Egyptian Journal of Medical Microbiology Volume 27 / No.1 / January 2018 17-24 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Incidence of Vancomycin Resistant Enterococci Colonization in Zagazig University Pediatric ICU

Mahmoud Shokry[1], Ahmed Elhewala[2], Amr Megahed[2], Ahmed Elsadek Fakhr[3]*
[1]Pediatric Department, Benha Educational Hospital
[2]Pediatric Department, Faculty of Medicine, Zagazig University
[3]Medical Microbiology & Immunology, Faculty of Medicine, Zagazig University

Key words:
Rectal, Nasal, VRE, Children, Colonization, Egypt

Corresponding Author:
Ahmed Elsadek Fakhr[1]
Tel: +2 0552302809 / Mobile: 00201005356630
ahmed_fakhr@yahoo.com
amfakhr@zu.edu.eg

ABSTRACT

Background: *Multidrug resistant (MDR) bacteria inside hospitals are a major health problem which prolongs the hospital stay, increases the treatment costs and worsen the prognosis of patients. Vancomycin resistant enterococci (VRE) represent a group of MDR bacteria which came into the spotlight in the last decades. In Egypt, the problem is not well estimated especially in pediatric settings.* ***Objectives****: The objective of this work was to estimate the rate of VRE colonization inside Pediatric intensive care units (PICU) of Zagazig University Hospitals.* ***Methodology****: A total of 168 children admitted to PICU were surveyed for nasal and perirectal colonization by VRE. Phenotypic identification of the genus was performed and further species identification was done using PCR technique. VanA and VanB resistance genes were screened using specific primers for each.* ***Results****: Among the 168 children investigated, 47 (27.98%) were colonized by enterococci; 41 rectal and 6 nasal colonization. Only 7 isolates (14.9%) were identified as vancomycin resistant. The overall VRE colonization rate was 4.17% among the PICU children investigated. The colonization rate was significantly higher among children exceeded 7 days of hospital stay inside PICU. Thirty one (65.96%) isolates were identified as E. fecalis and 10 (21.28%) were identified as E. faecium. Six isolates (12.76%) were not identified by PCR reaction. VanA gene was detected in 4 of the VRE isolates while none of the isolates revealed VanB gene product.* ***Conclusion:*** *Generally the incidence of VRE colonization in the current study is considered low in comparison to other groups of MDR bacteria and to other geographic distribution.*

INTRODUCTION

Enterococci are known to be a normal inhabitant of GIT in Human and other mammals [1]. They have emerged recently to be a leading cause of multidrug resistance inside hospitals and different medical settings. The enterococci taxonomy has expanded in the last decade to include more than forty different species with variable habitats and distinct clinical and microbiologic characters [2].

Among the different species of enterococci, *E. faecalis* and *E. faecium* are responsible for more than 80 % of human enterococcal infections[3].

The GI tract of human, the skin, the upper respiratory tract, the vagina and the oral cavity are colonized by different microbial communities which collectively constitute the normal human microbiota[4]. The enterococcal colonization inside the intestinal tract is hindered by many barriers including other intestinal microbiota, low pH in the stomach, duodenum, and jejunum, which limit the presence of Enterococcus species to the ileum and the colon. The intestinal epithelial cells produce a protein termed lectin REGIIIγ which blocks Gram-positive bacteria in response to the abundant gram negative bacteria in the lumen. Heavy exposure to antibiotic treatment inside hospitals lessens the load of Gram-negative bacteria and, consequently, decreases the production of REGIIIγ. This supports the massive colonization of the gut with Gram positive bacteria such as enterococci and *Clostridium difficile* especially drug-resistant strains such as vancomycin-resistant enterococci (VRE)[5]. The unusual sticking between the enterococci and the epithelial cells may prime the translocation of the bacteria to the blood or the lymphatic system leading to human infections [6]. Currently, enterococci rank among chief causes of bloodstream, urinary tract and surgical wound nosocomial infections [7]. They are also commonly isolated from mixed infections in association with obligate anaerobes from deep seated infections [8]. In the US, among 84,050 isolates of enterococci from different infection sites in different hospitals, 20.6% were vancomycin-resistant. However, 75% of the resistant isolates were *E. faecium*[6]. In another US study, an overall VRE prevalence of 30.4% were reported, with also significant difference between species where 76% of *E. faecium* isolates were resistant and only 4.5% for *Enterococcus faecalis* showed vancomycin resistance [9].

