ANTIBIOTIC OPTIONS FOR ENTEROCOCCUS FAECALIS INFECTIONS

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ABSTRACT

Objective: Escalating resistance of enterococci to many antimicrobials poses a major therapeutic challenge; vancomycin-resistant enterococci (VRE), in particular, exhibit variation in resistance patterns, demanding tailored drug prescription. Hence we screened the *in vitro* sensitivity profiles of 112 local clinical isolates using 13 antibiotics for their possible therapy options biased by the site of infection.

Setting: Specimens yielding enterococci referred by local hospitals (n=103) and private physicians (n=09) were processed during a two-year period ending September 2005 at Dr. Essa's Lab in Karachi, Pakistan.

Methods: Consecutive *Enterococcus faecalis* strains cultured from urine (n=78), pus (n=18), blood (n=12), HVS (n=2) and ascitic fluid (n=2) were challenged *in vitro* with locally available antibiotics using the standard disc diffusion method; MICs of one VRE isolate were also performed.

Results: Vancomycin (99.1%) and teicoplanin (99.1%), followed by nitrofurantoin (97.3), fosfomycin (91.0%) and chloramphenicol (78.5%), were more effective than pipericillin-tazobactam (72.3%), ampicillin (54.4%), meropenem (45.5%) and ciprofloxacin (35.7%). Gentamicin and cotrimoxazole offered ineffective zones of inhibition. Only a single VRE strain, also teicoplanin-resistant, was encountered.

Conclusion: Increasing drug resistance of enterococci warrants concern and the search for possible therapeutic options prejudiced by local patterns of resistance and the site of infection. Vancomycin, teicoplanin, nitrofurantoin and chloramphenicol are also significantly effective on our current isolates and fosfomycin, typically considered a urinary drug, but encouraged by reports of its successful use in diverse sites, is discussed as a possible alternative.

KEY WORDS: Enterococci, VRE, Treatment options, Nitrofurantoin, Fosfomycin.

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INTRODUCTION

Enterococci comprise a significant portion of the normal intestinal flora, with some also being found on the skin, in oropharyngeal and vaginal secretions and in the perineal area.[1] Nevertheless, they have become increasingly important nosocomial pathogens in recent years as a result of increased incidence of resistance to many antimicrobials.[2]

Enterococcal infections most commonly occur in the urinary tract, but the organism also crops up in bacteremia, wound infections, intra-abdominal abscesses, infective endocarditis and infrequently, meningitis.[3] The intrinsic or relative resistance of the isolates to aminoglycosides, cephalosporins and cotrimoxazole, and the ability to acquire resistance to erythromycin and tetracycline is well known.[4] Ampicillin was the drug of choice for enterococcal UTI, but resistance to this agent has been rapidly emerging. Also, the fluoroquinolones are said to be marginally

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effective on enterococci, clindamycin is generally considered to be inactive at clinically achievable levels, and the action of cotrimoxazole is compromised *in vivo* due to endogenous sources of folate.[2]

The recent disturbing trait to emerge is indifference to vancomycin[5] necessitating the search for alternative drugs[6] and the exploitation of various synergistic combinations of selected antibiotics. While deep tissue and bloodstream infections have been traditionally treated with ampicillin-gentamicin, or with vancomycin-gentamicin, high level aminoglycoside resistance has encouraged alternative therapy. A beta-lactam with daptomycin has been employed,[7] as has quionopristin/dalfopristin.[8] But a review of 69 cases of VRE found that none of the 42 different combinations of antimicrobials used were absolutely effective,[9] which illustrates the extent of the problem in handling relevant clinical infections.

The final prescription, then, is prejudiced by the drug-sensitivity profile of local isolates, as well as the site of the enterococcal infection involved. Hence, our study screening 112 consecutive clinical isolates was designed to fathom the extent of multidrug-resistance of enterococci in our environment to easily available antibiotics, and thereby suggest possible options and limitations of drug prescription.

MATERIAL AND METHODS

A total of 103 isolates identified as *Enterococcus faecalis* from specimens submitted for culture by city hospitals, and 09 referred by private physicians, were processed on sheep-blood agar (Merck, Hamburg) at Dr. Essa's Lab which has its branches in key areas of Karachi Metropolis, during a 2-year period ending September, 2005. The isolates from urine (n=78), pus (n=18), blood (n=12), HVS (n=2) and ascitic fluid (n=2) also grew in broth containing 6.5% NaCl incubated at 35C for 3 days, fermented aesculin on Nutrient agar with 4% bile (Oxoid, UK), and were arabinose negative, pyruvate and sorbitol positive on API-20 STREP strips (BioMerieux, France). The patients (n=112) included adults of both sexes aged 19-72, a male neonate and a 6 year-old boy.

