JOURNAL OF CLINICAL MICROBIOLOGY, Nov. 1991, p. 2595–2598
0095-1137/91/112595-04$02.00/0

Vol. 29, No. 11

NOTES

Detection of High-Level Aminoglycoside Resistance in Enterococci Other Than *Enterococcus faecalis*

DANIEL F. SAHM,[1,2]* S. BOONLAYANGOOR,[1] AND J. E. SCHULZ[1]

Clinical Microbiology Laboratories[1] and the Department of Pathology,[2] The University of Chicago Medical Center, Chicago, Illinois 60637

Received 2 May 1991/Accepted 29 July 1991

The ability of six screening methods to detect high-level aminoglycoside resistance in enterococcal species other than *Enterococcus faecalis* was investigated. The 85 *Enterococcus* isolates, which included 55 *E. faecium*, 11 *E. gallinarum*, 9 *E. casseliflavus*, 5 *E. raffinosus*, 4 *E. avium*, and 1 *E. mundtii*, were tested by using aminoglycoside-supplemented brain heart infusion agar (BHI), Remel EF Synergy Quad plates, high-content aminoglycoside diffusion disks, standard (prepared in-house) microdilution panels, Pasco MIC Gram Positive microdilution panels, and Vitek GPS-TA cards. When tested on BHI, 32 and 35 strains showed resistance to gentamicin and streptomycin, respectively. Resistance profiles obtained with Remel EF Synergy Quad plates were in complete agreement with those obtained on BHI. However, growth on Mueller-Hinton agar-based plates was not as heavy. Some isolates showed only weak growth and required 48 h for resistance to become evident, especially with swab inoculation of quadrants containing 2,000 μg of gentamicin per ml. Profiles obtained by use of the agar-based screens were used as the basis for evaluating the other methods. Disk diffusion showed complete agreement. No false resistance occurred by either microdilution method, but 48 h of incubation was needed for detection of some gentamicin-resistant isolates, and 14% of the streptomycin-resistant strains were not detected by standard microdilution. The Vitek GPS-TA card detected 81 and 100% of the gentamicin- and streptomycin-resistant isolates, respectively. In general, most methods used to detect high-level aminoglycoside resistance in *E. faecalis* appear to be reliable for the testing of the other enterococcal species. However, further investigations with a greater number of resistant *E. raffinosus*, *E. avium*, and *E. mundtii* isolates, when they are available, will be useful for establishing the full range of enterococci that can reliably be tested by the various methods.

The initial report by Eliopoulos et al. (1) of transferable high-level gentamicin resistance in *Enterococcus faecium* predicted the spread of this resistance to other isolates and species of enterococci. Subsequent reports have confirmed the dissemination of high-level aminoglycoside resistance, including resistance to gentamicin, among *E. faecium* and other enterococcal species (4, 8, 15). The consequences that such resistance may have for effective synergistic therapy of serious enterococcal infections emphasizes the need to establish in vitro testing accuracy for resistance detection. However, most previous investigations of various testing methods have focused primarily on *Enterococcus faecalis* (3, 6, 9–13, 16, 17). *E. faecium* has been the only other species included in some of these studies, but the number of strains tested has been limited and few have exhibited gentamicin resistance (3, 6, 12, 13, 16). A report by Metchock and McGowan (6) included 14 streptomycin-resistant and 1 gentamicin-resistant *E. faecium* tested by Vitek GPS-TA. Louie et al. (5) evaluated MicroScan microdilution panels by using 103 *E. faecium* isolates; 37 were streptomycin resistant and only 7 exhibited gentamicin resistance.