Similar trends have been also reported for the European Union[6].

In pediatric settings, colonization and infection by VRE is not an uncommon event. In a Turkish study, 9.5% of the children admitted to PICU were found to be colonized with VRE over the study period. Systemic infections by VRE developed later in 10.2% of the colonized patients[10].

Enterococci have an intrinsically resistance to numerous antibiotics. In addition, it can acquire gene mutations and extra-genes via conjugation plasmids and high-frequency recombination or conjugative transposons that increase its ability to resist antimicrobials[11].

The mechanism of Glycopeptide resistance is often caused by either replacement of the terminal D-Ala of peptidoglycan precursors with D-lactate (D-Lac), or with D-Ser. The genetic basis involves VanA, VanB, VanC, VanD, VanE, VanG and VanM genes[12], however VanA and VanB are the most likely encountered globally[13].

Although many studies in Arab countries investigated VRE from different nosocomial infections[14,15,16], to our knowledge no published study investigated the colonization of VRE in pediatric age. The objectives of this work were to determine the prevalence of colonization with VRE in children admitted to PICU inside Zagazig University Hospitals and to identify the genetic basis of resistance in these children.

METHODOLOGY

This study included (168) children 99 males and 69 females, their ages ranged from 1 month to 12 years. Patients with duplicate admissions during the study period were excluded.

Collection of Samples and Enterococcal isolation:

After taking written consent from parents, nasal and perirectal swab samples were collected from each patient under complete aseptic conditions and directly transferred to the Microbiology laboratory. Samples were cultivated on Azide Dextrose Broth (Oxoid, England) over night at 37°C. Subculture on Bile esculin agar (BEA) (Oxoid, England) was later performed and incubated at 44° C for 24 hours. Sodium azide inhibits growth of gram-negative bacteria, allowing enterococci to grow. Characteristic pinpoint colonies on BEA with black zone around were subcultured on Mueller Hinton Agar (MHA) with 6% NaCl (Oxoid, UK) at 45°C for confirmation.

Antibiotic susceptibility tests:

All Enterococcal isolates were tested for antibiotic susceptibility on Muller-Hinton Blood agar by disk diffusion method using the following antibiotic discs (Oxoid, England): Vancomycin 30µg, Ampicillin 10µg, Tetracycline 30µg, Ciprofloxacin 5µg, Imipenem 10µg, Cefoxitin 30µg, Cephalexin 30 µg, Erythromycin 15 µg, Oxacillin 1 µg, Penicillin G 10 IU and amoxacillin 30 µg. Diameters of inhibition zones were measured and isolates were identified as sensitive, intermediate or resistant according to CLSI standards (2014)[17].

Strains revealed to be resistant or intermediate to vancomycin by disc diffusion method were further tested by E test (BioMerieux, France) technique for determining the MIC (in µg/mL). The vancomycin MIC value was read from the graduated scale in terms of µg/mL where the ellipse edge intersects the strip. The cutoff MIC used for detection of VRE was ≥ 32 µg/mL [18].

Molecular identification

Since more than 80-90% of medically important enterococci are either *E. faecalis* or *E. faecium*, a separate PCR reaction targeting D-ala:D-ala ligase (*ddl*) genes of *E. faecalis* and *E. faecium* was done to identify the enterococci species. The vancomycin-resistance genes were investigated using specific primers for *van*A and *van*B genes. All the primer sequences used have been described previously [19] and were obtained from Biolegio (Netherlands) (Table 1).

