

## Evaluation of errors during susceptibility reporting of glycopeptide antibiotics for enterococcal isolates on sole basis of widely used disk diffusion test

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### Abstract

In resource limited settings, very often susceptibility reports glycopeptide antibiotics are released only on the basis of disk diffusion test. Determination of MIC by either E-strips or microbroth dilution tests can be of paramount importance in checking the errors that may have incurred with the singular use of disk diffusion tests. Distribution of errors in Vancomycin and Teicoplanin susceptibility reporting by disk diffusion test was evaluated in comparison with MIC determination with reference method of E-strip. A total of 40 isolates of *Enterococcus* species were obtained during the study period. In vancomycin susceptibility reporting, very major errors, major errors and minor errors were observed in 33.3%, 5.4% and 5% instances respectively. For teicoplanin, major errors were observed in 5.3% of instances. E test can be used in conjunction with disk diffusion where resources are limited. We have observed that by using a combination of both disk diffusion and MIC determination by E-strip methods for glycopeptide antibiotics, a majority of reporting errors can be addressed.

### Introduction

Enterococci were previously considered commensal organisms of little clinical importance but have emerged as serious nosocomial pathogens responsible for infections of bloodstream, meninges, urinary tract, biliary tract, wounds and endocarditis.<sup>1</sup> Commonly used glycopeptide antibiotics of vancomycin and teicoplanin have a crucial role in the management of severe infections due to enterococci in cases where patients are resistant or allergic to beta-lactam group of antibiotics.<sup>2-4</sup> Plasmid-mediated resistance to these glycopeptide

antibiotics, vancomycin and teicoplanin, was first detected in 1986.<sup>5,6</sup> Resistant enterococcal strains responsible for colonization<sup>7-9</sup> or infection<sup>10</sup> have been isolated with increased incidence in current times.

*In vitro* Vancomycin and teicoplanin susceptibility can be determined by disk diffusion, agar dilution, E-test, broth microdilution and automated antimicrobial susceptibility testing methods.<sup>11</sup> The disk diffusion test is done by measuring the zones of growth inhibition that result when fixed concentrations of an antibiotic diffuse from impregnated disk onto an agar plate, which has previously been inoculated with the test organism. After proper incubation the inhibitory zones around the disk are measured and interpreted as resistant, intermediate and sensitive.

The E-test combines diffusion with the ability to establish a Minimum Inhibitory Concentration (MIC) or breakpoint result. It is based on diffusion of an antimicrobial gradient from coated strips onto an agar surface inoculated with the test organism. MIC value is recorded directly from a scale on the strip in terms of  $\mu\text{g}/\text{mL}$  at the point where zone of growth inhibition intersects the strip.<sup>12</sup>

In resource limited settings, very often the susceptibility reports to these glycopeptide antibiotics are released only on the basis of disk diffusion test. Determination of MIC by either E-strips or microbroth dilution tests can be of paramount importance in checking the errors that may have incurred with the singular use of disk diffusion tests. The use of E-strips is less labor intensive and can be easily incorporated in routine testing even in high volume centers with restricted logistics. This study was planned to evaluate the errors during susceptibility reporting of glycopeptide antibiotics on sole basis of widely used disk diffusion test.

### Material and Methods

This prospective study was conducted in the Department of Microbiology of a tertiary care hospital of Delhi over a period of six months extending from January 2022 to June 2022. All isolates of *Enterococcus* species obtained from wound aspirates were included in the study.