The purpose of this investigation was to establish the reliability of several screening methods previously evaluated for testing *E. faecalis* (3, 6, 9–13, 14, 16, 17) for detecting aminoglycoside resistance in other enterococcal species. The 85 strains used included 55 *E. faecium*, 11 *E. gallinarum*, 9 *E. casseliflavus*, 5 *E. raffinosus*, 4 *E. avium*, and 1 *E. mundtii*. Kathryn Ruoff, Massachusetts General Hospital, provided 10 isolates, and 20 other isolates were provided by the Antimicrobics Investigation Branch, Centers for Disease Control. The remaining 55 isolates were blood culture isolates from single patients obtained in the Clinical Microbiology Laboratories at the University of Chicago Medical Center. Species identification of all isolates was based on the conventional scheme reported by Facklam and Collins (2). *E. faecalis* ATCC 29212 and UC 73, an isolate previously shown to be highly resistant to both gentamicin and streptomycin, were used as susceptible and resistant controls, respectively.

Four to five colonies of each isolate were selected from overnight growth on Trypticase soy agar supplemented with 5% sheep blood (Becton-Dickinson Microbiology Systems, Cockeysville, Md.) and were suspended in 0.85% saline to a turbidity matching a 0.5 McFarland standard. Aliquots of this suspension (ca. 10^8 CFU/ml) were diluted appropriately to achieve final desired inoculum sizes for each of the screening tests. These tests included brain heart infusion agar (BHI; Becton-Dickinson Microbiology Systems, Cockeysville, Md.) quadrant plates prepared at the University of Chicago Medical Center, EF Synergy Quad plates containing Mueller-Hinton agar (Remel, Lenexa, Kan.), standard Mueller-Hinton broth microdilution panels prepared by using a Dynatech MIC 2000 microdilution system (Dynatech

* Corresponding author.

TABLE 1. Growth in the presence of gentamicin and streptomycin on BHI and Remel EF Synergy Quad plates

Medium[a]	Incubation time (h)	No. of strains growing in the presence of aminoglycoside[b]		
		Gentamicin		Streptomycin (2,000 μg/ml)
		500 μg/ml	2,000 μg/ml	
BHI	24	32 (0)	32 (1)	35 (0)
	48	32 (0)	32 (0)	35 (0)
EF spot	24	32 (0)	31 (6)	35 (1)
	48	32 (0)	32 (3)	35 (0)
EF swab	24	32 (0)	22 (17)	34 (1)
	48	32 (0)	32 (14)	35 (1)

[a] EF spot, Remel EF Synergy Quad Plate inoculated by use of a micropipette; EF swab, Remel EF Synergy Quad Plate inoculated by use of a swab (see text).

[b] Values in parentheses are the number of growing isolates for which growth was considered positive, but weak (light haze or individual colonies).

Laboratories, Alexandria, Va.), Pasco MIC Gram Positive panels (Difco Laboratories, Detroit, Mich.), Vitek GPS-TA cards (Vitek Systems, Inc., Hazelwood, Mo.), and disk diffusion with high-content gentamicin and streptomycin disks as described previously (11). By using a micropipette, the BHI and EF Synergy Quad plates were spot inoculated with 10^6 CFU. As described previously for *E. faecalis* testing (9), each quadrant of the EF Synergy Quad plates was also inoculated with the tip of a swab that had been immersed in the organism suspension. After inoculation, standard and Pasco microdilution wells contained 10^4 CFU and Vitek GPS-TA cards had 10^5 CFU per well, as recommended by the manufacturers. All screens were incubated at 35°C in an ambient atmosphere and were examined for growth after 24 and 48 h. The aminoglycoside concentrations tested are given in Tables 1 and 2. To establish consistency and minimize false susceptibility, weak growth (light haze or individual colonies) on the agar surface was interpreted as resistance (9, 14).

