Scand J Infect Dis 35: 12–14, 2003

Taylor & Francis
healthsciences

Antimicrobial Susceptibility of Vancomycin-Resistant Enterococcus faecium: Potential Utility of Fosfomycin

NABIN K. SHRESTHA[1,2], JIMMY D. CHUA[2], MARION J. TUOHY[2], DEBORAH A. WILSON[2], GARY W. PROCOP[2], DAVID L. LONGWORTH[1], CARLOS M. ISADA[1] and GERRI S. HALL[2]

From the [1]Department of Infectious Diseases and [2]Section of Clinical Microbiology, The Cleveland Clinic Foundation, Cleveland, Ohio, USA

The increasing prevalence of vancomycin-resistant enterococcal (VRE) infections has necessitated a search for drugs that are effective in treating these infections, and a need to determine whether currently available antimicrobials are effective. 75 consecutive clinical isolates of vancomycin-resistant Enterococcus faecium (VRE faecium) (40 blood and 35 urine isolates) isolated over 1 y at the Cleveland Clinic Foundation were tested for susceptibility to linezolid, quinupristin–dalfopristin, fosfomycin and nitrofurantoin using the Etest®. The minimum inhibitory concentrations were read independently by 3 observers and compared, and a final reading was obtained by predetermined criteria. The proportion of isolates susceptible to linezolid, quinupristin–dalfopristin, fosfomycin and nitrofurantoin was 100%, 98.7%, 98.7% and 78.7%, respectively. No single isolate was resistant to more than 1 of the 4 drugs tested. Etest presented significant unexpected difficulties in testing for VRE faecium susceptibility to nitrofurantoin. Fosfomycin may be a useful alternative to linezolid and quinupristin–dalfopristin in the treatment of VRE infections in certain clinical situations, e.g. uncomplicated urinary tract infections. In addition, the use of fosfomycin could limit the use of newer agents, thus reducing the chance of development of further resistance in the enterococci.

G. S. Hall, PhD, Department of Clinical Pathology, The Cleveland Clinic Foundation, 9500 Euclid Avenue/L-40, Cleveland, OH 44195, USA (Tel. +1 216 444 5990, fax. +1 216 445 6984, e-mail. hallg@ccf.org)

INTRODUCTION

Therapeutic options for the treatment of vancomycin-resistant enterococcal (VRE) infections are limited. Enterococci can cause various types of infections, including urinary tract infections (UTI), intra-abdominal infections, line-related infections, cellulitis, bacteremia and endocarditis (1). Ampicillin and vancomycin, with or without aminoglycosides, are the drugs of choice for the treatment of infections caused by enterococci (1). Quinupristin–dalfopristin and linezolid represent newer agents that possess good activity against VRE; however, their cost can be a limiting factor. In the case of the former, the lack of an oral preparation is also a practical disadvantage. Furthermore, the use of alternative agents for VRE and other enterococci may help to minimize the prescribing of these newer agents and, thus, development of resistance against these. Most enterococcal isolates remain susceptible to nitrofurantoin (2), but there are limited susceptibility data on vancomycin-resistant strains. Fosfomycin also has good in vitro activity against enterococci (2), and there has been a report of a patient with VRE prostatitis successfully treated with fosfomycin (3). Nevertheless, susceptibility data on vancomycin-resistant strains are limited, and comparative studies with other antimicrobial agents have not been reported. This study compared the in vitro activity of fosfomycin and nitrofurantoin with that of linezolid and quinupristin–dalfopristin against vancomycin-resistant Enterococcus faecium (VRE faecium).

MATERIALS AND METHODS

75 consecutive clinical isolates of VRE faecium (40 blood and 35 urine isolates) from 75 patients were obtained. These had been isolated over a period of 1 y between 18 January 1999 and 17 January 2000 at the Cleveland Clinic Foundation. All of the vancomycin-resistant enterococcal isolates were E. faecium. They were speciated using the Vitek® GPI card (bioMerieux, Hazelwood, MO, USA) and vancomycin minimum inhibitory concentrations (MIC) were determined using the Vitek GPS-101 card (bioMerieux, Hazelwood, MO). Pulsed-field gel electrophoresis of the blood isolates of VRE faecium had previously shown no evidence of clonality (data not shown). Genetic analyses to determine the type of vancomycin resistance were not done.

The isolates were tested for susceptibility to linezolid, quinupristin–dalfopristin, fosfomycin and nitrofurantoin, using the Etest® (Biodisk, Solna, Sweden). The microorganisms were cultured on blood–agar plates and incubated for 18 h. A 0.5 McFarland suspension of the microorganism in 0.85% saline was inoculated into Mueller–Hinton agar. Etest strips were placed on the culture plates and the MIC read after 20 h, as instructed by the manufacturer. The Etest strips for fosfomycin contained glucose-6-phosphate and did not require supplementation of the compound in the culture medium. Staphylococcus aureus strain ATCC 29213 and Enterococcus faecalis ATCC strain 29212 were used as the quality-control organisms.

