Jpn. J. Infect. Dis., 62, 158-159, 2009

Short Communication

High-Level Aminoglycoside Resistance and β-Lactamase Production in Enterococci at a Tertiary Care Hospital in India

Jyotsna Agarwal*, Rajkumar Kalyan, and Mastan Singh

Department of Microbiology, CSM Medical University, Lucknow, India

(Received August 11, 2008. Accepted January 19, 2009)

SUMMARY: Enterococci, a family of important opportunistic pathogens, exhibits intrinsic resistance to a number of antimicrobial agents in addition to acquired multidrug resistance. The present study was conducted to determine whether enterococci at a tertiary care hospital in India exhibit high-level aminoglycoside resistance and β-lactamase production. Enterococci were isolated from various clinical specimens and identified phenotypically. High-level resistance (HLR) to gentamicin, kanamycin, and streptomycin was determined by disc diffusion tests. β-lactamase production was detected using three methods: iodometric, acidometric, and chromogenic β-lactamase assays. Among the 86 enterococci isolated, 34 were found to have HLR to one or more aminoglycosides; HLR to kanamycin was most common. Vancomycin resistance was present in four of the isolates. Only one enterococcus produced β-lactamase, and it was sensitive to ampicillin on routine disc diffusion testing. β-lactamase production among enterococci, though not very common, may be missed on routine susceptibility testing. Frequent occurrence of HLR to kanamycin makes amikacin a poor choice for inclusion in combination therapy with cell wall-active agents.

In recent years the incidence of nosocomial enterococcal infections has increased markedly (1). This genus is intrinsically resistant to a number of antimicrobial agents, including low concentrations of aminoglycosides (2). None of the currently available cephalosporins exhibit clinically useful activity against them. In addition, enterococcus is rapidly acquiring resistance to many commonly used antibiotics in hospitals. Of particular concern is the rapid dissemination of enterococci with high-level aminoglycoside-resistance. The synergistic effect obtained by the combination of aminoglycosides with cell wall-active agents disappears in strains that show high-level resistance (HLR) to aminoglycoside (2).

The present study was designed with the aim of isolating and characterizing *Enterococcus* spp. from clinical specimens and studying the prevalence of drug resistance among these isolates, especially with regard to high-level aminoglycoside resistance (HLAR) and β-lactamase production.

The study was conducted between May 2004 and April 2005, and included 5,890 different clinical specimens referred for bacteriological culture (5,516 inpatients department [IPD] samples and 374 outpatients department [OPD] samples). There were 3,277 urine, 1,666 blood, 344 pus, and 183 cerebrospinal fluid (CSF) samples, 175 vaginal swabs, 70 throat swabs, and 175 samples of other body fluids such as ascitic fluid, pleural fluid, tissue aspirates, tissue pieces, and catheter tips. The isolated enterococci were identified to the species level using phenotypic methods (3). An antibiotic sensitivity test was performed by Kirby-Bauer's method on Muller-Hinton sheep blood agar. The following discs at the following concentrations were used (Hi-Media Pvt. Ltd., Mumbai, India): ampicillin, 10 μg/disc; chloramphenicol, 30 μg/disc; ciprofloxacin, 5 μg/disc; erythromycin, 15 μg/disc; teicoplanin, 30 μg/disc; tetracycline, 30 μg/disc; vancomycin, 30 μg/disc. Aminoglycoside discs were used at a high-level concentration: diffusion in solid medium using discs impregnated with 120 μg of gentamicin (which also predicts susceptibility to tobramycin and netilmicin) and kanamycin (which also predicts response to amikacin), and 300 μg of streptomycin was performed (3,4). Before use, each lot of discs was checked with the standard strain of *Enterococcus faecalis* ATCC 29212. Inhibition zones were interpreted according to NCCLS guidelines. β-lactamase production was detected by three methods: iodometric, acidometric, and chromogenic β-lactamase assays (5).

