ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 1991, p. 1943–1945
0066-4804/91/091943-03$02.00/0

Vol. 35, No. 9

Species Identification and Antibiotic Susceptibility Testing of Enterococci Isolated from Hospitalized Patients

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Received 12 April 1991/Accepted 13 June 1991

A total of 236 enterococci from hospitalized patients were identified to the species level, and their susceptibilities to 11 antibiotics were determined. Overall, 195 (82.6%) and 38 (16.1%) isolates were identified as *Enterococcus faecalis* and *E. faecium*, respectively, but the species distribution as determined from blood culture isolates differed markedly. A total of 27 (63.2%) *E. faecium* isolates, but no *E. faecalis* strains, were ampicillin resistant (MIC, >8 μg/ml). High-level gentamicin resistance (MIC, >500 μg/ml) was found in 8.2% of *E. faecalis* isolates but was not seen in other species.

Enterococci have recently emerged as an important cause of serious nosocomial infection (13). The Royal Victoria Infirmary is a 650-bed teaching hospital and tertiary referral center in northeast England. During 1990, enterococci were the fourth most common significant blood culture isolate, occurring in 26 of 314 (8.3%) bacteremic episodes. In the Intensive Care Unit, enterococci were isolated in 6 of 27 (22.6%) episodes of bacteremia and were the second most common blood culture isolate (11).

Serious enterococcal infections can be difficult to treat. Enterococci are intrinsically resistant to many antibiotics, including clindamycin, the penicillinase-resistant antistaphylococcal penicillins, and most cephalosporins (19). Acquired resistance to chloramphenicol, erythromycin, and tetracycline is relatively common (1). Enterococci are relatively resistant to aminoglycosides (29), while antibiotics which attack the cell wall, such as ampicillin and vancomycin, usually lack bactericidal activity at achievable concentrations in serum (17). However, the combination of an aminoglycoside with penicillin, ampicillin, or a glycopeptide achieves a synergistic bactericidal effect, and such combinations are the mainstay of treatment of serious enterococcal infections (6).

Studies have shown that enterococci highly resistant to aminoglycosides (21) or ampicillin (23, 28) are becoming increasingly common. Vancomycin resistance has also emerged, but it is uncommon at present (7). Resistant isolates are no longer susceptible to synergistic killing (18) and present a serious therapeutic problem. There are relatively few data on the prevalence of antibiotic resistance among enterococci in the United Kingdom. The purposes of this study were to determine the species distribution of enterococci isolated from hospitalized patients and to determine their susceptibilities to a range of antibiotics.

A total of 205 consecutive clinical isolates of enterococci from hospitalized patients were collected between 8 May and 3 September 1990. Of these, 106 were isolated from urine, 18 were isolated from gastrointestinal or genital tract specimens, and most of the remainder were isolated from skin and soft tissue sites. A total of 31 blood culture isolates collected between 1 August 1989 and 1 January 1991 were also examined. In both cases, care was taken to exclude duplicate isolates.

Enterococci were identified as bile-tolerant esculin-positive gram-positive cocci which grew in 6.5% NaCl. Identification to the species level was carried out according to the criteria of Facklam and Collins (10). Isolates were inoculated with a multipoint inoculator onto a series of Columbia agar plates (BBL Microbiology Systems, Oxford, England) containing 1% peptone and 1% arabinose, lactose, mannitol, sorbitol, or sorbose, with bromothymol blue (0.2%) as a pH indicator. The plates were incubated in air at 37°C for 48 h. Isolates which produced acid from lactose, mannitol, and sorbitol, but not from arabinose or sorbose, were identified as *Enterococcus faecalis*. Isolates which produced acid from arabinose, lactose, and mannitol, but not from sorbitol or sorbose, were identified as *E. faecium*. Isolates not identified with this system were identified by using API 20 Strep identification strips (API Bio-merieux, Basingstoke, England). MICs of 11 antibiotics were determined by an agar incorporation technique in Iso-Sensidisc agar (BBL Microbiology Systems). An inoculum of approximately 10^4 CFU was delivered to the surface of the plates with a multipoint inoculator. The MIC was defined as the lowest concentration of antibiotic which completely inhibited visible surface growth of the inoculum after 18 h of incubation at 37°C. The Oxford strain of *Staphylococcus aureus* (NCTC 6571; National Collection of Type Cultures, London, England) was used as a control.

