Staphylococcus Efflux msr(A) Gene Characterized in Streptococcus, Enterococcus, Corynebacterium, and Pseudomonas Isolates†

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The staphylococcal msr(A) gene, coding for a macrolide efflux protein, was identified in three new gram-positive genera and one gram-negative genus. These msr(A) genes shared 99 to 100% identity with each other and the staphylococcal gene. This study demonstrates that the msr(A) gene has a wider host range than previously reported.

Macrolide resistance is typically due to the acquisition of genes coding for rRNA methylases, macrolide efflux proteins, and/or inactivation enzymes (10, 12, 15) (http://faculty.washington.edu/marilynvr/). The use of macrolide-lincosamide-streptogramin (MLS) antibiotics has increased over the last 20 years and has been correlated with an increase in bacterial resistance to macrolides, due primarily to acquisition of a new gene(s), usually on mobile elements (12, 15). Fifty-four different acquired MLS resistance genes have been identified, with 14 of these genes found in more than two genera and 50 identified in one or two genera. The msr(A) gene codes for an ATP transporter that transports erythromycin and streptogramin B from the cell using energy from ATP hydrolysis and has been identified only in Staphylococcus spp. (9, 12–15).

Recently another gene, msr(D), with similarities to the msr(A) gene, was found downstream of another macrolide efflux gene, mef(A), and both genes have a wide host range encompassing both gram-negative and gram-positive genera (1, 7, 10) (http://faculty.washington.edu/marilynvr/). Therefore, we hypothesized that the msr(A) gene may also have a wider host range. To test this hypothesis, 1,125 Streptococcus spp., 226 Staphylococcus spp., 193 Enterococcus spp., and 100 gram-negative isolates from oral samples, as well as 566 Staphylococcus spp., 160 Enterococcus spp., and 100 gram-negative isolates from urine samples (3, 7, 8), were screened using DNA-DNA hybridization (4, 7). The isolates were randomly selected commensal bacteria collected between 1997 and 2004 from healthy children in Lisbon, Portugal, in a randomized study aimed at assessing the safety of low-level mercury exposure from dental amalgam restorations (3, 7, 8). The isolates were previously identified using biochemical methods (3, 5). We also included 17 macrolide-resistant (Emr) Corynebacterium spp. isolated from skin cultures in 1997 from patients attending an acne clinic at the University of Leeds, United Kingdom (2).

Forty-two Staphylococcus spp., 2 Staphylococcus aureus spp., 5 Enterococcus spp., 10 Streptococcus spp., 7 Pseudomonas spp., and 1 Corynebacterium spp. were positive for the msr(A) gene.

All the positive Enterococcus spp., Streptococcus spp., Corynebacterium spp., Pseudomonas spp., and a selected number of the Staphylococcus spp. were used as templates in an msr(A) PCR assay with S. aureus RN4220 carrying a cloned msr(A) gene as the positive control (Table 1) (13, 14). All of the isolates tested produced PCR products of the correct size which hybridized with an internal probe. Thus, the msr(A)-positive staphylococci represented 5.6% of the isolates, the one msr(A)-positive Corynebacterium spp. represented 5.9% of the small number of Emr Corynebacterium spp., and the msr(A)-positive Enterococcus spp. and Streptococcus spp. represented 1.7% and 0.9% of the isolates, respectively. We also identified 7 (10%) msr(A)-positive Pseudomonas spp., including 2 Pseudomonas aeruginosa spp., out of 69 urine isolates examined. A similar number of oral Pseudomonas spp. from the same population were also screened, but none were positive for the msr(A) gene.

We had previously examined these isolates for other MLS genes (2, 3, 7) and found that 57% of the Staphylococcus spp. carried at least one emr gene, which codes for an rRNA methylase enzyme that confers resistance to macrolides, lincosamides, and streptogramin B (2, 3, 12, 15), and that all of the other msr(A)-positive isolates carried at least one emr gene and/or mef(A) genes. The seven Pseudomonas spp. carried from one to five other MLS genes (Table 2).

To determine whether these genes were closely related to the Staphylococcus msr(A) genes, one msr(A)-positive isolate from each of the four genera, Corynebacterium, Enterococcus, Streptococcus, and Pseudomonas, was selected, and the complete structural msr(A) gene was sequenced using PCR assays and primers indicated in Table 1. The PCR products were confirmed using an internal 32P-labeled probe, cloned into the pCRT/NT-TOPO vector (Invitrogen, Carlsbad, CA), and transformed into Escherichia coli TOP10 following the manufacturer’s instructions. All sequencing was carried out at the University of Washington, Department of Biochemistry Sequencing Facility, as previously described (7, 8). The msr(A) genes from the Corynebacterium sp. (AY591760), Enterococcus sp. (DQ068449), and Streptococcus sp. (DQ131177) were indistinguishable at the DNA and amino acid levels from each other and the Staphylococcus msr(A) gene (GenBank accession no. AB016613). The Pseudomonas msr(A) gene (DQ068450) shared 99% identity at the DNA and amino acid
level with this *Staphylococcus msr(A)* gene and had two amino acid changes at positions 59 and 430 (see Fig. S1 in the supplemental material). The variability found in the *Pseudomonas msr(A)* gene is within the range (98 to 100%) found among different *Staphylococcus msr(A)* genes (http://faculty.washington.edu/marilynr/).

The upstream region of the *msr(A)* genes in staphylococci is thought to be involved with regulation. Therefore, it was of interest to determine whether this upstream region was also present upstream of the *Pseudomonas msr(A)* structural gene. A PCR assay, consisting of the primer *msr*A-F3 located 328 bp upstream of the ribosome binding site and the primer *msr*A-REV located 544 bp downstream from the start codon of the *msr(A)* structural gene, was used to generate the sequences upstream of the start codon of the *Pseudomonas msr(A)* gene, which were indistinguishable from the upstream sequences of the *Staphylococcus msr(A)* gene (see Fig. S2 in the supplemental material). The upstream and complete *msr(A)* gene sequences are at GenBank, accession no. DQ066450.

A second *Pseudomonas* sp. *msr(A)* gene has now been partially sequenced and has the same high level of homology with the *Staphylococcus msr(A)* gene as the first gene sequence. We have also recently verified the presence of an *msr(A)* gene in an *Enterobacter* isolate from this same collection, suggesting that other gram-negative genera may also carry this gene. The *Enterobacter* isolate has an erythromycin MIC of 128 μg/ml using standard CLSI (formerly NCCLS) methods (6) and does not carry any of the other known gram-negative MLS resistance genes (7).

There have been 64 different acquired MLS genes identified in bacteria; however, only 17 (27%) of these genes are found in more than a single genus (http://faculty.washington.edu/marilynr/). As illustrated in this and other studies (3, 7, 10, 11), if MLS genes are screened in new genera, they are often found. Thus, screening for the presence of the *msr(A)* gene should be considered when examining the distributions of MLS genes in either gram-positive or gram-negative isolates.

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