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The Prevalence of Fecal Colonization of Enterococci, the Resistance of the Isolates to Ampicillin, Vancomycin, and High-Level Aminoglycosides, and the Clonal Relationship Among Isolates

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ABSTRACT

The gastrointestinal tract carriage of enterococci was searched in 150 hospitalized patients and 100 outpatients, and clonal relatedness of the isolates and their resistance to ampicillin, vancomycin, and high-level streptomycin and gentamicin were investigated. A stool sample or rectal swab collected from each patient was inoculated into appropriate media within an hour. *Enterococcus* species were identified by using conventional biochemical tests, API-20 Strep assay, and BBL crystal kit. Antibiotic susceptibility tests were performed using Kirby-Bauer disk diffusion method. A polymerase chain reaction (PCR) was used to detect *vanA* and *vanB* genes. Pulsed-field gel electrophoresis (PFGE) and arbitrarily primed-polymerase chain reaction (AP-PCR) methods were used for molecular typing of the strains. Enterococci were isolated from 90 (60%) of the specimens collected from 150 inpatients. Of these 90 isolates, 37 (41%) had high-level gentamicin resistance, 36 (40%) had high-level streptomycin resistance, and 50 (55.6%) had ampicillin resistance. Fecal colonization was found in 30% of the outpatients. Resistances to ampicillin, high-level streptomycin, and gentamicin were 13%, 10%, and 3%, in these patients' isolates, respectively. No vancomycin-resistant enterococci were detected by both agar diffusion and PCR assays in our study. Both typing procedures were applied on 78 *Enterococcus* strains isolated from inpatients. AP-PCR typing showed that 30 (50.8%) of the 59 *E. faecium* and 5 (50%) of the 10 *E. faecalis* strains were clonally related. These values were found to be 12 (20.3%) and two (20%) by PFGE, respectively. The typing procedures did not find any clustered strains in the six *E. durans* and three *E. avium* isolates. Neither PFGE nor AP-PCR result was significantly different among the sensitive and resistant strains. Our results indicate that the high prevalence of colonization with ampicillin and high-level aminoglycoside-resistant enterococci is an important problem in our medical center. The high clonal diversity among the isolates indicates limited spread of antibiotic-resistant strains between patients.

INTRODUCTION

ENTEROCOCCI, which are normal residents of the human gastrointestinal tract, cause urinary tract infections, bacteremia, endocarditis, intraabdominal and pelvic infections, wound and soft tissue infections, and neonatal sepsis.[16] The source of the enterococcal infections is usually endogenous.[14]

These bacteria are resistant to many antibiotics used for the treatment of infections caused by gram-positive microorganisms.[16] They are intrinsically resistant to a majority of antimicrobial agents, while some express acquired resistance through plasmids, transposons, and chromosomal modification.[20] Aminoglycosides are critical components of the combination therapy of enterococcal infections with penicillin, ampicillin, or one of the glycopeptides.[4,22] Aminoglycoside resistance occurs mainly through different aminoglycoside modifying enzymes, and gentamicin resistance is a good predictor of resistance to other aminoglycosides except streptomycin. The major mechanism in developing resistance to beta lactam antibiotics is the production of low-affinity penicillin-binding proteins.[3]

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Vancomycin, linezolid, quinupristin/dalfopristin, and daptomycin are used for the treatment of severe infections caused by multi-drug-resistant enterococci. However, resistance to vancomycin has begun to spread among enterococci since the first vancomycin-resistant enterococci (VRE) had been isolated in England in 1986.[3,10,26,28]

Enterococci colonized in the gastrointestinal tract of hospitalized patients play an important role in nosocomial infections.[14] The spread of infections caused by resistant strains was mainly due to the existence of unrecognized silent carriage, environmental contamination, transfer of colonized patients among hospitals, and ineffective precautions regarding hand washing.[18] Typing of the strains isolated the infectious agent. In this way, more effective control measures can be improved.

This study was done to detect vancomycin, ampicillin, and high-level aminoglycoside resistance rate of enterococci colonized in the gastrointestinal tract of patients in high-risk wards of our hospital and to determine the clonal relatedness among the isolates.

