Dissemination of Clonally Unrelated Erythromycin- and Glycopeptide-Resistant *Enterococcus faecium* Isolates in a Tertiary Greek Hospital

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Between September 1999 to February 2001, 25 glycopeptide-resistant *Enterococcus faecium* (GRE) isolates were recovered from a Greek hospital. The isolates exhibited 13 distinct chromosomal macrorestriction types by pulsed-field gel electrophoresis, and all were erythromycin and vancomycin resistant, carrying the genes *vanA* and *ermB*. Vancomycin resistance, always linked with erythromycin resistance, was transferable from 17 isolates. The dissemination of erythromycin-resistant GRE strains may, at least in part, reflect the extensive use of macrolides in husbandry in Greece.

Glycopeptide-resistant *Enterococcus faecium* (GRE) was first detected in Europe in 1986 (9). Since that time, its prevalence has dramatically increased in several countries, especially in the United States (11). European studies regarding the origin of GRE found such isolates circulating in the community, potentially due to the use of glycopeptide antibiotics as growth promoters in animals (3). It has also been suggested that the use of macrolides for treatment or growth promotion of farm animals may coselect for erythromycin- and vancomycin-resistant enterococci (1). In the United States, GRE species appear to be concentrated in health care settings, rather than the community, and are thought to spread primarily by cross-contamination. However, the possibility of dissemination through animal-based food products cannot be excluded (4, 7). GRE isolates are only sporadically recovered in our region (5, 14). We report the characteristics of an outbreak due to erythromycin- and glycopeptide-resistant *Enterococcus faecium* (EGRE) isolates from a tertiary-care hospital in Greece.

The study included all GRE nonrepetitive isolates that were recovered in the Clinical Microbiology Laboratory of Sismanoglion General Hospital, Athens, Greece, from September 1999 (the month when GRE were first identified) through February 2001. A total of 22 GRE isolates were recovered from clinical samples, and another 3 isolates were recovered from fecal surveillance cultures of specimens from separate patients. Seventeen isolates were derived from the intensive care unit (ICU) of the hospital, six were derived from the urology department, one was derived from a medical department, and one was derived from a pneumology department (Table 1). All patients were hospitalized for more than 5 days and treated with multiple antimicrobials, such as broad-spectrum cephalo-

sporins, imipenem, vancomycin, or clindamycin, prior to the isolation of these organisms.

The identification of isolates to the species level was performed with the automated Vitek system (bioMérieux, Hazelwood, Mo.) as specified by the manufacturer. The same system was also used to determine susceptibility to a range of antimicrobials (ampicillin, gentamicin, streptomycin, nitrofurantoin, ciprofloxacin, tetracycline, and vancomycin). Susceptibility status was defined according to the National Committee for Clinical Laboratory Standards guidelines (13). When GRE isolates were recovered, the MICs of vancomycin and teicoplanin, as well as erythromycin, rifampin, and tetracycline, were also determined by an agar dilution method (13).

A multiplex PCR assay for the detection of *vanA* and *vanB* genes was performed using primers and conditions described previously (18). A PCR assay was also used for amplification of the *ermB* gene (10). Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA was performed with a contour-clamped homogeneous electric field apparatus (CHEF DRII; Bio-Rad Laboratories, Hemel Hempstead, England) (12), and banding patterns of the strains were compared visually by following the criteria of Tenover et al. (16).

The GRE isolates were used as donors in filter mating experiments (8) with *E. faecium* GE-1 (Fus^r and Rif^r), as the recipient strain. Transconjugant colonies were selected on brain heart infusion agar plates containing rifampin (100 µg/ml), fusidic acid (25 µg/ml), and vancomycin (10 µg/ml). Plasmid DNA was extracted by an alkali lysis procedure (19) and separated by electrophoresis in 0.8% (wt/vol) agarose gels.