Since there is a potential for subjective differences in the reading of the MIC values, the MIC values were read independently by 3 observers. The independent readings were compared and a final reading was determined in the following manner: if 2 or 3 observers had identical MIC readings, that reading was taken as the final MIC. If all 3 readings differed, the median MIC reading was taken as the final MIC. The readings were tabulated and the MIC_{50} and MIC_{90} values determined. The breakpoint criteria to determine susceptibility were based on the National Committee for Clinical

 ISSN 0036-5548

DOI: 10.1080/0036554021000026985

Laboratory Standards (NCCLS) guidelines (4) whenever possible. The NCCLS does not provide breakpoint criteria for determining susceptibility of E. faecium to fosfomycin; instead, the breakpoint criteria for E. faecalis were used, which the NCCLS does provide. The NCCLS also did not provide breakpoint criteria for determining susceptibility of enterococci to linezolid at the time of the study; the breakpoint criteria recommended by the manufacturer of linezolid were used, and subsequent NCCLS guidelines recommended these same breakpoints for interpretations (5).

RESULTS

Table I shows the susceptibility of 75 VRE faecium isolates to fosfomycin, nitrofurantoin, linezolid and quinupristin–dalfopristin. All isolates were susceptible to linezolid. Fosfomycin and quinupristin–dalfopristin had good in vitro activity against VRE faecium, approaching 100%. Of the 75 isolates, 1 isolate each was resistant to quinupristin–dalfopristin, fosfomycin and nitrofurantoin. An additional 20% had intermediate susceptibilities to nitrofurantoin. Table II gives an estimate of the interobserver variability noted in reading the MIC values. With quinupristin–dalfopristin and fosfomycin, there was good correlation between the readings obtained by different observers, with at least 2 of the 3 observer readings giving an identical MIC approximately 99% of the time. There was less interobserver concordance with linezolid, but all isolates were found to be susceptible by all observers. Remarkable difficulty was encountered in reading the nitrofurantoin test strips, with any 2 observers agreeing on the MIC only 71% of the time. When individual observer MIC readings for nitrofurantoin were compared, a wide range of MIC values was noted, and 1 specific observer had a higher reading than the others 60% of the time. Although the latter did not contribute to the interobserver variability, the wide range of MIC readings made it abundantly clear that reading the nitrofurantoin test strips was a significant problem.

DISCUSSION

Fosfomycin was as effective as quinupristin–dalfopristin and linezolid, and more effective than nitrofurantoin in the inhibition of VRE faecium in vitro. The present fosfomycin susceptibility results are consistent with the findings of other studies. Perri et al. found that only 1 of 52 VRE faecium isolates were resistant to fosfomycin (6). Allerberger and Klare (7) found an MIC_{90} of 64 μg/ml for fosfomycin against different species of VRE (including E. faecium), and this was interpreted as being in the intermediately susceptible range based on breakpoint criteria by Andrews et al. (8). There are no NCCLS breakpoint criteria for determining the susceptibility of E. faecium to fosfomycin, but for E. faecalis, MIC values that are ≤ 64 μg/ml are interpreted as indicating susceptibility (4). In the present study, the MIC_{90} for fosfomycin against VRE faecium was 48 μg/ml, and 98.7% of the isolates had a MIC ≤ 64 μg/ml and would have been classified as susceptible using the NCCLS breakpoints for E. faecalis.

Linezolid and quinupristin–dalfopristin have been shown to have clinical efficacy in the treatment of VRE infections (9, 10). Although the current investigation did not find much resistance to quinupristin–dalfopristin, the SENTRY Antimicrobial Surveillance program found that only 83% of the VRE isolates in the USA were susceptible (11). Resistance to linezolid was not observed in this study, but such resistance has been described recently (12). These findings demonstrate the potential for increased resistance to these

Table I. *Susceptibility of the 75 isolates of vancomycin-resistant Enterococcus faecium to 4 drugs*

Drugs tested	Susceptible *n* (%)	Intermediate *n* (%)	Resistant *n* (%)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Linezolid[a]	75 (100)	0 (0.0)	0 (0.0)	1.0	1.5
Quinupristin–dalfopristin[b]	74 (98.7)	0 (0.0)	1 (1.3)	0.50	0.75
Fosfomycin[c]	74 (98.7)	0 (0.0)	1 (1.3)	32	48
Nitrofurantoin[b]	59 (78.7)	15 (2.0)	1 (1.3)	24	48

[a] Breakpoints used: ≤ 2.0 μg/ml, susceptible; 4.0 μg/ml, intermediately susceptible; ≥ 8.0 μg/ml, resistant.
[b] NCCLS breakpoint criteria used (4).
[c] Breakpoints used: ≤ 64.0 μg/ml, susceptible; 128.0 μg/ml, intermediately susceptible; ≥ 256.0 μg/ml, resistant.
MIC_{50}: the MIC at which 50% of the strains were susceptible; MIC_{90}: the MIC at which 90% of the strains were susceptible.

