ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2008, p. 2994–2995 Vol. 52, No. 8
0066-4804/08/$08.00+0 doi:10.1128/AAC.00230-08

Detection of the Erythromycin rRNA Methylase Gene *erm*(A) in *Enterococcus faecalis*▽

Erythromycin, a macrolide antibiotic, is used for the treatment of diseases caused by gram-positive bacteria (7). Resistance to macrolides, lincosamides, and streptogramin B in many isolates is due to so-called *erm* genes, encoding RNA methylases (6). Several classes of rRNA methylases in gram-positive cocci, in *Bacillus* spp., and in erythromycin-producing actinomycetes have been identified previously (1, 10). However, the detection of *erm*(A) in *Enterococcus faecalis* has not been reported to date.

In this study, a total of 296 *E. faecalis* strains isolated from animals ($n = 147$) and humans ($n = 149$) were screened for the presence of the *erm*(A) gene by PCR.

The 147 *E. faecalis* strains of animal origin were originally isolated from pig manure and identified in a previous study (3). The 149 human isolates were provided from hospitals of maximal and regional care, as well as from resident microbiological laboratories in Bavaria, Germany. Species identifications were verified by API20Strep (bioMérieux). All strains proved to be resistant to tetracycline (MIC > 4 μg/ml).

All isolates were tested by the broth microdilution method as prescribed by DIN 58940-81:2002-10 from the Deutsches Institut für Normung (2) and as described elsewhere (4), except that 100 μl of the dilution was added to 12 ml of Mueller-Hinton bouillon (Table 1). *E. faecalis* DNA was extracted using the QIAquick PCR purification kit (Qiagen). Primers and PCR conditions were chosen according to the guidelines of Khan et al. (6). The 609-bp products were stored at 4°C until their analysis on agarose gel. Each PCR cycle included one positive and one negative control. Reaction products were visualized in UV light after electrophoresis through a 1.5% agarose gel (Bio-Rad) containing 0.25 μl of ethidium bromide (Sigma). To verify the identities of bands of the expected sizes, the presumably positive PCR products were sent for sequencing (Sequiserve). Sequence similarity was evaluated using the NCBI BLAST database (http://www.ncbi.nlm.nih.gov/). Two isolates of animal origin and two isolates of human origin showed bands of the expected molecular sizes upon gel electrophoresis. The MICs of erythromycin for each of the two isolates from animals were >8 μg/ml, whereas the MICs for the strains of human origin were 1 and 4 μg/ml, respectively. The nucleotide sequences of the amplicons were 100% identical to a section of the *Staphylococcus aureus* transposon Tn*554* (X03216), which harbors the *erm*(A) gene, as stated by Khan et al. (6). These findings indicate a horizontal gene transfer of this mobile DNA element to *E. faecalis*—however, to prove this thesis, further molecular biological investigations are required.

TABLE 1. Phenotypic resistance properties of examined *E. faecalis* isolates

Origin (no.) of isolates	No. of isolates for which MIC of erythromycin[a] (and susceptibility status) was:		
	≤1 μg/ml (susceptible)	>1 and <4 μg/ml (intermediately resistant)	>4 μg/ml (resistant)
Pig (147)	10	24	113
Human (149)	4	130	15

[a] Examined concentration range, 0.0625 to 8 μg/ml.

Jensen et al. (5) found *erm*(A) in 16% of 68 staphylococci but not in the strains of *Enterococcus* spp. examined ($n = 113$). The only published evidence of *erm*(A) in enterococci was reported by Portillo et al. (9), who examined 87 isolates. However, in that study *erm*(A) was detected in only one *E. faecium* strain.

To our knowledge, the present study is the first report demonstrating the presence of the *erm*(A) gene in *E. faecalis*. Since enterococci are third among the bacterial pathogens most frequently associated with nosocomial infections, after staphylococci and *Escherichia coli* (8), consequent limited treatment options are a cause for concern.

This work was supported by the Bavarian State Ministry of the Environment, Public Health and Consumer Protection.

We are grateful to R. R. Reinert and assistants (Nationales Referenzzentrum für Streptokokken, Universitätsklinikum Aachen, Institut für Medizinische Mikrobiologie) for kindly providing the reference strain *Streptococcus pyogenes* RRR 335.

REFERENCES

1. **Arthur, M., A. Brisson-Noël, and P. Courvalin.** 1987. Origin and evolution of genes specifying resistance to macrolide, lincosamide and streptogramin antibiotics: data and hypotheses. J. Antimicrob. Chemother. **20:**783–802.
2. **Deutsches Institut für Normung.** 2002. Empfindlichkeitsprüfung von mikrobiellen Krankheitserregern gegen Chemotherapeutika, Teil 81. Mikrodilution: spezielle Anforderungen an die Testung von nicht anspruchsvollen Bakterien. DIN 58940-81:2002-10. Beuth-Verlag, Berlin, Germany.
3. **Hoelzel, C.** 2006. Antibiotikaresistente Bakterien und Resistenzgene in Schweinegülle. Dissertation in veterinary medicine. Ludwig-Maximilians-Universität München, Munich, Germany.
4. **Hölzel, C. S., and J. Bauer.** 2008. *Salmonella* spp. in Bavarian liquid pig manure: occurrence and relevance for the distribution of antibiotic resistance. Zoonoses Public Health **55:**133–138.
5. **Jensen, L. B., N. Frimodt-Møller, and F. M. Aarestrup.** 1999. Presence of *erm* gene classes in gram-positive bacteria of animal and human origin in Denmark. FEMS Microbiol. Lett. **170:**151–158.
6. **Khan, S. A., M. S. Nawaz, A. A. Khan, and C. E. Cerniglia.** 1999. Simultaneous detection of erythromycin-resistant methylase genes *ermA* and *ermC* from *Staphylococcus* spp. by multiplex-PCR. Mol. Cell. Probes **13:**381–387.
7. **Leclercq, R., and P. Courvalin.** 1991. Intrinsic and unusual resistance to macrolide, lincosamide, and streptogramin antibiotics in bacteria. Antimicrob. Agents Chemother. **35:**1273–1276.
8. **Peters, J., K. Mac, H. Wichmann-Schauer, G. Klein, and L. Ellerbroek.** 2003. Species distribution and antibiotic resistance patterns of enterococci isolated from food of animal origin in Germany. Int. J. Food Microbiol. **88:**311–314.
9. **Portillo, A., F. Ruiz-Larrea, M. Zarazaga, A. Alonso, J. L. Martinez, and C. Torres.** 2000. Macrolide resistance genes in *Enterococcus* spp. Antimicrob. Agents Chemother. **44:**967–971.

10. **Roberts, M. C., J. Sutcliffe, P. Courvalin, L. B. Jensen, J. Rood, and H. Seppala.** 1999. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. Antimicrob. Agents Chemother. **43:**2823–2830.

Karin Schwaiger*
Johann Bauer
Technische Universität München
Weihenstephaner Berg 3
85354 Freising-Weihenstephan, Germany

*Phone: 49 81 61/71-33 14
Fax: 49 81 61/71-45 16
E-mail: Karin.Schwaiger@wzw.tum.de

▽ Published ahead of print on 27 May 2008.