Original Article

Vancomycin-resistant enterococci (VRE) isolated from hospitalized patients: Molecular characterization of the *van B* gene

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ABSTRACT

Vancomycin-resistant enterococci (VRE) are widespread bacteria that emerged as major multi-resistant pathogens responsible for nosocomial infections, current study aimed to determine the molecular characterization of vanB genes among Sudanese hospitalized patients. A total of 10^{Y} samples were collected from hospitalized patients , *Enterococcus* species were isolated and identified using colonial morphology, Gram stain, and biochemical tests in conformity with standard microbiological techniques. Antimicrobials susceptibility of enterococci was tested to 8 antibiotics based on the modified Kirby-Bauer disc diffusion method on Muller Hinton agar. Resistance to the vancomycin (van B) gene was molecularly identified among vancomycin-resistant isolates using the conventions PCR method. Seventy-one (66.4%) of patients were colonized with enterococci, where 38.4% were diagnosed as *Enterococcus faecalis* while 28 % of patients were infected with *Enterococcus faecium*. Resistant of *E. faecalis*. to other anti-microbial includes (68.3%) to ampicillin, (87.8%) ciprofloxacin, (70.7%) to penicillin is resistant among, and (56.6%) to ampicillin, (53.3%) to ciprofloxacin, (80%) to penicillin, and 70 % to Penicillin is resistant among *E. faecium*, among whole isolated Enterococci 39 (54.9%) were VRE, Majority of vancomycin-resistant enterococci (VRE) 33 (84.6%) were multi-drug resistant to ciprofloxacin, penicillin, ampicillin, and chloramphenicol. PCR analysis of VRE samples showed that 12 (16.9%) enterococci isolates had the *van B* gene. The prevalence of VRE was significantly documented with a relative increase in the frequency of the *van B* gene which plays a crucial role in nosocomial infections, as a consequence, antibiotic resistance in enterococci to glycopeptide antibiotics is regarded as a threat to nosocomial infections.

Keywords: *Enterococci*, *vanB* gene, Nosocomial infection, Antimicrobial resistance

Introduction

VRE has emerged as a significant multidrug-resistant (mdr pathogen causing nosocomial infections [1, 2], its infections have been linked with a longer hospital stay and leftover in-hospital mortality, posing a growing serious threat to public health [3].

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VRE was identified as among the most vital resistant organisms in the World Health Organization's "Worldwide Priority list of antibiotic-resistant bacteria" in 2017 [4].

Hence within the past few decades, enterococci have emerged as important nosocomial pathogens. Only several antimicrobials are ongoing against enterococcal species, and they have an inherent resistance to several therapeutically used antimicrobial drugs, making them significant nosocomial pathogens [5]. *E. faecalis* can confer resistance through diverse types of conjugation and spread these genes via conjugative transposons, pheromone-responsive plasmids, or broad-host-range plasmids [5]. The predominance of VRE varies significantly depending on the epidemic circumstances, *E. faecium* represents the majority of VRE infections in nosocomial environments, while *E. faecalis* accounts for only 2-20% of VRE isolates, based on geographic location and

care setting [6, 7].

The epidemiology of vancomycin-resistant *E. faecalis* (VREF) is poorly understood [8, 9]. PCR-based assessment can identify the existence of VRE easily and aid in the prevention strategies of VRE spread [10]. To prevent and control VRE transmission, screening critically ill patients at high risk of VRE colonization is strongly suggested [10]. There are currently eight phenotypic different types of acquired glycopeptide resistance in enterococci (*VanA, VanB, VanD, VanE, VanG, VanL, VanM,* and *VanN*) and one form of intrinsic resistance (VanC) in Enterococcus gallinarum and Enterococcus casseliflavus [11]. *VanA* and v**anB** phenotypes given this potential vancomycin resistance (MIC > 64 g/mL) are more frequent than other phenotypic traits. So current study aimed to study the molecular characterization of the *van B* gene of *Enterococci species* isolated from hospitalized patients in Khartoum states.

