

Prevalence of the *erm*(T) Gene in Clinical Isolates of Erythromycin-Resistant Group D *Streptococcus* and *Enterococcus*[∇]

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Received 15 October 2007/Returned for modification 9 January 2008/Accepted 10 February 2008

Among 48 erythromycin-resistant group D streptococci (GDS), 36 had the *erm*(T) resistance gene. *erm*(T) was also found in 4 of 31 erythromycin-resistant *Enterococcus faecium* isolates. This is the first report of the *erm*(T) gene in U.S. GDS isolates and the first report of the *erm*(T) gene in enterococci.

Group D streptococci (GDS) include members of the *Streptococcus bovis* group. Proposed nomenclature changes for the *S. bovis* group would establish several new species, including *Streptococcus gallolyticus* subsp. *gallolyticus*, *S. gallolyticus* subsp. *pasteurianus*, *Streptococcus infantarius* subsp. *infantarius*, and *Streptococcus lutetiensis* (7, 19, 21). GDS are commensal organisms of the human gut and have been found in 14% of fecal samples from normal controls (6). GDS bacteremia is often associated with underlying gastrointestinal diseases, such as colon cancer (9, 15). *Enterococcus faecalis* and *Enterococcus faecium* are found in the feces of most adults but can cause infections such as bacteremia and endocarditis (14). GDS, *E. faecalis*, and *E. faecium* can have erythromycin (ERY) resistance rates as high as 75% (11, 20, 23).

ERY ribosomal methylases (coded for by *erm* genes) methylate the bacterial ribosome, impairing the binding of macrolide, lincosamide, and streptogramin B antibiotics and resulting in resistance (MLS_B phenotype) (10). Another common ERY resistance mechanism is macrolide efflux, coded for by *mef* genes, which are common in streptococci (17). *mef* genes cause resistance to 14- and 15-membered macrolides (such as ERY) but not 16-membered macrolides, lincosamides, and streptogramin B (M phenotype).

Recently, we reported high levels of ERY (54%) and clindamycin (CLI) (33%) resistance in clinical group B *Streptococcus* (GBS) isolates (4). Sixty-one of 66 (92%) MLS_B GBS isolates were shown to contain *erm*(A) or *erm*(B), but 5 MLS_B *erm*(A)- and *erm*(B)-negative GBS isolates and 1 *erm*(B)-positive isolate were found to contain the *erm*(T) gene (5). Since the *erm*(T) gene has been found in MLS_B *S. bovis* isolates in Asia (12, 23), we investigated the prevalence of the *erm*(T) gene in clinical isolates of GDS. In addition, since *E. faecalis* and *E. faecium* can be highly resistant to ERY, we also studied the prevalence of *erm*(T) in these organisms.

(This work was presented in part at the 107th General Meeting of the American Society for Microbiology, Toronto, Canada, 21 to 25 May 2007.)

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[∇] Published ahead of print on 19 February 2008.

One hundred twenty-seven clinical GDS isolates were evaluated. Thirty-three GDS isolates were collected from north-east Ohio and 12 from Chicago. Twenty-nine additional ERY-resistant GDS isolates were obtained from the U.S. SENTRY program from a collection of 82 GDS isolates. GDS were originally isolated from blood, urine, skin, and soft tissue samples. Isolates were identified by the laboratories of origin as members of the *S. bovis* group or simply GDS. Forty-one *E. faecalis* and 35 *E. faecium* blood isolates collected at Summa Health System were also studied. Identifications were performed using standard laboratory methods.

Disk diffusion testing for ERY and CLI resistance in GDS from northeast Ohio and Chicago and 29 ERY-resistant SENTRY isolates was performed and interpreted according to the CLSI (3). ERY and CLI disks were placed 15 mm apart edge to edge (D test) to detect inducible CLI resistance in GDS (26). Additionally, *E. faecalis* and *E. faecium* isolates were tested for susceptibility to ERY by disk diffusion. MIC testing of selected isolates was done by Etest according to the manufacturer's guidelines (AB Biodisk, Solna, Sweden).

Isolates were tested for macrolide resistance genes *erm*(A), *erm*(B), *erm*(C), *erm*(T), *mef*(A), and *mef*(E) by PCR as previously described (4, 5). *erm*(T) PCR products from seven *erm*(T)-positive GDS isolates and four *erm*(T)-positive *E. faecium* isolates were DNA sequenced by Sequetech (Mountain View, CA) as previously described (5). DNA database searches and DNA sequence identity analysis used Basic Local Alignment Tool (BLASTn) (1).

Forty-four of 127 (35%) GDS isolates had the MLS_B phenotype, while 4 had the M phenotype (Table 1). Twenty-seven *E. faecalis* and 31 *E. faecium* isolates were ERY resistant (Table 2).

erm(T) was the predominant macrolide resistance gene found among the ERY-resistant GDS (Table 1). Of the 44 MLS_B GDS, 36 (82%) contained *erm*(T), while 8 (18%) contained *erm*(B). Thirty-three of the 36 (92%) *erm*(T)-positive isolates were inducibly CLI resistant (iMLS_B phenotype), while 3 (8%) were constitutively CLI resistant (cMLS_B phenotype), one of which also contained *erm*(B). Two GDS isolates with the M phenotype contained the efflux gene *mef*(A), and two contained *mef*(E) (Table 1). The *erm*(A) and *erm*(C) genes were not found in this collection of GDS.

