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DETECTION OF ANTIMICROBIAL RESISTANCE AMONG ENTEROCOCCI ISOLATES BY VITEK SYSTEM

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ABSTRACT

The clinical significance of *Enterococci* is directly related to its antimicrobial resistance. Enterococci are inherently resistant to many antibiotics and can acquire resistance by mutation, or acquisition of plasmids or transposons. Fifty six Enterococci isolates from various clinical samples were identified to species level and their antimicrobial sensitivity pattern was determined by conventional method and Vitek system. Forty four (78.57%) were *Enterococcus faecalis*, nine (16.07%) were *Enterococcus faecium* and, three (5.36%) were identified as *Enterococcus avium*. Of the total isolates, 11(19.64%) were resistant to penicillin (MIC ≥16µg/ml). Among *E.faecium* isolates, 4(44.44%) were with MIC of 16 µg/ml for vancomycin and one isolate was resistant to both vancomycin (MIC ≥ 32µg/ml) and teicoplanin (MIC ≥32µg/ml). Out of 49 isolates from urine, 39(79.59%) were sensitive to nitrofurantoin (MIC ≤ 16µg/ml). The antimicrobial susceptibility testing by Vitek was concordant with the results of disk diffusion for penicillin and vancomycin. Two minor errors (discrepancy between results of reference method and test method, that differed by one interpretation category) were detected for erythromycin and nitrofurantoin and one minor error was detected for ciprofloxacin. Five very major errors (Isolates resistant by reference method, identified as susceptible by test method) were detected in teicoplanin resistance. Vitek analyser provides Enterococcus susceptibility data, significantly faster than conventional methods used in diagnostic laboratory.

KEYWORDS: *Vitek, Enterococci, Penicillin resistance, Glycopeptides resistance, Fluoroquinolone resistance.*

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INTRODUCTION

Enterococci as normal commensals of human gastrointestinal tract are gram positive facultative anaerobes and are considered as low grade pathogens of little clinical significance. The incidence of infection with these organism is on rise over the past decade. [1-2] Enterococci infection are caused by more than a dozen of Enterococcus species, but most clinical infections are due to *Enterococcus faecalis* (85-90%) and *Enterococcus faecium* (5 -10 %). The remarkable increase in the use of antimicrobials in clinical practice in the latter half of 20th century provided selective environment for these organisms to evolve by resistant determinants to multiple antibiotics. Their intrinsic resistance to several antibiotics, malleability of genomes, and the ability to recruit and disseminate antibiotic resistant determinants make these organisms a real challenge to clinicians in the 21st century.[3] Enterococci expresses low affinity Penicillin Binding Proteins (PBP s); Penicillin Binding Protein- 5 in *E.faecium* and Penicillin Binding Protein- 4 in *E.faecalis* that binds weakly to β-lactam antibiotics. Resistance against penicillin and ampicillin have been described in *E.faecalis* and *E.faecium* by β-lactamase production. Glycopeptide resistant enterococci produce altered penicillin binding precursors, render them unable to inhibit cell wall biosynthesis.[2] Enterococci exhibits moderate level of intrinsic resistance to aminoglycosides. High level resistance to aminoglycosides are due to aminoglycoside modifying enzymes, decreasing the binding to ribosomal targets. Enterococci can acquire high level resistance to quinolones through mutation of target genes.[3] Macrolide Lincosamide, Streptogramin B (MLSB) phenotypes are common in Enterococci due to the modification of 23 s rRNA target by methylase gene(ermB). Resistance to tetracyclines was described in enterococci by multiple genes, efflux pump and ribosomal protection.[3,4]Linezolid and quinopristin/dalfopristin are the two drugs approved by FDA to treat VRE infection. Resistance to linezolid has been reported in Enterococcal outbreaks. Daptomycin , a lipopeptide antibiotic showed *invitro* bactericidal activity against Vancomycin Resistant Enterococci, however failure have been reported after prolonged treatment. Tigecycline, a glycylcycline antibiotic exhibit broad spectrum activity against Vancomycin Resistant Enterococci.[3,5] VITEK is an automated method for rapid identification of bacteria and antimicrobial sensitivity testing.[6] VITEK system is easy to use and provide accurate results with less handling time as compared to conventional test procedure. Hence the present study was done to evaluate the performance of VITEK to identify Enterococci to species level and to detect their antibiotic resistance pattern.