Table 1: Oligonucleotides primers used for identification of enterococcal species and mechanism of resistance

Primer designation	Sequences (5 →3)	Product size (bp)
E. faecium *ddl* F	TTGAGGCAGACCAGATTGACG	658
E. faecium *ddl* R	TATGACAGCGACTCCGATTCC	
E.faecalis *ddl* F	ATCAAGTACAGTTAGTCT	941
E.faecalis *ddl* R	ACGATTCAAAGCTAACTG	
***van*A F**	GGGAAAACGACAATTGC	732
***van*A R**	GTACAATGCGGCCGTTA	
***van*B F**	GTGCTGCGAGATACCACAGA	635
***van*B R**	CGAACACCATGCAACATTTC	

DNA extraction from isolated colonies was done using I-genomic BYF DNA Extraction Mini Kit (iNTRON Biotechnology, Inc) following manufacturer's instructions.

DNA amplification was done using 2x Taq PCR Master Mix (Qiagen). For each reaction, a total volume of 20 µl was obtained using 10 µl of 2x Taq PCR Master Mix, 1 µl (10 pmole) of each of forward and reverse gene specific primers, 4 µl of template DNA and 4 µl of RNAse free water into PCR tubes. Thermal cycler (Applied Biosystems, Veriti™ 96-Well) was programmed to perform a one-step initial denaturation at 94ºC for 5 min, followed by 35 cycles of (94ºC for 45 sec, 50ºC for VanA and *E. faecalis* $_{ddl}$ or 55ºC for VanB and *E. faecium* $_{ddl}$ for 1 min, and 72ºC for 1 min), with a final elongation step of 7 min at 72ºC.

10 µl of the negative control and 10 µl of each PCR product were loaded on agarose gel after addition of loading dye and electrophoresis was performed. For each gene a separate PCR reaction was performed. All isolates phenotypically identified as enterococci were subjected to molecular identification. Only isolates which revealed to be resistant or intermediate to vancomycin was further investigated for VanA and VanB detection by PCR. The amplified DNA products were identified by appearance of a sharp band at the corresponding molecular weight size as shown in table 1.

RESULTS

A total of 336 nasal and perirectal samples were obtained from 168 pediatric ICU admitted patients. Only 47 samples (13.99%) (39 perirectal and ٨ nasal samples) showed growth of enterococci on BEA. The antibiotic sensitivity testing of the isolated enterococcal strains (Table 2) revealed that the highest incidence of resistance was towards Oxacillin 97.9% and Erythromycin 97.9% followed by Cefoxitin 93.6%. Fortunately, the least incidence was against vancomycin 14.9%. This points that vancomycin is still an effective choice for treatment of Enterococcal infection in our hospitals. The resistance to Ciprofloxacin and to Cephalexin was also high 87.2% and 89.4% respectively. Penicillin G, ampicillin, amoxicillin, tetracycline and imipenem resistance rates were 70.2, 63.8, 55.3, 61.7% respectively.

Table 2: Disc diffusion results of the 47 enterococcal strains in the studied group:

Antibiotic	Antimicrobial Susceptibility Patterns Of HAI Cases			
	Susceptible		Resistant	
	No	%	No	%
Ciprofloxacin	6	12.8	41	87.2
Cephalexin	5	10.6	42	89.4
Oxacillin	1	2.1	46	97.9
Cefoxitin	3	6.4	44	93.6
Ampicillin	17	36.2	30	63.8
Amoxacillin	21	44.7	26	55.3
Tetracycline	18	38.3	29	61.7
Erythromycin	1	2.1	46	97.9
Penicillin G	14	29.8	33	70.2
Vancomycin	40	85.1	7	14.9
Imipenem	17	36.2	30	63.8

Many socio-epidemiologic, clinical and laboratory criteria were investigated in all patients including age, sex, residence, breast or artificially feeding, total parenteral nutrition (TPN), length of stay in PICU, vancomycin intake and other antibiotics intake in addition to laboratory investigations "CBC and CRP". Except for a statistically significant difference regarding length of PICU stay between VRE and VSE (Table 3), there was no significant correlation between enterococcal colonization and enterococcal vancomycin resistance with any of the clinical or laboratory criteria investigated in the studied subjects.