When tested on BHI, 32 of the 85 isolates (38%) grew in the presence of gentamicin and 35 (41%) grew in the presence of streptomycin after 24 h of incubation (Table 1). Only one *E. faecium* isolate showed weak growth on 2,000 μg of gentamicin per ml at 24 h and was considered resistant. The same strains that showed high-level resistance on BHI also demonstrated resistance when tested on EF Synergy Quad plates. However, growth on these Mueller-Hinton agar base plates was not as heavy as that on BHI. Several isolates gave only weak growth when tested against 2,000 μg of gentamicin per ml, especially when the swab inoculation technique was used. With 500 μg of gentamicin per ml, spot and swab inoculation results were the same. Prolonging of incubation to 48 h substantially increased the number of gentamicin-resistant strains detected by swab inoculation of the quadrant with 2,000 μg of gentamicin per ml, but the effect on the results obtained with 500 μg gentamicin per ml and streptomycin was negligible.

Because there was complete agreement in the results obtained by BHI and EF Synergy Quad plates after 48 h of incubation, the gentamicin and streptomycin resistance profiles of the 85 enterococcal species were taken as those obtained by these screening methods. The profiles were as follows: 22 *E. faecium* isolates and 1 *E. casseliflavus* isolate were resistant to both gentamicin and streptomycin; 5 *E. faecium* isolates, 2 *E. raffinosus* isolates, and 1 isolate each of *E. gallinarum* and *E. casseliflavus* were resistant to only gentamicin; 9 *E. faecium* isolates and 1 isolate each of *E. avium*, *E. gallinarum*, and *E. raffinosus* were resistant to only streptomycin; and 19 *E. faecium* isolates, 9 *E. gallinarum* isolates, 7 *E. casseliflavus* isolates, 3 *E. avium* isolates, 2 *E. raffinosus* isolates, and 1 *E. mundtii* isolate were susceptible to both gentamicin and streptomycin. These profiles were used to compare and evaluate the results obtained by the other four methods.

The results obtained by disk diffusion, standard microdilution, and Pasco microdilution and with Vitek cards are

TABLE 2. Comparison of four methods for detection of high-level aminoglycoside resistance[a]

Method[b]	Aminoglycoside (concn)[c]	No. (%) of isolates exhibiting:							
		False resistance		Agreement for susceptible isolates		False susceptibility		Agreement for resistant isolates	
		24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
Disk	Gent (120 μg/disk)	0 (0)	0 (0)	53 (100)	53 (100)	0 (0)	0 (0)	32 (100)	32 (100)
	Strep (300 μg/disk)	0 (0)	0 (0)	50 (100)	50 (100)	0 (0)	0 (0)	35 (100)	35 (100)
Std Md	Gent (500 μg/ml)	0 (0)	0 (0)	53 (100)	53 (100)	1 (3)	1 (3)	31 (97)	31 (97)
	Gent (2,000 μg/ml)	0 (0)	0 (0)	53 (100)	53 (100)	5 (16)	1 (3)	27 (84)	31 (97)
	Strep (2,000 μg/ml)	0 (0)	0 (0)	50 (100)	50 (100)	5 (14)	5 (14)	30 (86)	30 (86)
Pasco Md	Gent (2,000 μg/ml)	0 (0)	0 (0)	53 (100)	53 (100)	1 (3)	0 (0)	31 (97)	32 (100)
	Strep (2,000 μg/ml)	0 (0)	0 (0)	50 (100)	50 (100)	2 (6)	1 (3)	33 (94)	34 (37)
Vitek	Gent (500 μg/ml)	0 (0)	—[d]	51[e] (100)	—	6 (19)	—	26 (81)	—
	Strep (2,000 μg/ml)	3 (6)	—	45[e] (94)	—	0 (0)	—	35 (100)	—

[a] As determined by BHI agar screen and EF Synergy Quad plates (Table 1).

[b] Disk, disk diffusion (an inhibition zone diameter of <10 mm was considered resistance); Std Md, standard microdilution; Pasco Md, Pasco microdilution; Vitek, Vitek GPS-TA card.

[c] Gent, gentamicin; Strep, streptomycin.

[d] —, Vitek results were obtained only at 6 h.

