Colonization by vancomycin-resistant enterococci of the intestinal tract of patients in intensive care units from French general hospitals

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Objective: To evaluate the prevalence of fecal carriage of vancomycin-resistant enterococci (VRE) by patients hospitalized in intensive care units from 24 French general hospitals.

Methods: Rectal swabs were obtained from 647 patients hospitalized in intensive care units during the month of June 1994 and plated on agar medium selective for vancomycin-resistant enterococci. The glycopeptide resistance phenotypes and genotypes of the enterococci detected were characterized.

Results: Thirty-two of 647 patients (4.9%) carried VRE. Thirteen strains (2%) were identified as *Enterococcus faecium* and 19 (2.9%) as *Enterococcus gallinarum* or *Enterococcus casseliflavus*. None of these strains was highly resistant to gentamicin. The *E. gallinarum* and *E. casseliflavus* strains contained the *vanC1* and *vanC2* genes, respectively. The *E. faecium* strains were highly resistant to vancomycin and teicoplanin and carried the *vanA* gene. No infection due to VRE was observed during the study period. Pulsed-field gel analysis of total DNA following digestion with *Sma*I or *Ksp*I from 13 VanA-type *E. faecium* strains revealed intra- and inter-hospital strain heterogeneity. However, the finding of isolates with indistinguishable pulsed-field types within the same ward and in two medical centers suggests patient-to-patient transmission or a common source. Four *E. faecium* strains were isolated within 48 h after admission of patients.

Conclusions: These results indicate that VRE form part of the normal flora of patients and that, despite the actual scarcity of infections due to VRE, there is a potential risk for dissemination of these strains in French hospitals.

Key words: Vancomycin, *Enterococcus*, antibiotic resistance, fecal carriage

Many reports have drawn attention to the emerging problem of vancomycin-resistant enterococci (VRE) in hospitalized patients [1,2]. VRE are increasingly isolated in certain hospitals in the UK and the USA, especially in large, tertiary-care hospitals and in intensive care units (ICUs) [1,3,4]. In the USA, vancomycin resistance in enterococcal isolates from ICU patients increased from 0.4% in 1989 to 13.6% in 1993 [1]. In contrast, the figures are different in most European countries. Belgian [5] and French [6] multicenter studies

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carried out in 1993 reported low rates of glycopeptide resistance in enterococci (2.5% and 0.76% respectively), and only sporadic cases of infection or colonization have been reported from Italy [7], The Netherlands [8] and Spain [9]. In cases of outbreaks, the source of enterococci is usually considered to be endogenous, although patient-to-patient transmission is being increasingly reported [2, 10–12,13,14]. The patient's gastrointestinal tract appears to be a reservoir for VRE [15] and risk factors for acquisition of such strains include gastrointestinal colonization [16]. We wished to assess the fecal carriage of VRE in patients in French general hospitals where VRE infections are rare, to evaluate the risk for potential hospital dissemination of these organisms. For this purpose, the prevalence of fecal carriage of VRE in the ICUs of 24 French general hospitals was studied and the relationship between the isolates was analyzed.

MATERIALS AND METHODS

Study design

The study was performed in June 1994 in the ICUs of 24 general hospitals located in Aix-en-Provence, Alès, Annemasse, Arras, Aulnay-sous-Bois, Aurillac, Avranches, Compiégne, Charleville-Mézières, Le Havre, Le Havre-Monod, La Roche-sur-Yon, Mantes-la-Jolie, Meaux, Montceau-les-Mines, Montreuil-sous-Bois, Mulhouse, Neufchâteau, Paris (Clinique de la Porte de Choisy), Saint-Brieuc, Saint-Germain-en-Laye, Villeneuve-Saint-Georges, and Villeneuve sur Lot. The hospital size varied from 200 to 1600 beds. The mean number of ICU beds was nine (from four to 20) and the total number 233. The mean duration of ICU stay was 6.5 days (from 3 to 10.9). In order to obtain a rectal swab per patient hospitalized in June 1994, the study was performed as follows. First, rectal swabs were taken from all patients in all the ICUs on 1 June 1994 and transported to the microbiology laboratory for selective culture of VRE. Then, a rectal swab was obtained for all new patients hospitalized in the ICUs until 30 June 1994. The day of sampling was chosen as the most convenient for the investigator between the patient's admission and discharge.