Materials and Methods

Design of study

A prospective, analytical study was conducted among Sudanese hospitalized patients admitted at different governmental hospitals in Khartoum Sudan during the period from May to December 2021. Before participation, each study subject provided verbal informed consent. The authorities of governmental health facilities approved specimen collection. The inclusion criteria are as follows all hospitalized patients regardless of their age and gender, any patients who had been admitted to a hospital for more than one week; patients who were on long-term vancomycin medication; and those who had undergone medical interventions, or had implants such as catheterization and cannulas were included in the study. While patients who disagreed to participate, and who those were unconscious, also debited or severely ill patients were not eligible for the study.

Sample collection and processing

A total of 10^{V} samples including urine, sputum, and wound specimens were collected from each study subject based on the non-probability sampling technique, each sample was processed as soon as possible and in case of delay samples were placed into bile esculin broth media. Classical culture media such as bile esculin agar, brain heart infusion (BHI) agar, and blood agar were used for the cultivation of clinical specimens, and then plates were incubated at 37°C overnight. The isolates were sub-cultured onto nutrient agar and subsequently for purity, incubated at 37°C for close to eighteen to twenty-four h.

Using standard microbiological techniques, enterococci strains were isolated and determined by Gram's stain, colonial morphology, and different biochemical tests (such as catalase, Culture in MSA containing 6.5% NaCl, carbohydrates fermentation, motility, pigment production in blood agar and vancomycin screening. according to the standard microbiological procedures. All gram-positive non-motile, catalase-negative dipliococci Enterococci genus, but hydrolyze esculin were regarded as *E. faecalis,* and other species that ferment lactose sugar and produce pigment on blood agar medium were considered as *E. faecium* and other enterococci species.

Antimicrobial susceptibility technique

The modified Kirby-Bauer disc diffusion technique on Muller Hinton agar by following the standard zone sizes of inhibition to the assessment of antimicrobial resistance pattern of isolated enterococci species, which was performed on 7 types of commonly used antimicrobial agent includes, including ampicillin (10 µg), vancomycin (30 µg), gentamicin (10 µg), chloramphenicol (30 µg), erythromycin (15 µg), penicillin G (10 µg), and ciprofloxacin (5 µg), following the instruction of Clinical and Laboratory Standards Institute (CLSI) [12]. Vancomycin screening test carried out in BHI agar (Merck) containing 6 g/mL vancomycin was used to evaluate phenotypical vancomycin-resistant isolates., positive result on vancomycin agar screen test, had been tested for MIC determination using broth microdilution method to determine vancomycin MIC.

Molecular technique

Using the bacterial DNA extraction kit (Cat. No. K-3032-2, Bioneer Company, Korea), DNA extraction was performed. The extracted DNA was stored at -20°C until use. The technique for the *vanB* gene was carried out by conventional PCR technique, 5-mins for the pre-denaturation step at 94°C, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 59°C, and 45 seconds at 72°C. A final 5 mins extension step was carried out at 72°C.

The following primers were used:

Forward primer: *vanB*-
F:
5′GTGACAAACCG
GAGGCGAGGA3′;
Reverse primer: *vanB*-
R:
5′CCGCCATCCTC
CTGCAAAAAA3′

Certainly a 25 µL container containing: 2.5 µL of bacterial DNA, 10 pM of each primer, 1.5 mM of $MgCl_2$, 250 µM of each dNTP, 10 mM of Tris-HCl (pH = 9.0), 30 mM of KCl, and 1U of Taq DNA polymerase. The reaction was carried out on Techne thermocycler (Bibby Scientific Limited, Beacon Road, Stone, Staffordshire ST15 0SA, UK). Amplification thermal cycling conditions for *vanB were carried out* with the by the mentioned steps which are, 10 mins at 95°C and 30 cycles of amplification comprising of 1 min at 94°C, one min at 60°C, one min at 72°C, followed by ten mins at 72°C for the final step of extension. Using electrophoresis on 1.5% agarose gel and stained with ethidium bromide, final amplified products were separated. The PCR product bands (433-bp) were inspected under ultraviolet light using a UV gel documentation system.

Statistical analysis

The Statistical Package of Social Science (SPSS) were used to analyze Data.

