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Short Communication

Antimicrobial Resistance of Enterococcal Blood Isolates at a Pediatric Care Hospital in India

Lata Kapoor*, V. S. Randhawa and Monorama Deb

Department of Microbiology, Lady Hardinge Medical College, New Delhi 110001, India

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SUMMARY: Enterococci are one of the leading causes of nosocomial infections. In recent years, enterococci have become increasingly resistant to a wide range of antimicrobial agents. From April to October 2001, a study was conducted to speciate and determine the antimicrobial susceptibility of 50 isolates of enterococci from bacteremic children. These isolates were tested for antimicrobial susceptibility to the commonly used antibiotics. Screening for vancomycin resistance was done by the agar screen method, and the results were confirmed by determining the minimum inhibitory concentration (MIC) using the agar dilution method. It was observed that 33 isolates were *Enterococcus faecium*, followed by *E. faecalis* (10), *E. durans* (4), and *E. dispar* (3). Seventy-two percent of strains were resistant to ampicillin, 46% to amoxicillin + clavulanic acid, 72% to ciprofloxacin, 54% to doxycyclin, and 74% to erythromycin. Sixty-six percent of isolates showed high-level gentamicin resistance and 42% showed high-level streptomycin resistance. Four strains showed raised MIC to vancomycin (8 μg/ml). It was concluded that multidrug resistant *E. faecium* is emerging as an important agent of bacteremia in children.

Once regarded as a bacterial genus of little consequence, enterococci, in the past several years, have rapidly emerged as important nosocomial and community acquired pathogens. These organisms can cause serious invasive infections including endocarditis, bacteremia, meningitis, and urinary tract infections. *Enterococcus faecalis* and *Enterococcus faecium* are responsible for the majority of human infections, and until recently, *E. faecalis* was the predominant enterococcal species isolated from clinical samples (1,2). Enterococci are intrinsically resistant to a wide range of antibiotics that most notably include beta-lactams and aminoglycosides, frequently used to treat infections with Gram-positive cocci. In addition, enterococci have the ability to acquire resistance to antimicrobial agents through transfer of plasmids and transposons and chromosomal exchange or mutations (3). Further, acquisition of vancomycin resistance leaves few options for therapeutic management (1).

The present study was undertaken to speciate and determine the antimicrobial susceptibility of enterococci isolated from blood cultures in children.

Enterococci isolated in pure culture from blood specimens of pediatric in-patients from April to October 2001 were included in the study. The isolates were identified by colony morphology, Gram stain, catalase reaction, growth on bile esculin agar, esculin hydrolysis, and tolerance to 6.5% NaCl. Species identification was done using the conventional test scheme (4). Antimicrobial susceptibility to amoxicillin + clavulanic acid (20 μg + 10 μg), ampicillin (10 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycyclin (30 μg), erythromycin (15 μg), penicillin (10 U), and vancomycin (30 μg) (Hi-media laboratories, Mumbai, India) was determined by the Kirby-Bauer disc diffusion method and interpreted according to the guidelines of National Control Committee for Laboratory Standards (NCCLS) (5).

High-level aminoglycoside resistance (HLAR) was determined by the agar dilution screening method (6). In brief, 10^6 CFU of the test strain was inoculated onto two brain heart infusion (BHI) agar plates containing 500 μg/ml of gentamicin and 2,000 μg/ml of streptomycin, respectively, and incubated at 37°C for 24 h for gentamicin and 48 h for streptomycin. The presence of more than 1 colony or a haze of growth denoted resistance. Vancomycin resistance was determined by both disc diffusion and the agar screen method. BHI agar supplemented with 6 μg of vancomycin per ml were inoculated with 10^6 CFU of the test strain and interpreted after 24 h as for HLAR. The minimum inhibitory concentration (MIC) of vancomycin was determined by the agar dilution method (7). *E. faecalis* ATCC 29212 was included as control.

A total of 50 isolates of *Enterococcus* spp. were recovered from blood cultures. Eighteen isolates were from patients in the age group of 0 - 28 days, 12 from those 29 days to 1 year, 16 from those 2 to 5 years, and 4 from those 6 to 10 years. Thirty isolates were from the non-intensive care units (non-ICU), 7 from the nursery, 9 from pediatric ICU, and 4 from the neonatal ICU. The following species were identified: *E. faecium* (n = 33), *E. faecalis* (n = 10), *E. durans* (n = 4), and *E. dispar* (n = 3).

All the isolates were resistant to penicillin. Thirty-six (72%) strains were resistant to ampicillin and 23 (46%) to amoxicillin + clavulanic acid, 37 (74%) to erythromycin and 27 (54%) to doxycyclin. Ciprofloxacin resistance was seen in 36 (72%) isolates (Table 1). HLAR and vancomycin resistance for the different species of enterococci isolated are depicted in Table 2.