MATERIALS AND METHODS

Identification of Enterococci and determination of antimicrobial susceptibility by conventional method

Fifty six isolates of Enterococci (44 Enterococcus *faecalis*, 9 *Enterococcus faecium*, 3 *Enterococcus avium*) from various clinical samples such as urine, pus, blood etc were inoculated onto Blood agar and Mac Conkey agar and incubated at 37 ^{0}C for 24 to 48 hours. The colonies were identified by gram staining, catalase test, bile esculin agar, 6.5% sodium chloride and heat tolerance test. Speciation of enterococci were done according to conventional test scheme (Facklam and Collin's). [7] Antimicrobial susceptibility on these isolates were determined by Kirby Bauer disk diffusion method on Muller Hinton agar and the results were interpreted as per Clinical Laboratory Standard Institute.[8] Penicillin (10units), Ciprofloxacin (5µg), Teicoplanin (30µg), High Level Gentamicin disc (120µg), Nitrofurantoin (300µg), Erythromycin (15µg), Tetracycline (30µg), Levofloxacin (5µg), Linezolid (30µg), Vancomycin (30µg) were used. *Enterococcus faecalis* 29212 was used as quality control strain. A zone diameter of ≤ 6mm was considered as resistant, 7-9 mm as intermediate and ≥10mm as sensitive for High Level Gentamicin.

Identification of Enterococci species by Vitek

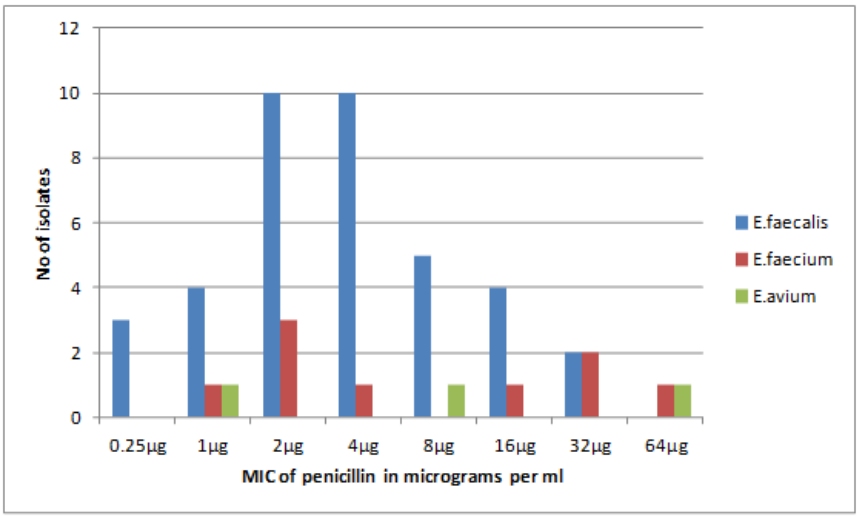
Turbidometrically controlled bacterial pure growth were suspended into sterile physiological saline and this suspension was used to fill Vitek 2 Compact system and antimicrobial susceptibility testing cards. For biochemical identification in Vitek system, the following parameters were used: Growth in 6.5 % Nacl, β-glucuronidase, trehalose, arginine dihydrolase, D-sorbitol, urease, raffinose, D-galactose, D-mannitol, sucrose, β-galactosidase, salicin, L-pyrrolidonyl arylamidase, D-xylose, D-maltose, methyl- β-D-glycopyranoside, D-ribose, α-glucosidase, α-mannosidase ,phosphatase etc.

Detection of Antimicrobial susceptibility by Vitek

Antimicrobial susceptibility testing of test organisms were determined according to manufacturer 's instruction. Minimum inhibitory concentration for the following antibiotics were tested: Penicillin, Erythromycin, Vancomycin, Teicoplanin, Ciprofloxacin, Tigecycline, Tetracycline, Nitrofurantoin, Linezolid, Daptomycin.[8]

RESULTS

Out of 56 Enterococci, 44(78.57%) were identified as *E.faecalis*, 9(16.07%) were *E.faecium*, 3(5.36%) were *E.avium* by both conventional method and Vitek analyser. Among 44 isolates of *E.faecalis*,40 were from urine samples, 3 from pus and 1 from body fluid. Out of 9 *E.faecium* isolates, 7 were from urine, 1 from pus and 1 from blood sample. Two isolates of *E.avium* were obtained from urine and 1 from pus sample. Of the total isolates, 45(80.36%) isolates were with MIC ≤ 8µg/ml (susceptible) and 11 (19.64%) isolates with MIC ≥16µg/ml (resistant) for penicillin.(Fig:1)