Results and Discussion

Fifty-Three patients who fulfilled the inclusion criteria were recruited for this study. Almost of clinical samples 75 (70%) were isolated from urine, followed by 18 (16.8%) from sputum. The majority of participants were male, 61 (57%) and 46 (43%) were females; their age range was between 35-65 years old with a mean age of 32.6 ± 22.4 months, and about 40.2% of admitted participants had the complication of diabetes. Seventy-one (66.4%) of patients were colonized with enterococci, where 38.4% were diagnosed as *E. faecalis* and 28 % of patients infected with *E. faecium* among whole isolated Enterococci 39 (54.9%) were VRE, all clinical and demographic data were summarized in **Table 1**.

Table 2 displays the frequency of Enterococcus isolates based on the Vancomycin screening test, there is a non-significant result related to gender, interestingly 24 (33.8%) of study subjects in the age group 56-65 years old were carried out VRE strain, and the result is statistically significant (p value≤0.003), as well as 19 (26.8%) of admitted patients, had VRE strain were diabetic patients, the result is statistically significant (P value 0.023).

The frequency of resistance of enterococci isolates to antibacterial drugs is assessed as illustrated in **Table 3**, in which (68.3%) of ampicillin., (87.8%) ciprofloxacin, (70.7%) penicillin is resistant to *E. faecalis*. The majority of vancomycin-resistant enterococci (VRE) 33 (84.6%) were multi-drug resistant to ciprofloxacin, penicillin, ampicillin, and chloramphenicol. A total of 63.3% of *E. faecium* was sensitive to Gentamycin and Erythromycin. PCR analysis of VRE samples showed that only 12 (16.9%) samples had the *vanB* gene **(Figure 1)**.

Table 1. Baseline and clinical data of the study participants

Patients characteristics	Frequency n=107	Percentage (%)
Gender		
- Male	61	57%
- Female	46	43%
Age group		
- 35-45	18	16.8 %
- 46-55	38	35.5 %
- 56-65	51	47.7%
Chronic diseases		
- Diabetes	43	40.2%
- Renal diseases	41	38.3%
- Abdominal surgery	23	21.5%
Bacterial isolates		
Enterococci		
- *E. faecalis*	41	38.4%
- *E. faecium*	30	28 %
Total	71	66.4%
Other bacteria	36	33.6%

Table 2. Frequency of Enterococcus isolates based on Vancomycin screening test

	Enterococcus isolates n=71	VRE (n = 39)	VSE (n = 32)	P. value
Gender				
- Male	40 (56.3%)	19 (26.8%)	21 (29.6%)	0.06
- Female	31 (43.7%)	20 (28.2%)	11 (15.5%)	
Age group				
- 35-45	12 (16.9%)	3 (4.2%)	9(12.7%)	0.003
- 46-55	21 (29.6%)	12 (16.9%)	9 (12.8%)	
- 56-65	38 (53.5%)	24 (33.8%)	14 (19.7%)	
Chronic diseases				
- Diabetes	33 (46.5%)	19 (26.8%)	14 (19.7%)	0.023
- Renal diseases	21 (29.6%)	12 (16.9%)	9 (12.8%	
- Abdominal surgery	17 (23.9%)	8 (11.3%)	9 (12.8%)	

Table 3. Frequency of Antimicrobial Pattern among *Enterococcus faecalis* and Enterococcus isolates

Antimicrobial tested	Interpretation	*Enterococcus faecalis* n=(41)	*Enterococcus faecium* n=(30)
Ampicillin 10 µg	S	13 (31.7%)	13(43.3%)
	R	28 (68.3%)	17 (56.6%)
Gentamycin 10 µg	S	15(36.6%)	19 (63.3%)
	R	26 (63.4%)	11 (36.7%)
Chloramphenicol 30 µg	S	19 (46.3%)	9 (30%)
	R	22 (53.7%)	21 (70%)
Erythromycin 15 µg	S	33 (80.5%)	19 (63.3%)
	R	8 (19.5%)	11 (36.7%)
Penicillin 10 µg	S	12 (29.3%)	6(20%)
	R	29 (70.7%)	24 (80%)
Ciprofloxacin 5 µg	S	15(12.2%)	14(46.7%)
	R	36 (87.8%)	16 (53.3%)

