ORIGINAL ARTICLE

Frequency and co-colonization of vancomycin-resistant *Enterococci* and *Candida* in ICU-hospitalized children

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Abstract

In the time span between January 2018 and September 2020, 205 patients were enrolled in a prospective cohort study at Mofid Children's Hospital. Demographic information and clinical data on all the participating children were collected and rectal swabs were performed for the sampling method. All samples were analysed so as to identify the presence of *Enterococcus* and *Candida* colonization by the use of conventional biochemical tests. Resistance to vancomycin in *Enterococcus* isolates was phenotypically identified using an E-test kit and MIC value, interpreted according to the CLSI criteria. The presence of *vanA* and *vanB* genes, which encode the resistance to vancomycin, was screened by PCR assay. *Candida* species were detected in 21.5% of rectal swab samples. *Candida glabrata* (56.8%) and *Candida albicans* (43.2%) were the only *Candida* species detected. *Enterococcus* species were detected in 29.3% of rectal swab samples. Out of 60 *Enterococcus* isolates, 33 (55%) were resistant to vancomycin. Moreover, *vanA* was detected in 84.8% and *vanB* was detected in 3% of the 33 vancomycin-resistant *Enterococcus* isolates. *Enterococcus* and *Candida* species were frequently detected in the <1 year and 1–3 years age groups, respectively. Central venous access catheter and brain tumour were the main reasons for hospital admissions, 32.2% and 20.1% of total admissions, respectively. Furthermore, it must be noted that the most frequent underlying medical conditions in participating patients were esophageal atresia and hydrocephalus. The results of the present study demonstrated the necessity of determining the susceptibility of *Enterococcus* isolates to vancomycin before prescribing antibiotics.

Keywords: *Candida* spp., *Enterococcus* spp., intensive care unit, Iran, vancomycin
Original Submission: 25 November 2020; **Revised Submission:** 3 April 2021; **Accepted:** 7 April 2021
Article published online: 14 April 2021

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Introduction

Healthcare-associated infections (HAI) carry substantial costs to hospitals and are considered serious challenges to patient care all over the world [1,2]. According to a national update for the year 2015 on HAI in Iran, approximately 21% of patients hospitalized in intensive care units (ICU) for HAI die every year [3]. Therefore, HAI in ICU are increasingly associated with high mortality and morbidity rates [4]. Among HAIs, polymicrobial infections are associated with severe clinical outcomes, frequently leading to aggressive infections. In most cases, polymicrobial infections exert an impact on therapeutic procedures and display increased clinical resistance to prescribed antibiotics [5,6]. Globally, the fungus *Candida* species and the Gram-positive bacterium *Enterococcus* species are ranked among the top microorganisms that frequently co-exist in polymicrobial infections [2]. Among the *Candida* species, *Candida albicans* is a serious challenge to public health and is considered the third most frequently isolated hospital-acquired bloodstream pathogen [7]. Both microorganisms (*Candida* species and *Enterococcus* species) are usually normal colonizers of human urogenital and gastrointestinal tracts. However, these

https://doi.org/10.1016/j.nmni.2021.100881

organisms could be isolated from the oral cavity or the moist skin as well. Mucosal surfaces are the leading opportunistic pathogens causing infections in humans [8–10]. Under several conditions, such as immune dysfunction and antibiotic therapy, both pathogens could cause opportunistic disseminated infections including invasive candidiasis; bloodstream, central nervous system, urinary tract, intra-abdominal and pelvic infections; endocarditis and localized mucosal and skin infections [11,12]. *Enterococcus* species, or *Enterococcus faecium* in particular, have the highest frequency of all enterococcal infections (80%), leaving approximately 20% of enterococcal infections to be caused by other *Enterococcus* species [8]. Vancomycin is one of the most important antibiotics used to treat infections caused by multidrug-resistant enterococci [13]. However, enterococci develop a high level of resistance against this antibiotic. Vancomycin-resistant enterococci (VRE) are frequently isolated from hospitalized patients [14]. Furthermore, having an increased mortality rate, VRE is a growing issue concerning health care [5]. Among *Enterococcus* species, *E. faecium* and *Enterococcus faecalis* constitute the majority of VRE cases [13]. Based on WHO reports, vancomycin-resistant *E. faecium* is a high-priority pathogen, urgently needing antimicrobial research and development [8]. In the health-care setting, asymptomatically colonized patients with VRE might act as reservoirs, leading to an increase in the spread of VRE [15]. Although prevention and control are critical, a better knowledge of the risk factors that prognosticate VRE and *Candida* species colonization is necessary. The present cross-sectional study has been conducted to analyse the frequency of *Candida* species, alone and in conjunction with *Enterococcus* species, and VRE isolates. Finally, risk factors for rectal colonization by both pathogens in the case of ICU-hospitalized children at Mofid Children's hospital have been examined.