MIC of penicillin in micrograms per ml
Penicillin MIC: ≤ 8µg/ml (susceptible) ≥16µg/ml (resistant)

Figure 1
Detection of Minimum Inhibitory Concentration (0.25µg/ml to ≥64µg/ml for Penicillin by Vitek

Among the fluoroquinolones studied, 17(30.36%) isolates were sensitive (MIC ≤ 1µg/ml), 2 (3.57%) isolates were intermediate (MIC 2µg/ml), and 37(66.07 %) were resistant (MIC≥4µg/ml) to ciprofloxacin. 21(37.50%) isolates were with MIC ≤ 2µg/ml (sensitive), 4(7.14%) isolates with MIC of 4µg/ml (intermediate) and 31 (55.36%)isolates with MIC ≥8µg/ml (resistant) for levofloxacin.(Table:1)

Table 1
Detection of fluoroquinolone MIC among Enterococci by Vitek

Isolate	Ciprofloxacin (µg/ml)					Levofloxacin (µg/ml)					
	≤0.5 (S)	1 (S)	2 (I)	4 (R)	≥8 (R)	≤0.25 (S)	0.5 (S)	1 (S)	2 (S)	4 (I)	≥8 (R)
E.faecalis (44)	4	9	2	2	27	4	2	3	8	4	23
E.faecium (9)	2	-	-	-	7	-	2	-	-	-	7
E.avium (3)	2	-	-	-	1	-	2	-	-	-	1

Ciprofloxacin: MIC ≤ 1µg/ml (Susceptible) ≥4µg/ml (Resistant)
Levofloxacin : MIC ≤ 2µg/ml (Susceptible) ≥8µg/ml(Resistant)

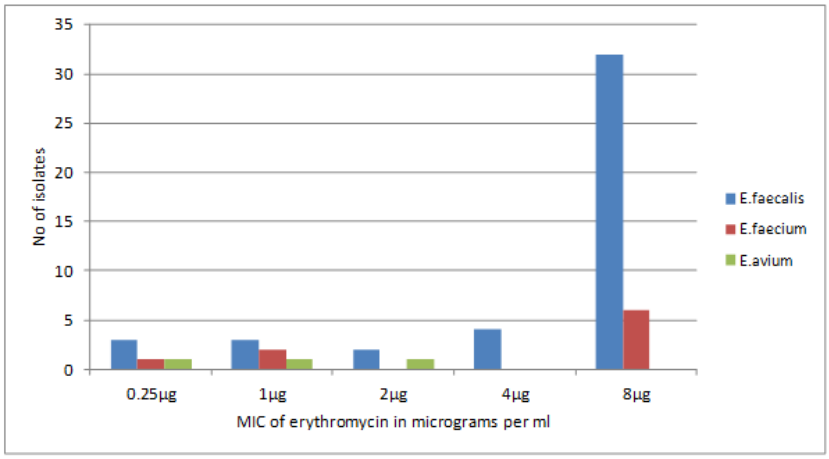
Among the glycopeptides studied, 50(89.29%) isolates were susceptible(MIC≤ 4µg/ml),5(8.93%) were intermediate (MIC 8-16µg/ml),and one isolate was resistant with MIC ≥32µg/ml for vancomycin. 55(98.21%) isolates were sensitive to teicoplanin with MIC of ≤ 8µg/ml and one isolate was resistant to teicoplanin (MIC ≥32µg/ml) (Table:2)

Table 2
Determination of Glycopeptide MIC by Vitek

Isolate	Vancomycin (µg/ml)						Teicoplanin (µg/ml)						
	≤0.5 (S)	1 (S)	2 (S)	4 (S)	8 (I)	≥32 (R)	≤0.5 (S)	1 (S)	2 (S)	4 (S)	8 (S)	16 (I)	≥32 (R)
E.faecalis (44)	4	30	9	-	1	-	34	3	1	6	-	-	-
E.faecium (9)	-	4	-	-	4	1	8	-	-	-	-	-	1
E.avium (3)	1	2	-	-	-	-	3	-	-	-	-	-	-

Vancomycin MIC: ≤ 4µg/ml (Susceptible), ≥32 µg/ml (Resistant)
Teicoplanin MIC : ≤ 8µg/ml (Susceptible), ≥32µg/ml (Resistant)