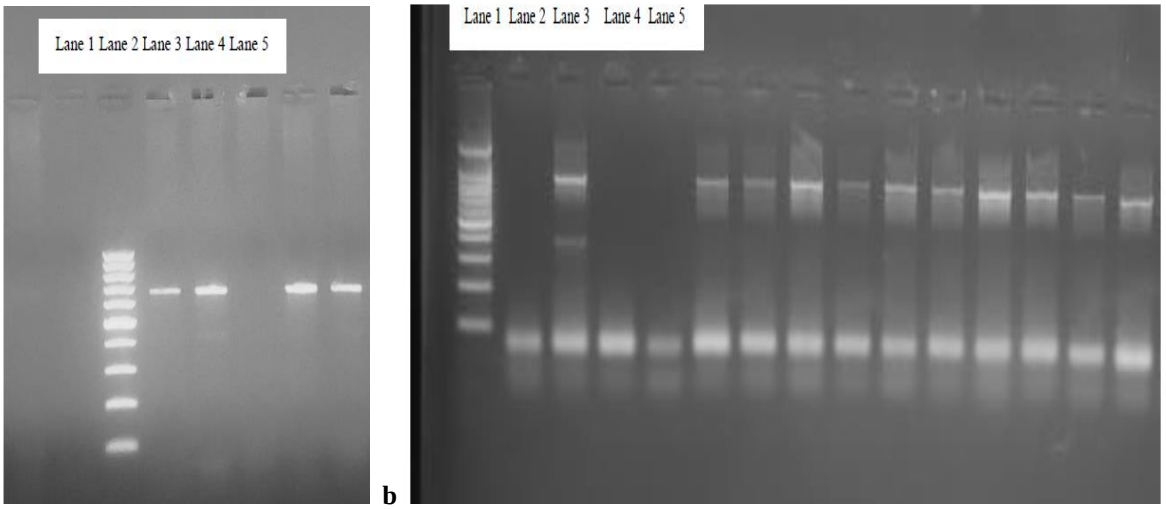
Table 3: Comparison of the clinical data between VRE and VSE enterococci in the studied group:

Variable	VRE (7)		VSE (40)		χ^2	P
	Perirectal (6) No (%)	Nasal (1) No (%)	Perirectal (35) No (%)	Nasal (5) No (%)		
PICU stay						
<1 week	1 (16.77%)	0 (0.0%)	30 (85.7%)	5 (100%)	26	0.001**
>1 week	5 (83.33%)	1 (100.0%)	5 (14.28%)	0 (00.0%)		
Ventilation						
No	2 (33.33%)	1 (100.0%)	18 (51.42%)	2 (40.00%)	2.6	0.8
Yes	4 (66.67%)	00 (00.0%)	17 (48.57%)	3 (60.00%)		
Vomiting						
No	1 (16.67%)	00 (00.0%)	18 (51.42%)	1 (20.00%)	6.1	0.4
Yes	5 (83.33)	1 (100.0%)	17 (48.57%)	4 (80.00%)		
Diarrhea						
No	5 (83.33%)	1 (100.0%)	30 (85.7%)	4 (80.00%)	2.5	0.8
Yes	1 (16.67%)	0 (00.0%)	5 (14.28%)	1 (20.00%)		
Vancomycin intake						
No	2 (33.33%)	1 (100.0%)	22 (62.86%)	3 (60.00%)	10.3	0.08
Yes	4 (66.67%)	0 (00.0%)	13 (37.14%)	2 (40.00%)		

** Statistically significant difference ($P \leq 0.05$)

Regarding molecular identification of the enterococcal species using specific gene primers for *E. fecalis* $_{ddl}$ and *E. faecium* $_{ddl}$, a specific band at sizes 941 bp, 658 bp was identified as *E. fecalis* and *E. faecium* respectively (Fig. 1-A, 1-B). Among the 47 isolates of enterococci isolated, 31 (65.96%) were identified as *E. fecalis* and 10 (21.28%) were identified as *E. faecium* using PCR reaction (Table 4). Six isolates (12.76%) were not identified by PCR reaction. Four isolates of the *E. faecium* were VRE, where 3 of *E. fecalis* were resistant to vancomycin (Table 4).