The following available antimicrobial agents (n=9) were obtained as PDM Epsilometers (AB Biodisk, Solna, Sweden) for Etest MIC estimation: ampicillin, chloramphenicol, ciprofloxacin, cotrimoxazole, erythromycin, gentamicin, nitrofurantoin, teicoplanin, and vancomycin. Isolated colonies of the single VRE isolate from a 24hr plate were homogenized in suspension medium to achieve 0.5 McFarland turbidity and spread on Mueller Hinton agar (Oxoid, UK). Etest strips were promptly positioned using a 6-strip template, the plates incubated at 35C for 24hrs, and the inhibition intersection in the ellipses recorded. Isolates with MIC breakpoints of >16ug/mL for ampicillin and >08ug/mL for vancomycin were considered to be resistant; nitrofurantoin susceptibility was taken as <32 ug/mL. *E.faecalis* ATCC 29212 was used as the control strain.

Sensitivity screening with commercial discs (Oxoid, UK) included the above nine antibiotics along with doxycycline (30ug), fosfomycin (50ug), meropenem (10ug) and pipericillin–tazobactam (110ug) were performed on Mueller-Hinton agar (Oxoid, UK) using the standard disc diffusion technique.[10] However, for gentamicin, along with the usual 10ug disc, an additional 120ug disc was added to check for high-level aminoglycoside resistance, with the resultant zone diameter <06mm considered resistant, and >10mm sensitive.[11] Results of ampicillin tests (disc content: 10ug, with zones <16mm= resistant and >17mm= sensitive) also predicted the susceptibility to amoxicillin and pipericillin. For testing against vancomycin, a disc of 30mg was used and the zones of inhibition measured after an incubation time of 24hrs using transmitted light.[11] Presence of any growth within the zone indicated possible resistance, and MICs of the implicated isolate were determined.

RESULTS

Comparison of disc diffusion zone diameters: Results (Table-I) indicate that vancomycin and

Table-I: Disc contents and percentage of 112 isolates susceptible

Drug	*Disc content (ug)*	*No. Resistant*	*% Sensitive*
Vancomycin	30	1	99.1
Teicoplanin	30	1	99.1
Nitrofurantoin	300	3	97.3
Fosfomycin	50	10	91.0
Chloramphenicol	30	24	78.5
Pipericillin-tazobactam	110	31	72.5
Ampicillin	10	51	54.4
Meropenem	10	61	45.5
Ciprofloxacin	05	72	35.7
Gentamicin	10	86	23.2
Cotrimoxazole	25	89	20.5
Erythromycin	15	90	19.6
Doxycycline	30	92	17.8

teicoplanin were significantly effective on all but one of the 112 *E.faecalis* isolates tested. The single VRE strain, encountered in the urine of an elderly hospitalized male, was unaffected by the 30ug vancomycin disc and was also indifferent to teicoplanin. All the other isolates were sensitive to vancomycin, and "Intermediate" vancomycin resistance was also not seen. Nitrofurantoin and fosfomycin inhibited 109 and 102 of the isolates respectively, followed in effectiveness by chloramphenicol and pipericillin-tazobactam. Ampicillin, meropenem and ciprofloxacin offered unsatisfactory inhibition zones. Dalbamycin, daptomycin, linezolid, pristinamycin, ramoplanin and quinopristin/dalfopristin discs and Etest Epsilometers were not available in the local market for evaluation.

MICs of the VRE isolate and related sensitivity profiles: Table-II illustrates the Etest MIC sensitivity profile of the single VRE strain encountered. It showed "low level" resistance to vancomycin (08ug/mL) and was resistant to ampicillin, ciprofloxacin, erythromycin, gentamicin and cotrimoxazole. It was also indifferent to teicoplanin (MIC >32ug/mL), a drug which was effective on all the other strains (MICs <2ug/mL). However, it was sensitive to chloramphenicol and nitrofurantoin in Etest MIC evaluation, and in addition also to the standard fosfomycin disc which gave an excellent zone of inhibition (>20mm) in the disc-diffusion assessment. Also, the VRE isolate was unaffected by routine discs of doxycycline, meropenem and pipericillin-tazobactam which afforded poor inhibitory zones well below the required resistance cut-off diameters of <12mm, <13mm and <17mm respectively.

DISCUSSION

Illness caused by the genus Enterococcus (notably *E. faecalis*, which accounts for almost 80% of all infections)[1] include, among others, UTI and bacteremia. In our study, the isolate was grown mainly from urine (69.6%), followed by pus and blood. All the 112 isolates were challenged with 13 representative drugs irrespective of the site of infection to ascertain their general susceptibility profiles in our environment where "over-the-counter" drug vending and empirical prescribing abound.

Vancomycin insusceptibility is an acquired resistance mediated by transposons or plasmids in bacteria, and these can develop in hospital wards that regularly use the drug and initiate serious infections. The single VRE encountered in our study was grown from the urine of an elderly male following cardiac bypass surgery and catheterization, suggesting that the mechanism of genetic exchange was likely at play in his sickbay.