The antibiotic resistance phenotypes of the 25 GRE isolates are shown in Table 1. All isolates exhibited resistance to penicillin, ampicillin, erythromycin, ciprofloxacin, vancomycin, and teicoplanin. In addition, some organisms exhibited high-level resistance to gentamicin or streptomycin (9 and 19 isolates, respectively). The MICs of vancomycin for the isolates ranged from 128 to >256 µg/ml, and those of teicoplanin ranged from

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TABLE 1. Department, date, and clinical source of isolation, resistance phenotype, and PFGE profile of 25 glycopeptide-resistant *E. faecium* isolates

Isolate no.	Department collected	Date of isolation (mo/yr)	Source of isolate	Resistance pattern of clinical isolates ^a	Resistance transferred	PFGE type
14	ICU	09/99	Urine	Am, Em, Cp, Sm, Rf, Va, Tc	Em, Sm, Va, Tc	I
16	Urology	10/99	Urine	Am, Em, Cp, Sm, Rf, Va, Tc, Nf		I
19	Urology	10/99	Pus	Am, Em, Cp, Gm, Sm, Rf, Va, Tc	Em, Gm, Sm, Va, Tc	II
20	Urology	10/99	Feces	Am, Em, Te, Cp, Cm, Sm, Rf, Va, Tc, Nf	Em, Sm, Va, Tc	III
26	ICU	11/99	Wound	Am, Em, Cp, Gm, Sm, Rf, Va, Tc, Nf		IV
27	ICU	11/99	Urine	Am, Em, Cp, Gm, Sm, Rf, Va, Tc, Nf	Em, Gm, Sm, Va, Tc	II
28	ICU	11/99	Intravenous catheter	Am, Em, Cp, Gm, Sm, Rf, Va, Tc, Nf	Em, Gm, Sm, Va, Tc	II
30	ICU	11/99	Urine	Am, Em, Cp, Sm, Rf, Va, Tc, Nf	Em, Va, Tc	I
33	Urology	11/99	Feces	Am, Em, Cp, Gm, Sm, Rf, Va, Tc		II
38	ICU	12/99	Blood	Am, Em, Te, Cp, Gm, Sm, Rf, Va, Tc, Nf		II
54	ICU	03/00	Urine	Am, Em, Te, Cp, Cm, Sm, Rf, Va, Tc	Em, Te, Cm, Sm, Va, Tc	V
56	Urology	03/00	Wound	Am, Em, Cp, Sm, Rf, Va, Tc	Em, Va, Tc	VI
57	ICU	03/00	Urine	Am, Em, Cp, Cm, Sm, Rf, Va, Tc		VII
60	ICU	03/00	Bronchial specimen	Am, Em, Cp, Sm, Rf, Va, Tc	Em, Sm, Va, Tc	VII
62	ICU	04/00	Bronchial specimen	Am, Em, Te, Cp, Gm, Va, Tc	Em, Gm, Va, Tc	VIII
69	D-Medicine	05/00	Intravenous catheter	Am, Em, Cp, Gm, Va, Tc	Em, Gm, Va, Tc	II
74	ICU	05/00	Feces	Am, Em, Cp, Va, Tc	Em, Va, Tc	IX
77	ICU	05/00	Urine	Am, Em, Cm, Cp, Rf, Va, Tc	Em, Va, Tc	IX
81	ICU	06/00	Blood	Am, Em, Te, Cp, Cm, Rf, Va, Tc, Nf	Em, Va, Tc	X
103	ICU	10/00	Wound	Am, Em, Cm, Cp, Rf, Sm, Va, Tc		III
112	Pneumology	12/00	Urine	Am, Em, Cp, Gm, Sm, Rf, Va, Tc		XI
124	ICU	02/01	Urine	Am, Em, Te, Cp, Cm, Sm, Va, Tc	Em, Va, Tc	XII
126	ICU	02/01	Blood	Am, Em, Cp, Sm, Va, Tc	Em, Va, Tc	XIII
131	ICU	02/01	Urine	Am, Em, Te, Cp, Sm, Va, Tc	Em, Sm, Va, Tc	XII
133	Urology	02/01	Urine	Am, Em, Cm, Cp, Rf, Va, Tc		IX

^a Am, ampicillin; Em, erythromycin; Te, tetracycline; Cp, ciprofloxacin; Cm, chloramphenicol; Gm, high-level gentamicin resistance; Sm, high-level streptomycin resistance; Rf, rifampin; Va, vancomycin; Tc, teicoplanin; Nf, nitrofurantoin.