All isolates of *Enterococcus* were identified and tested for their antimicrobial susceptibility patterns according to Clinical Laboratories Standards Institute (CLSI) guidelines. Antimicrobial susceptibility to Vancomycin & Teicoplanin in enterococcal isolates were performed using two methods, Kirby-Bauer disk diffusion technique and E-test method of Minimum Inhibitory

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Concentration (MIC) testing. CLSI has defined Susceptible (S), Intermediate (I), and Resistant (R) breakpoints for Vancomycin against enterococci for both disk diffusion ( $\geq 17$  mm, S; 15-16 mm, I;  $< 14$  mm, R) dilution MIC testing ( $\leq 4$   $\mu\text{g}/\text{ml}$ , S; 8-16  $\mu\text{g}/\text{ml}$ , I;  $\geq 32$   $\mu\text{g}/\text{ml}$ , R) and for teicoplanin by disk diffusion ( $\geq 14$  mm, S; 11-13 mm, I;  $\leq 10$  mm, R) and dilution MIC testing ( $\leq 8$   $\mu\text{g}/\text{ml}$ , S; 16  $\mu\text{g}/\text{ml}$ , I;  $\geq 32$   $\mu\text{g}/\text{ml}$ , R).<sup>13</sup> E-test (Himedia Laboratories, Mumbai, India) was performed following CLSI guidelines.<sup>13</sup>

Standard Kirby Bauer disk diffusion method was also used for determining the susceptibility of the isolates to the commonly used antibiotics against *Enterococcus* spp. The antibiotic disks that were used to identify the susceptibility pattern of the *Enterococcus* spp. were ampicillin (10 µg), ciprofloxacin (5 µg), high level gentamicin (120 µg), tetracycline (30 µg), vancomycin (30 µg), teicoplanin (30 µg), linezolid (30 µg; Himedia Laboratories, Mumbai, India) and results were interpreted as per the latest CLSI guidelines.<sup>13</sup>

Distribution of errors in Vancomycin and Teicoplanin susceptibility reporting by disk diffusion test was evaluated in comparison with MIC determination with reference method of E-strip. Very major errors were defined as method in which the test result was susceptible and the reference method result was resistant. Major errors were defined as errors in which the test method result was resistant and the reference method result was susceptible, and minor

errors were defined as those in which either method reported a result as intermediate and the other method reported the result as susceptible or resistant.<sup>14</sup>

## Results

A total of 40 isolates of *Enterococcus* species were obtained during the study period. Table 1 shows the demographic profile of the patients with enterococcal isolates. Male to female ratio was 0.67, showing a female preponderance. Over 50% of patients belonged to 20-40 years age-group.

Out of 40 isolates, 38 isolates were *Enterococcus faecalis* and 2 isolates were *E. faecium*. Figure 1 depicts the distribution of susceptibility to commonly used antibiotics among *Enterococcus* spp. isolates. 92.5% of *Enterococcus* spp. isolates were susceptible to Vancomycin and 95% were susceptible to teicoplanin by disk diffusion

method. Tables 2 and 3 show the distribution of errors in Vancomycin and teicoplanin susceptibility reporting by singular use of disk diffusion method respectively. In vancomycin susceptibility reporting, very major errors, major errors and minor errors were observed in 33.3%, 5.4% and 5% instances respectively (Table 2). For teicoplanin, major errors were observed in 5.3% instances (Table 3).

## Discussion

Drug resistance has far reaching impacts on global healthcare. Various studies have reported widely prevalent drug resistance among gram positive as well as gram negative organisms.<sup>15,16</sup> In line with other studies, *Enterococcus faecalis* was predominant among enterococcal species in the present study.<sup>17,18</sup> Though widely used, issue of false vancomycin resistance reporting with disk diffusion method is a concern,<sup>19</sup> prompting several manufacturers to issue alerts regarding the detection of vancomycin and teicoplanin resistant organisms.