Materials and methods

Study population and sample collection

Aiming at investigating the frequency and co-colonization of VRE and *Candida* species, a cross-sectional, single-centre study was conducted in children hospitalized in surgical or medical ICUs at Shahid Beheshti University of Medical Sciences, Mofid Hospital between January 2018 and September 2020. First, written informed consent forms were signed by parents and the aims of the study were explained to all participating patients. Sampling was subsequently performed using rectal swabs (Amies agar gel; Copan Diagnostics, Murrieta, CA, USA), simultaneously testing the children for rectal *Enterococcus* species and *Candida* colonization. Within 24 hours, all specimens were transported to the central laboratory at the Paediatric Infectious Diseases Research Centre, where they were subsequently cultured on different bacterial and fungal media within 4 hours of their arrival. Finally, demographic information and clinical data on participating patients were collected, including age, sex, residence (urban or rural), reason for admission, length of ICU stay, medical records, underlying medical conditions and malignancy, antimicrobial drug exposure, duration of antimicrobial therapy.

The study was approved by the Ethics Committee of the Paediatric Infectious Diseases Research Centre, Shahid Beheshti University of Medical Sciences (SBMU), Tehran, Iran (IR.NIMAD.REC1396.103).

Growth conditions and microbiological processing

When examining the presence of *Candida* species, rectal swabs were inoculated into microbiology media, such as Sabouraud dextrose agar containing chloramphenicol and CHROM agar *Candida* (Merck, Darmstadt, Germany). *Candida* species identification was conducted using a range of conventional tests, such as direct microscopic observation, culture on cornmeal agar with polysorbate 80, and germ tube formation in fetal calf serum at 37°C. Identification of *Enterococcus* species and VRE was performed using different conventional tests, such as culture on bile esculin broth and bile esculin agar with 6 μg/mL vancomycin. The plates were then incubated for 24–48 hours and subsequently examined for the growth of yeast isolates and Gram-positive cocci using a Gram staining method as well as catalase and pyrrolidonyl arylamidase tests.

VRE laboratory screening

The vancomycin MIC of *Enterococcus* isolates was determined by an E-test kit (Biomérieux, Geneva, Switzerland). For this purpose, *Enterococcus* isolates were initially subcultured on a Müller–Hinton agar medium and then the plates were incubated for 24 hours at 37°C. After 24 hours, bacterial colonies were picked to suspend McFarland 2 standards, being streaked on a plate containing Müller–Hinton agar medium. At the next step, vancomycin strips were located at the centre of the plates, incubated for 48 hours at 37°C. The MIC value was interpreted according to the CLSI criteria. *Enterococcus faecalis* ATCC 29212 strains were used as quality control for the E-test method.

Molecular detection of *vanA* and *vanB* genes

Resistance to vancomycin was confirmed by the detection of *vanA* and *vanB* resistance genes. To isolate genomic DNA from VRE strains, a DNA extraction kit (GeNet Bio Company, Daejeon, Korea; Cat. No. K-3000) was used in accordance with the manufacturer's guidelines. The presence of *vanA* and *vanB* genes encoding the resistance to vancomycin was screened by

