DIAGNOSTIC ACCURACY OF IN-HOUSE BIOCHEMICAL TESTING FOR IDENTIFICATION OF ENTEROCOCCUS SPECIES ISOLATED FROM VARIOUS CLINICAL SPECIMENSAGAINST VITEK 2 SYSTEM

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ABSTRACT

Objective: To determine the diagnostic accuracy of in-house biochemical testing for identification of enterococcus species isolated from various clinical specimens against gold standard i.e., automated Vitek 2 system. This study also includes the antimicrobial susceptibility testing of enterococci against various antimicrobials.

Study Design: Cross-sectional comparative study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from Apr 2017 to Mar 2018.

Methodology: A total of 218 isolates from various clinical specimens suspected to be *Enterococcus spp.* were checked by in-house biochemical testing including bile esculin, 6.5% NaCl and 1% arabinose and results were compared with Vitek 2 compact system. The frequencies were determined by both systems and antimicrobial susceptibility testing was performed by disk diffusion as per clinical and laboratory standards institute guidelines.

Results: Comparing the results of in-house testing with gold standard i.e., Vitek 2 system, the statistical data was calculated. Sensitivity turned out to be 100%, Specificity was found to be 68.75%. Positive and negative predictive values were 97.58% and 100% respectively. Accuracy turned out to be 97.71%.

Conclusion: The in-house biochemical testing can be quite a useful method for identification of enterococci in resource-limited settings. However, it requires overight incubation and cannot identify other enterococcal species and non-enterococcal species. Vitek 2 is an automated system that is easy-to-handle, provides a rapid and reasonably accurate identification of enterococci alongwith accurate AST results. Enterococcal isolates from various clinical specimens in our setup showed least resistance to linezolid, followed by teicoplanin and vancomycin. Nitrofurantoin and fosfomycin have less than 50% resistance for urinary isolates.

Keywords: Enterococcus spp, In-house biochemical testing, Vitek 2 system.

INTRODUCTION

Enterococci are part of the normal intestinal flora of humans and animals. They are considered important human pathogens. The genus Enterococcus includes more than 17 species, although only a few cause clinical infections in humans. Most of the times, these infections are difficult to treat because of the high rate of intrinsic and acquired resistance of enterococci to multiple antimicrobials[1,2]. *Enterococcus faecalis* and *Enterococcus faecium* are the most frequently isolated species from clinical specimens. About 90-95% of enterococcal infections in humans are caused by these two species and the remaining 5-10% are caused by other members of the genus[3].

The mainstay of the treatment of enterococcal infection over the years was penicillin with gentamicin due to their synergistic action. By 1979, resistance to high-level gentamicin was reported due to genetically acquired mechanisms. Today acquired resistance has rendered many of the circulating strains of enterococci resistant to other available therapeutic options as well. Presently many circulating strains are reported to have acquired resistance to most of the remaining therapeutic options including vancomycin and linezolid which are thought to be antibiotics of last resort in enterococcal infections[4,5]. *Vancomycin-resistant enterococci* (VRE) first appeared in

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Europe in the late 1980s. Nowadays, six types of acquired vancomycin resistance in enterococci are known; however, only Van A and to a lesser extent Van B are mainly prevalent[6]. VRE are resistant to most of the commonly used antibiotics, providing a selective advantage over other intestinal flora organisms thus posing a major therapeutic challenge[7].

The Vitek 2 system is an automated system that provides rapid and accurate identification and antimicrobial susceptibility testing (AST) results for most clinical isolates including *enterococci.* Biochemical reactions are used for identification purpose, and minimum inhibitory concentrations (MICs) are determined by applying an algorithm to the growth kinetics monitored by the Vitek 2 system[8-10].

The objective of this study is to determine the diagnostic accuracy of in-house biochemical testing for identification of Enterococcus species isolated from various clinical specimensagainst gold standard i.e., automated Vitek 2 system. This study also includes the antimicrobial susceptibility of enterococci against various antimicrobials.

METHODOLOGY

This cross-sectional comparative was carried out at the department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from April 2017 to March 2018.

A total of 218 isolates from various clinical specimens suspected to be *Enterococcus spp*. (gram positive cocci, catalase negative, Lancefield group D) were checked by in-house biochemical testing and Vitek 2 compact system.