MATERIALS AND METHODS

Study groups

This study was performed on 150 inpatients and 100 outpatients at Inonu University, Turgut Ozal Medical Center, from October 2001 to April 2002. The samples were collected from inpatients hospitalized at least for 1 week and from outpatients on admitted days. Fifty of the inpatients and outpatients were in the pediatric age group, whereas the others were in the adult age group. Of the inpatients, 32 were in the intensive care unit, 18 were in hematology-oncology, 14 were in gastroenterology, 17 were in cardiovascular surgery, 19 were in orthopedic, and 50 were in pediatric wards.

Age and gender of the patients, duration of hospitalization, underlying diseases such as diabetes mellitus and malignancy, gastrointestinal manipulations (colonoscopy, endoscopy, enteral feeding, ileostomy, and colostomy), the existence of diarrhea, used antibiotics, and the duration of antibiotherapy were recorded in follow-up forms.

Isolation and identification of Enterococci

The stool samples and rectal swabs were inoculated into media within 1 h. For agar screen test, Campylobacter agar containing 5% sheep blood and 10 μg/ml vancomycin, Campylobacter agar containing 5% sheep blood and 8 μg/ml ampicillin, and blood agar plates without antibiotic were used.[17] After incubation at 35°C for 24 h, a presumptive identification of the *Enterococcus* species was made on the basis of colony morphology, Gram stain, catalase test, hydrolyze esculin in the presence of 40% bile salts, and growth in broth containing 6.5% NaCl. Definitive identification was done by the API-20 Strep assay (Bio Merieux, France) and BBL Crystal kit (gram-positive ID system, Becton Dickinson Microbiology Systems, France).

Antibiotic susceptibility testing

All isolates were tested for vancomycin (30 μg), ampicillin (10 μg), high-level gentamicin (120 μg), and high-level streptomycin (300 μg) resistance by disk diffusion according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS).[21] Aminoglycoside disks were obtained from Bioanalyse company (Ankara, Turkey); others were Oxoid (Oxoid, Ltd., Hamshire, UK). Minimal inhibitory concentrations (MICs) of ampicillin with concentrations ranging from 2 to 64 μg/ml and β-lactamase activity were assessed for all ampicillin-resistant isolates that were not *E. faecium*. The strains having a MIC value of ≥16 μg/ml were confirmed as ampicillin resistant.[21]

PCR for assessing glycopeptides resistance

A PCR assay described by Dutka-Malen et al.[6] was used to simultaneously amplify *vanA* and *vanB* genes in the 78 isolates having available stock culture. DNA isolation by using lysozyme and proteinase K, and extraction were performed by following the protocol of Welsh and McClelland.[30] DNA concentration was measured by UV absorbance at A_{260}. PCR was performed in a total volume of 50 μl containing 200 ng of DNA; 50 pmol of each oligodeoxynucleotide primer (primer A1 and A2 for *vanA*, B1 and B2 for *vanB* gene); 250 μmol (each) deoxynucleoside triphosphates; 4 mM $MgCl_2$; 2 U of Taq DNA polymerase; and 5 μl of 10 × amplification buffer. Amplification was performed with MJ Research PTC-200 Peltier thermal cycler by using the following profile: 1 cycle for 94°C for 2 min, and 30 cycles of 94°C for 1 min, 54°C for 1 min, and 72°C for 2 min and a final extension at 72°C for 10 min. PCR products were electrophoresed by 1.5% agarose gel with ethidium bromide. Expected PCR products were 732 base pair (bp) for *vanA* gene and 635 bp for *vanB* gene.[6] DNA samples prepared from *E. faecium* NCTC 12202 and *E. faecalis* ATCC 51299 were used as positive control for *vanA* and *vanB* gene, respectively.

Molecular typing of the strains

AP-PCR and PFGE typing studies were performed on a total of 78 Enterococci isolated from inpatients. These isolates included 59 *Enterococcus faecium*, 10 *E. faecalis*, six *E. durans*, and three *E. avium* strains. Stock cultures were not available for 12 strains.

