E) element shares a higher degree of identity with DNA host than the *erm*(B)-*erm*(B) transposon.

The nasopharynx is one of the environments most colonized in humans. While in the nasopharynx, VGS and *Gemellae* spp. come into contact with other bacteria, such as *S. pneumoniae* and *S. pyogenes*. This work demonstrates that VGS and Gemella spp. are important reservoirs of genes conferring resistance to macrolides and related antibiotics. The genetic pattern found in VGS and *Gemella* spp. [i.e., the presence of the *erm*(B) gene; the absence of the *erm*(A) subclass *erm*(TR), *erm*(A), and *erm*(C) determinants; and the prevalence of the *mef*(E) subclass], together with evidence of the presence of Tn*916*-related transposons and the transfer of the *mef*(E) by transformation, suggests that these groups of bacteria can exchange genetic information, especially with *S. pneumoniae*. These results emphasize the need to monitor the epidemiology and genetic basis of antibiotic resistance in VGS and *Gemella* spp. from the normal flora, not only because they are reservoirs of antibiotic resistance genes but also because of the serious invasive diseases that they can cause.

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