[e] Two susceptible *E. avium* isolates failed to grow on the Vitek GPS-TA card.

presented in Table 2. For both gentamicin and streptomycin testing there was complete agreement between disk diffusion results and those obtained by use of BHI and EF Synergy Quad plates. The inhibition zone diameters for all gentamicin and streptomycin resistant isolates were 6 mm. For susceptible strains, zones ranged from 15 to 29 mm for gentamicin and from 11 to 28 mm for streptomycin. Prolonging of incubation to 48 h had no notable effect on the inhibition zone diameters that were obtained. These findings are consistent with those of previous studies that have shown high-content disk diffusion to be a reliable method for detecting resistance in *E. faecalis* (9, 11, 13).

False resistance did not occur by either the standard or Pasco microdilution methods (Table 2). With the standard microdilution screen, five strains tested against 2,000 μg of gentamicin per ml showed false susceptibility at 24 h, as did one strain tested against 500 μg/ml. At 48 h, only one isolate was falsely susceptible, regardless of the drug concentration tested. False streptomycin susceptibility occurred with 5 (14%) of the streptomycin-resistant isolates tested, and this did not change with prolonged incubation. Only one isolate that showed resistance at 48 h had exhibited weak growth (fine haze on the bottom of the microdilution well) at 24 h. Use of the Pasco microdilution panel resulted in one isolate that gave false gentamicin susceptibility at 24 h, with no false susceptibility observed at 48 h. Similarly, the Pasco screen detected 94 and 97% of the streptomycin-resistant isolates with 24 and 48 h of incubation, respectively. These agreement percentages are comparable to those reported by Weissmann et al. (14) for testing *E. faecium* by using MicroScan MIC panels containing either Mueller-Hinton or dextrose phosphate broth, but they were higher than the 43% of resistant *E. faecium* detected by MicroScan as reported by Louie et al. (5).

No false streptomycin susceptibility occurred with Vitek GPS-TA cards, but 19% of the gentamicin-resistant isolates were not detected (Table 2). These findings are in contrast to the 36% false streptomycin susceptibility reported for *E. faecium* by Metchock and McGowan (6), but are more comparable to the 6% false streptomycin susceptibility and 18% false gentamicin susceptibility reported by Weissmann et al. (14). In our study, three strains showed false resistance to streptomycin, but upon retesting they were susceptible. Additionally, two susceptible *E. avium* strains failed to grow in the Vitek system, but these organisms also grew poorly on EF Synergy Quad plates. Further work to enhance resistance detection by Vitek GPS-TA cards is in advanced stages of investigation (7).

Testing problems previously reported for *E. faecalis* (9), such as the need for prolonged incubation to detect resistance, weak growth by some resistant isolates, and false susceptibility to streptomycin, were evident in this study. Prolonging of incubation to 48 h increased the number of gentamicin-resistant isolates detected by EF Synergy Quad plates and microdilution screens, especially when 2,000 μg of drug per ml was used (Tables 1 and 2). Additionally, with swab inoculation, several resistant isolates showed weak growth in the presence of 2,000 μg of gentamicin per ml. As has been recommended for *E. faecalis* (9, 14), prolonging of incubation to 48 h when weak or light growth, in comparison with the growth control, is observed at 24 h enhances resistance detection in some isolates. BHI results indicate that some of these problems might be related to the growth medium. Whether or not BHI contained aminoglycoside, growth of resistant and susceptible strains was notably heavier than that on the Mueller-Hinton-based EF plates, weak growth occurred with only one resistant isolate tested on BHI, and all resistant isolates were detected without prolonging incubation to 48 h (Table 1). False streptomycin susceptibility among these species of enterococci, which in this study was most evident with the standard microdilution screen, is a problem that also has been reported for *E. faecalis* (3, 6, 9, 12–14).