The in-house tests included a set of 3 parameters.

Bile esculin agar [prepared by adding 5.0 grams of bile esculin agar base to 100 mL distilled water, then dispensed in individual bottles (5 mL each) in a slanting position to obtain agar with a slant] to differentiate group D and non-group D *streptococci* (group D *streptococci* and *enterococci* (formerly group D *streptococci*) cause esculin hydrolysis and are tolerant to the presence of bile, whereas non-group D streptococci do not). This provides a way to presumptively identify group D streptococci.

About 6.5% saline broth [prepared by adding 1.5 grams of nutrient broth and 6.5 grams of sodium chloride in 100 mL distilled water, then dispensed in individual bottles (5 mL each)] to differentiate salt-tolerant enterococci from group D *streptococci* (*enterococci* cause turbidity in the broth because they are salt-tolerant whereas group D *streptococci* do not).

1% arabinose [prepared by adding 1 gram arabinose to 100 mL buffered peptone water, 1 mL Andrade indicator is added to see for color change] to differentiate *Enterococcus faecium* (medium turns pink because of arabinose fermentation) from *Enterococcus faecalis* (does not ferment arabinose).

The in-house biochemical testing can help identify only 2 species of enterococci i.e., *E. faecalis* and *E. faecium*. The rest of the species cannot be identified. So the results of this in-house testing were compared with those of Vitek 2 compact, automated ID/AST instrument (bioMerieux Diagnostics). The antimicrobial susceptibility testing for the isolates was also performed against various antimicrobials via disk diffusion method, as per CLSI guidelines.

The Vitek 2 system (bioMérieux) is an automated system that consists of a filling-sealer unit, a reader-incubator, a computer control module, a data terminal, and a multicopy printer. The system detects bacterial growth and metabolic changes in the microwells of thin plastic cards by using a fluorescence-based technology. Different microwell cards contain antibiotics or biochemical substrates. We used the ID-GPC card of the Vitek 2 system for identification and the AST-P516 card of the Vitek 2 system for the antimicrobial susceptibility testing of *enterococci*.

Each organism suspension was prepared from the growth of pure cultures of bacteria cultivated for 18 to 24 h on blood agar. The suspensions were prepared in sterile saline (0.45% NaCl)

to a turbidity equivalent to that of a 0.5 Mc Farland standard. These suspensions were used for the inoculation of both cards (ID-GPC and AST-P516). The cards were manually situated, as were the suspensions, in plastic racks that were inserted in the Vitek 2 system's reader-incubator module (incubation temperature, 35.5°C). The cards were automatically filled by a vacuum device and were automatically sealed and subjected to a kinetic fluorescence measurement every 15 min. The results were interpreted by the ID-GPC database after an incubation period of 4h, and final results were obtained automatically after a minimum of 4h and a maximum of 15h of incubation. All cards used were automatically discarded in a waste container. The ID-GPC database contained data on the following species of *Enterococcus: E. faecalis, E. faecium, E. durans, E. avium, E. hirae, E. casseliflavus,* and *E. gallinarum*.

Table-I: The scheme of in-house biochemical testing for enterococcal identification.

Biochemical Parameters	Group D Streptococci	Enterococci		Non-Group D Streptococci
		Enterococcus Faecium	Enterococcus Faecalis	
Bile esculin (hydrolysis)	Positive	Positive	Positive	Negative
6.5% NaCl (turbidity)	Negative	Positive	Positive	Negative
1% arabinose (fermentation)	Negative	Positive	Negative	Negative

Ethics: Institutional Review Board (IRB) of Armed Forces Institute of Pathology Rawalpindi approved the study. IRB approval certificate number: FC-MIC16-4/READ-IRB/17/414.

RESULTS

Two hundred and eighteen isolates were included in the study, out of which 127 (58.3%) samples were from male patients and 91 (41.7%) samples were from female patients, with age of patients ranging from 13 to 69 years.

Isolates included in the study were from various clinical specimens as shown in table-II.

Table-II: Distribution of clinical specimens included in study.

