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Antimicrobial susceptibility pattern of vancomycin resistant enterococci to newer antimicrobial agents

Sir,

Enterococci are recognized as opportunistic pathogens and are natural inhabitants of the oral cavity, gastrointestinal tract (GIT) and the female genital tract in both humans and animals[1]. They have emerged as important nosocomial pathogens[2]. There are two main species - *Enterococcus faecalis* and *E. faecium* responsible for human enterococcal infections[3]. The most frequent infections caused by these organisms include urinary tract infections, intra-abdominal and intra-pelvic abscesses. These are increasingly being isolated from bacteraemia and meningitis cases mainly from hospitalized patients[4].

The emergence of resistance to the most common anti-enterococcal antibiotics which include the β-lactam antibiotics like ampicillin, aminoglycosides and most importantly glycopeptides like vancomycin besides being inherently resistant to many others like cephalosporins and clindamycin has made the treatment of these infections a real challenge for clinicians[5]. With the increase in emergence of resistance in enterococci to vancomycin, treatment of these infections has become difficult especially in serious infections[6]. Since options for the treatment of patients with vancomycin resistant enterococci (VRE) are very limited, this study was aimed to assess the potential usefulness of compounds, which have come into recent use.Newer antibiotics such as linezolid, daptomycin and tigecycline have shown good *in vitro* activity against VRE[7]. Quinupristin-dalfopristin (Q/D) is another agent that has potent *in vitro* activity against *E. faecium* but poor activity against *E. faecalis*[8].

This study was conducted in the department of Microbiology, Government Medical College and Hospital, Chandigarh, India. In this study, the *in vitro* activity of vancomycin, teicoplanin, tigecycline, daptomycin, linezolid and quinupristin/dalfopristin has been evaluated against 75 non-repeat clinical isolates of vancomycin resistant *E. faecalis* (60) and *E. faecium* (15) by MIC (minimum inhibitory concentration) testing with Epsilometer test (E-test) method (E-test, AB Biodisk, Solna, Sweden). These 75 VRE isolates were obtained over a period of three years (2009-2011) from various samples namely, urine (56), blood (8) and pus (11). All these isolates were identified as *Enterococcus* according to standard methods and species identification was done using the conventional test scheme[9]. Initially the vancomycin resistant isolates were collected based on disc diffusion results as per Clinical and Laboratory Standards Institute (CLSI) guidelines using vancomycin disc (30 µg)[10]. All culture media, antibiotics discs and standard strains of bacteria used in this study were procured from Hi-media Laboratories Pvt. Ltd., Mumbai, India. *E. faecalis* ATCC 29212 and *E. faecalis* ATCC 51299 were used for quality control. E-test strips of vancomycin, teicoplanin, tigecycline, daptomycin, linezolid and quinupristin/dalfopristin were obtained from AB BioDisk, Solna, Sweden. E-test to daptomycin was done on Mueller-Hinton agar supplemented with 50 mg/l calcium (Difco, USA) due to daptomycin's dependence on calcium. MIC values were interpreted according to the CLSI guidelines[10] except for tigecycline for which EUCAST was followed[11].

Based on the MIC values for glycopeptides - vancomycin and teicoplanin, in our study majority of strains (49 *E. faecalis* and 15 *E. faecium*) belonged to the vanA resistance phenotype having high level vancomycin and teicoplanin resistance (MIC values being in the range of 64 to 256 µg/ml). We had eight *E. faecalis* isolates of van B type having variable levels of vancomycin resistance but were susceptible to teicoplanin (MIC values being in the range of 64 to

128 µg/ml for vancomycin and 0.064-0.50 µg/ml for teicoplanin). Three *E. faecalis* isolates had vancomycin MIC as 64 and teicoplanin MIC to be 4.0, 4.0, and 8.0 µg/ml, so probably these were van D type. Van D-type strains are characterized by moderate levels of resistance to both vancomycin and teicoplanin. Earlier reports from India have shown mainly vanA and vanB phenotypes[12].The phenotypic classification of VRE in to various types is solely based on the vancomycin and teicoplanin breakpoints and is not a reliable method, and has some limitations also. Earlier report has shown that mutations in van B strains can exhibit resistance to teicoplanin and such strains become phenotypically indistinguishable from van A resistant phenotypes[13]. These strains need to be confirmed by molecular characterization.