Arbitrarily primed polymerase chain reaction (AP-PCR). Total DNA was prepared by following the protocol of Welsh and McClelland.[30] AP-PCR optimized previously[7] was performed with M13 primer (5′-GAG GGT GGC GGT TCT-3′). Amplification products were electrophoresed by 1.5% agarose gel with ethidium bromide and were visualized under UV illumination. The band patterns of the strains were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium). Dice coefficients of similarity were calculated for every pair of isolates. Strains with a similarity coefficient value of ≥70% were grouped in a cluster. The isolates with a similarity coefficient of ≥90% were evaluated as the same type, those having a similarity coefficient of ≥70% and <90% were classified as a subtype, and the isolates showing a similarity coefficient of <70% were identified as a different genotype.[1]

Pulsed field gel electrophoresis of genomic DNA. Isolation and deproteinization of the genomic DNA were done by fol-

lowing the protocol of Elliott et al.[8] with the modification that lysostaphin (5 U/ml) was used instead of mutanolysin. The genomic DNA in the plugs was incubated with 24 U of *SmaI* (Promega Corp., USA) for 24 h at 25°C in a water bath. DNA fragments were separated on 1% agarose gels run in 0.5× Tris-borate-EDTA buffer by using a CHEF-DR II system (Bio-Rad Laboratories, Nazareth, Belgium). The electrophoresis conditions were 14°C at 6 V/cm for 24 h. The initial and final switch times were 5 and 40 sec, respectively. The gel was stained with ethidium bromide and photographed under UV light. The fingerprinting patterns of the strains were analyzed with GelCompar software (version 3.0; Applied Maths, Sint-Martens-Latem, Belgium), and a dendrogram was formed. The results were evaluated according to the interpretative criteria for DNA restriction patterns of Tenover et al.[27]

Statistical analysis

Statistical analysis was performed using the Epi-Info 2002 package version 6.0 (Centers for Disease Control and Prevention, Atlanta, GA). χ^2 test was used to analyze the rates of carriage, resistance of the isolates from inpatients and outpatients, and risk factors. A p value of <0.05 was considered significant.

RESULTS

Enterococcal strains were isolated from 90 (60%) of the specimens collected from 150 inpatients. Forty percent of the strains were isolated from pediatric ward, 20% from intensive care unit, and the remaining 40% from other wards (9% from hematology-oncology, 12% from gastroenterology, 9% from cardiovascular surgery, and 10% from orthopedic ward). Of the 90 enterococci identified, 64 (71.1%) were *E. faecium*, 15 (16.7%) were *E. faecalis*, seven (7.8%) were *E. durans*, and four (4.4%) were *E. avium*. Of the 44 enterococcal strains isolated in 50 pediatric patients, 31 (70.4%) were *E. faecium*, eight (18.2%) *E. faecalis*, three (6.8%) *E. avium*, and two (4.5%) *E. durans*, while of 46 enterococcal strains isolated from 100 adult patients, 33 (71.7%) were *E. faecium*, seven (15.2%) were *E. faecalis*, five (10.9%) were *E. durans*, and one (2.2%) was *E. avium*. Enterococcal strains were isolated in the 30 (30%) specimens of the outpatients. Twenty-one (70%) of these strains were identified to be *E. faecium*, while 9 (30%) were *E. faecalis*.

Agar diffusion assay did not detect vancomycin-resistant enterococci among the isolates tested. Although *vanA* and *vanB* genes were successfully amplified in DNA samples of the control strains, all clinical isolates yielded a negative result for *vanA* and *vanB* gene. The rates of the resistance to ampicillin and aminoglycosides of the 90 enterococci isolated from hospitalized patients are shown in Table 1. All strains with high-level aminoglycoside resistance were also resistant to ampicillin. MICs and β-lactamase tests performed for one *E. faecalis*, three *E. durans*, and two *E. avium* isolates that were resistant to ampicillin showed that all isolates were β-lactamase producers and had ampicillin MICs of ≥ 16 μg/ml. Ampicillin and high-level gentamicin and streptomycin resistances were found to be 13%, 3%, and 10% in the isolates of outpatients, respectively. There were statistically significant differences regarding enterococcal colonization and the rates of ampicillin and high-level aminoglycoside resistance between the isolates of two groups ($p < 0.05$).