Specimen	Number of Samples
Urine	81 (37.2%)
Pus	43 (19.3%)
Pus swab	29 (13.3%)
Stool	16 (7.4%)
Blood	15 (6.9%)
Fluid	10 (4.6%)
Tissue	9 (4.2%)
Drain fluid	5 (2.4%)
Cerebrospinal fluid	4 (1.9%)
Pleural fluid	4 (1.9%)
Bile	2 (0.9%)
Total	218 (100%)

Keeping Vitek 2 as gold standard, in-house biochemical testing was found to have a good diagnostic accuracy for enterococcal identification. Out of the total 218 isolates, Vitek 2 reported 202 as enterococci and 16 as *non-enterococci.* In-house biochemical testing reported 207 as *enterococci* and 11 as *non-enterococci*. Comparing the results of in-house testing with gold standard i.e., Vitek 2 system, the statistical data was calculated and found as follows:

Sensitivity: 100%

Specificity: 68.75%

Positive predictive value: 97.58%

Negative predictive value: 100%

Accuracy: 97.71%

Antimicrobial susceptibility testing was performed for the enterococcal isolates against various antimicrobials as per CLSI guidelines, using disk diffusion method. The resistance of isolates against various antimicrobials is shown in percentage in the figure.

DISCUSSION

In this study, we determined the diagnostic accuracy of in-house biochemical testing for identification of enterococci isolated from various clinical specimens. The in-house biochemical testing used 3 parameters i.e., bile esculin agar, 6.5% saline broth and 1% arabinose. These three parame-

ters were used for identification of the 218 isolates suspected to be enterococci on Gram reaction and morphology (gram positive cocci), catalase non-reactivity, and Lancefield grouping (group D).

Table-III: Comparison of results by in-house and Vitek 2 testing.

In-house Biochemical Testing		Vitek 2 System	
Enterococcus faecium	135 (62%)	Enterococcus faecium	107 (79.4%)
		Enterococcus faecalis	11 (8.2%)
		Enterococcus avium	8 (5.9%)
		Enterococcus casseliflavus	2 (1.5%)
		Aerococcus viridans	1 (0.7%)
		Enterococcus hirae	1 (0.7%)
		Enterococcus raffinosus	1 (0.7%)
		Kocuria kristinae	1 (0.7%)
		Lactococcus garvieae	1 (0.7%)
		Pediococcus pentosaceus	2 (1.5%)
Enterococcus Faecalis	72 (33%)	Enterococcus faecalis	56 (77.8%)
		Enterococcus faecium	14 (19.4%)
		Enterococcus gallinarum	2 (2.8%)
Non-Enterococci	11 (5%)	Pediococcus pentosaceus	4 (36.3%)
		Aerococcus viridans	3 (27.3%)
		Kocuria kristinae	2 (18.2%)
		Lactococcus garvieae	2 (18.2%)
		Vitek 2	
		Enterococci (202)	**Non-Enterococci (16)**
In-house biochemical testing	Enterococci (207)	202	5
	Non-enterococci (11)	-	11

Out of the total 218 isolates, the in-house biochemical testing reported:

1. 135 isolates (62%) to be *Enterococcus faecium*
2. 72 isolates (33%) to be *Enterococcus faecalis.*
3. 11 isolates (5%) to be *non-enterococci*

The 218 isolates were identified using the automated Vitek 2 system as gold standard along with the in-house biochemical identification. Vitek 2 system led to identification of 202 isolates as seven enterococcal species including *E. faecium, E. faecalis, E. avium, E. casseliflavus, E. gallinarum, E. hirae, E. raffinosus,* and 16 isolates turned out to be *non-enterococci.*

1. Enterococcus faecium : 121 (55.6%)
2. Enterococcus faecalis : 67 (30.8%)
3. Enterococcus avium : 8 (3.7%)
4. Pediococcus pentosaceus : 6 (2.7%)
5. Aerococcus viridans : 4 (1.8%)
6. Kocuria kristinae : 3 (1.4%)
7. Lactococcus garvieae : 3 (1.4%)
8. Enterococcus casseliflavus : 2 (0.9%)
9. Enterococcus gallinarum : 2 (0.9%)
10. Enterococcus hirae : 1 (0.4%)
11. Enterococcus raffinosus : 1 (0.4%)

The frequencies of various Enterococcus species in our setup thus turned out to be:

1. Enterococcus faecium : 121 (59.9%)
2. Enterococcus faecalis : 67 (33.2%)
3. Enterococcus avium : 8 (3.9%)
4. Enterococcus casseliflavus : 2 (1%)
5. Enterococcus gallinarum : 2 (1%)
6. Enterococcus hirae : 1 (0.5%)
7. Enterococcus raffinosus : 1 (0.5%)

The most frequently isolated species in our setup was found to be *Enterococcus faecium,* followed by *Enterococcus faecalis*. Sixteen isolates were found to be *non-enterococci,* however 5 out of

these were diagnosed as *enterococci* by in-house biochemical testing.