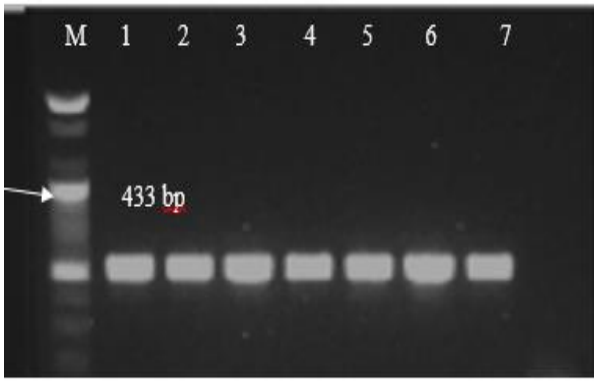

Figure 1. Electrophoresis of the amplified products of Enterococcus spp. By a duplex PCR in a 1.5% agarose gel. Lane M, 100 bp DNA ladder; lanes 1 to 7 *vanB* VRE

VRE was becoming a common nosocomial opportunistic pathogen due to an enormous rise in vancomycin usage. Vancomycin in combination with an aminoglycoside can successfully treat severe Enterococcus infections, but unfortunately, vancomycin resistance is dramatically increasing among enterococci species [13]. So the pattern of antibiotic resistance and the prevalence of vancomycin resistance, as well as molecular characterization of enterococci species among Sudanese patients were assessed in the current study.

The present study revealed that seventy-one (66.4%) enterococci species were isolated from hospitalized patients, *E. faecalis* is the predominate enterococcus species at 38.4%, which is in agreement with Kateete *et al*. [14], Labibzadeh *et al*. [15], and Boccella *et al*. [16] who noted that the frequency rate of *E. faecalis* was predominate Enterococci species among Patients. Although its contrast result was documented by Moosavian *et al*. [17]. These disparities and conflict in research results could be attributed to the different factors, such as different climatic environments, ethnic groups, non-representative samples, and types of samples utilized, such as urine, blood, and sputum collected in our study while some authors collect rectal swabs, but in their studies. And since enterococci colonization in the digestive system is the most frequent cause of VRE spread [18], we used rectal swabs for sampling in this study.

Interestingly out of 39 (54.9%) were VRE, the majority of (VRE) strains 33 (84.6%) were multi-drug resistant to ciprofloxacin, penicillin, ampicillin, and chloramphenicol. On the other hand, *Enterococcus faecium* is resistant to other anti-microbial (68.3%) ampicillin, (87.8%) ciprofloxacin, and (70.7%) penicillin-resistant to *E. faecalis*. while (56.6%) ampicillin, (53.3%) ciprofloxacin, (80%) penicillin, and 70 % Penicillin are resistant to *E. faecium*, these findings are inconsistent with findings reported by Salem-Bekhit *et al*. [19], and Toru *et al*. [20]. Such results indicate that antimicrobial-resistant enterococci have a geographically diverse distribution. The high rate of resistance to a diverse range of antibacterial drugs may be due to antimicrobial agent abusive behavior.

Vancomycin-resistant phenotypes in enterococci were subdivideds as *vanA, vanB, vanC, vanD,* and *vanE,* according their ability of resistance, and/or levels of resistance. The strains of VRE have been described globally with an increase in the frequency rates, even though the total incidence of these strains differs greatly in various populations and geographical areas [21]. The present study noted that 12 (30.8%) of the Enterococci isolates carry the *vanB* gene, and it now the predominate *vanB* gene in Enterococci isolates is responsible for resistant mechanisms among studied subjects. The frequency rate was consistent with findings of Karki *et al*. [22] in Melbourne, Australia, as they encountered 58 (17.5%) VRE with the *vanB* gene among 331 rectal specimens, but none with the *vanA* gene. The justification cited in their study was that the *vanB* gene in VRE was ubiquitous in the region. Moreover, the *vanB* gene is primarily linked to outbreaks and food contamination, whereas the *vanA* gene is connected to clinical strains [23], and this justifies the predominant presence of *vanB* among Sudanese patients.

The present study shows a discrepancy with Talebi *et al*. [24] and Shirvani *et al*. [25] who noted the presence of the vanA gene but not the vanB gene in vancomycin-resistant Enterococcus isolates. These variation can either be because of the study population (hospitalized patients are more susceptible to VRE), study procedures, clinical and immunological status of participants, differences in hospital hygiene, and/or vancomycin abuse. The amalgamation of aminoglycosides (gentamycin and streptomycin) and β lactam (ampicillin) are first line treatment choice for managing enterococcal infections[11]. The relatively high

resistance of the isolates in this study to these antibiotics is of remains a major concern.