In summary, high-level aminoglycoside resistance has disseminated among several enterococcal species and will undoubtedly continue to spread. Therefore, regardless of the species, there is a need to perform resistance screening on isolates that cause serious infections. Results of this study show that several methods previously shown to be reliable for aminoglycoside resistance detection in *E. faecalis* performed well for the testing of other enterococci. However, as larger numbers of resistant strains are encountered and the variety of species exhibiting resistance increases, the reliabilities of these and other methods will need to be monitored.

We thank Kathryn Ruoff of Massachusetts General Hospital and Carolyn Baker of the Antimicrobics Investigation Branch, Centers for Disease Control, for kindly providing a number of the isolates used in this study.

This work was supported in part by grants from Difco Laboratories, Remel, and Vitek Systems, Inc.

REFERENCES

1. **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.
2. **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.
3. **Fuller, S. A., D. E. Low, and A. E. Simor.** 1990. Evaluation of a commercial microtiter system (MicroScan) using both frozen and freeze-dried panels for detection of high-level aminoglycoside resistance in *Enterococcus* spp. J. Clin. Microbiol. **28**: 1051–1053.
4. **Kaufhold, A., D. Guse, and P. Ferrieri.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 58.
5. **Louie, M., S. Szeto, and D. E. Low.** 1990. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 691.
6. **Metchock, B. G., and J. E. McGowan, Jr.** 1990. Evaluation of the Vitek GPS-TA card for the detection of high-level resistance to gentamicin and streptomycin in enterococci, abstr. C-168, p. 372. Abstr. 90th Annu. Meet. Am. Soc. Microbiol. 1990. American Society for Microbiology, Washington, D.C.
7. **Mulder, R.** Personal communication.
8. **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.
9. **Sahm, D. F., S. Boonlayangoor, P. Iwen, J. Baade, and G. Woods.** 1991. Factors influencing determination of high-level aminoglycoside resistance in *Enterococcus faecalis*. J. Clin. Microbiol. **29**:1934–1939.
10. **Sahm, D. F., and C. Torres.** 1988. Effects of medium and inoculum variations on screening for high-level aminoglycoside resistance in *Enterococcus faecalis*. J. Clin. Microbiol. **26**:250–256.
11. **Sahm, D. F., and C. Torres.** 1988. High-content aminoglycoside disks for determining aminoglycoside-penicillin synergy against *Enterococcus faecalis*. J. Clin. Microbiol. **26**:257–260.
12. **Solliday, J., R. Mulder, and J. Scott.** 1989. Evaluation of detection of high-level aminoglycoside-aminocyclitol resistance in enterococci using the Vitek GPS-TA card, abstr. C-272, p.

438. Abstr. 89th Annu. Meet. Am. Soc. Microbiol. 1989. American Society for Microbiology, Washington, D.C.
13. **Spiegel, C. A.** 1988. Laboratory detection of high-level aminoglycoside-aminocyclitol resistance in *Enterococcus* spp. J. Clin. Microbiol. **26**:2270–2274.
14. **Weissmann, D., J. Spargo, C. Wennersten, and M. J. Ferraro.** 1991. Detection of enterococcal high-level aminoglycoside resistance with MicroScan freeze-dried panels containing newly modified medium and Vitek Gram-Positive susceptibility cards. J. Clin. Microbiol. **29**:1232–1235.
15. **Woodford, N., R. C. George, E. McNamara, E. Smyth, S. Namnyak, and A. H. C. Uttley.** 1991. *Enterococcus faecium* with high-level resistance to gentamicin. Lancet **337**:1356.
16. **Yagupsky, P., S. Petry, and M. A. Menegus.** 1990. Comparison of four methods for testing high-level aminoglycoside resistance in enterococci. Eur. J. Clin. Microbiol. Infect. Dis. **9**:133–135.
17. **Zervos, M. J., J. E. Patterson, S. Edberg, C. Pierson, C. A. Kauffman, T. S. Mikesell, and D. R. Schaberg.** 1987. Single-concentration broth microdilution test for detection of high-level aminoglycoside resistance in enterococci. J. Clin. Microbiol. **25**:2443–2444.