Antimicrobial susceptibility testing was performed for the *enterococcal isolates* against various antimicrobials as per CLSI guidelines, using disk diffusion method.

Penicillin susceptibility was performed for a total of 201 isolates. One hundred forty seven

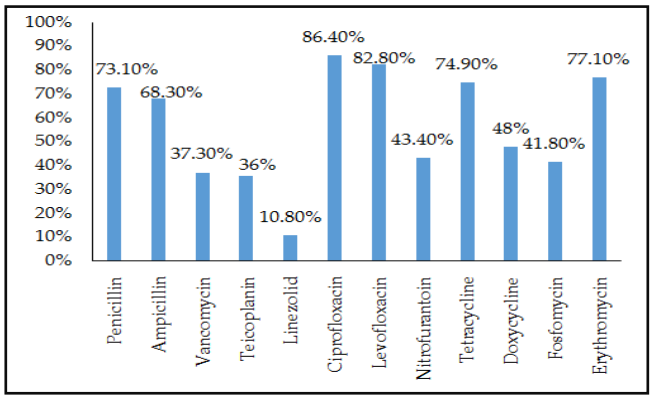

Figure: Resistance of isolates against various antimicrobials.

(73.1%) were found to be resistant and 54 (26.9%) were found to be susceptible. Ampicillin susceptibility was performed for 202 isolates which showed 138 (68.3%) isolates to be resistant and 64 (31.7%) to be susceptible. Thus, comparing the susceptibility of penicillin and ampicillin, out of the total 147 penicillin resistant isolates, 137 were resistant to ampicillin as well, but 10 isolates were susceptible to ampicillin; and all of the 54 isolates susceptible to penicillin were also susceptible to ampicillin. 93.2% of penicillin resistant isolates were resistant to ampicillin as well. A study conducted by Grayson *et al* showed a significant increase in resistance to penicillin and ampicillin during years[11]. Franz *et al* carried out a study on antibiotic resistance among *enterococci isolated* from food. This study showed that 45.8% of *E. faecium* strains were resistant to penicillin whereas none were resistant to ampicillin. In contrast, 12.8% of the *E. faecalis* strains were resistant to penicillin and 2.1% were resistant to ampicillin[12].

Vancomycin susceptibility was performed for 204 isolates, 128 (62.7%) turned out to be susceptible and 76 (37.3%) turned out to be resistant to vancomycin. Teicoplanin susceptibility was performed for 203 isolates, 130 (64%) turned out to be susceptible whereas 73 (36%) were found to be resistant to teicoplanin. Other than the species showing intrinsic resistance to vancomycin because of van C gene (*E. gallinarum, E. casseliflavus*)[13], susceptibility of vancomycin and teicoplanin was compared. Out of the total 76 vancomycin resistant enterococci (VRE), 71 (93.4%) were found resistant to teicoplanin as well, however 5 (6.6%) VRE isolates were found susceptible to teicoplanin. All of the vancomycin susceptible isolates were found susceptible to teicoplanin. The isolates which show resistance to both vancomycin and teicoplanin are likely to have van A gene whereas van B gene confers resistance to vancomycin and not to teicoplanin. Thus, van A seems to be much more prevalent in our setup than van B gene on the basis of susceptibility pattern, however this study does not include the molecular detection of vancomycin resistance genes, so this assumption cannot be confirmed. A study by O 'Driscoll *et al* showed that certain characteristics of VRE like its colonization strategy, persistence in the environment, and genome plasticity, make it a major nosocomial pathogen worldwide, typically in immunocompromised[14]. In a study by Matar *et al* conducted in cancer patients, it was seen that VRE fecal colonization was documented in 4.7% of patients screened[15].

