APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Jan. 1995, p. 374–376 Vol. 61, No. 1
0099-2240/95/$04.00+0

Occurrence of High-Level Aminoglycoside Resistance in Environmental Isolates of Enterococci

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High-level resistance to aminoglycosides was observed in environmental isolates of enterococci. Various aquatic habitats, including agricultural runoff, creeks, rivers, wastewater, and wells, were analyzed. Strains of *Enterococcus faecalis*, *E. faecium*, *E. gallinarum*, and other *Enterococcus* spp. demonstrated multiple antibiotic resistance patterns to aminoglycosides.

There is a growing awareness of the public health concerns associated with the occurrence of drug-resistant strains of bacteria. The emergence of multiple antibiotic-resistant bacteria has become a major challenge in the treatment of infectious diseases (2). Enterococci are recognized as important human pathogens in both community- and nosocomial-acquired infections (3, 8). Enterococci exhibit inherent low-level aminoglycoside resistance; the MICs are between 2 and 16 μg/ml (8). In recent years, a number of enterococcal strains have acquired high-level aminoglycoside resistance (HLAR); the MICs are ≥2,000 μg/ml (3, 8, 12). The occurrence of HLAR in enterococci is an example of acquired resistance as opposed to intrinsic resistance. *Enterococcus faecalis* infections are often treated with synergistic combinations of a cell wall-active agent and an aminoglycoside. The addition of a cell wall-active agent, such as penicillin or vancomycin, typically results in enhanced killing of enterococci. However, when *E. faecalis* isolates acquire HLAR, the synergism with a cell wall-active antibiotic is lost (8). In these instances, medical treatment options are limited.

Although a considerable amount of information regarding HLAR among clinical isolates of enterococci exists, there have been few reports on the occurrence of these strains outside of the hospital setting (4, 5). The presence of enterococcal strains which exhibit HLAR in the environment is of particular interest in reference to the occurrence of community-acquired enteroccal infections (8, 9). This report summarizes the results of a survey in which enterococcal isolates from various environmental sources were screened for HLAR to four aminoglycosides.

The environmental samples included groundwater samples collected from wells in Alabama, Florida, Maryland, Oregon, and the Virgin Islands and wastewater, creek, river, and agricultural runoff samples collected within a 30-mile radius of Cincinnati, Ohio. The single agricultural runoff sample was from a local dairy farm. All samples were transported on ice and analyzed within 24 h of collection.

The samples were analyzed for enterococci by the membrane filtration method by using mE agar and standard verification procedures (1, 6). Individual isolates were subcultured to brain heart infusion agar (BHIA) and further characterized on the basis of salt tolerance, characteristic growth on bile esculin agar, and catalase activity (1). Selected isolates were stored at 5°C on BHIA slants.

Screening for HLAR was conducted after the manner of Sahm et al. (12). Briefly, individual isolates were spot inoculated at a concentration of approximately 10^6 CFU by using a sterile micropipette onto BHIA supplemented with a known concentration of aminoglycoside. Unsupplemented BHIA served as the control. Gentamicin at concentrations of 500 and 2,000 μg/ml and streptomycin, kanamycin, and tobramycin at concentrations of 2,000 μg/ml were the aminoglycosides used in the screening process. In accordance with previously reported recommendations, cultures were examined after 24 and 48 h of incubation (7). To minimize false susceptibility, weak growth was interpreted as resistance (12). Control cultures were incorporated in each assay. An environmental isolate of *Enterococcus faecium* which lacked HLAR served as the negative control culture. The positive control was a clinical isolate of *E. faecalis* HH22 (kindly provided by B. E. Murray and K. Zscheck, University of Texas Health Science Center, Houston) which was resistant to gentamicin, streptomycin, kanamycin, and tobramycin (MICs of >2,000 μg/ml). Gentamicin and streptomycin MICs were determined for those isolates with HLAR to these two antibiotics (10). All isolates which exhibited HLAR were subcultured to sheep blood agar and further identified by using the API 20 Strep system (bioMerieux Vitek, Inc., Hazelwood, Mo.).

A total of 248 isolates of enterococci were screened for HLAR. The highest percentage of resistance was seen for kanamycin, closely followed by tobramycin and to a lesser degree by streptomycin and gentamicin (Table 1). Increasing the incubation time from 24 to 48 h and recording weak growth as part of the screening procedure did not have an appreciable effect upon the detection of high-level resistance for gentamicin or streptomycin; however, the effect was more pronounced for kanamycin and tobramycin. Resistance to 500 μg of gentamicin per ml accurately predicted resistance at the 2,000-μg/ml level.

Multiple antibiotic resistance patterns were observed in 95% of HLAR isolates. The most frequently occurring multiple resistance pattern was HLAR to both kanamycin and tobramycin, followed by multiple resistance to streptomycin, kanamycin, and tobramycin (Table 2). All isolates that exhibited gentamicin resistance were also resistant to kanamycin and tobramycin but not to streptomycin. Both *E. faecalis* and *E. faecium* isolates exhibited HLAR to gentamicin. All isolates produced gamma hemolysis on blood agar.