PCR. Primers used for *vanA* and *vanB* genes are presented in Table 1. The PCR was performed with a final volume of 25 µL reaction mixture containing 2.5 µL of 10 × PCR buffer without $MgCl_2$, 2.5 mmol/L $MgCl_2$, 250 µM of each dNTP, 10 pM of each primer, 1 IU *Taq* DNA polymerase (Cinnagene, Tehran, Iran), 2.5 µL of bacterial DNA, and sterile distilled water up to 25 µL. Amplification reactions were carried out on a thermal cycler (Eppendorf, Mastercycler Gradient; Eppendorf, Hamburg, Germany) with the condition previously reported by Daghighi *et al.* [16]. After staining with the DNA-safe stain, PCR products were visualized on a 1.5% agarose gel under UV light (SinaClon, Tehran, Iran). *Enterococcus faecalis* ATCC 51299 (*vanB* positive) and *E. faecium* ATCC 700221 (*van A* positive) were used as positive controls.

Statistical analysis

All demographic information and clinical data of patients were collected, and a descriptive analysis was conducted with SPSS version 23 (IBM, Armonk, NY, USA).

Results

Study population

The present study surveyed a total of 205 children hospitalized in ICU at Mofid Children's Hospital, Tehran, Iran from January 2018 to September 2020. Clinical data and demographic information of these patients are summarized in Table 2. In total, 118 (57.6%) and 87 (42.4%) rectal swab samples were collected from male and female patients, respectively. A total of 185 (90.2%) and 20 (9.8%) patients were resident in urban and rural areas, respectively. Fifty-seven patients had at least one underlying medical condition. The most frequent underlying medical conditions among the included patients were oesophageal atresia (13/205), hydrocephalus (11/205), craniosynostosis (10/205) and skull malformation (9/205). Overall, out of 205 patients, 124 (60.8%) and 99 (48.3%) had records of surgery and hospitalization, respectively. Among hospitalized children, the main reasons for hospitalization were central venous access catheter (66/205; 32.2%), surgery to remove a brain tumour (40/199; 20.1%), acute respiratory distress syndrome (37/199; 18.6%), seizure (18/199; 9%), craniosynostosis (10/199; 5%), shunting (7/199; 3.5%), lethargy (6/199; 3%) and thoracotomy (6/199; 3%). Types of prescribed antibiotics as well as the duration of their therapy are summarized in Table 3. Most of the patients (95%) had a record of antibiotic therapy and cephalosporins were used in 84.9% (174/205). Vancomycin (75/205; 36.6%) was, in particular, the most frequently prescribed antibiotic. The duration of antibiotic use was 2–5 days on average. Moreover, out of the 205 participants in the present study, only three (1.5%) and five (2.4%) made use of antifungal and antiviral drugs, respectively. The duration of hospitalization in most cases of either *Enterococcus* or *Candida* infection was ≤3 days. However, co-colonization of *Enterococcus* and *Candida* species extended the duration of therapy to 4–6 days. An illustration of the duration of hospitalization is shown in Table 4.

TABLE 1. Primers used for the detection of the *vanA* and *vanB* resistance genes in *Enterococcus* species

Primer name		Sequence (5'→3')	PCR product size (bP)
vanA	F	CATGAATAGAATAAAAGTTGCAATA	1030
	R	CCCCTTTAACGCTAATACGATCAA	
vanB	F	GTGACAAACCGGAGGCGAGGA	433
	R	CCGCCATCCTCCTGCAAAAAA	

Frequency of *Candida* species in rectal swab samples

The frequency of *Candida* species and *Enterococcus* species, and of co-colonization of *Candida* and *Enterococcus*, are presented in Table 2. Overall, as indicated by conventional microbiological tests, *Candida* species were detected in 44 (21.5%) rectal swab samples. The frequency rates of *Candida* species among male and female participants were 22.9% (27/118) and 19.5% (17/87), respectively. Among patients with *Candida* infection, 16 patients had a central venous access catheter and eight patients had acute respiratory distress syndrome. Of patients with *Candida* species infections, 24% and 21% had medical records of hospitalization and surgery, respectively. Moreover, 21% of the previously mentioned patients had undergone different antibiotic therapies. The frequency of the *Candida* species isolated from rectal swab samples in different age groups is shown in Table 5. *Candida* species showed the highest frequencies in the age groups <1 year and 1–3 years with 47.7% and 34%, respectively. *Candida glabrata* (25/44; 56.8%) and *C. albicans* (n = 19/44; 43.2%) were the only *Candida* species detected in rectal swab samples. The frequency of *Candida* species was as follows: *C. glabrata* (25/205; 12.2%) and *C. albicans* (19/205; 9.3%).