32 to 256 µg/ml. PCR analysis revealed that all the isolates were positive for the *vanA* and *ermB* genes and negative for the *vanB* gene. The glycopeptide resistance phenotype was transferable from 17 isolates (Table 1). Transfer frequencies varied between 4.7×10^{-4} and 3.1×10^{-8} transconjugants per donor cell. With all 17 donor isolates, transfer of resistance to erythromycin was linked to glycopeptide resistance. In addition, high-level resistance to gentamicin or streptomycin was co-transferred with glycopeptide resistance in five and eight transconjugants, respectively. One transconjugant also exhibited resistance to chloramphenicol and tetracycline. Plasmid analysis of the transconjugants revealed (i) for 10 isolates, a single plasmid, ranging in size from 70 to 110 kb, (ii) in three isolates, two or three plasmids (data not shown), and (iii) in four isolates, no plasmids, suggesting either integration into the recipient chromosome or the transfer of plasmids too large to be visualized by conventional agarose gel electrophoresis.

The clinical isolates were classified into 13 clonal types on the basis of their macrorestriction profiles (PFGE types I to XIII) (Table 1). Approximately 25% of the isolates (6 of 25) exhibited a common profile (type II), while the remaining patterns were more sporadic (one to three isolates each) (Fig. 1).

It has been shown previously that food animals can serve as a reservoir for GRE and that transfer of GRE isolates between animals and humans does take place (15, 17). In Western Europe, the extensive use (more than 20 years) of the glycopeptide avoparcin has been related to the dissemination of clinical GRE isolates, resulting in the ban of its use in 1997. In Greece, avoparcin has never been extensively used as a growth promoter for animals, and this may have contributed to the relatively delayed emergence of glycopeptide resistance, com-

pared with the early dissemination of GRE isolates in other European countries. It has also been suggested that the linkage of erythromycin and vancomycin resistance genes in enterococci of farm animals could play a major role in the coselection or persistence of glycopeptide resistance when macrolides are used for treatment or growth promotion (1, 2). In Denmark, the proportion of GRE isolates among isolates from pigs remained constant after the ban of avoparcin and did not decrease until the decrease in the use of tylosin (2). While most of the GRE isolates in this study were genetically unrelated, all exhibited erythromycin resistance, which was always linked to glycopeptide resistance in conjugal transfer experiments. The macrolide tylosin is heavily used as a growth promoter in food animals in Greece, and this may have exerted a selective pressure toward the dissemination of EGRE strains or glycopeptide resistance determinants. Plasmid analysis suggests that the simultaneous integration of the *vanA* and *ermB* gene clusters into different conjugative plasmids and strains might have contributed to the increased isolation of EGRE isolates in our hospital. Nevertheless, the reasons for the spread of GRE isolates in the hospital environment may be more complex.

It has been suggested that if GRE isolates are not controlled soon after introduction into a hospital, the first sporadic cases may evolve into a monoclonal outbreak and then to polyclonal endemicity, which can be especially difficult to control (7). It should be noted that the percentage of *Staphylococcus aureus* strains in Greek hospitals that are methicillin resistant is among the highest in Europe, reaching 50%. Thus, the extensive use of vancomycin for treating methicillin-resistant *S. aureus* infections probably also selects for GRE isolates. The results of this study suggest that if effective infection control

FIG 1. PFGE patterns of *Sma*I-restricted genomic DNA of representative GRE isolates tested in this study. The origin and the PFGE types of the isolates are shown in Table 1. M, molecular mass markers.

approaches are not stringently applied at this early stage and the antibiotic consumption in husbandry is not restricted, the apparently low prevalence of GRE isolates in Greece might dramatically increase. Future studies to determine if similar GRE clones or *vanA* determinants exist among food animals, fecal samples from the community, and hospital isolates will be important in continuing to monitor this situation.

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