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Letters to the Editor

The Newly Described *msrC* Gene Is Not Equally Distributed among All Isolates of *Enterococcus faecium*

Two recent reports have discussed the role of a newly described enterococcal gene, *msrC* (3, 5). The *msrC* gene possesses 53 to 62% identity with a staphylococcal gene, *msr(A)*, encoding an ABC porter for macrolide and streptogramin B antibiotics (3–5). Portillo et al. (3) found identical *msrC* genes distributed among different macrolide resistance phenotypes of *Enterococcus faecium*, suggesting a role other than involvement in antibiotic resistance. However, disruption of *msrC* by insertion mutagenesis was associated with two- to eightfold decreases in MICs of 14-, 15-, and 16-membered macrolides and streptogramins B (5). The *msrC* gene was detected in all of the investigated 256 isolates of *E. faecium* in both studies (3, 5). In none of the other seven investigated enterococcal species was the corresponding gene found, suggesting *msrC* to be an intrinsic property of *E. faecium*. With the exception of two isolates from animals all other tested *E. faecium* isolates possessing *msrC* were from humans.

As reported previously, we have already examined a collection of streptogramin-resistant *E. faecium* isolates of the *vat*(E) type for the presence of *msrC* by PCR and found it in only 45 of 77 isolates (6). However, the primers were based on the gene sequence for *msrC* reported by Portillo et al. (3), which is only 95% identical to the recently reported sequence of *msrC* for the two U.S. isolates (5). New primers corresponding to sites of 100% identity between all three identified *msrC* alleles were constructed (primer msrC3, 5′ AAGGAATCCTTCTCT CTCCG; primer msrC4, 5′ GTAAACAAAATCGTTCCCG; product, 343 bp). With these primers we screened seven isolates of *E. hirae*, *E. durans*, and *E. faecalis* as negative controls and a collection of 139 unrelated *E. faecium* isolates of sewage, animal, food, and human origins including 10 macrolide-susceptible *E. faecium* isolates for which erythromycin MICs were ≤4 mg/liter (including *E. faecium* ATCC19434). A total of 121 *E. faecium* isolates, including all 10 erythromycin-susceptible isolates, gave a product for *msrC*. The nucleotide composition of the PCR product from six isolates of different origins was, despite two nucleotide changes, identical to the allele of *msrC* described by Portillo et al. (3).

Eighteen *E. faecium* isolates (as well as the seven negative controls) were negative for *msrC*. The species *E. faecium* was confirmed for the 18 isolates by a PCR specific for an unknown fragment of the *E. faecium* chromosome (1) and for the *E. faecium* specific ligase gene *ddl* (2). Dot blot hybridizations of genomic DNA from the *msrC* PCR-negative isolates with a labelled *msrC* gene probe were negative for all but three *E. faecium* isolates. The 15 *E. faecium* isolates negative for *msrC* in PCR and hybridization experiments consisted of 10 of 32 from poultry, 0 of 16 from pigs, 1 of 21 from sewage, and 4 of 70 from humans.

The results of our study show that the *msrC* gene is not an intrinsic property of all *E. faecium* isolates. The data also suggest that antibiotic use in animals has not been a selective force for *msrC*.

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Authors' Reply (reference 5)

The letter from Werner and colleagues reports the interesting finding that a gene, *msrC*, previously identified in 100% of over 250 isolates of *Enterococcus faecium*, was not found in 15 (4 of which were from humans) of 139 isolates of *E. faecium* currently studied. Overall, in the three studies combined, *msrC* was documented by PCR or hybridization in 98.7% of approximately 320 human isolates. While one could question whether the *msrC* gene probe covered the entire gene or if it might have missed evidence suggesting a partial deletion of the gene, it nonetheless appears that a small percentage of *E. faecium* isolates are not able to make MsrC. So, while *msrC* still appears to be specific for identifying *E. faecium*, it is not sufficiently sensitive for identifying all isolates of this species, which could well be a limitation with any single-gene species identification method, particularly for a nonessential gene, such as this one.

Whether *msrC* represents a gene that has been acquired horizontally by *E. faecium* at some time in the past, or whether it represents an endogenous gene that has been lost by a small percentage of isolates during the evolution of the species, remains to be seen. If the former is true, the presence of the gene in >95% isolates suggests either that it was acquired very early

in the species' history or that it offers some substantial advantage. Since the gene is not essential for survival in vitro, as shown in our study (nor did disruption result in a difference in growth curves), it would seem that whatever function *msrC* normally provides to the cell is ancillary to cell survival and/or can be assumed by another cell component. The presence of multiple potential ABC transporters in the genome of *Enterococcus faecalis* (1), and of at least some in our preliminary sequence of *E. faecium* (www.hgsc.bcm.tmc.edu/microbial/efaecium), suggests that there may be many other candidates for products that may provide a similar function. Sequencing in the regions surrounding *msrC* may reveal the presence of elements suggesting horizontal movement of this gene between strains or, alternatively, may find remnants of *msrC*, suggesting deletion of what once was a gene endogenous to this species. Such studies, and similar ones with other genes, should help shed light on how enterococcal species have evolved.

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Ed. Note: *The authors of the other published article, Portillo et al. (reference 3), declined to respond.*