A number of relatively new agents that possess clinical data and ultimately clinical utility in the treatment of more serious infections due to VRE have been studied[7]. There is limited information reported from India[14]. In our study, all our isolates had MIC for linezolid within susceptibility range, MIC values ≤ 2 µg/ml except for one *E. faecium* showing linezolid MIC to be 4 µg/ml which is intermediate susceptibility. A study from India has shown 100 per cent susceptibility of VRE to linezolid[14]. Yasliani *et al*[15] reported two isolates of *E. faecium* resistant to linezolid from Tehran with MIC 32 µg/ml. Susceptibility of VRE to linezolid was shown to decrease to 83 per cent six months after inclusion of linezolid on the hospital antibiotic policy[16]. A study from India showed daptomycin to be the most active agent against VRE, highlighting the importance of the drug as an excellent therapeutic option[17]. We also found all VREs to be 100 per cent susceptible to daptomycin (MIC ≤ 4.0 µg/ml). A surveillance of US hospitals showed that more than 99.5 per cent of VRE isolates were susceptible to daptomycin[18-21]. But empiric daptomycin therapy for VRE infections should be used with caution and be based on susceptibility data[19]. Daptomycin resistance in enterococci was observed in a previously sensitive *E. faecalis* isolate, while on therapy[21,22]. Quinupristin-dalfopristin is a streptogramin antibiotic active only against *E. faecium*. Resistance to quinupristin/dalfopristin has been reported in 1.3-2.4 per cent of patients with VRE[23]. We found three isolates of *E. faecium* resistant to Q/D(MIC=4.0 µg/ml) (Table). Quinupristin/dalfopristin is advocated for vancomycin-resistant *E. faecium* infections in critically ill patients with serious underlying diseases[23]. In our study, all enterococcal isolates were found to be

Table. Distribution of MIC values for various antibiotics in vancomycin resistant enterococci isolates

Species (n)	Vancomycin			Teicoplanin			Daptomycin			Tigecycline			Linezolid			Quinupristin-Dalfopristin*		
MIC	50	90	Range (µg/ml)	50	90	Range (µg/ml)	50	90	Range (µg/ml)	50	90	Range (µg/ml)	50	90	Range (µg/ml)	50	90	Range (µg/ml)
E. faecalis 49, *E. faecium* 15	64	128	64-256	32	192	12-256	1.5	2.0	0.064-3.0	0.023	0.125	0.016-0.19	1.0	2.0	0.75-4.0	0.75	3.0	0.25-4.0
													1 *E. faecium* intermediate MIC = 4.0 µg/ml			3 *E. faecium* resistant MIC = 4.0 µg/ml		
E. faecalis 8	8	64	6-128	0.094	0.38	0.064-0.5	1.5	2.0	0.064-3.0	0.023	0.125	0.016-0.19	1.0	2.0	0.75-4.0	0.75	3.0	0.25-4.0
E. feacalis 3	16	48	16-128	4.0	8.0	2-16	1.5	2.0	0.064-3.0	0.023	0.125	0.016-0.19	1.0	2.0	0.75-4.0	0.75	3.0	0.25-4.0

MIC_{50} and MIC_{90} - MIC at which 50 and 90 per cent of the isolates are inhibited, respectively.
*Tested only for 15 *E. faecium*

susceptible to tigecycline (MIC≤ 0.25 µg/ml), which was in agreement with a study from south India[24]. Cases of *E. faecalis* isolates with MIC 6 µg/ml for tigecycline have also been described[25]. A study from Korea showed high resistance to all the newer drugs and out of all tigecycline was found to be an effective drug[26]. Of all these antibiotics considered, daptomycin is the only bactericidal drug while linezolid, tigecycline and quinupristin/dalfopristin are bacteriostatic drugs. All have their side effects also[27]. Further, linezolid, tigecycline, and daptomycin, have activity against both vancomycin-resistant *E. faecalis* and *E. faecium,* whereas quinupristin-dalfopristin has activity against *E. faecium* only. Daptomycin is found to be inhibited by pulmonary surfactant so should not be used for pneumonias[28].

Vancomycin still remains the mainstay of treatment for serious enterococcal infections, if the strain is found susceptible. However, with the emergence of resistance to vancomycin other antibiotics like linezolid, quinupristin-dalfopristin, tigecycline and daptomycin can also be considered. The data on local patterns of susceptibility of VRE to newer antimicrobial agents can help in guiding the treatment of these pathogens.

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