Eighty-eight percent of the inpatients from which enterococci were isolated had a history of antibiotic usage during their current hospitalization, and 49% of these patients had taken antibiotics for more than 10 days. Of the carrier of the inpatients, 74% had hospitalization for at least 2 weeks. There was no significant difference between the patients having high-level aminoglycosides and ampicillin resistance, and those patients without resistance with regard to risk factors such as diabetes, malignancy, chemotherapy, the existence of diarrhea, endoscopy, colonoscopy, duration of stay in intensive care unit, and previous antibiotic usage.

Both typing procedures were applied on 78 *Enterococcus* strains. PFGE yielded a total of 56 different patterns among the 59 *E. faecium* isolates. This procedure found that six (10.2%) of the isolates were clustered in three groups (each cluster had two isolates), four (6.8%) were closely related, two (3.4%) were possibly related, and 47 isolates (79.7%) were clonally unrelated. PFGE performed on 10 *E. faecalis* strains did not find any strain that was clustered or possibly related, but two of the strains were closely related. Fifty-four AP-PCR banding patterns were obtained among the 59 *E. faecium* isolates. PCR-based typing showed that 14 (23.7%) of the isolates had similarity coefficients of ≥90%, 16 (27.1%) had similarity coefficients of 70–90%, and 29 (49.2%) were different.

TABLE 1. RATES OF AMINOGLYCOSIDES AND AMPICILLIN RESISTANCE IN THE ISOLATES OF HOSPITALIZED PATIENTS

	Antimicrobial/No. (n) of resistant isolates and their percentages (%)					
	Gentamicin (120 μg)		*Streptomycin (300 μg)*		*Ampicillin (10 μg)*	
	n	%	n	%	n	%
Enterococcus species (*n*)						
E. faecium (64)	34	53	33	52	44	69
E. faecalis (15)	2	13.3	1	6.7	1	6.7
E. durans (7)	1	14.3	1	14.3	3	42.9
E. avium (4)	0	0	1	25	2	50
Total (90)	37	41.1	36	40	50	55.6