Frequency of *Enterococcus* species and *vanA* and *vanB* genes

The frequency of *Enterococcus* species isolated from rectal swab samples in different age groups is shown in Table 5. Overall, according to conventional microbiological tests, *Enterococcus* species were detected in 60 (29.3%) rectal swab samples. *Enterococcus* species were isolated from 29.7% and 28.7% of rectal swab samples collected from male and female patients, respectively. Briefly, 33.3% of patients with a medical record of

TABLE 2. Clinical data, demographic information, and frequency of *Candida* and *Enterococcus* species alone and in co-colonization condition among hospitalized patients in intensive care units

Characteristic	*n.*	Total with characteristic *n* (%)	With *Candida* *n* (%)	Without *Candida* *n* (%)	With *Enterococcus* *n* (%)	Without *Enterococcus* *n* (%)	With *Candida* and *Enterococcus* *n* (%)
Gender (male)	118 (57.6%)	—	27 (22.9%)	91 (77.1%)	35 (29.7%)	83 (71.3%)	11 (3.4%)
Gender (female)	87 (42.4%)	—	17 (19.5%)	70 (80.5%)	25 (28.7%)	62 (71.3%)	12 (8%)
Resident (urban)	185 (90.2%)	—	40 (21.6%)	145 (78.4%)	54 (29.2%)	131 (70.8%)	21 (5.4%)
Resident (village)	20 (9.8%)	—	4 (20%)	16 (80%)	6 (30%)	14 (70%)	2 (20%)
Underlying medical condition	205	57 (27.8%)	—	—	—	—	—
Surgical history	204	124 (60.8%)	26 (21%)	98 (79%)	35 (28.2%)	89 (71.8%)	13 (10.5%)
Hospitalization history	205	99 (48.3%)	24 (24.2%)	75 (75.8%)	33 (33.3%)	66 (66.7%)	12 (12.1%)
Antibiotic usage	205	195 (95.1%)	41 (21%)	154 (79%)	54 (27.6%)	141 (72.4%)	21 (10.8%)
Pneumonia	199	4 (2%)	—	—	1(25%)	3(75%)	—
Oesophageal atresia	199	3 (1.5%)	1 (33.3%)	2 (66.7%)	0 (0%)	3 (100%)	—
Acute respiratory distress syndrome	199	37 (18.6%)	8 (21.6%)	29 (78.4%)	10 (27%)	27 (73%)	4 (10.8%)
Brain tumour	199	40 (20.1%)	4 (10%)	36 (90%)	9 (22.5%)	31 (77.5%)	2 (5%)
Congenital heart disease	199	2 (1%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Guillain–Barré	199	1 (0.5%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	—
Gastroenteritis	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Diarrhoea	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Pelvic fracture	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Seizure	199	18 (9%)	3 (16.7%)	15 (83.3%)	7 (38.9%)	11 (61.1%)	2 (11.1%)
Transrectal ultrasound	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Abdominal mass	199	2 (1%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Endoscopy	205	9 (4.4%)	3 (33.3%)	6 (66.7%)	2 (22.2%)	7 (77.8%)	2 (22.2%)
Bronchoscopy	205	10 (4.9%)	3 (30%)	7 (70%)	3 (30%)	7 (70%)	3 (30%)