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TABLE 1. Environmental isolates of enterococci which exhibited growth in the presence of high levels of aminoglycoside antibiotics

Sample type (no.)	No. of isolates examined	Incubation time (h)	No. of resistant isolates[a]				
			Gentamicin		Streptomycin (2,000 μg/ml)	Kanamycin (2,000 μg/ml)	Tobramycin (2,000 μg/ml)
			500 μg/ml	2,000 μg/ml			
Well (8)	90	24	0	0	2 (2)	24 (3)	21 (5)
		48	0	0	2 (2)	32 (10)	31 (8)
Sewage (4)	76	24	3 (0)	3 (0)	6 (1)	21 (10)	24 (1)
		48	3 (0)	3 (0)	6 (0)	27 (5)	28 (4)
Creek (1)	32	24	1 (0)	1 (0)	2 (0)	12 (3)	12 (2)
		48	1 (0)	1 (0)	2 (0)	13 (1)	12 (1)
River (1)	30	24	0	0	2 (1)	9 (8)	8 (8)
		48	0	0	2 (0)	10 (2)	8 (0)
Agricultural runoff (1)	20	24	0	0	0	1 (1)	2 (2)
		48	0	0	0	2 (0)	2 (0)
Total [%]	248		4 [1.6]	4 [1.6]	12 [4.8]	84 [33.9]	81 [32.7]

[a] Values in parentheses are the numbers of isolates which exhibited weak growth (light haze or individual colonies).

Two *E. faecalis* and two *E. faecium* isolates were resistant to high levels of gentamicin, with MICs for all four isolates of >64,000 μg/ml. Nine of the twelve isolates resistant to high levels of streptomycin were *E. faecium*; the MICs were between 4,000 and 16,000 μg/ml. The MICs of streptomycin for two *E. faecalis* isolates were 4,000 and 16,000 μg/ml. For one enterococcal isolate which could not be identified to the species level, the MIC of streptomycin was >16,000 μg/ml.

Resistance to gentamicin and streptomycin was of particular interest because most of the recent clinical data has been associated with these two antibiotics (8). The range of samples which exhibited HLAR to streptomycin was larger than that seen for gentamicin, with resistant isolates found in all samples except for the dairy barn runoff sample. The gentamicin-resistant isolates were from a large municipal sewage treatment plant and a creek which is located in a densely populated urban area and known to be subject to sewage contamination. Sewage samples subjected to both primary and secondary treatment harbored gentamicin-resistant strains. The large municipal sewage treatment plant receives wastewater from several large hospital complexes. Gentamicin-resistant isolates were not obtained from sewage samples from another treatment plant, known to receive primarily domestic sewage.

HLAR patterns were observed in isolates of *E. faecalis*, *E. faecium*, *E. gallinarum*, and other *Enterococcus* spp. (Table 2). This finding confirms previous reports regarding transferable high-level gentamicin resistance in *E. faecalis* isolates and subsequent dissemination of HLAR to other species of enterococci (12). Consistent with previous reports, all strains isolated in this study which exhibited high-level resistance to gentamicin were also resistant to kanamycin and tobramycin but not necessarily resistant to streptomycin (8).

TABLE 2. Species of *Enterococcus* with multiple HLAR patterns

Source	Species	No. of isolates with multiple HLAR[a]			
		Gen-Kan-Tob	Str-Kan-Tob	Kan-Tob	Str-Kan
Well	*E. faecium*	0	2	29	0
Sewage	*E. faecium*	2	4	15	0
	E. faecalis	1	0	3	0
	Enterococcus spp.	0	1	1	0
Creek	*E. faecium*	0	1	9	0
	E. faecalis	1	0	0	1
	Enterococcus spp.	0	0	1	0
River	*E. faecium*	0	1	6	0
	E. faecalis	0	0	0	1
	E. gallinarum	0	0	1	0
Agricultural runoff	*E. faecium*	0	0	2	0

[a] Gen, gentamicin; Kan, kanamycin; Tob, tobramycin; Str, streptomycin.

The results of this survey indicate that enterococcal isolates which exhibit HLAR are not limited to the clinical setting and may be recovered from a variety of aquatic environmental sources. Both the well water and river water which were sampled serve as source water for drinking water. The river is also used extensively for recreational activities. The presence of gentamicin-resistant isolates in secondary sewage effluent is of particular interest since enterococci have been shown to be more resistant than *Escherichia coli* to chlorine disinfection (11).

In a study conducted in Germany, one sewage isolate of *E. faecium* was reported to exhibit high-level resistance to gentamicin (4). In a survey of 49 enterococcal water isolates in the United States, none of the isolates exhibited high-level resistance to gentamicin on the basis of gentamicin synergy screening tests (5). In this study, two sewage isolates of *E. faecium* were found to exhibit high-level resistance to gentamicin. Furthermore, the occurrence of high-level gentamicin resistance was also seen for two isolates of *E. faecalis*, one from sewage and one from an urban stream.

The occurrence of community-acquired HLAR enterococcal infections is a matter of continuing concern. Nachamkin et al. (9) noted that 7% of infections in their study were community acquired. A careful review of patients to exclude those who had recently resided in a hospital or chronic care facility prompted these investigators to conclude that a reservoir for gentamicin-resistant enterococci existed in the community. Our findings suggest that environmental sources may contribute to the dissemination of HLAR enterococci. The increasing prevalence of HLAR enterococci associated with nosocomial disease and the current finding of these organisms in environmental samples should be a matter of concern for both clinicians and public health authorities.

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