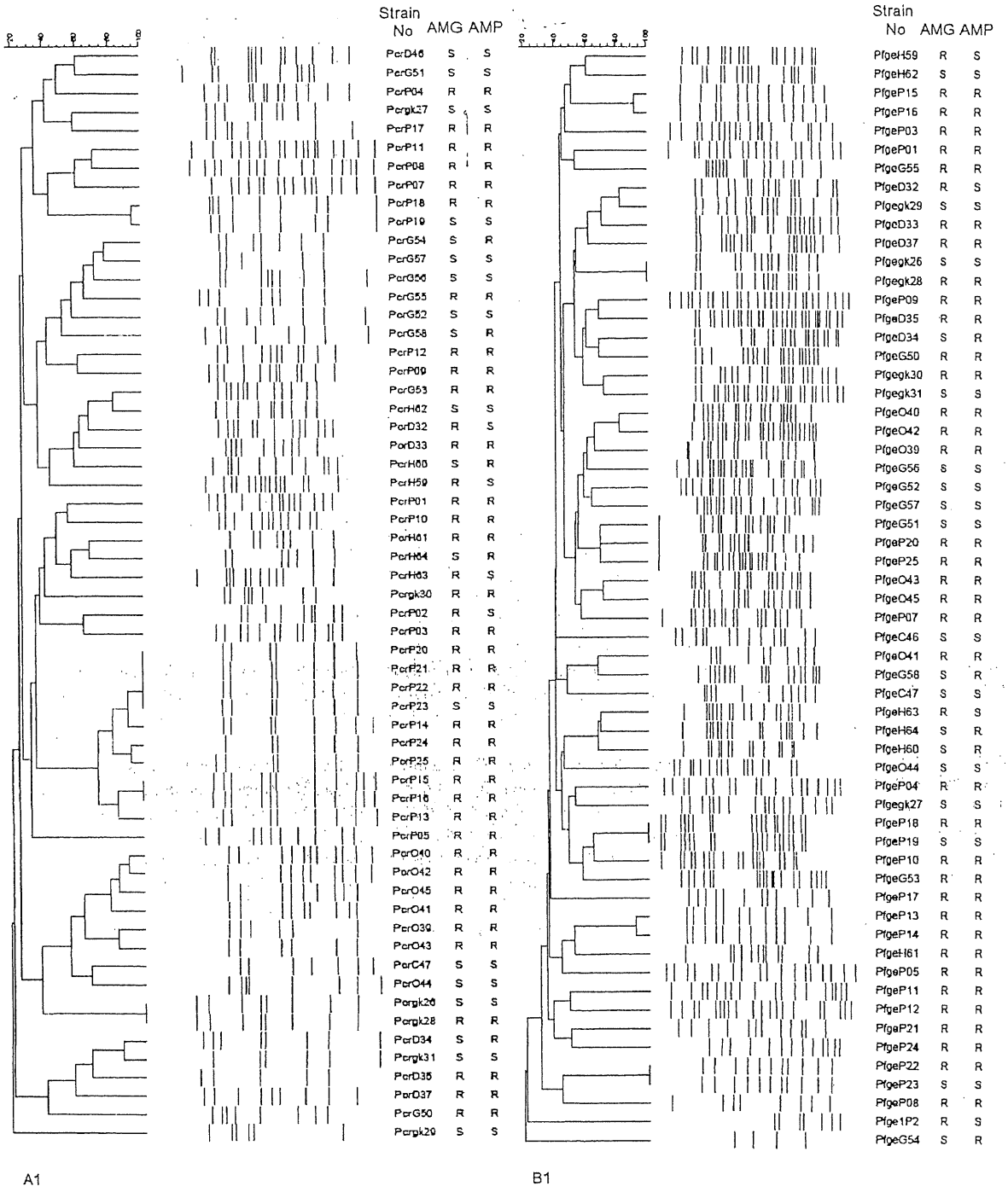


FIG. 1. A dendrogram showing clonal relatedness among the 59 *E. faecium* and 10 *E. faecalis* strains. (**A1**) *E. faecium*. (**A2**) *E. faecalis* results of AP-PCR. (**B1**) *E. faecium*. (**B2**) *E. faecalis* results of PFGE. S, susceptible; R, resistant; AMG, aminoglycosides; AMP, ampicillin; P, pediatric ward; H, hematology-oncology; G, gastroenterology; O, orthopedic ward; gk, cardiovascular surgery; C, surgical intensive care unit, D, internal intensive care unit. (*Continued on p. 163*)

AP-PCR revealed that two of the 10 *E. faecalis* strains were identical, and three were classified as subtypes. In total, 30 (50.8%) of the 59 *E. faecium* and five (50%) of the 10 *E. faecalis* strains were found clonally related with AP-PCR typing. These values were found to be 12 (20.3%) and two (20%) by PFGE, respectively. Figure 1 provides dendrograms showing clonal relatedness among *E. faecium* and *E. faecalis* strains by AP-PCR and PFGE. Both typing procedures did not find

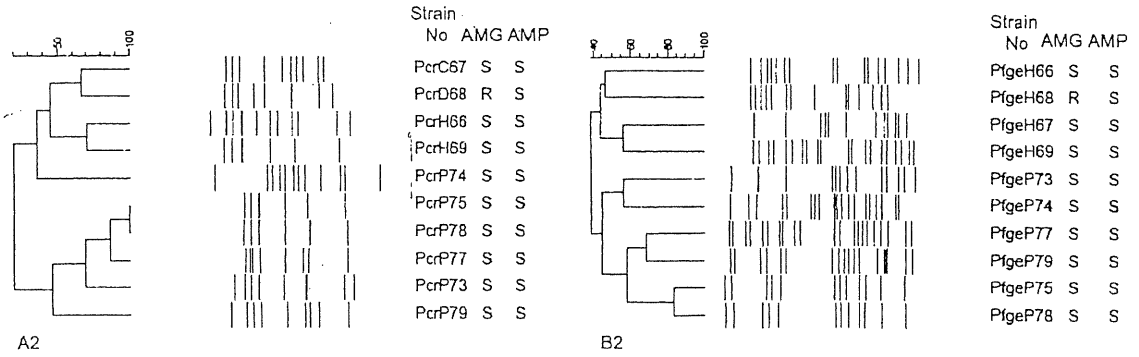


FIG. 1. (*continued*)

any clustered strains in the six *E. durans* and three *E. avium* isolates.