The current study reported very major, major and minor errors of 33.3%, 5.4% and 5% instances respectively in vancomycin susceptibility reporting by disk diffusion method. However, Tenover *et al.* observed

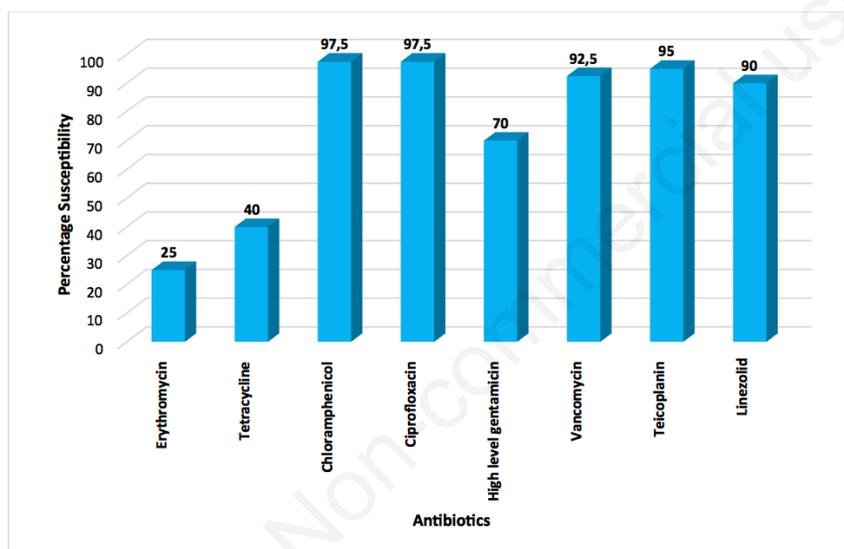


Figure 1. Distribution of susceptibility to commonly used antibiotics among *Enterococcus* spp. isolates.

Table 1. Demographic profile of patients with enterococcal isolates (n=40).

Age group	n (%)
0-10 years	2(5)
10-20 years	6(15)
20-30 years	13(32.50)
30-40 years	8(20)
40-50 years	3(7.5)
50-60 years	5(12.50)
>60 years	3(7.5)

Table 2. Distribution of errors in Vancomycin susceptibility reporting by singular use of disk diffusion method (n=40).

Method (no. reported)	No. of very major errors/no. of resistant isolates (%)	Enterococci (n = 40)	
		No. of major errors/no. of susceptible isolates (%)	No. of minor errors/total no. of isolates (%)
Disk diffusion	1/3 (33.3)	2/37 (5.4)	2/40 (5)

Table 3. Distribution of errors in teicoplanin susceptibility reporting by singular use of disk diffusion method (n=40).

Method (no. reported)	No. of very major errors/no. of resistant isolates (%)	Enterococci (n = 40)	
		No. of major errors/no. of susceptible isolates (%)	No. of minor errors/total no. of isolates (%)
Disk diffusion	0	2/38 (5.26)	0

no very major or major errors in their study while reporting and minor errors were limited to  $\leq 6\%$ .<sup>20</sup> The inception of intermediate and resistant breakpoints for vancomycin in enterococci (e.g., designating MIC of 8–16  $\mu\text{g}/\text{mL}$  as intermediate and 32  $\mu\text{g}/\text{mL}$  as resistant) would not address the high rates of very major errors as these breakpoints would still fail to capture several of the non-susceptible strains. Meanwhile, it would be difficult to lower the zone diameter for vancomycin to reclassify 17 mm (which currently is in the susceptible range) as intermediate unless adequate data is available that demonstrates a lack of clinical efficacy. Thus, optimizing the reading of these tests particularly disk diffusion and E-test for enterococci is necessary to improve the accuracy of vancomycin and teicoplanin susceptibility results. E-test is superior to the disc diffusion method in detecting resistance among enterococcal isolates against glycopeptide antibiotics of vancomycin and teicoplanin.<sup>20</sup>

Though the gold standard test for detection of resistance in *Enterococcus* spp. is broth dilution, its use in routine testing by manual antimicrobial susceptibility reporting is difficult. E test can be used in conjunction to disc diffusion in where resources are limited. In present study we have observed that by using a combination of both disk diffusion and MIC determination by E-strip methods for glycopeptide antibiotics, a majority of reporting errors can be inscribed. However, research involving wider population and large sample sizes is required to further back our findings.

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