Anoplasty	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Keratinocytes	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Hepatoblastoma	199	2 (1%)	—	—	0 (0%)	2 (100%)	—
Lethargy	199	6 (3%)	2 (33.3%)	4 (66.7%)	3 (50%)	3 (50%)	2 (33.3%)
Haemolytic–uraemic syndrome	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Meningitis	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Tracheostomy	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Tachypnoea	199	2 (1%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Pleural effusion	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Gastrostomy	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Laparoscopy	199	1 (0.5%)	—	—	1 (100%)	0 (0%)	—
Cough	199	4 (2%)	2 (50%)	2 (50%)	3 (75%)	1 (25%)	1 (25%)
Vomiting	199	2 (1%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)
Ataxia	199	1 (0.5%)	1 (100%)	0	0 (0%)	1 (100%)	—
Headache	199	1 (0.5%)	0	0	0 (0%)	1 (100%)	—
Laparotomy	199	6 (3%)	2 (33.3%)	4 (66.6%)	1 (16.6%)	5 (83.4%)	—
Pelvis osteosarcoma	199	1 (0.5%)	—	—	1 (100%)	0 (0%)	—
Hirschsprung's disease	199	1 (0.5%	—	—	1 (100%)	0 (0%)	—
Leucodystrophy	199	1 (0.5%)	—	—	1 (100%)	0 (0%)	—
Posterior urethral valve	199	1 (0.5%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	—
Gastric polyps	199	7 (3.5%)	1 (14.3%)	6 (85.7%)	2 (28.5%)	5 (71.5%)	1 (14.3%)
Shunting	199	7 (3.5%)	1 (14.3%)	6 (85.7%)	2 (28.6%)	5 (71.4%)	—
Burns	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Hydatid cyst	199	2 (1%)	—	—	1 (50%)	1 (50%)	—
Thoracotomy	199	6 (3%)	2 (33.3%)	4 (66.6%)	2 (33.3%)	4 (66.6%)	1 (16.7%)
Craniosynostosis	199	10 (5%)	2 (20%)	8 (80%)	2 (20%)	8 (80%)	1 (10%)
Colostomy	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Leucotomy	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Craniotomy	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Skull malformation	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Status epilepticus	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Undescended testicle	199	1 (0.5%)	1 (100%)	0 (0%)	1 (100%)	0 (0%)	1 (100%)
Neonatal jaundice	199	2 (1%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	—
Hydrocephalus	199	2 (1%)	2 (100%)	0 (0%)	1 (50%)	1 (50%)	1 (50%)
Trauma	199	1 (0.5%)	—	—	1 (100%)	0 (0%)	—
Teratomas	199	1 (0.5%)	1 (100%)	0 (0%)	0 (0%)	1 (100%)	—
Rectorrhagia	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Intracerebral haemorrhage	199	1 (0.5%)	—	—	0 (0%)	1 (100%)	—
Central venous access catheter	205	66 (32.2%)	16 (24.2%)	50 (75.8%)	22 (33.3%)	44 (66.7%)	11 (16.7%)
Bronchoalveolar lavage	205	8 (3.9%)	2 (25%)	6 (75%)	2 (25%)	6 (75%)	2 (25%)