Comparison of the clustering rates of the isolates resistant to high-level aminoglycosides and of the ampicillin to those of the susceptible groups showed that neither PFGE nor the AP-PCR result was significantly different among the susceptible and resistant strains. According to the results of PFGE, only three of the 40 *E. faecium* strains that were resistant to high-level aminoglycoside were in three clusters which were shared by a susceptible isolate. The highest clustering rates were found among the strains isolated from pediatric ward. Six of the eight identical strains defined by AP-PCR and four of the six strains clustered by PFGE were among the pediatric isolates.

DISCUSSION

Fecal colonization is the most common source of enterococcal infections.[23] The use of antibiotics such as penicillin, third generation cephalosporins, and quinolones as well as prolonged hospitalization increase the colonization and resistance of enterococci.[11,29] In agreement with this data, we found that the colonization rate was significantly higher in inpatients than in outpatients (60% versus 30%, respectively; $p < 0.05$).

High-level aminoglycoside-resistant *E. faecalis* and *E. faecium* strains are increasingly encountered throughout the world. European SENTRY Antimicrobial Surveillance program found that high-level gentamicin resistance was 38.7% in enterococcal strains, and resistant strains were commonly identified in Belgium, France, Greece, Italy, Poland, Portugal, and Spain. In *E. faecium* isolates, high-level gentamicin resistance was found to be 22% and streptomycin resistance was 49%, while in *E. faecalis* isolates these values were 32% and 41%, respectively.[24] High-level gentamicin resistance had been detected in 40% and 25% of *E. faecalis* and *E. faecium* strains isolated at intensive care units of 23 hospitals in the United Kingdom.[13] In the studies conducted in Turkey, it was found that high-level gentamicin and streptomycin resistances was 21–67.6% and 19–38.2% in *E. faecium* strains, respectively, while these rates for *E. faecalis* strains were 11–42.7% and 20–42.7%.[5,19] The high-level gentamicin (41%) and streptomycin (36%) resistances found in the current study were more than those of European countries and were in the upper values detected in Turkey. The fact that more than half of the strains showed ampicillin resistance is an important point that should be investigated further in our hospital.

Fecal colonization of vancomycin-resistant enterococci (VRE) in European countries is as low as 1.4%.[2,25] VRE isolation in Turkey is rather low just like the results in Europe.[9] Similar to these data, no vancomycin resistance was detected in enterococcal strains isolated in our study.

Molecular typing studies providing information about the clonal relationship among the isolates have been widely used to improve strategies to prevent spread of antibiotic-resistant bacteria. PFGE typing performed on the strains collected from the hospitals in Norway had found that ampicillin-resistant and/or high-level gentamicin-resistant strains were clustered in larger group, while susceptible isolates were genetically more diversified.[12,15] Contrary to these results, the clustering rates found between ampicillin-resistant and high-level aminoglycoside-resistant strains were not significantly different from the susceptible ones in our study.

We found that, in total, 14 (17.9%) and 35 (44.9%) of the 78 *Enterococcus* isolates typed by PFGE and AP-PCR were clonally related, respectively. Both typing procedures indicate that spread of *Enterococcus* strains is common among the pediatric patients, and more effective control precautions are necessary for protection of these critical groups. The comparison of both typing procedures showed that 80% of the strains defined as clonally related by PFGE had also related AP-PCR profiles; however, 71.4% of the strains defined in the same AP-PCR profile were found unique by PFGE. From these results, it can be suggested that, although AP-PCR typing is cheaper, easier, and more rapid than PFGE, it cannot be used alone to find clonal relationships among the *Enterococcus* species collected in prolonged period.