38(67.86%) isolates were resistant to Erythromycin with MIC ≥8µg/ml, 13(23.21%) were intermediate (MIC 1-4µg/ml) and 5(8.93%) were sensitive (≤ 0.5µg/ml) .(Fig :2)



MIC of Erythromycin in microgram per ml
Erythromycin MIC : ≤ 0.5µg/ml (Susceptible) ,1-4 µg/ml (Intermediate)
≥8µg/ml (Resistant)

Figure 2
Detection of Erythromycin MIC(≤ 0.25 µg/ml to≥8 µg/ml)by Vitek

Out of 49 urinary isolates ,39 (79.59%)were sensitive to nitrofurantoin with MIC ≤ 16µg/ml and 6(12.24%) isolates were resistant with MIC of 128µg/ml. Of the total isolates, 44 (78.57%) were resistant to tetracycline (MIC ≥ 16µg/ml) and 8 (14.29 %)were sensitive (≤ 0.5µg/ml). The most effective antibiotics tested were linezolid ,tigecycline and daptomycin. Of the total isolates,50(89.29%) isolates were sensitive(≤ 2µg/ml) to linezolid,49(87.50%) were susceptible (≤ 0.12µg/ml) to tigecycline and 35(62.50%) were sensitive (≤ 4µg/ml) to daptomycin. The results of antimicrobial susceptibility testing for Enterococci by Vitek was concordant with the results obtained by Kirby Bauer disk diffusion method for penicillin, vancomycin,linezolid,tetracycline. Minor errors were observed for nitrofurantoin, ciprofloxacin and erythromycin. Two isolates identified as intermediate to Erythromycin by disk diffusion method found to be resistant by Vitek analyser. one isolate detected by disk diffusion as resistant for ciprofloxacin ,was identified by Vitek as intermediate. For nitrofurantoin , two isolates were identified as intermediate by Vitek, of these one isolate was resistant and the other was detected as sensitive by Kirby bauer method. Very major error was observed in detecting teicoplanin resistance.5 isolates identified by Kirby Bauer s method as resistant were detected to be sensitive by Vitek. By vitek, β lactamase enzyme production was observed in 28 isolates of *Enterococcus faecalis* and 7 isolates of *E.faecium*. High Level Gentamicin synergy was detected in 14 isolates of *Enterococcus faecalis* and 2 isolates of *E.faecium.*

DISCUSSION

The usefulness of Vitek system for Enterococcus species identification and for determination of resistant phenotypes have already been reported.[9,10] Vitek provides enterococcal susceptibility data in approximately 8 hours which is significantly faster than conventional methods used in laboratory. A study by Chen *et al* [11] reported , vitek reduces turn over time for blood cultures in diagnostic laboratories. In the present study Vitek ID and AST system reported results within 6 hours and 8-10 hours respectively. Enterococci isolates, correctly identified by Vitek ID system were evaluated for antimicrobial susceptibility testing. VITEK showed accurate results in detecting resistance of Enterococcus to penicillin in our study. High level resistance to β lactams are observed in E.faecium due to overproduction of Penicillin binding proteins .As a result minimum inhibitory concentration for penicillins are typically 2-8µg/ml for E.faecalis and 8-16µg/ml for E.faecium.[2] As per CLSI guidelines, [8] resistance to β lactams can be tested by disc diffusion method using penicillin (10 units) and Ampicillin 10 µg disc with MIC interpretive criteria as <8 µg/ml (susceptible) and >16µg/ml (resistance) for both penicillin and ampicillin.Enterococci susceptible to penicillin are predictably susceptible to amoxicillin-clavulanate, ampicillin-sulbactam, amoxicillin, ampicillin, piperacillin and piperacillin-tazobactam for non β lactamase producing enterococci .In the present study, 38(86.36%) isolates of E.faecalis were with MIC <8 µg/ml and 6 (13.64%)isolates were with >16µg/ml for penicillin. Out of E.faecium isolates 5 were with MIC <8 µg/ml and 4 isolates with MIC of >16µg/ml. The use of Vitek analyser in the rapid identification of Enterococci and detection of penicillin resistance was reported in a study by I. Kobayashi et al. [12] The MIC of penicillin obtained by Vitek system showed good concordance with the reference method in a study reported by Hae Kyung Lee et al. [13] High level aminoglycoside resistance is most frequently mediated by aminoglycoside modifying enzymes and these mechanism abolishes the synergistic bactericidal activity of aminoglycosides in combination with cell wall active agents. [2] HLG synergy was detected in 16 isolates of Enterococci studied. Quinolone resistance in Enterococci is due to mutation in the quinolone resistance determining regions of the genes that encodes gyrase and topoisomerase IV. [2-3] In the present study, 35(62.50 %) isolates were resistant to ciprofloxacin (MIC ≥8µg/ml) and 31(55.36%) isolates to levofloxacin (MIC≥8µg/ml) The most common form of