Linezolid susceptibility was performed for 204 isolates. One hundred and eighty one (88.7%) were susceptible, 22 (10.8%) were resistant and 1 (0.5%) was intermediate. The results of a study conducted by Scheetz *et al* established an ecological link between linezolid consumption and increasing incidence of enterococci with decreased susceptibility to linezolid[16].

Ciprofloxacin susceptibility was performed for 198 isolates. One hundred seventy one (86.4%) were found to be resistant, 25 (12.6%) were susceptible and 2 (1%) were intermediate. Levofloxacin susceptibility was performed for 198 isolates. One hundred and sixty four (82.8%) were found to be resistant, 31 (15.7%) were susceptible

and 3 (1.5%) were intermediate. The susceptibility of cipro-floxacin and levofloxacin was compared for 195 isolates. Out of the total 170 ciprofloxacin resistant isolates, 162 (95.3%) were also resistant to levofloxacin, 6 (3.5%) were susceptible to levofloxacin, 2 (1.2%) were found to be intermediate. Twnty five ciprofloxacin susceptible isolates were also found to be susceptible to levofloxacin. Schouten *et al* showed that over 90% of *E. faecalis* isolates were susceptible to sparfloxacin, trovafloxacin, and moxifloxacin. The activities of these towards *E. faecium*, however, were much lower[17].

One hundred and ninety eight isolates were tested for nitrofurantoin susceptibility. Ninety One (46%) were found susceptible, 86 (43.4%) were resistant and 21 (10.6%) were intermediate. Zhanel *et al* tested the activity of nitrofurantoin against 300 isolates of *E. faecium, E. faecalis, and E. gallinarum*. No isolates tested were resistant to nitrofurantoin, including vancomycin-resistant isolates. This study concluded that nitrofurantoin may provide effective treatment of urinary tract infections caused by VRE[18].

E. faecalis isolates were tested for fosfomycin susceptibility. About 58.2% were found susceptible, 41.8% turned out to be resistant (however fosfomycin was reported only for the urinary isolates). Butcu *et al* conducted a study that showed 2.3% of *E. faecalis* strains to be resistant to fosfomycin[19].

Tetracycline susceptibility was performed for 199 isolates. One hundred fory nine (74.9%) were found to be resistant, 48 (24.1%) were susceptible and 2 (1%) were intermediate. Doxycycline susceptibility was performed for 198 isolates. Ninety five (48%) were found to be resistant, 68 (34.3%) were susceptible and 35 (17.7%) were intermediate. The susceptibility of tetracycline and doxycycline was compared for 196 isolates. Out of the total 147 tetracycline resistant isolates, 95 (63.8%) were also resistant to doxycycline, 35 (23.5%) were intermediate, and 17 (11.4%) were found to be susceptible. All the 47 tetracycline susceptible and the 2 tetracycline intermediate isolates were found susceptible to doxycycline. Reinert *et al* described antimicrobial susceptibility among bacterial isolates associated with hospital infections. In this study, tigecycline was found to be the only antimicrobial to maintain activity against all Gram-positive isolates including *E. faecium* and *E. faecalis*[20].

One hundred ninety two isolates were tested for erythromycin susceptibility. One hundred forty eight (77.1%) were found resistant, 34 (17.7%) were susceptible and 10 (5.2%) were intermediate.

CONCLUSION

The in-house biochemical testing can identify the 2 most frequent enterococcal species involved in human enterococcal infections (*E. faecium* and *E. faecalis*), therefore in resource-limited settings, can be quite a useful method for identification of *enterococci*. However, it requires overight incubation and cannot identify other enterococcal species and *non-enterococcal* species.

Vitek 2 is an automated system that is easy tohandle andprovides a faster (4 to 15 h) and reasonably accurate identification of the most commonly isolated Enterococcus species alongwith the rarely isolated species and gives accurate AST results. It improves the work flow of the clinical microbiology laboratory by significantly reducing the handling time.