Twenty-four ERY/CLI-susceptible GDS isolates were tested

TABLE 1. Distribution of ERY resistance phenotypes and genes in 48 ERY-resistant clinical GDS isolates

Resistance phenotype	No. of isolates	No. (%) of isolates with ERY resistance gene(s)					Negative
		<i>erm</i> (B)	<i>erm</i> (T)	<i>erm</i> (B) and <i>erm</i> (T)	<i>mef</i> (A)	<i>mef</i> (E)	
iMLS _B	33		33 (69)				
cMLS _B	11	7 (15)	2 (4)	1 (2)			1 (2)
M	4				2 (4)	2 (4)	
Total	48	7 (15)	35 (73)	1 (2)	2 (4)	2 (4)	1 (2)

for the presence of *erm*(A), *erm*(B), *erm*(C), *erm*(T), *mef*(A), and *mef*(E). One ERY/CLI-susceptible isolate (ERY MIC, 0.064 µg/ml; CLI MIC, 0.125 µg/ml) was found to contain the *erm*(T) gene; all others were negative for the genes tested.

erm(T) was found along with *erm*(B) in only four ERY-resistant *E. faecium* isolates (Table 2). The ERY resistance genes *erm*(A), *erm*(C), *mef*(A), and *mef*(E) were not found in *E. faecalis* or *E. faecium* isolates. Although *erm*(B) has often been found in ERY-resistant enterococci, efflux genes *mef*(A/E) and *msr*(C) have also been reported (13, 16). Our enterococcal isolates were negative for *mef*(A/E), and *msr*(C) was not examined in this study. There may be other not-yet-characterized macrolide resistance mechanisms in enterococci, as recently suggested (8).

The *erm*(T) gene PCR product was sequenced from seven *erm*(T)-positive GDS isolates. Six GDS isolates had the MLS_B phenotype (ERY MIC, ≥256 µg/ml), and the seventh was the ERY/CLI-susceptible but *erm*(T)-positive isolate. In addition, the four *erm*(T) gene PCR products from *E. faecium* were sequenced. All 11 *erm*(T) PCR products had 99 to 100% sequence identity with the previously published *erm*(T) sequence from a clinical *Streptococcus gallolyticus* subsp. *pasteurianus* (*Streptococcus bovis*) isolate (GenBank database accession number AY894138; <http://www.ncbi.nlm.nih.gov/Genbank/index.html>) (23, 24). The single ERY/CLI-susceptible GDS isolate which tested positive for *erm*(T) had 100% DNA sequence identity with the published *erm*(T) gene sequence mentioned above. ERY-susceptible *erm*-positive isolates are uncommon. There may be a mutation in the portion of the gene not sequenced in our study or in the promoter region of the gene. This is presently under study in our laboratory.

Studies in Europe found the *erm*(B) gene in ERY-resistant clinical isolates of *S. bovis* (11, 18). However, in two Asian studies, the *erm*(T) gene was found in 46% and 37% of ERY-resistant clinical *S. bovis* isolates, respectively (12, 23); the remaining isolates contained *erm*(B). In the present study of 44 MLS_B U.S. GDS isolates, 36 (82%) contained the *erm*(T) gene and 8 (18%) contained *erm*(B).

The *erm*(T) gene (originally called *ermGT*) was first found in a poultry isolate of *Lactobacillus reuteri* in 1994. The *erm*(T) gene was shown to have 81% nucleotide sequence identity with the *erm*(C) gene (22). In 2001, an *erm*(T) gene discovered in a swine feces isolate, *Lactobacillus* strain 121B, showed 99% DNA sequence identity with the *erm*(T) gene found in *Lactobacillus reuteri* (25). Also in 2001, an *erm*(T) gene was found in MLS_B clinical *S. bovis* isolates in Taiwan (23). This gene demonstrated 98.5% nucleotide sequence identity with the *erm*(T)

TABLE 2. Distribution of ERY resistance phenotypes and genes among 41 clinical *Enterococcus faecalis* and 35 clinical *Enterococcus faecium* isolates

Species (no. of isolates)	ERY resistance phenotype	No. of isolates	No. (%) of isolates with ERY resistance gene(s)		
			<i>erm</i> (B)	<i>erm</i> (B) and <i>erm</i> (T)	Negative
<i>Enterococcus faecalis</i> (41)	Resistant	27	24 (59)		3 (7)
	Intermediate	13			13 (32)
	Susceptible	1			1 (2)
<i>Enterococcus faecium</i> (35)	Resistant	31	25 (71)	4 (11)	2 (6)
	Susceptible	4			4 (11)

gene found in *L. reuteri* (22, 23). Recent studies involving *erm* genes in livestock manure and manure management systems found *erm*(T) to be the second most abundant *erm* gene found in swine manure (2).

Given the high prevalence of *erm*(T) in MLS_B GDS in the United States, this organism may represent a major reservoir for the transfer of *erm*(T) to other human enteric commensals and pathogens such as GBS and enterococci. To our knowledge, this is the first report of the *erm*(T) gene in U.S. GDS and the first report of *erm*(T) in enterococci.

This work is supported by a research grant from Merck & Co., Inc., and by the Summa Health System Foundation.

We thank Gerri Hall, Tom Thomson, and JMI Laboratories for the contribution of GDS isolates.

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