Furthermore, VRE organisms are typically resistant to further than one antibiotic and can also be transmitted from person to person which is increasingly prevalent in hospitals and long-term care facilities. To preclude VRE transmission from person to person, it is critical to wash or decontaminate hands regularly, even before and after inspecting the patients or their surrounding environment. When trying to care for a VRE patient in the hospital, staff should also wear gowns and gloves.

Limitation of the study

The study has some limitations, including a limited sample size, a sampling technique followed was a non-probability sampling technique in which the sample size didn't represent the whole community. Then the study failed to investigate the Enterococci *vanA* gene due to limited funds and resources. Further research with constructed cohort study design and representative sampling technique should be followed.

Conclusion

Along with a wide range of other antimicrobial agents among study subjects, there is a high-frequency rate of vancomycin resistance; together with an increase in multi-drug resistant strains of enterococci, and a relative increase in the frequency of *vanB* gene which play a crucial role in nosocomial infections, as a consequence, antibiotic resistance in enterococci to glycopeptide antibiotics is regarded as a threat to nosocomial infections.

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References

1. Mercuro NJ, Davis SL, Zervos MJ, Herc ES. Combatting resistant enterococcal infections: a pharmacotherapy review. Expert Opin Pharmacother. 2018;19(9):979-92. doi:10.1080/14656566.2018.1479397
2. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill. 2008;13(47):19046. Available from: https://www.eurosurveillance.org/content/10.2807/ese.13.47.19046-en
3. Chiang HY, Perencevich EN, Nair R, Nelson RE, Samore M, Khader K, et al. Incidence and outcomes associated with infections caused by vancomycin-resistant enterococci in the United States: systematic literature review and meta-analysis. Infect Control Hosp Epidemiol. 2017;38(2):203-15. doi:10.1017/ice.2016.254
4. World Health Organization (WHO): Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. Available from: http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf?ua=1. Accessed 03 Mar 2017. 2017.
5. Remschmidt C, Schröder C, Behnke M, Gastmeier P, Geffers C, Kramer TS. Continuous increase of vancomycin resistance in enterococci causing nosocomial infections in Germany - 10 years of surveillance. Antimicrob Resist Infect Control. 2018;7(1):54. doi:10.1186/s13756-018-0353-x
6. Barnes AM, Frank KL, Dale JL, Manias DA, Powers JL, Dunny GM. Enterococcus faecalis colonizes and forms persistent biofilm microcolonies on undamaged endothelial surfaces in a rabbit endovascular infection model. FEMS Microbes. 2021;2. doi:10.1093/femsmc/xtab014
7. Tripathi A, Shukla S, Singh A, Prasad K. Prevalence, outcome and risk factor associated with vancomycin-resistant Enterococcus faecalis and Enterococcus faecium at a Tertiary Care Hospital in Northern India. Indian J Med Microbiol. 2016;34(1):38-8.
8. Jahansepas A, Ahangarzadeh Rezaee M, Hasani A, Sharifi Y, Rahnamaye Farzami M, Dolatyar A, et al. Molecular Epidemiology of Vancomycin-Resistant Enterococcus faecalis and Enterococcus faecium Isolated from Clinical Specimens in the Northwest of Iran. Microb Drug Resist. 2018;24(8):1165-73. doi:10.1089/mdr.2017.0380
9. Mamishi S, Shalchi Z, Mahmoudi S, Hosseinpour Sadeghi R, Haghi Ashtiani MT, Pourakbari B. Antimicrobial Resistance and Genotyping of Bacteria Isolated from Urinary Tract Infection in Children in an Iranian Referral Hospital. Infect Drug Resist. 2020;13:3317-23. doi:10.2147/IDR.S260359
10. Ulu-Kilic A, Özhan E, Altun D, Perçin D, Güneş T, Alp E. Is it worth screening for vancomycin-resistant Enterococcus faecium colonization? Financial burden of screening in a developing country. Am J Infect Control. 2016;44(4):e45-9. doi:10.1016/j. ajic.2015.11.008
11. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015;8:217.
12. Clinical and Laboratory Standards Institute. M100S Performance Standards for Antimicrobial Susceptibility Testing. M02-A12, M07-A10, and M11-A8; 26th Edition. 2016;36(1):82-6.
13. George SK, Suseela MR, El Safi S, Elnagi EA, Al-Naam YA, Adam AA, et al. Molecular determination of van genes among clinical isolates of enterococci at a hospital setting.