hospitalization experienced *Enterococcus* infection, and 28.2% of those with *Enterococcus* infection had a medical record of surgery. As indicated by the results, 27.6% (54/195) of patients under antibiotic therapy were positive for *Enterococcus* species. Among patients with *Enterococcus* species, 33.3% (22/66) had central venous access catheter and 27% (10/27) had acute respiratory distress syndrome. Similar to *Candida* species, *Enterococcus* species showed the highest frequency in the <1

year and 1–3 years age groups, respectively. The results of the antibiotic susceptibility test by E-test kits indicated that the frequency of VRE isolates was 16% (33/205). Fifty-five per cent (33/60) of *Enterococcus* species isolates were resistant to vancomycin. In this study, resistance to vancomycin was confirmed by the detection of *vanA* and *vanB* resistance genes. The results demonstrated that the frequency rates of *vanA* and *vanB* resistance genes in *Enterococcus* species were 46.7% and 1.7%, respectively. Moreover, the results indicated that out of 33 VRE isolates, 28 (84.8%) and 1 (3%) contained *vanA* and *vanB* genes, respectively. In contrast, 4 (12.2%) isolates lacked *vanA* and *vanB* genes.

Prevalence of co-colonization

The frequency rates of co-colonization of *Enterococcus* species, VRE and *Candida* species are presented in Table 2. The results indicated that *Enterococcus* species and *Candida* species were simultaneously isolated from 23 (11.2%) patients. The frequency of co-colonization of VRE isolates and *Candida* species was 33.3% (11/33). The frequency of co-colonization of different *Candida* species was as follows: *C. glabrata* (6/11; 54.5%) and *C. albicans* (5/11; 45.5%). The highest frequency of co-colonization among *Enterococcus* species and *Candida* species and VRE isolates and *Candida* species was seen in the <1 year age group.

Discussion

Healthcare-associated infections in hospitalized patients have a wide range of mortality and morbidity rates across the globe [17]. *Candida* species could cause different HAIs. Other pathogens such as *Staphylococcus aureus* could result in several adverse outcomes as well [18]. *Candida* and *Enterococcus* species are important pathogens in health-care settings and cause several infections, especially in patients hospitalized in ICU [2,7]. The present study attempted to identify the frequency of *Candida* species, *Enterococcus* species and VRE isolates alone in co-colonization conditions. Moreover, risk factors for rectal colonization by these pathogens in ICU-hospitalized children have also been analysed. The results demonstrated that out of 205 children included in the present study, 60 (29.3%) were positive for *Enterococcus* infections. It must be noted that the results of several studies were not in agreement with those of the present research. In the studies carried out by Daghighi *et al.* [16], Moosavian *et al.* [19] and Gonzalez *et al.* [20], *Enterococcus* species had a high frequency in patients' rectal samples. Furthermore, Azimi *et al.* demonstrated that the frequency of *Enterococcus* species in the ICU ward was low [1]. Several factors, including the diversity of specimen types, could properly justify the detected differences in the proportions of *Enterococcus* species. Although the present study made use of rectal swab samples, Azimi *et al.* applied samples from the wound, blood, cerebrospinal fluid and pleural fluid [1]. Moreover, exposure to dissimilar antibiotics, duration of antibiotic therapy, different detection methods and different sample sizes could possibly affect the prevalence rate of *Enterococcus* species [1,19]. In the current study, 95.1% of patients were under antibiotic therapy and most patients had used different antibiotics such as cephalosporins, aminoglycoside, meropenem and vancomycin for 1–5 days. The frequency of VRE isolates in rectal swab samples was 16% (33/205), which is in agreement

TABLE 4. The duration of hospitalization of patients with *Candida* and *Enterococcus* colonization

Agents	Duration of hospitalization (no. of patients)						
	≤3 days	4–6 days	7–10 days	11–15 days	16–20 days	>20 days	Total
Enterococcus	24	18	11	3	1	3	60
VRE	14	8	5	3	1	2	33
Candida species	17	14	7	3	—	3	44
Candida glabrata	11	6	4	1		3	25
Candida albicans	6	8	3	2	—	—	19
Enterococcus and *Candida*	6	10	4	1	—	2	23
Enterococcus and VRE	3	5	1	1	—	1	11

Abbreviation: VRE, vancomycin-resistant enterococci.

TABLE 3. The type of antibiotics with duration of usage among patients hospitalized in intensive care units

Antibiotics	N[a]	With usage	Without usage	Duration of antibiotic usage			
				0–1 day	2–5 days	6–10 days	>10 days
Cephalosporin	205	174 (84.9%)	31 (15.1%)	65 (37.3%)	89 (51.2%)	17 (9.8%)	3 (1.7%)
Aminoglycoside	205	16 (7.8%)	189 (92.2%)	5 (31.3%)	6 (37.5%)	1 (6.2%)	4 (25%)
Meropenem	205	43 (21%)	162 (79%)	8 (18.6%)	24 (55.8%)	7 (16.3%)	4 (9.3%)
Vancomycin	205	75 (36.6%)	130 (63.4%)	15 (20%)	39 (52%)	14 (18.7%)	7 (9.3%)
Other antibiotics[b]	205	76 (37%)	129 (63%)	25 (32.9%)	14 (18.4%)	24 (31.6%)	13 (17.1%)

[a]N, number of all patients.
[b]Antibacterials: cotrimoxazole, azithromycin, clindamycin, ampicillin, metronidazole, piperacillin/tazobactam, colistin, rifampicin, ampicillin/sulbactam; antivirals: aciclovir, and oseltamivir; antifungals: fluconazole and voriconazole.