All isolates were tested for β-lactamase production by the rapid chromogenic-cephalosporin method (nitrocefin; Oxoid Ltd., Basingstoke, England) (26).

Table 1 compares the species distribution of enterococci

TABLE 1. Distribution of species of enterococci isolated from blood cultures and other sites

Source of isolates	No. (%) of isolates				Total
	E. faecalis	*E. faecium*	*E. raffinosus*	*E. avium*	
Blood cultures	14 (45.2)	15 (48.4)	1 (3.2)	1 (3.2)	31
Other sites	181 (88.3)	23 (11.2)	1 (0.5)		205
Total	195 (82.6)	38 (16.1)	2 (0.8)	1 (0.4)	236

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TABLE 2. Susceptibilities of *E. faecalis* and *E. faecium* to nine antibiotics

Antibiotic	MIC[a] (μg/ml)					
	E. faecalis			*E. faecium*		
	50%	90%	Range	50%	90%	Range
Ampicillin	1.0	2.0	≤0.25–4.0	16.0	≥64.0	0.5–>64.0
Ciprofloxacin	1.0	2.0	≤0.25–16.0	2.0	8.0	≤0.25–16.0
Erythromycin	2.0	≥64.0	≤0.25–>64.0	4.0	≥64.0	≤0.25–>64.0
Imipenem	1.0	2.0	≤0.25–4.0	16.0	≥64.0	0.5–>64.0
Meropenem	4.0	8.0	≤0.25–16.0	≥64.0	≥64.0	2.0–>64.0
Ofloxacin	2.0	4.0	0.5–16.0	4.0	8.0	1.0–16.0
Teicoplanin	0.5	0.5	≤0.25–4.0	1.0	2.0	≤0.25–2.0
Temafloxacin	1.0	4.0	0.5–16.0	4.0	8.0	1.0–16.0
Vancomycin	1.0	2.0	≤0.25–4.0	≤0.25	0.5	≤0.25–2.0

[a] 50% and 90%, MIC for 50 and 90% of the isolates, respectively.

isolated from blood cultures and other sites. Our observations concur with other recent studies (4, 15, 22), which have found that 80 to 90% of clinical isolates are *E. faecalis*, with *E. faecium* accounting for most of the remainder. Many studies have shown that *E. faecalis* is also the predominant blood culture isolate (12, 24, 28). However, of the 31 blood culture isolates in our study, 15 (48.4%) were *E. faecium* and 14 (45.2%) were *E. faecalis*. These results accord well with those of some other recent studies: of 45 Lancefield group D streptococcal bacteremias occurring in liver transplant patients, 23 were due to *E. faecium* and 21 were due to *E. faecalis* (27). Of 10 blood culture isolates identified by Ruoff and colleagues (22), five each were *E. faecalis* and *E. faecium*.

A total of 15 (7.7%) *E. faecalis* and 4 (10.5%) *E. faecium* strains were highly resistant to streptomycin (MIC, >2,000 μg/ml). A total of 16 (8.2%) *E. faecalis* isolates were highly gentamicin resistant (MIC, >500 μg/ml). High-level gentamicin resistance is uncommon in other enterococcal species (9); no isolates with such resistance were found in this study. Studies in the United States have shown high-level gentamicin resistance in up to 55% of *E. faecalis* isolates (21). In previous British studies, high-level gentamicin resistance had been found in 7% of *E. faecalis* isolates in a London study (25) and in 13% of all enterococci in a Nottingham study (8).