A total of 86 enterococci were isolated (70/5,516 IPD samples; 16/374 OPD samples). The isolation rate of enterococci was 1.46%. The spectrum of infections associated with enterococci was diverse (Table 1). Seventy-nine percent (68/86) of enterococci were sensitive to ampicillin (Table 2). Resistance to ampicillin was found to be significantly higher among *E. faecium* than among *E. faecalis* (11/33 versus 3/42, respectively), as reported earlier (6). Resistance to tetracycline was the highest noted in the present study, followed by resistance to erythromycin and ciprofloxacin. For all other antibiotics there was no significant difference between the resistance pattern of *E. faecalis* and *E. faecium*. In another study (7) from the subcontinent, among the 444 enterococcal isolates tested 66% were found to be resistant to ampicillin, 85% to erythromycin, and 88% to ciprofloxacin, and 26% of isolates had HLAR. In the present study, 40% (34/86) of enterococci were found to have HLR to one or more aminoglycosides. The HLR was greatest for kanamycin, followed by streptomycin and gentamicin (48.8, 20.9, and 8.3%, respectively). Species-wise there was no significant difference for the presence of HLAR (Table 2). Seven patterns of HLR were found; the most common was HLR to kanamycin alone in 26 isolates, followed by HLR to kanamycin and streptomycin together in 5 isolates. HLR to gentamicin was not found alone in any enterococci; it was always associated with resistance to kanamycin, streptomycin or both. The low prevalence of HLR to gentamicin suggests that gentamicin

*Corresponding author: Mailing address: Department of Microbiology, CSM Medical University (Former KGMC), Lucknow-226 003, India. Tel: +91-522-2257569, E-mail: jyotsna_govil@yahoo.co.in

Table 1. Distribution of *Enterococcus* spp. in various clinical specimens

Sample	No. of enterococci	*E. faecalis*	*E. faecium*	*E. avium*	*E. durans*	*E. dispar*	*E. casseliflavus*	*E. cecorum*	*E. hirae*
Urine	54	34	13	3	1	–	1	1	1
Blood	21	5	14	1	–	1	–	–	–
Pus	4	1	3	–	–	–	–	–	–
Vaginal swab	3	–	1	1	–	–	1	–	–
Catheter tip	3	1	2	–	–	–	–	–	–
Tissue	1	1	–	–	–	–	–	–	–
Total	86	42	33	5	1	1	2	1	1

Table 2. Antibiotic sensitivity of enterococci by disc diffusion method

Species	A		T		Km*		Gm*		Sm*		Cp		E		C		Va		Te	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
E. faecalis (*n* = 42)	39	3	10	32	27	15	38	4	34	8	15	27	15	27	14	28	38	4	42	–
E. faecium (*n* = 33)	22	11	9	24	18	15	31	2	27	6	16	17	11	22	16	17	33	–	33	–
Others (*n* = 11)	7	4	4	7	3	8	9	2	7	4	2	9	5	6	4	7	11	–	11	–
Total (*n* = 86)	68	18	23	63	48	38	78	8	68	18	33	53	31	55	34	52	82	4	86	–

A, ampicillin; T, tetracycline; Km, kanamycin; Gm, gentamicin; Sm, streptomycin; Cp, ciprofloxacin; E, erythromycin; C, chloramphenicol; Va, vancomycin; Te, teicoplanin; S, sensitive; R, resistant.
*Aminoglycoside disks used are high level.

should maintain a synergistic effect when combined with cell wall-active agents in the treatment of serious enterococcal infections. The frequent occurrence of HLR to kanamycin confirms that amikacin is a poor choice when attempting to achieve synergistic therapy. Higher HLAR rates of 73.3 and 68% have been reported from other Indian centers and may reflect antibiotic usage practices (8,9).