Our findings of the high genetic diversity among the enterococcal isolates suggest the dissemination of a limited number of resistant strains between the patients in our hospital. A restrictive antibiotic prescription practice and universal precautions may be effective in limiting the spread of high-level aminoglycoside-resistant and ampicillin-resistant enterococci.

REFERENCES

1. **Ayan, M., R. Durmaz, E. Aktas, and B. Durmaz.** 2003. Bacteriological, clinical and epidemiological characteristic of hospital-acquired *Acinetobacter baumannii* infection in a teaching hospital. J. Hosp. Infect. **54**:39–45.
2. **Braak, N., A. Ott, A. Belkum, J.A.J.W. Kluytmans, J.G.M. Koeleman, L. Spanjaard, A. Voss, A.J.L. Weersink, C.M.J.E. Vandenbroucke Grauls, A.G.M. Buiting, H.A. Verbrugh, and H.P. Endtz.** 2000. Prevalence and determinants of fecal colonization with vancomycin resistant enterococcus in hospitalized patients in the Netherlands. Infect. Control Hosp. Epidemiol. **21**:520–524.
3. **Cetinkaya, Y., P. Falk, and C.G. Mayhall.** 2000. Vancomycin resistant enterococci. Clin.Microbiol. Rev. **13**:686–707.
4. **Chow, J.W.** 2000. Aminoglycoside resistance in enterococci. Clin. Infect. Dis. **31**:586–589.
5. **Cinar, T., H. Leblebicioglu, M. Sunbul, C. Eroglu, S. Esen, and M. Gunaydin.** 1999. Determination of high level gentamicin and streptomycin resistance in enterococci. Flora **4**:114–119.
6. **Dutka-Malen, S., S. Evers, and P. Courvalin.** 1995. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J. Clin. Microbiol. **33**:24–27.
7. **Durmaz, R., B. Durmaz, M. Bayraktar, I.H. Ozerol, M.T. Kalcioglu, E. Aktas, and Z. Cizmeci.** 2003. Prevalence of group A streptococcal carriers in asymptomatic children and clonal relatedness among isolates in Malatya, Turkey. J. Clin. Microbiol. **41**:5285–5287.
8. **Elliot, I.A., K.D. Farmer, and R.R. Facklam.** 1998. Sudden increase in isolation of group B streptococci, serotype V, is not due to emergence of a new pulse field gel electrophoresis type. J. Clin. Microbiol. **36**:2115–2116.
9. **Ersoy, Y., E. Sonmez, H.J. Young, E. Agel, and B. Durmaz.** 2001. Investigation of glycopeptide resistance in enterococcal strains isolated from Malatya, Kayseri and Elazig medical Faculty hospitals. Mikrobiol. Bul. **35**:197–209.
10. **Goossens, H.** 1998. Spread of vancomycin resistant enterococci: differences between the United States and Europe. Infect. Control Hosp. Epidemiol. **19**:546–551.
11. **Harthug, S., G.E. Eide, and N. Langeland.** 2000. Nosocomial outbreak of ampicillin resistant *Enterococcus faecium*: risk factors for infection and fatal outcome. J. Hosp. Infect. **45**:135–144.
12. **Harthug, S., R. Jureen, S.C. Mohn, A. Digranes, G.S. Simonsen, A. Sundsfjord, N. Langeland, and The Norwegian Enterococcal Study Group.** 2002. The prevalence of faecal carriage of ampicillin resistant and high level gentamicin resistant enterococci among inpatients at 10 major Norwegian hospitals. J. Hosp. Infect. **50**:145–154.
13. **Johnson, A.P., C. Henwood, S. Mushtaq, D. James, M. Warner, D.M. Livermore, and The ICU Study Group.** 2003. Susceptibility of gram-positive bacteria from ICU patients in UK hospitals to antimicrobial agents. J. Hosp. Infect. **54**:179–187.
14. **Jordens, J.Z., J. Bates, and D.T. Griffiths.** 1994. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J. Antimicrob. Chemother. **34**:515–528.
15. **Jueen, R., J. Top, S.C. Mohn, S. Harthug, N. Langeland, and J.L. Willems.** 2003. Molecular characterization of ampicillin-resistant *Enterococcus faecium* isolates from hospitalized patients in Norway. J. Clin. Microbiol. **41**:2330–2336.