Investigating the genes specific for vancomycin resistance was done using VanA and VanB genes specific primers. Among the 7 VRE isolates, only 4 were identified to harbor VanA gene while none of the isolates revealed VanB gene product. Three isolates were negative for both genes.



a **b**

Fig. 1-A: Lane 1 contains the negative control (no template). Lane 2 contains a 100 bp DNA size marker (Thermo Fisher Scientific), Lanes 3, 4 and 6-7 contain *E. faecium* $_{ddl}$ gene product at approximately 658 bp. Lane 4 contains a negative PCR sample for the gene. Fig. 1-B: Lane 1 contains a 100 bp DNA size marker (iNTRON Biotechnology, Inc). Lane 2 contains the negative control (no template). Lanes 3, 6-15 contain *E. fecalis* $_{ddl}$ gene product at approximately 941 bp. Lanes 4 and 5 contain a negative PCR for the gene.

Table 4: Frequency of VRE and VSE and non-enterococci in nasal and perirectal samples

	VRE faecium No (%)	VRE faecalis No (%)	VSE faecium No (%)	VSE faecalis No (%)	Non-typable No (%)	No enterococci No (%)
Nasal (n= 168)	1 (0.59)	1(0.59)	1(0.59)	3(1.78)	2(1.19)	160(95.24)
Perirectal (n=168)	3(1.48)	2(1.19)	5(2.98)	25(14.88)	4(2.38)	129(76.78)
Total (n=336)	4(1.61)	3(0.89)	6(1.79)	28(8.33)	6(1.79)	288(85.71)

Table 4 shows the distribution of isolates whether VRE or VSE among the nasal and rectal samples in addition to the species type according to the molecular identification performed. Most of enterococci isolates were obtained from rectal swab samples (39 out of 47). *E. fecalis* were the predominant species, however VRE faecium isolates (4) were more than VRE fecalis (3).

DISCUSSION

VRE is responsible for resistant infections in susceptible populations, including children especially hospitalized groups. This is usually preceded by colonization either in GIT or in the URT. In the current study, we investigated a PICU admitted children for VRE colonization and possible associated factors. Out of 168 children investigated, 47 were recognized to have enterococcal colonization with an overall incidence of 27.98%. However, only 7 isolates (14.9%) were identified as vancomycin resistant. The overall VRE rate of colonization among the pediatric population of our study was 4.17%. The VRE rate was significantly higher among children exceeded 7 days of hospital stay inside PICU (Table 3). This rate is less than that described in different adult population previously reported rates which ranged from 10 to 40%[20,21,22]. These higher rates in the other groups may be attributed to the different age group investigated, and the different co-morbidities such as neutropenia, immune-compromisation, disturbance of the GIT mucosal lining by intake of chemotherapeutic agents, use of different invasive devices and frequent hospitalization.

In a British children Hospital study, GIT colonization with VRE was identified in 38.3% of Hematology/Oncology and 11.1% of Hepatology and Gastroenterology patients. However, in the Pediatric Intensive Care and Renal Units, VRE gastrointestinal colonization was found in only 2.3% and 1.5% of children respectively. Six children only were colonized at extra-intestinal sites[23]. In a Turkish study, Flokas et al. (2017) reported an overall VRE colonization in a paediatric intensive care unit (PICU) of 5% but the rate in the hematology and oncology units was 23% [24]. The low incidence of the VRE colonization in PICU in our study (Table 4) and in these studies in comparison to other units suggested a possible role of the type of patients and the disease involved since patients in Hematology, oncology and hepato-gastroentrology units are expected to stay for longer time and to have more frequent hospitalization.

The geographic distribution of the study also could have its role. Different rates from different geographic localities were reported. In another Turkish study, 9.8% of the PICU patients were found to be colonized with vancomycin resistant enterococci.[10]

In an Iranian study, out of 180 *E. faecalis* isolates obtained from children with different types of infection, only 8 cases were isolated from PICU. In their study 16% of the isolates were resistant to vancomycin[1].

The only risk factor revealed to be associated with vancomycin resistance in our study was the prolonged stay more than 7 days (Table 3). The same factor was almost prevalent in similar studies[1,10,24].