Enterococcal isolates from various clinical specimens in our setup showed least resistance to linezolid, followed by teicoplanin and vancomycin. Nitrofurantoin and fosfomycin have less than 50% resistance for urinary isolates.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

REFERENCES

1. French G. Enterococci and vancomycin resistance. Clin Infect Dis 1998; 27(Suppl-1): S75-83.
2. Garcia-Garrote F, Cercenado E, Bouza E. Evaluation of a new system, VITEK 2, for identification and antimicrobial susceptibility testing of enterococci. Clin Microbiol Infect 2000; 38(6): 2108-11.
3. d'Azevedo PA, Siquiera I, Gugel J, Antunes ALS, Secchi C, Pasternak J, et al. Evaluation of the automated system Vitek2

for identification and antimicrobial susceptibility testing of Brazilian Gram-positive cocci strains. Braz J Infect Dis 2009; 13(2): 107-10.
4. Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and Vancomycin Resistant Enterococci: A Therapeutic Problem. J Clin Diagn Res 2017; 11(8): GC07-11.
5. Marothi YA, Agnihotri H, Dubey D. Enterococcal resistance--an overview. Indian J Med Microbiol 2005; 23(4): 214-19.
6. Werner G, Coque T, Hammerum A, Hope R, Hryniewicz W, Johnson A. Emergence and spread of vancomycin resis-tance among enterococci in Europe. Euro Surveill 2008; 13(47): 19046.
7. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant entero-cocci. Clin Microbiol Rev 2000; 13(4): 686-707.
8. Ligozzi M, Bernini C, Bonora MG, de Fatima M, Zuliani J, Fontana R. Evaluation of the VITEK 2 system for identification and antimicrobial susceptibility testing of medically relevant gram-positive cocci. J Clin Microbiol 2002; 40(5): 1681-86.
9. Fang H, Ohlsson AK, Ullberg M, Özenci V. Evaluation of species-specific PCR, Bruker MS, VITEK MS and the VITEK 2 system for the identification of clinical Enterococcus isolates. Eur J Clin Microbiol Infect Dis 2012; 31(11): 3073-77.
10. Eigner U, Schmid A, Wild U, Bertsch D. Analysis of the com-parative workflow and performance characteristics of the VITEK 2 and Phoenix systems. J Clin Microbiol 2005; 43(8): 3829-34.
11. Grayson M, Eliopoulos G, Wennersten C, Ruoff K, De Girolami P, Ferraro M, et al. Increasing resistance to beta-lactam anti-biotics among clinical isolates of Enterococcus faecium: a 22-year review at one institution. Antimicrob Agents Chemother 1991; 35(11): 2180-84.
12. Franz CM, Muscholl-Silberhorn AB, Yousif NM, Vancanneyt M, Swings J, Holzapfel WH. Incidence of virulence factors and antibiotic resistance among enterococci isolated from food. Appl Environ Microbiol 2001; 67(9): 4385-89.
13. Eliopoulos GM, Gold H. Vancomycin-resistant enterococci: mec-hanisms and clinical observations. Clin Infect Dis 2001; 33(2): 210-19.
14. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist 2015; 8(1): 217.
15. Matar MJ, Tarrand J, Raad I, Rolston KV. Colonization and infec-tion with vancomycin-resistant Enterococcus among patients with cancer. Am J Infect Control 2006; 34(8): 534-36.
16. Scheetz MH, Knechtel SA, Malczynski M, Postelnick MJ, Qi C. Increasing incidence of linezolid-intermediate or-resistant, van-comycin-resistant Enterococcus faecium strains parallels increa-sing linezolid consumption. Antimicrob Agents Chemother 2008; 52(6): 2256-59.
17. Schouten M, Voss A, Hoogkamp-Korstanje J. Antimicrobial sus-ceptibility patterns of enterococci causing infections in Europe. Antimicrob Agents Chemother 1999; 43(10): 2542-46.
18. Zhanel GG, Hoban DJ, Karlowsky JA. Nitrofurantoin is active against vancomycin-resistant enterococci. Antimicrob Agents Chemother 2001;45(1):324-6.
19. Butcu M, Akcay SS, Inan AS, Aksaray S, Engin DO, Calisici G. In vitro susceptibility of enterococci strains isolated from urine samples to fosfomycin and other antibiotics. J Infect Chemother 2011; 17(4): 575-78.
20. Reinert RR, Low DE, Rossi F, Zhang X, Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the asia/ pacific rim, europe and latin and north america collected as part of TEST and the in vitro activity of tigecycline. J Antimicrob Chemother 2007; 60(5): 1018-29.