Selective culture and identification of VRE

Samples were plated on bile azide aesculin agar (Sanofi Diagnostics Pasteur, Marnes la Coquette, France) or on D coccosel agar (bioMérieux, La-Balme les-Grottes, France), both supplemented with 8 mg/L of vancomycin. Two control strains were used, *E. faecium* BM4147, which contains the *vanA* gene (MIC of vancomycin: 512 mg/L) [17] and *E. faecium* D366, which harbors the *vanB* operon (MIC of vancomycin: 32 mg/L) [18]. Colonies which grew on agar with a dark brown halo were further identified by Gram staining, tolerance to tellurite and the API 20 STREP system (bioMérieux). Motility was searched for on mannitol motility agar incubated at 30 °C for 48 h. The presence of a pigment was assessed on trypticase soya agar. Species identification of *E. faecalis*, *E. faecium*, *E. casseliflavus* and *E. gallinarum* was confirmed by specific PCR amplification of the $ddl_{E.\ faecalis}$, $ddl_{E.\ faecium}$, *vanC1* and *vanC2* genes, respectively [19].

Antibiotic susceptibility

MICs of vancomycin and teicoplanin were determined by the agar dilution technique in Mueller-Hinton medium (Sanofi Diagnostics Pasteur). Susceptibility to ampicillin, erythromycin, ciprofloxacin, gentamicin, kanamycin and tetracycline was determined by the disk diffusion technique, using high-content disks for detection of high-level streptomycin, kanamycin and gentamicin resistance [20]. The breakpoints used are those of the CA-SFM [21].

Molecular analysis of VRE

The *vanA* and *vanB* resistance genotypes were identified by PCR, as described [19]. Enterococcal strains, including *E. faecium* PON from our laboratory collection as a control, were analyzed by pulsed-field gel electrophoresis (PFGE) of macrorestriction fragments. Genomic DNA was prepared in agarose plugs [22], and digested with *Sma*I or *Ksp*I (Boehringer Mannheim, Germany), and the DNA fragments generated were separated in a 1.2% agarose gel prepared and run in ×0.5 Tris–Borate–EDTA buffer in a contour clamped homogeneous field apparatus (CHEF-DR2, Biorad, Ivry sur Seine, France). The parameters for electrophoresis were 200 V for 18 h with pulse times ranging from 5 to 35 s for plugs digested with *Sma*I, and 150 V for 16 h using mode I 10 s–10 s and then for 8 h using mode II 6 s–6 s for plugs digested with *Ksp*I. Isolates having identical PFGE patterns with the two enzymes were assigned to the same type, whereas organisms differing by three restriction fragments or more were considered to be epidemiologically unrelated [23]. Calculation of Dice indexes and resulting dendrograms were done using the software Gel-Compar (Applied Maths, Kortrijk, Belgium).

RESULTS

Prevalence and characteristics of VRE

Rectal swabs were obtained from 173 patients (95% of hospitalized patients) on the first day of the survey and from 474 patients (44% of hospitalized patients) during the following 29 days. Thirty-two patients

(4.9%) carried VRE, including 13 *E. faecium* strains (2%) and 19 *E. gallinarum* and *E. casseliflavus* strains (2.9%) intrinsically resistant to vancomycin. Species identification by biochemical tests was in agreement with that based on PCR. Gene amplification was particularly useful in a difficult case of identification of an atypical *E. gallinarum* strain that did not ferment raffinose. All *E. faecium* strains were highly resistant to both vancomycin and teicoplanin (MICs ≥ 64 mg/L) and carried the *vanA* gene, as shown by PCR. Only one vancomycin-resistant *E. faecium* strain was resistant to ampicillin. Six of 13 *E. faecium* strains were resistant to high levels of streptomycin and kanamycin but not to high levels of gentamicin. Most *E. faecium* strains were resistant to erythromycin (12/13) and tetracycline (11/13), and none to ciprofloxacin. The 19 strains of *E. gallinarum* and *E. casseliflavus* were moderately resistant to vancomycin (MICs 8–16 mg/L) and susceptible to teicoplanin (MICs 0.5–1 mg/L) .