MIC of 2 isolates of *E. faecalis* and 1 of *E. faecium* moderately susceptible to vancomycin by disc diffusion test were determined as 8 μg/ml (sensitive = $\leq$ 4 μg/ml, intermediate = 8 - 16 μg/ml, resistant = $\geq$ 32 μg/ml) (5). MIC of 8 μg/ml was observed in another strain of *E. faecium* that was sensitive by disc diffusion method. These four isolates were also detected by the agar screen method. The isolates were from the pediatric ICU (1), the non-ICU wards (2) and the nursery (1).

*Corresponding author: Mailing address: 128, Jailakshmi Apartments, 59 I. P. Extension, Delhi, India. PIN-110092. Tel: +91-11-22449751, E-mail: latakapoor@yahoo.co.in

Table 1. Antimicrobial resistance of Enterococcal isolates (n = 50)

Antimicrobial agent	*E. faecium* (n = 33)		*E. faecalis* (n = 10)		*E. dispar* (n = 4)		*E. durans* (n = 3)	
	No.	(%)	No.	(%)	No.	(%)	No.	(%)
Ampicillin	23	(70)	7	(70)	3	(75)	3	(100)
Amoxicillin + clavulanic acid	14	(42)	4	(40)	3	(75)	2	(67)
Chloramphenicol	0	(0)	0	(0)	0	(0)	0	(0)
Ciprofloxacin	22	(67)	7	(70)	4	(100)	3	(100)
Doxycyclin	17	(51)	4	(40)	3	(75)	3	(100)
Erythromycin	24	(73)	6	(60)	4	(100)	3	(100)
Penicillin	33	(100)	10	(100)	4	(100)	3	(100)

Table 2. High-level aminoglycoside and vancomycin resistance of Enterococcal isolates (n = 50)

Organism (No. of isolates)	No. (%) of isolates showing resistance to			
	HLGR	HLSR	HLGR + HLSR	Vancomycin
E. faecium (33)	20 (61)	13 (39)	12 (36)	0
E. faecalis (10)	8 (80)	5 (50)	5 (50)	0
E. dispar (4)	3 (75)	2 (50)	2 (50)	0
E. durans (3)	2 (67)	1 (33)	1 (33)	0

HLGR, high-level gentamicin resistance; HLSR, high-level streptomycin resistance.

Studies from various parts of India (8-10) and elsewhere (11,12) have shown *E. faecalis* as the predominant species isolated in humans. A study of hospitalized patients from the United States reported *E. faecalis* to be two times more common than *E. faecium* among blood culture isolates (12). A multicenter study in Colombia reported 82% of enterococcal isolates as *E. faecalis* (11). A study from North India reported 56% urinary enterococcal isolates as *E. faecalis*, while 2 studies from South India reported 85% and 87% of clinical isolates of enterococci to be *E. faecalis* (9,10). In our study, the predominant enterococcal isolate was *E. faecium*, which is in concurrence with a recent report from India that described 81% isolates as *E. faecium* (13). Iwen et al. have also reported an increase in *E. faecium* isolates from 12.9 to 36.3% over a period of 8 years during 1987 to 1995 (14).

Multidrug-resistant enterococci are being increasingly reported from all over the world. Many studies have also demonstrated that *E. faecium* is comparatively more resistant than *E. faecalis* (10,11). However, in our study, resistance rates for ampicillin, amoxicillin + clavulanic acid, ciprofloxacin, and penicillin were comparable in *E. faecium* and *E. faecalis*. *E. faecium* showed higher rates of resistance to erythromycin and doxycyclin, while *E. faecalis* showed higher rates of resistance to aminoglycosides. Chloramphenicol showed the highest activity towards all isolates. Arias et al. reported 11% chloramphenicol resistance among *E. faecalis* and 0% among *E. faecium* isolates (11).

Indian studies have reported vancomycin resistance in 0-5% of enterococci (8-10,13). Gray and George reported 15% of colonizing enterococcal strains in a pediatric hospital to be vancomycin-resistant. In the present study, disc diffusion and agar screen method were used to detect vancomycin resistance (15). On comparing the MIC values, the agar screen method detected all 4 isolates with MIC 8 μg/ml, whereas the disc diffusion method placed one of these isolates in the susceptible category.

Iwen et al. reported vancomycin resistance in 22% of blood isolates of *E. faecium* as compared to 0% in *E. faecalis* (14). A Colombian study showed 39% of *E. faecium* and 5% of *E. faecalis* to be vancomycin-resistant (11). In the present study, two isolates each of *E. faecalis* and *E. faecium* showed raised MIC values for vancomycin.

In conclusion, *E. faecium* was observed as the predominant isolate from enterococcal bacteremia in children at a pediatric hospital in India. Enterococci revealed an alarming rate of resistance to the standard antimicrobial agents used for therapy and raised MIC values to vancomycin. The importance of rational use of antimicrobials in patient management and infection control is stressed.

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