Saudi J Biol Sci. 2021;28(5):2895-9. doi:10.1016/j.sjbs.2021.02.022

14. Kateete DP, Edolu M, Kigozi E, Kisukye J, Baluku H, Mwiine FN, et al. Species, antibiotic susceptibility profiles and van gene frequencies among enterococci isolated from patients at Mulago National Referral Hospital in Kampala, Uganda. BMC Infect Dis. 2019;19(1):486. doi:10.1186/s12879-019-4136-7
15. Labibzadeh M, Kaydani GA, Savari M, Ekrami A. Emergence of High-level Gentamicin Resistance among Enterococci Clinical Isolates from Burn Patients in South-west of Iran: Vancomycin Still Working. Pol J Microbiol. 2018;67(4):401-6. doi:10.21307/pjm-2018-043
16. Boccella M, Santella B, Pagliano P, De Filippis A, Casolaro V, Galdiero M, et al. Prevalence and Antimicrobial Resistance of Enterococcus Species: A Retrospective Cohort Study in Italy. Antibiotics (Basel). 2021;10(12):1552. doi:10.3390/antibiotics10121552
17. Moosavian M, Ghadri H, Samli Z. Molecular detection of vanA and vanB genes among vancomycin-resistant enterococci in ICU-hospitalized patients in Ahvaz in southwest of Iran. Infect Drug Resist. 2018;11:2269-75. doi:10.2147/IDR.S177886
18. Sun HL, Liu C, Zhang JJ, Zhou YM, Xu YC. Molecular characterization of vancomycin-resistant enterococci isolated from a hospital in Beijing, China. J Microbiol Immunol Infect. 2019;52(3):433-42. doi:10.1016/j.jmii.2018.12.008
19. Salem-Bekhit MM, Moussa IM, Muharram MM, Alanazy FK, Hefni HM. Prevalence and antimicrobial resistance pattern of multidrug-resistant enterococci isolated from clinical specimens. Indian J Med Microbiol. 2012;30(1):44-51.
20. Toru M, Beyene G, Kassa T, Gizachew Z, Howe R, Yeshitila B. Prevalence and phenotypic characterization of Enterococcus species isolated from clinical samples of pediatric patients in Jimma University Specialized Hospital, Ethiopia. BMC Res Notes. 2018;11(1):281.
21. Mousavi SH, Peeri-Doghaheh H, Mohammadi-Ghalehbin B, Teimourpour R, Maleki D, Khademi F, et al. High-level resistance to aminoglycosides and ampicillin among clinical isolates of Enterococcus species in an Iranian referral hospital. Iran J Microbiol. 2020;12(4):319-24. doi:10.18502/ijm.v12i4.3935
22. Karki S, Houston L, Land G, Bass P, Kehoe R, Borrell S, et al. Prevalence and risk factors for VRE colonization in a tertiary hospital in Melbourne, Australia: a cross-sectional study. Antimicrob Resist Infect Control. 2012;1(1):31.
23. Shokoohizadeh L, Mobarez AM, Alebouyeh M, Zali MR, Ranjbar R. Genotyping of clinical and environmental multidrug-resistant Enterococcus faecium strains. Indian J Pathol Microbiol. 2017;60(1):74.
24. Talebi M, Eshraghi SS, Pourshafie MR, Pourmand MR, Eshraghian MR. Characterization of vancomycin-resistant faecium. Iranian J Publ Health. 2007;36(4):20-5.
25. Shirvani F, Behzad A, Abdollahi N, Mohkam M, Sharifian M, Esfandiar N, et al. Frequency and co-colonization of vancomycin-resistant Enterococci and Candida in ICU-hospitalized children. New Microbes New Infect. 2021;41:100881. doi:10.1016/j.nmni.2021.100881