Epidemiology of VanA-type *E. faecium*

The 13 VanA-type *E. faecium* strains were isolated in eight hospitals (33%). A single *E. faecium* strain was isolated in four hospitals, two strains were isolated in three hospitals, and three strains in one hospital. The isolates differed in their pulsed-field type, except for two hospitals (Aix-en-Provence and Villeneuve-Saint-Georges) where two strains in each displayed closely related restriction profiles with a Dice index equal to 95% (Figure 1). In the Villeneuve-Saint-Georges hospital, located in the suburbs of Paris, two of the three isolates, obtained from two patients suffering from asthma and myocardial infarction, respectively, were indistinguishable by PFGE (Figure 1, lanes 3 and 5). In addition, these latter two strains could not be distinguished by PFGE after *Sma*I (Figure 1) or *Ksp*I (data not shown) digestion from an isolate from a Parisian hospital. In all, 10 different clones were observed (Figure 1). Four of the 13 *E. faecium* strains were isolated within 48 h after the patient's admission, and included, surprisingly, the two clonally related strains from Villeneuve-Saint-Georges. The two other strains were isolated from a patient with epilepsy related to a glioblastoma and a patient with a postpartum complication. None of these patients had received antimicrobials prior to hospitalization. A fifth strain was recovered on the day of a patient's admission for a cardiac arrest. The patient had been hospitalized for the 10 previous days in another hospital where he was given perioperative oxacillin prophylaxis for cardiac surgery. At the time of isolation of the eight remaining strains, the patients had been hospitalized for from 4 to 44 days. None of the patients colonized with VRE had received vancomycin in the ward or during the previous 6 months. The mean duration of hospital stay of VRE carriers did not significantly differ from that of the other patients. No VRE were isolated from clinical specimens during the study period.

DISCUSSION

Despite the fact that our study was carried out in ICUs where a high incidence of VRE can be observed [1], the prevalence of fecal colonization by vancomycin-

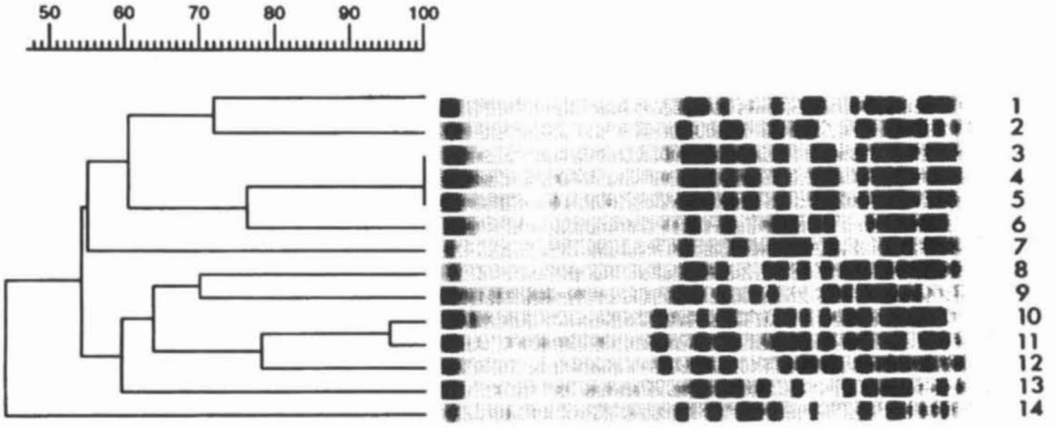

Figure 1 PFGE patterns of *Sma*I-digested genomic DNA obtained from vancomycin-resistant *E. faecium* strains. *E. faecium* PON (lane 1) was used as a control. Clinical isolates were from the hospitals of Compiègne (lanes 2 and 8), Villeneuve-Saint-Georges (lanes 3 and 5), Porte de Choisy (lanes 4 and 9), Montreuil-sous-Bois (lane 6), Neufchâteau (lane 7), Aix-en-Provence (lanes 10, 11 and 13), and Avranches (lane 12), Saint-Brieuc (lane 14). Dice indexes are indicated.