Generally, the frequency of resistance to different groups of antibiotics such as Methicillin Resistant *Staphylococcus aureus* (MRSA), extended spectrum □-lactamases (ESBL), and multidrug resistant organisms is high in Egyptian Hospitals and in many developing countries due to non-rationalized use of antibiotics[25]. However, it seems that the situation is different regarding the prevalence of VRE. This may be attributed to the high cost, low availability of the drug in pharmacies and the absence of oral or intramuscular forms of the drug. This makes it only available in infusion form and restricts its use to hospitals. Fortunately, all these factors may be involved in a relative low incidence of enterococcal resistance to vancomycin in Egypt.

Although previous intake of vancomycin has been linked with development of VRE colonization in adult and pediatric populations[24,26–28] However, in this study this correlation was not proved. The low number of VRE detected could be a reason. Further studies with larger number of the patients are needed to accurately evaluate the association of intake of vancomycin or other anti-bacterial drugs on the prevalence of VRE colonization and infection in pediatric population in our hospitals.

Enterococci generally and VRE strains frequently show resistance to other antimicrobials[29]. In our study, almost all strains were resistant to at least 3 antibiotics. However, the least incidence of resistance was against vancomycin (Table 2). This points that vancomycin is

still an effective choice for treatment of Enterococcal infection in our hospitals. Apart from low vancomycin resistance, these rates of resistance against most of the other antibiotics are higher than levels of enterococcal resistance observed in many other studies[30,31].

The Respiratory diseases were the commonest cause of PICU admission in our study. Similar finding was reported previously[32]**.** However neither of the clinical conditions predisposed to admission nor the invasive techniques investigated were associated with increased risk of VRE colonization.

Molecular identification to species level and determination of Glycopeptide resistance of enterococci was used before and revealed to be an efficient method[33]. In this study, using PCR revealed that most of isolates were *E. fecalis* (65.96%) and 21.28% were identified as *E. faecium*. However six isolates (12.76%) were non-typable by PCR reaction. The non typable strains by PCR are most probably other species of enterococci since the primers used were specific for *E. fecalis* and *E. faecium ddl* genes. The other possibility is that one or more of these isolates were incorrectly identified as enterococci using phenotypic methods. The species distribution is similar to that reported in another Egyptian study[34]. However, the prevalence of *E. faecium* was higher in a comparable PICU study in Pakistan[35]. Four out of 10 *E. faecium* isolates were VRE (40%), while only 3 out of 31 *E. fecalis* showed resistance to vancomycin (9.68%) (Table 4). This is consistent with many reports from different geographical locations and in different studies which confirmed that the frequency of vancomycin resistance among *E. faecium* isolates are much higher than its presence in *E. fecalis*[9,36].

In conclusion, we can say that unlike the high frequency of resistance to different antimicrobials which could be met in our hospitals, it seems that colonization with vancomycin resistant enterococci is not much high as expected, at least in pediatric ICU settings. However, similar or more comprehensive studies are needed to cover other units especially oncology and hematology units. As a last resort in many severe infections by gram positive bacteria, the use of vancomycin should be restricted to hospitals and to only those with resistant infection by *Staphylococcus aureus* and enterococci not responding to other treatment. Strengthening infection control measures should be taken into consideration to stop the spread of these resistant microbes among pediatric populations in our ICUs.

Conflicts of interest:

The authors declare no conflicts of interest.

Acknowledgement:

The authors pay special gratitude to the Zagazig Scientific and Medical Research Center (ZSMRC) for the continuous help to complete the molecular part of this work.