While we confirmed only one VRE strain among our isolates, noteworthy in reviewing the literature was that the Aga Khan University Hospital clinical lab in Karachi reported seven VRE among inpatients in 1997, none in outpatients,[12] and again none among both

Table-II: MIC estimates of the Vancomycin resistant Isolate

Drug	*MIC (ug/mL)*	*Result*
Ampicillin	128	Resistant
Chloramphenicol	08	Sensitive
Ciprofloxacin	08	Resistant
Erythromycin	>16	Resistant
Gentamicin	1,024	Resistant
Nitrofurantoin	<32	Sensitive
Cotrimoxazole	>32	Resistant
Teicoplanin	>32	Resistant
Vancomycin	08	Resistant (Low level)

inpatients and outpatients in two subsequent susceptibility profile cards distributed to local hospitals and diagnostic labs as information updates that covered all their isolates encountered during Jan-Jun 2001, and between Jul-Dec 2001.

The intrinsic ruggedness of enterococci also confers an unusual ability to acquire resistance to such diverse groups of drugs as aminoglycosides, beta-lactams, macrolides, fluoroquinolones and tetracyclines.[2,8] Accordingly, ampicillin, ciprofloxacin and erythromycin, along with cotrimoxazole, doxycycline and meropenem performed poorly, as did gentamicin. Though not indicated, we included erythromycin and doxycycline profiles as a note in particular for dermatologists who empirically prescribe these drugs.

Nitrofurantoin affected all but 3 of the 112 isolates, suggesting that it could be a drug of choice for treating enterococcal UTI. Indeed, nitrofurantoin levels in urine are said to reach concentrations of 200-400 ug/mL, which are well above the MICs of susceptible uropathogens, and in one report was said to be active on all 300 enterococcal_isolates from a hospital, a third of which were resistant to vancomycin, substantiating its effectiveness *in vivo*.[13]

While nitrofurantoin is primarily a urinary antiseptic, chloramphenicol can be employed for treating diverse infected sites in the body; it influenced as many as 78.5% of our strains *in vitro* including the VRE isolate, and may well be noted as a possible choice for general single-drug therapy. Others have suggested daptomycin,[14] dalbavancin,[15] and ramoplanin[16] especially for VRE strains, but these drugs are hardly available in Pakistan, and, being expensive, often beyond reach of the unaffording patient.

Also exorbitant in cost are combination drugs which include mixtures of cell wall agents, of daptomycin and gentamicin,[7] and of quinopristin/dalfopristin.[8] Linezolid, usually considered for treating Methicillin-resistant *Staph.aureus,* has also been suggested as a therapeutic option for VRE.[17] Although these agents are possibly appropriate for most enterococcal infections, *in vitro* testing of isolates is desirable to determine whether an alternate therapy is necessary.

Fosfomycin, known to have a wide spectrum of action which includes *E.faecalis, E.coli* and other gram-negative pathogens, is said to be useful even as a 3gm cachet for single-dose treatment of UTI, but, like nitrofurantoin, is not advised for systemic infection.[17] However, fosfomycin is available in capsule, suspension, and injectable forms, and recently has been reported by diverse authors to be effective in treating neonatal septicemia,[18] diabetic foot syndrome,[19] CSF infection[20] as well as urethritis, UTI, enterocolitis, osteomyelitis, chronic otorrhoea and meningitis.[21] Moreover the drug is said to cure patients with enteric fever either as a single agent[22] or in association with chloramphenicol or ampicillin,[23] and also, in synergy with ciprofloxacin, is active on pseudomonas strains in cystic fibrosis,[24] making the counsel that it should not be used for treating systemic infections a debatable one. Furthermore, fosfomycin has been reported to be active on VRE,[25] and has also affected as many as 91% of our isolates. Judging from such encouraging performance, the drug is a possible candidate for therapy, irrespective of site, and warrants clinical evaluation.

While the treatment of multidrug-resistant enterococcal infection remains controversial and undefined, current therapies should be best based on the local pattern of resistance. These organisms are not highly infectious by most measures, but can cause serious human disease. They are also well adapted for survival and persistence in the site of infection and on inanimate hospital surfaces.[6] Instruments used in patient care such as stethoscopes and blood glucose monitors may also be contaminated with nosocomial strains. Logically the way to reduce these thorny infections may lie not in the development of new antibiotics, but rather in early detection and the prudent use of antimicrobials. Accordingly the first step is to discourage clinicians from using last-ditch drugs like vancomycin promiscuously.