Table 2 shows the results of MIC testing with nine other antibiotics. A total of 24 (63.2%) *E. faecium* isolates were resistant to ampicillin (MIC, >8 μg/ml), while ampicillin resistance was not found in *E. faecalis* isolates. β-Lactamase production was not detected in any of the isolates. The overall ampicillin resistance rate in enterococci in our hospital (10.6%) is considerably higher than many previous studies have shown, although Oster and colleagues (20) found that the ampicillin MICs for 9.0% of enterococci isolated from hospitalized patients were ≥16 μg/ml. In other studies in the United States, Bush and colleagues (4) reported that the penicillin MICs for 4.7% of clinical isolates were ≥200 μg/ml, whereas Sapico and colleagues (23) found ampicillin resistance in <1% of enterococci. Watanakunakorn (28) reported that the ampicillin MICs for 34 of 180 (18.9%) blood culture isolates collected from 1985 to 1989 were ≥4 μg/ml. In our study, the ampicillin MICs for 29.0% of the blood culture isolates were ≥4 μg/ml. In a Spanish study (5), the ampicillin MICs for 4.2% of enterococci were ≥16 μg/ml. In a previous British study (8), ampicillin resistance had been detected in 8 of 144 (5.6%) enterococci.

No glycopeptide resistance was seen in our study. As in previous studies (3), teicoplanin was more active than vancomycin against *E. faecalis*. However, vancomycin was more active than teicoplanin against *E. faecium*. Only 35 (17.9%) *E. faecalis* and 4 (10.5%) *E. faecium* isolates were sensitive to erythromycin (MIC, ≤0.5 μg/ml).

Of the newer antibiotics, imipenem was as active as ampicillin, but meropenem was considerably less active, bearing out the results of previous reports (14). Ciprofloxacin was the most active of the three quinolone antibiotics, confirming previous findings (2, 16). *E. faecium* was more resistant to the quinolones than *E. faecalis*.

In conclusion, the two most notable findings in this study are the high proportion of blood culture isolates identified as *E. faecium* and the high frequency of ampicillin resistance in these isolates. The prevalence of high-level gentamicin resistance is lower than that reported in many previous surveys. Further studies on the epidemiology of infection by ampicillin-resistant *E. faecium* are required.

REFERENCES

1. **Acar, J. F., and A. Y. Buu-Hoi.** 1988. Resistance patterns of important Gram-positive pathogens. J. Antimicrob. Chemother. **21**:41–47.
2. **Barry, A. L., and R. N. Jones.** 1989. *In-vitro* activities of temafloxacin, tosufloxacin (A-61827) and five other fluoroquinolone agents. J. Antimicrob. Chemother. **23**:527–535.
3. **Bartoloni, A., M. G. Colao, A. Orsi, R. Dei, E. Giganti, and F. Parenti.** 1990. *In-vitro* activity of vancomycin, teicoplanin, daptomycin, ramoplanin, MDL 62873 and other agents against staphylococci, enterococci and *Clostridium difficile*. J. Antimicrob. Chemother. **26**:627–633.
4. **Bush, L. M., J. Calmon, C. L. Cherney, M. Wendler, P. Pitsakis, J. Poupard, M. E. Levison, and C. C. Johnson.** 1989. High-level penicillin resistance among isolates of enterococci. Implications for treatment of enterococcal infections. Ann. Intern. Med. **110**:515–520.