REFERENCES

1. Sabouni F, Movahedi Z, Mahmoudi S, Pourakbari B, Keshavarz Valian S, Mamishi S. High frequency of vancomycin resistant Enterococcus faecalis in children: An alarming concern. J Prev Med Hyg. 2016;57(4):E201–4.
2. Parte AC. LPSN - List of prokaryotic names with standing in nomenclature. Nucleic Acids Res. 2014;42(D1).
3. Ogier J-C, Serror P. Safety assessment of dairy microorganisms: the Enterococcus genus. Int J Food Microbiol. 2008 Sep;126(3):291–301.
4. Tannock GW. The normal microflora: new concepts in health promotion. Microbiol Sci. 1988 Jan;5(1):4–8.
5. Brandl K, Plitas G, Mihu CN, Ubeda C, Jia T, Fleisher M, et al. Vancomycin-resistant enterococci exploit antibiotic-induced innate immune deficits. Nature. 2008 Oct;455(7214):804–7.
6. Gilmore MS, Clewell DB, Ike Y, Shankar N. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. Enterococci From Commensals to Lead Causes Drug Resist Infect [Internet]. 2014; Available from: http://www.ncbi.nlm.nih.gov/books/NBK190424/%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/24649510
7. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol. 2008 Nov;29(11):996–1011.
8. Brook I. Hot Topics in Infection and Immunity in Children VII [Internet]. Vol. 697. 2011. Available from: http://link.springer.com/10.1007/978-1-4419-7185-2
9. Sader HS, Moet GJ, Farrell DJ, Jones RN. Antimicrobial susceptibility of daptomycin and comparator agents tested against methicillin-resistant Staphylococcus aureus and vancomycin-resistant enterococci: trend analysis of a 6-year period in US medical centers (2005-2010). Diagn Microbiol Infect Dis. 2011 Jul;70(3):412–6.
10. Sutcu M, Akturk H, Acar M, Salman N, Aydin D, Akgun Karapinar B, et al. Impact of vancomycin-resistant enterococci colonization in critically ill pediatric patients. Am J Infect Control [Internet]. 2016;44(5):515–9. Available from: http://dx.doi.org/10.1016/j.ajic.2015.11.026

11. Arias CA, Murray BE. The rise of the Enterococcus: Beyond vancomycin resistance. Nat Rev Microbiol. 2012;10(4):266–78.
12. Xu X, Lin D, Yan G, Ye X, Wu S, Guo Y, et al. vanM, a new glycopeptide resistance gene cluster found in Enterococcus faecium. Antimicrob Agents Chemother. 2010;54(11):4643–7.
13. Kristich CJ, Rice LB, Arias CA. Enterococcal Infection—Treatment and Antibiotic Resistance. In: Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. 2014. p. 1–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24649502
14. Hashem YA, Yassin AS, Amin MA. Molecular characterization of Enterococcus spp. clinical isolates from Cairo, Egypt. Indian J Med Microbiol. 2015;33 Suppl:80–6.
15. Shorman M, Al-Tawfiq JA. Risk factors associated with vancomycin-resistant enterococcus in intensive care unit settings in Saudi Arabia. Interdiscip Perspect Infect Dis. 2013;2013.
16. Hijazi N, Elmanama AA, Al-Hindi A. Vancomycin-resistant enterococci in fecal samples from hospitalized patients and non-hospitalized individuals in Gaza City. J Public Health (Bangkok). 2009;17(4):243–9.
17. CLSI, Institute C and LS. M100-S23 Performance Standards for Antimicrobial Testing. Vol. 33, Clinical and Laboratory Standards Institute. 2014. 1-206 p.
18. CLSI . Performance standards for antimicrobial susceptibility testing. CLSI supplement M100S. CLSI supplement M100S. Wayne, PA :Clinical and Laboratory Standards Institute. 2016.
19. Kariyama R, Mitsuhata R, Chow JW, Clewell DB, Kumon H. Simple and reliable multiplex PCR assay for surveillance isolates of vancomycin-resistant enterococci. J Clin Microbiol. 2000;38(8):3092–5.
20. Gedik H, Yıldırmak T, Şimşek F, Kantürk A, Arıca D, Aydın D, et al. Vancomycin-resistant enterococci colonization and bacteremia in patients with hematological malignancies. J Infect Dev Ctries [Internet]. 2014;8(9):1113–8. Available from: http://www.jidc.org/index.php/journal/article/view/4451
21. Mioljevic V, Markovic-Denic L, Vidovic A, Jovanovic M, Tosic T, Tomin D. Risk factors for vancomycin-resistant Enterococcus colonization in hematologic patients. Vojnosanit Pregl. 2013;70(12):1109–16.
22. Kamboj M, Chung D, Seo SK, Pamer EG, Sepkowitz KA, Jakubowski AA, et al. The Changing Epidemiology of Vancomycin-Resistant Enterococcus (VRE) Bacteremia in Allogeneic Hematopoietic Stem Cell Transplant (HSCT) Recipients. Biol Blood Marrow Transplant. 2010;16(11):1576–81.
23. Gray JW, George RH. Experience of vancomycin-resistant enterococci in a children's hospital. J Hosp Infect. 2000;45(1):11–8.
24. Flokas ME, Karageorgos SA, Detsis M, Alevizakos M, Mylonakis E. Vancomycin-resistant enterococci colonisation, risk factors and risk for infection among hospitalised paediatric patients: a systematic review and meta-analysis. Int J Antimicrob Agents [Internet]. 2017;49(5):565–72. Available from: http://dx.doi.org/10.1016/j.ijantimicag.2017.01.008
25. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis [Internet]. 2005;5(8):481–93. Available from: http://www.sciencedirect.com/science/article/pii/S1473309905701894
26. Olivier CN, Blake RK, Steed LL, Salgado CD. Risk of Vancomycin-Resistant Enterococcus (VRE) Bloodstream Infection Among Patients Colonized With VRE. Infect Control Hosp Epidemiol [Internet]. 2008;29(5):404–9. Available from: https://www.cambridge.org/core/product/identifier/S0195941700026801/type/journal_article
27. Zacharioudakis IM, Zervou FN, Ziakas PD, Rice LB, Mylonakis E. Vancomycin-Resistant Enterococci Colonization Among Dialysis Patients: A Meta-analysis of Prevalence, Risk Factors, and Significance. Am J Kidney Dis [Internet]. 2015;65(1):88–97. Available from: http://linkinghub.elsevier.com/retrieve/pii/S027263861400907X
28. Tornieporth NG, Roberts RB, John J, Hafner a, Riley LW. Risk factors associated with vancomycin-resistant Enterococcus faecium infection or colonization in 145 matched case patients and control patients. Clin Infect Dis [Internet]. 1996;23(4):767–72. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8909842
29. O'Driscoll T, Crank CW. Vancomycin-resistant enterococcal infections: Epidemiology, clinical manifestations, and optimal management. Infect Drug Resist. 2015;8:217–30.
30. Boost M, Lai L, O'Donoghue M. Drug resistance in fecal enterococci in Hong Kong. J Infect Chemother [Internet]. 2004;10(6):326–30. Available from: http://dx.doi.org/10.1007/s10156-004-0337-Z
31. Gajan E, Shrimohammad A, Aghazadeh A, Alizadeh M, Sighari A, Ahmadpour F. Antibiotic Resistance in Enterococcus faecalis Isolated from