resistant *E. faecium* was low (2%). In addition, no infection due to VRE was observed during the study period. The isolates did not display high-level resistance to ampicillin and gentamicin in contrast to the majority of VRE isolated in the USA [3,11]. The figure in France is similar to that recently reported in a Belgian university hospital, in which no infection due to VRE was reported and where 3.5% of the patients were VRE fecal carriers [24]. A 1993 survey in the USA suggested a relationship between the size of the hospitals and the incidence of VRE, which was 3.6% in hospitals with more than 500 beds, as opposed to 1.8% in hospitals with 200–500 beds and none in hospitals with less than 200 beds [1]. We could not demonstrate any similar relationship for fecal carriage in patients admitted in French general hospitals. Close to one third of VRE were isolated on days 1 or 2 of hospital stay, in the absence of previous antibiotic therapy. These findings indicate that these organisms form part of the normal human fecal flora, and are consistent with the results of European studies conducted in Belgium, Germany, and the UK [15,24–26]. Thus, it is not surprising that PFGE profiles of total DNA of *E. faecium* showed diverse restriction patterns. However, the finding of isolates with indistinguishable PFGE patterns within the same ward and in two medical centers suggested patient-to-patient transmission or inter-hospital dissemination or a common source. Inter-hospital clonal transmission of VRE isolated from clinical samples has already been reported from certain US medical centers [14]. However, the fact that two clonally related strains were isolated from two patients on the day of admission in the same hospital favors a common source for these strains outside hospital. Indeed, the presence of VRE has recently been reported in the feces of farm animals and in animal foodstuffs, and a possible source for VRE could be the food chain [26–29]. Danish and German authors raised the possibility that the glycopeptide avoparcin used for nearly 20 years as a feed additive in animal husbandry in European countries could have selected for VRE in animals [28,29]. Intrinsically vancomycin-resistant *E. casseliflavus* and *E. gallinarum* strains were not rare. The vancomycin concentration (8 mg/L) used in the selective medium allowed detection of strains with MICs greater than or even equal to 8 mg/L. Possibly, certain VanB- or VanC-type enterococci could have been missed because of the low vancomycin MICs of some of these organisms [19]. Lack of isolation of VanB-type strains in the feces is probably more related to the rarity of these organisms in clinical samples in France [6] than to technical problems, since the selective medium used allowed the growth of a VanB-type control strain. Because of the presence of VRE in the feces of ICU patients, there is a potential risk for selection and further dissemination of such organisms in these wards. In particular, administration of oral and/or parenteral vancomycin is frequently reported as a risk factor for infection or colonization by VRE [2,30] and the extensive use of this antibiotic in hospitals could strongly contribute to the selection of these organisms. A recent study reported that administration of oral vancomycin or teicoplanin to healthy volunteers led to the selection of large numbers of VRE in the fecal flora of most of them [15]. Thus, the use of oral or parenteral vancomycin in many situations, e.g. routine prophylaxis and selective digestive tract decontamination, should be discouraged.

References

1. Centers for Disease Control and Prevention. Nosocomial enterococci resistant to vancomycin—United States, 1989–1993. MMWR Morb Mortal Wkly Rep 1993; 42: 597–9.
2. Korten V, Murray BE. The nosocomial transmission of enterococci. Curr Opin Infect Dis 1993; 6: 498–505.
3. Jones RN, Sader HS, Erwin ME, Anderson SC, the *Enterococcus* Study Group. Emerging multiply resistant enterococci among clinical isolates. I. Prevalence data from 97 medical center surveillance study in the United States. Diagn Microbiol Infect Dis 1995; 21: 85–93.
4. Wade JJ. The emergence of *Enterococcus faecium* resistant to glycopeptides and other standard agents—a preliminary report. J Hosp Infect 1995; 30(supp): 483–93.
5. Vercauteren E, Leclercq R, Goossens H. Belgian surveillance study of enterococcal susceptibility patterns [abstract E10]. In: Program and Abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, Washington, DC: American Society for Microbiology, 1994.
6. Schmit JL, Leclercq R, Scheimberg A, Landauer D. Approche épidémiologique et clinique des entérocoques: résultats d'une enquête. Med Mal Infect 1994; 24: 141–8.
7. Manso E, De Sio G, Biavasco F, Varaldo P, Sambo G, Maffei C. Vancomycin resistant enterococci. Lancet 1993; 342: 616–17.
8. Guiot H, Peetermans W, Sebens F. Isolation of vancomycin resistant enterococci in haematologic patients. Eur J Clin Microbiol Infect Dis 1991; 10: 32–4.
9. Lanzano F, Corzo J, Nogales C, Garcia-Bragado F. Bacteremia due to vancomycin resistant of Van B phenotype during prophylaxis with vancomycin. Clin Infect Dis 1992; 15: 752–3.
10. Bingen EH, Denamur E, Lambert-Zechovsky NJ, Elion J. Evidence for genetic unrelatedness of nosocomial outbreak of vancomycin-resistant *Enterococcus faecium* strains in a pediatric hospital. J Clin Microbiol 1991; 29: 1888–92.
11. Boyle JF, Soumakis SA, Rendo A, et al. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J Clin Microbiol 1993; 31: 1280–1285.
12. Handwerger S, Raucher B, Altarac D, et al. Nosocomial outbreaks due to *Enterococcus faecium* highly resistant to