All enterococci were sensitive to teicoplanin, whereas 4 (4.65%) were resistant to vancomycin by the disc diffusion method. The MIC for vancomycin for the 3 isolates was 32 μg/ml and the MIC of 1 isolate was 256 μg/ml by both the agar and broth dilution method. All were recovered from urine and identified as *E. faecalis*. All four patients were female, aged 30 to 65 years, and all four developed nosocomial urinary tract infection during a prolonged hospital stay. Based on the results of the MIC studies and their susceptibility to teicoplanin, the vancomycin-resistant enterococci (VRE) isolates appear to be of the vanB phenotype. Of these four VRE, three were sensitive to high-level gentamicin; all were sensitive to high-level streptomycin and two were resistant to kanamycin. Thus, high-level doses of aminoglycosides in combination with β-lactams may be helpful in the treatment of VRE. Studies (7,10,11) on VRE isolation from India have reported an incidence ranging from 1 to 5.6%, with both type A and B being observed, indicating that VRE is slowly emerging as a serious problem. Of the 86 enterococcal isolates in the present study, only one produced β-lactamase (positive by all three methods), which was *E. faecalis* isolated from blood, and by routine disc diffusion it was sensitive to ampicillin. This isolate was sensitive to vancomycin. β-lactamase production among enterococci is not very common (12). But what is important is that this can be missed on routine disc susceptibility testing due to the inoculum effect (5,13).

REFERENCES

1. Udo, E.E., Al-Sweih, N., Phillips, O.A., et al. (2003): Species prevalence and antibacterial resistance of enterococci isolated in Kuwait hospitals. J. Med. Microbiol., 52, 163-168.
2. Gilbert, D.N. (2000): Aminoglycosides. p. 307-336. *In* G.L. Mandell, J.E. Bennett and R. Dolin, (ed.), Principles and Practice of Infectious Diseases. vol. 1. Basic Principles in the Diagnosis and Management of Infectious Diseases. 5th ed. Churchill Livingstone, Philadelphia.
3. Facklam, R.R. and Collins, M.D. (1989): Identification of *Enterococcus* species isolated from human infections by conventional test scheme. J. Clin. Microbiol., 27, 731-734.
4. Swenson, J.M., Ferraro, M., Sahm, D., et al. (1995): Multilaboratory evaluation of screening methods for detection of high-level aminoglycoside resistance in enterococci. National Committee for Clinical Laboratory Standards Study Group on Enterococci. J. Clin. Microbiol., 33, 3008-3018.
5. Miles, R.S. and Amyes, S.G.B. (1996): Laboratory control of antimicrobial therapy. p. 151-178 *In* Collee, J.G., Marmion, B.P., Fraser, A.G., et al. (ed), Practical Medical Microbiology. 14th ed. Churchill Livingstone, New York.
6. Gordon, S., Swenson, J.M., Hill, B.C., et al. (1992): Antimicrobial susceptibility pattern of common and unusual species of enterococci causing infection in the United States. J. Clin. Microbiol., 30, 2373-2378.
7. Mathur, P., Kapil, A., Chandra, R., et al. (2003): Anti-microbial resistance in *Enterococcus faecalis* at a tertiary care center of northern India. Indian J. Med. Res., 118, 25-28.
8. Mohanty, S., Jose, S., Singhal, R., et al. (2005): Species prevalence and antimicrobial susceptibility of enterococci isolated in a tertiary care hospital of North India. Southeast Asian J. Trop. Public Health, 36, 962-965.
9. Randhawa, V.S., Kapoor, L., Singh, V., et al. (2004): Aminoglycoside resistance in enterococci isolated from paediatric septicaemia in a tertiary care hospital in north India. Indian J. Med. Res., 119, 77-79.
10. Taneja, N., Rani, P., Emmanuel, R., et al. (2004): Significance of VRE from urinary specimens at a tertiary care centre in northern India. Indian J. Med. Res., 119, 72-74.
11. Ghoshal, U., Garg, A., Tiwari, D.P., et al. (2006): Emerging vancomycin resistance in enterococci in India. Indian J. Pathol. Microbiol., 49, 620-622.
12. Toledo, C., Perez, M.E., Rocchi, M., et al. (2004): [Isolation of enterococci species causative of infections and sensitivity to antimicrobial drugs] Rev. Argent. Microbiol., 36, 31-35 (in Spanish).
13. Murray, B.E. (1990): The life and times of the enterococcus. Clin. Microbiol. Rev., 3, 46-65.