Hospitalized Patients. J Dent Res Dent Clin Dent Prospect Orig [Internet]. 2013;7(2):7–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23875089%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3713858

32. Lanetzki CS, Oliveira CAC de, Bass LM, Abramovici S, Troster EJ. The epidemiological profile of Pediatric Intensive Care Center at Hospital Israelita Albert Einstein. Einstein (São Paulo) [Internet]. 2012;10(1):16–21. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1679-45082012000100005&lng=en&tlng=en
33. Dutka-Malen S, Evers S, Courvalin P. Detection of glycopeptide resistance genotypes and identification to the species level of clinically relevant enterococci by PCR. J Clin Microbiol. 1995;33(1):24–7.
34. Elwakeel NA, Elwakeel AA IE and EH. Prevalence, phenotypic and Genotypic Characterization of Vancomycin Resistant Enterococci (VRE) isolated from Children with Infective Endocarditis in Mansoura University Children's Hospital Department of Medical Microbiology and Immunology , Faculty. Egypt J Med Microbiol. 2012;21(4):21–30.
35. Yameen MA, Iram S, Mannan A, Khan SA, Akhtar N. Nasal and perirectal colonization of vancomycin sensitive and resistant enterococci in patients of paediatrics ICU (PICU) of tertiary health care facilities. BMC Infect Dis [Internet]. 2013;13(1):156. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23536967%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC3621148
36. Godreuil S, Marchandin H, Boulier A, Boumzebra A, Campos J, Jean-Pierre H. [A 6-year study of glycopeptides-resistant enterococci in 3 intensive care units of the University Hospital of Montpellier]. Pathol Biol (Paris). 2007 Nov;55(8–9):418–23.