5. **Cercenado, E., M. E. García-Leoni, P. Rodeño, and M. Rodríguez-Créixems.** 1990. Letter. J. Clin. Microbiol. **28**:829.
6. **Chen, H. Y.** 1986. Resistance of enterococci to antibiotic combinations. J. Antimicrob. Chemother. **18**:1–8.
7. **Courvalin, P.** 1990. Resistance of enterococci to glycopeptides. Antimicrob. Agents Chemother. **34**:2291–2296.
8. **Edwards, R., and D. Greenwood.** 1990. Letter. J. Antimicrob. Chemother. **26**:155–156.
9. **Eliopoulos, G. M., C. Wennersten, S. Zighelboim-Daum, E. Reiszner, D. Goldmann, and R. C. Moellering, Jr.** 1988. High-level resistance to gentamicin in clinical isolates of *Streptococcus* (*Enterococcus*) *faecium*. Antimicrob. Agents Chemother. **32**:1528–1532.
10. **Facklam, R. R., and M. D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **27**:731–734.
11. **Gray, J. W., and S. J. Pedler.** Unpublished data.
12. **Gullberg, R. M., S. R. Homann, and J. P. Phair.** 1989. Enterococcal bacteraemia: analysis of 75 episodes. Rev. Infect. Dis. **11**:74–85.
13. **Hoffmann, S. A., and R. C. Moellering, Jr.** 1987. The enterococcus: "putting the bug in our ears." Ann. Intern. Med. **106**:757–761.
14. **Jones, R. N., A. L. Barry, and C. Thornsberry.** 1989. *In-vitro* studies on meropenem. J. Antimicrob. Chemother. **24**(Suppl. A):9–29.
15. **Kim, M. J., M. Weiser, S. Gottschall, and E. L. Randall.** 1987. Identification of *Streptococcus faecalis* and *Streptococcus faecium* and susceptibility studies with newly developed antimicrobial agents. J. Clin. Microbiol. **25**:787–790.
16. **King, A., K. Shannon, and I. Phillips.** 1985. The *in-vitro* activities of enoxacin and ofloxacin compared with that of ciprofloxacin. J. Antimicrob. Chemother. **15**:551–558.
17. **Krogstad, D. J., and A. R. Parquette.** 1980. Defective killing of enterococci: a common property of antimicrobial agents acting on the cell wall. Antimicrob. Agents Chemother. **17**:965–968.
18. **Moellering, R. C., Jr., O. M. Korzeniowski, M. A. Sande, and C. B. Wennersten.** 1979. Species-specific resistance to antibiotic synergism in *Streptococcus faecium* and *Streptococcus faecalis*. J. Infect. Dis. **140**:203–208.
19. **Murray, B. E., D. A. Church, A. Wanger, K. Zscheck, M. E. Levison, M. J. Ingerman, E. Abrutyn, and B. Mederski-Samoraj.** 1986. Comparison of two β-lactamase-producing strains of *Streptococcus faecalis*. Antimicrob. Agents Chemother. **30**: 861–864.
20. **Oster, S. E., V. A. Chirurgi, A. A. Goldberg, S. Aiken, and R. E. McCabe.** 1990. Ampicillin-resistant enterococcal species in an acute-care hospital. Antimicrob. Agents Chemother. **34**:1821–1823.
21. **Patterson, J. E., and M. J. Zervos.** 1990. High-level gentamicin resistance in *Enterococcus*: microbiology, genetic basis and epidemiology. Rev. Infect. Dis. **12**:644–652.
22. **Ruoff, K. L., L. de la Maza, M. J. Murtagh, J. D. Spargo, and M. J. Ferraro.** 1990. Species identities of enterococci isolated from clinical specimens. J. Clin. Microbiol. **28**:435–437.
23. **Sapico, F. L., H. N. Canawati, V. J. Ginunas, D. S. Gilmore, J. Z. Montgomerie, W. J. Tuddenham, and R. R. Facklam.** 1989. Enterococci highly resistant to penicillin and ampicillin: an emerging clinical problem? J. Clin. Microbiol. **27**:2091–2095.
24. **Shales, D. M., J. Levy, and E. Wolinsky.** 1981. Enterococcal bacteraemia without endocarditis. Ann. Intern. Med. **141**:578–581.
25. **Smythe, E. G., P. J. Stevens, and R. E. Holliman.** 1989. Prevalence and susceptibility of highly-gentamicin resistant *Enterococcus faecalis* in a south London teaching hospital. J. Antimicrob. Chemother. **23**:633–639.
26. **Sykes, R. B.** 1978. Methods for detecting beta-lactamases, p. 64–69. *In* D. S. Reeves, I. Phillips, J. D. Williams, and R. Wise (ed.), Laboratory methods in antimicrobial chemotherapy. Churchill Livingstone, Ltd., Edinburgh.
27. **Warren, R. E.** 1988. Difficult streptococci. J. Hosp. Infect. **11**(Suppl. A):352–357.
28. **Watanakunakorn, C.** 1990. Letter. J. Antimicrob. Chemother. **26**:602–604.
29. **Zervos, M. J., S. Dembinski, T. Mikesell, and D. R. Schaberg.** 1986. High-level resistance to gentamicin in *Streptococcus faecalis*: risk factors and evidence for exogenous acquisition of infection. J. Infect. Dis. **153**:1075–1083.