vancomycin, penicillin, and gentamicin. Clin Infect Dis 1993; 16: 750–5.
13. Plessis P, Lamy T, Donnio PY, et al. Epidemiologic analysis of glycopeptide-resistant *Enterococcus* strains in neutropenic patients receiving prolonged vancomycin administration. Eur J Clin Microbiol Infect Dis 1995; 14: 959–63.
14. Sader HS, Pfaller MA, Tenover FC, Hollis RJ, Jones RN. Evaluation and characterization of multiresistant *Enterococcus faecium* from 12 U.S. medical centers. J Clin Microbiol 1994; 32: 2840–2.
15. Van der Auwera P, Pensart N, Korten V, Murray BE, Leclercq R. Influence of oral glycopeptides on the fecal flora of human volunteers: selection of highly-glycopeptide-resistant enterococci. J Infect Dis 1996; 173: 1129–36.
16. Edmond MB, Ober JF, Weinbaum DL, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. Clin Infect Dis 1995; 20: 1126–33.
17. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N Engl J Med 1988; 319: 157–61.
18. Williamson RC, Al-Obeid S, Shlaes JH, Goldstein FW, Shlaes DM. Inducible resistance to vancomycin in *Enterococcus faecium* D366. J Infect Dis 1989; 159: 1095–104.
19. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol 1995; 33: 24–7.
20. Leclercq R, Bismuth R, Duval J. New high-content disks for determination of high-level aminoglycoside resistance in clinical isolates of *Enterococcus faecalis*. Eur J Clin Microbiol Infect Dis 1992; 11: 356–60.
21. Acar J, Chardon H, Choutet P, et al. Communiqué 1995 du Comité de l'Antibiogramme de la Société Française de Microbiologie. Path Biol 1995; 43: I–VIII.
22. Murray BE, Singh K, Health JD, Sharma BR, Weinstock GM. Comparison of genomic DNAs of different enterococcal isolates using restriction endonucleases with infrequent recognition sites. J Clin Microbiol 1990; 28: 2059–63.
23. Goering RV. Molecular epidemiology of nosocomial infection: analysis of chromosomal restriction fragment patterns by pulse-field gel electrophoresis. Infect Control Hosp Epidemiol 1993; 14: 595–600.
24. Gordts B, Van Landuyt H, Ieven M, Vandamme P, Goossens H. Vancomycin-resistant enterococci colonizing the intestinal tracts of hospitalized patients. J Clin Microbiol 1995; 33: 2842–6.
25. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother 1994; 34: 515–28.
26. Bates J, Jordens JZ, Griffiths DT. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. J Antimicrob Chemother 1994; 34: 507–16.
27. Torres C, Reguera JA, Sanmartin MJ, Perez-Diaz JC, Baquero F. *vanA*-mediated vancomycin-resistant *Enterococcus* spp. in sewage. J Antimicrob Chemother 1994; 33: 553–61.
28. Klare I, Heier H, Claus H, et al. *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb Drug Res 1995; 1: 265–72.
29. Aarestrup F. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb Drug Res 1995; 1: 255–7.
30. Leclercq R, Courvalin P. Emerging problems with enterococcal infections. Curr Opin Infect Dis 1996; 9: 115–19.