Table II. *Concordance of the minimum inhibitory concentration readings between the 3 observers for 75 isolates of vancomycin-resistant Enterococcus faecium*

Drugs tested	3 identical readings	2 identical readings	All discordant	≥ 1 identical reading
Linezolid	17 (22.7)	47 (62.7)	11 (14.7)	64 (85.3)
Quinupristin–dalfopristin	43 (57.3)	31 (41.3)	1 (1.3)	74 (98.7)
Fosfomycin	44 (58.7)	30 (40.0)	1 (1.3)	74 (98.7)
Nitrofurantoin	9 (12.0)	44 (58.7)	21 (28.0)	53 (70.7)

Data are *n* (%).

crucial drugs, and warrant their judicious use. Avoiding their use in situations where an alternative drug could be used would be a prudent approach to delay the development of resistance to their action.

A great deal of difficulty was encountered in reading the Etest strips for nitrofurantoin, owing to the lack of a clearly demarcated zone of inhibition. It is not clear why this phenomenon was observed. It is also not a known problem with the drug nitrofurantoin when tested against other organisms. Further experience will clarify whether this observation was a chance finding or is actually true. The difficulty encountered in reading the strips for nitrofurantoin raises questions about the validity of the Etest method as a measure of VRE susceptibility to that drug. With an acknowledgement of these limitations, it was found that 79% of the VRE faecium isolates were susceptible to nitrofurantoin, which is comparable to the 81% susceptibility of all enterococcal isolates in the USA in the SENTRY antimicrobial survey program (11). Susceptibility testing of these same isolates by the Vitek GPS-101 card revealed that only 64% were susceptible to nitrofurantoin (MIC ≤ 32 μg/ml; data not shown). It was concluded that nitrofurantoin was significantly less effective in vitro against VRE faecium than the other 3 drugs, and the Etest was not reliable for testing VRE faecium susceptibility to nitrofurantoin.

In summary, linezolid, quinupristin–dalfopristin and fosfomycin all had good in vitro activity against 75 isolates of VRE faecium. Fosfomycin is far less expensive than quinupristin–dalfopristin and linezolid (13), and can be administered orally. It has been used successfully to treat a patient with vancomycin-resistant enterococcal prostatitis (3). It is to be noted, however, that most of the data on the use of fosfomycin involves the treatment of uncomplicated UTI with single-dose therapy (14). Nevertheless, it may be a useful alternative to linezolid and quinupristin–dalfopristin for the treatment of VRE faecium UTI in some situations, thereby limiting the use of the other 2 drugs and the risk of emergence of resistance in the enterococci.

ACKNOWLEDGEMENT

The Etest® strips for linezolid were provided by Pharmacia & Upjohn.

REFERENCES

1. Moellering RC Jr. Enterococcus species, Streptococcus bovis, and Leuconostoc species. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, and Bennett's Principles and practice of infectious diseases, 4th edn. New York: Churchill Livingstone; 2000. pp. 2147–56.
2. Fuchs PC, Barry AL, Brown SD. Fosfomycin tromethamine susceptibility of outpatient urine isolates of Escherichia coli and Enterococcus faecalis from ten North American medical centers by three methods. J Antimicrob Chemother 1999; 43: 137–40.
3. Shrestha NK, Amud DA, Riebel JW, Goldman MP, Tomford JW. Treatment of a complicated vancomycin-resistant enterococcal urinary tract infection with fosfomycin. Infect Dis Clin Pract 2000; 9: 368–71.
4. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: Tenth International Supplement. Wayne, PA: National Committee for Clinical Laboratory Standards; 2000. M100–10.
5. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility testing: Twelfth International Supplement. Wayne, PA: National Committee for Clinical Laboratory Standards; 2002. M100–12.
6. Perri MB, Hershberger E, Ionescu M, Lauter C, Zervos MJ. In vitro susceptibility of vancomycin-resistant enterococci (VRE) to fosfomycin. Diagn Microbiol Infect Dis 2002; 42: 269–71.
7. Allerberger F, Klare I. In-vitro susceptibility of fosfomycin against vancomycin-resistant enterococci. J Antimicrob Chemother 1999; 43: 211–7.
8. Andrews JM, Baquero F, Beltran JM, Canton E, Crokaert F, Gobernado M, et al. International collaborative study on standardization of bacterial sensitivity to fosfomycin. J Antimicrob Chemother 1983; 12: 357–61.
9. Clemett D, Markham A. Linezolid. Drugs 2000; 59: 815–27, Discussion 828.
10. Murray BE. Vancomycin-resistant enterococcal infections. N Engl J Med 2000; 342: 710–21.
11. Low DE, Keller N, Barth A, Jones RN. Clinical prevalence, antimicrobial susceptibility and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program 1997–1999. Clin Infect Dis 2001; 32 (Suppl 2): S133–45.
12. Gonzales RD, Schreckenberger PC, Graham MB, Kelkar S, DenBesten K, Quinn JP. Infections due to vancomycin-resistant Enterococcus faecium resistant to linezolid. Lancet 2001; 357: 1179.
13. 2002 Drug topics red book. Montvale, NJ: Thomson Medical Economics; 2002. pp. 441, 551, 600.
14. Shrestha NK, Tomford JW. Fosfomycin: a review. Infect Dis Clin Pract 2001; 10: 255–60.

Submitted July 25, 2002; accepted October 22, 2002