16. **Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn.** 1997. Color atlas and text book of diagnostic microbiology. Lippincott, Philadelphia.
17. **Landman, D., J.M. Quale, E. Oydna, B. Willey, V. Ditore, M. Zaman, K. Patel, G. Saurina, and W. Huang.** 1996. Comparison of five selective media for identifying fecal carriage of vancomycin-resistant enterococci. J. Clin. Microbiol. **34**:751–752.
18. **Martone, W.J.** 1998. Spread of vancomycin-resistant enterococci: why did it happen in the United States? Infect. Control. Hosp. Epidemiol. **19**:539–545.
19. **Moaddab, S.R., and K. Toreci.** 2000. Species level identification and investigation of resistance to vancomycin and some other antibiotics in enterococcus strains. J. Turkish Microbiol. Soc. **30**:77–84.
20. **Mundy, L.M., D.F. Sahm, and M. Gilmore.** 2000. Relationships between enterococcal virulence and antimicrobial resistance. Clin. Microbiol. Rev. **13**:513–522.
21. **National Committee for Clinical Laboratory Standards.** 2003. Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard, 8th ed. M2-A8/Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, 6th ed. M7-A6. Wayne, PA.
22. **Patterson, J.E., and M. Zervos.** 1990. High-level gentamicin resistance in enterococcus: microbiology, genetic basis, and epidemiology. Rev. Infect. Dis. **12**:644–652.
23. **Robert, C., and J.R. Moellering.** 2000. *Enterococcus species, Streptococcus bovis*, and *Leuconostoc species*. In G.L. Mandell, J.E. Bennett, and R. Dolin (eds.), Principles and practice of infectious diseases. Churchill Livingstone, Philadelphia, pp. 2147–2156.
24. **Schmitz, F.J., J. Verhoef, A.C. Fluit, and SENTRY Participants Group.** 1999. Prevalence of aminoglycoside resistance in 20 European University hospitals participating in the European SENTRY antimicrobial surveillance programme. Eur. J. Clin. Microbiol. Infect. Dis. **18**:414–421.
25. **Simonsen, G.S., B.M. Andersen, A. Digranes, S. Harthug, T. Jacobsen, E. Lingaas, O.B. Natas, O. Olsvik, S.H. Ringertz, A. Skulberg, G. Syversen, and A. Sundsfjord.** 1998. Low faecal carrier rate of vancomycin-resistant enterococci in Norwegian hospital patients. Scand. J. Infect. Dis. **30**:465–468.
26. **Spera, R.V., and B.F. Farber.** 1994. Multidrug-resistant *Enterococcus faecium. Drugs* **48**:678–688.
27. **Tenover, F.C., R.D. Arbeit, R.V. Goering, P.A. Mickelsen, B.E. Murray, D.H. Persing, and B. Swaminathan.** 1995. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J. Clin. Microbiol. **33**:2233–2239.
28. **Toledano, H., Y. Schlesinger, D. Raveh, B. Rudensky, D. Attias, A.I. Eidelman, and A.M. Yimon.** 2000. Prospective surveillance of vancomycin resistant enterococci in a neonatal intensive care unit. Eur. J. Clin. Infect. Dis. **19**:282–287.
29. **Weinstein, J.W., M. Roe, M. Towns, L. Sanders, J.J. Thorpe, G.R. Corey, and D.J. Sexton.** 1996. Resistant enterococci: a prospective study of prevalence, incidence, and factors associated with colonization in a university hospital. Infect. Control Hosp. Epidemiol. **17**:36–41.
30. **Welsh, J., and M. McClelland.** 1993. Characterization of pathogenic microorganisms by genomic fingerprinting used arbitrarily primed PCR. In D.H. Persing, T.F. Smith, F. Tenover, and T.J. White (eds.), Diagnostic molecular microbiology. Principles and applications. American Society for Microbiology, Washington, DC, pp. 595–602.

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Microbial Drug Resistance

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