REFERENCES

1. Moellering RC Jr. Emergence of *Enterococcus* as a significant pathogen. Clin Infect Dis 1992; 14:1173-6.
2. Ardino RC, Murray BE. Enterococcus: antimicrobial resistance. In: Mandell GL, Douglas RG Jr, Bennett JE (Eds) Principles and Practice of Infectious Diseases (3rd edition), New York: Churchill Livingstone; 1990. Update Vol 2: 4.
3. Sharma A, Mong MS, Minamoto G. *Enterococcus faecalis*: An unusual cause of meningitis in HIV-infected patients. AIDS Read 2002; 12:540-2.
4. Kaplan AH, Gilligan PH, Facklam RR. Recovery of resistant Enterococci during Vancomycin prophylaxis. J Clin Microbiol 1988: 26:1216-8.
5. Shlaes DM. Vancomycin-resistant bacteria. Infect Control Hosp Epidemiol 1992; 13:193-4.
6. Hayden MK, Harris AA. Confronting antibiotic resistance in enterococcal infections. Contemp Intern Med 1994; 6:7-17.
7. Leclercq R, Bingen E, Su CH. Effects of combinations of beta-lactams, daptomycin, gentamicin, and glycopeptides against glycopeptide-resistant enterococci. Antimicrob Agents Chemother 1991; 35:92-8.
8. Moellering RC Jr, Cerwinka SL. Early clinical results with quinopristin/dalfopristin for the treatment of bacteremia due to resistant gram-positive bacteria. In: Zinner SH, Young LS, Acar JF, Neu HC, editors. Expanding Indications for the new Macrolides, Azalides, and Streptogramins. New York: Marcel Dekker; 1997:173-6.
9. Chernow JA. Studies expand fund of epidemiologic data on VRE. Infect Dis News 1994; 1-5.
10. Bauer AW, Kirby WMM, Sherris JC. Antibiotic susceptibility testing by a standardized single disc method. Am J Clin Pathol 1966; 45:493-6.
11. Malik N, Karamat KA, Butt T. Antimicrobial sensitivity testing for Enterococci by disk diffusion technique. Inf Dis J Pak 1998; 5:53-4.
12. AKUH Clinical Lab Antibiotic Sensitivity Report Jan-June 1997. J Pak Med Assoc 1998; 48(1):24.
13. Zhanel GG, Hoban DJ, Karlowsky JA. Nitrofurantoin is active against vancomycin-resistant Enterococci. Antimicrob Agents Chemother 2001; 45:324-6.
14. Sader HS, Streit JM, Fritsche TR, Jones RN. Antimicrobial activity of daptomycin against multidrug-resistant Gram-positive strains collected worldwide. Diagn Microbiol Infect Dis 2004; 50:201-4.
15. Seltzer E. Dalbavancin bests vancomycin for Gram-positive bacteremia. Clin Infect Dis 2005; 40:374-80.
16. Goosens H, Jabes D, Rossi R, et al. European survey of vancomycin-resistant enterococci in at-risk hospital wards and *in vitro* susceptibility testing of Ramoplanin against these isolates. J Antimicrob Chemother 2003; 52: 5-12.
17. Jacobs RA, Guglielmo BJ. Anti-infective Chemotherapetic & Antibiotic Agents. In: Tierney LM Jr, McPhee SJ, Papadakis MA, eds. Current Medical Diagnosis & Treatment. 44th edition. New York, Lange Medical Books/McGraw-Hill, 2005; p.1531.
18. Chico RE. Antibiotic treatment of respiratory sepsis. Antibiot Chemother 1976; 21:115-6.
19. Legat FJ, Maier A, Dittrich P, et al. Penetration of fosfomycin into inflammatory lesions in patients with cellulitis or diabetic foot syndrome. Antimicrob Agents Chemother 2003; 47 (1):371-4.
20. Pfausler B, Spiss H, Dittrich P. Concentrations of fosfomycin in the cerebrospinal fluid of neurointensive care patients with ventriculostomy-associated ventriculitis. J Antimicrob Chemother 2004; 53 (5):848-52.
21. Rodriguez A, Gallego A, Olay T, Mata JM. Bacteriological evaluation of fosfomycin in clinical studies. Chemotherapy 1977; 1:247-58.
22. Ali, SMI. Use of fosfomycin in treatment of Enteric fever. Abstract, 8th Annual Dow Medical College and Civil Hospital Symposium 1990.
23. Figueroa J, Baquero G, Otal C, Rodriguez A. Treatment of typhoid fever with fosfomycin alone and in association with chloramphenicol or ampicillin. Chemotherapy 1977; 23 (1):365-77.
24. Figueredo VM, Neu HC. Synergy of ciprofloxacin with fosfomycin *in vitro* against Pseudomonas isolates from patients with cystic fibrosis. J Antimicrob Chemother 1988; 22: 41-50.
25. Allerberger F, Klare I. *In vitro* activity of fosfomycin against vancomycin-resistant enterococci. J Antimicrob Chemother 1999; 211-7.