acquired resistance to macrolides is production of enzymes that methylase a specific adenine in the 23S rRNA of 50 S ribosomal subunit which reduces binding affinity of macrolides to ribosomes. [14] 32 (72.73%) isolates of E.faecalis and six (66.67%) *E.faecium* isolate were resistant to Erythromycin (MIC ≥8µg/ml) in the present study. The Van B locus confers moderate to high level resistance to vancomycin(MIC 4-1000µg/ml) and most of these strains remain sensitive to teicoplanin invitro.[1] In our study ,43(97.73%) isolates of *E.faecalis* were sensitive (MIC ≤4µg/ml) to vancomycin and these isolates were also sensitive to teicoplanin. Among the *E.faecium,* one isolate was resistant to both vancomycin (MIC ≥32µg/ml) and teicoplanin (MIC ≥32µg/ml),4(44.44%) isolates were with MIC 16µg/ml and were sensitive to teicoplanin(MIC ≤8µg/ml). Van Den Braak et a al [9] have reported sensitivity of Vitek system to correctly distinguish resistance phenotype and for glycopeptides susceptibility testing for Enterococci. Azevedo *et al* [15] have evaluated the performance of Vitek for the identification and detection of vancomycin resistance in Enterococci. In contrast to the present study Kohner *et al* [10] have reported major errors by Vitek system to detect vancomycin resistance in Enterococci. The failure of Vitek system to detect teicoplanin resistance has been reported by Garcia-Garotte *et al.*[16] Daptomycin is useful for treatment of infections caused by multidrug resistant Gram positive strains .As a result of mutation in chromosomal genes, daptomycin resistance has been observed in isolates following therapy.[3] The present study showed 35 isolates of Enterococci sensitive to daptomycin with MIC ≤4µg/ml. Out of 40 isolates of *E.faecalis* from urine sample,36(90.00%) were susceptible to nitrofurantoin (MIC≤16µg/ml). Three out of seven, *E.faecium* isolates were susceptible to nitrofurantoin. Hence nitrofurantoin may be an effective treatment for urinary tract infection caused by Enterococci. A study conducted by Zhanel et al [17] have reported that nitrofurantoin is active against urinary isolates of *E.faecalis* and *E.faecium.* The antimicrobial susceptibility results obtained by Vitek for linezolid, tetracycline and vancomycin were concordant with the disk diffusion testing. In the present study, 50(89.29%) isolates were sensitive to linezolid (MIC ≤4µg/ml).Very major error (Isolates that were resistant by disk diffusion appeared to the susceptible by Vitek) was observed in detecting teicoplanin resistance in our study. A study by Van Den Braak *et al 2001* [9] reported major errors in detection of teicoplanin resistance in Enterococci containing van A gene. Minor errors were found in the susceptibility testing for ciprofloxacin, erythromycin and nitrofurantoin. Minor error is the discrepancy between results of the reference method (Disk diffusion) and test method (Vitek), that differed only by one interpretation category.Minor errors in Vitek AST results was observed in a study conducted by Williams Bouyer *et al.*[18] A study by Stefaniuk. E. et al[19] has reported high concordance of Vitek results with reference method for Enterococci. Minor error for Erythromycin by Vitek susceptibility testing was reported by I Kobayashi *et al.*[12]

CONCLUSION

Vitek is an automated susceptibility method for rapid bacterial identification and antimicrobial susceptibility testing. There was a significant reduction in handling time compared to conventional method for the identification of Enterococcus species. Vitek detects resistance to penicillin accurately and is sensitive to identify in resistance phenotypes in Enterococci. Hence, this method can be used to determine antimicrobial susceptibility of enterococci over conventional methods.

CONFLICT OF INTEREST

Conflict of interest declared none.

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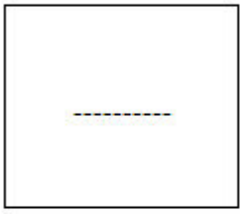

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