TABLE 5. The frequency of the *Enterococcus* species and *Candida* species alone and in co-colonization condition among by different age groups

Agents	Age groups (years)					Total
	<1	1–3	4–6	7–10	>10	
Candida spp.	21 (47.7%)	15 (34%)	5 (11.4%)	2 (4.5%)	1 (2.3%)	44 (100%)
Candida albicans	9 (47.4%)	7 (36.8%)	2 (10.5%)	1 (5.3%)	—	19 (100%)
Candida glabrata	12 (48%)	8 (32%)	3 (12%)	3 (12%)	1 (4%)	25 (100%)
Enterococcus spp.	34 (56.7%)	12 (20%)	7 (11.7%)	4 (6.7%)	3 (5%)	60 (100%)
VRE	19 (57.6%)	6 (18.2%)	4 (12.2%)	2 (6%)	2 (6%)	33 (100%)
Candida and enterococci	14 (60.9%)	7 (30.4%)	2 (8.7%)	—	—	23 (100%)
Candida and VRE	8 (72.7%)	3 (27.3%)	—	—	—	11 (100%)

Abbreviation: VRE, vancomycin-resistant enterococci.

with the studies conducted by Gonzalez *et al.* from the Netherlands [20], Melese *et al.* from Ethiopia [21], and Akpaka *et al.* from Trinidad and Tobago [22]. However, the results of several studies performed in different countries were not in agreement with the findings of the present research and it was proven that resistance to vancomycin in *Enterococcus* species was high [16,19,23]. According to an epidemiological analysis of bloodstream infections in European countries, the rate of VRE isolates increased from 8.1% in 2012 to 19.0% in 2018 [24]. It was revealed that infection with VRE isolates has several unfortunate consequences, such as (a) decrease in the success rate of the infection treatment, (b) increase in mortality rates, (c) increase in total admission days, and (d) increase in healthcare and ICU costs [19,25]. It was, in addition, demonstrated that 55% of *Enterococcus* species were VRE, an extremely serious issue in hospitals. Therefore, drastic measures including constant surveillance of hospitals, strict monitoring of antibiotic prescription and consumption, reliable preparation of guidelines, and use of strategies regarding antibiotic consumption are critically required. The PCR assay demonstrated that 46.7% (28/44) and 84.8% (28/33) of *Enterococcus* species and VRE isolates contained the *vanA* gene, respectively. These results were in approximate agreement with the findings of the studies carried out by Daghighi *et al.* (89.3%), Moosavian *et al.* (91.5%), Rahimi *et al.* (100%), Akpaka *et al.* (84%), Caiaffa Filho *et al.* (100%) and Marom *et al.* (100%) [4,9,16,19,22,26]. Overall, the results of the present study, in line with the findings of other studies carried out in different parts of the world, revealed that the *vanA* gene was the main resistance mechanism in *Enterococcus* isolates recovered in ICU. *Enterococcus* isolates positive for the *vanA* gene are generally resistant to both vancomycin and teicoplanin antibiotics. On the other hand, strains that are positive for *vanB* are susceptible to teicoplanin and show a low level of resistance to vancomycin. The *vanA* gene is associated with clinical strains and could be potentially introduced to conjugative plasmid. This gene is transferable and could quickly spread through conjugation between enterococci, staphylococci and other Gram-positive organisms [19,27,16]. The minor differences in the frequency of the *vanA* gene could be partially attributed to the duration of research and type of samples. As the richest source of the spread of VRE was the colonization of enterococci in the digestive system, rectal swab samples were employed in the present study. However, other studies made use of different clinical samples such as wound, blood and urine.

However, in the study carried out by Karki *et al.*, 58 (17.5%) VRE isolates were positive for the *vanB* gene, whereas none of the isolates contained the *vanA* gene [29]. As demonstrated by the results, out of 205 patients included in the present study, 44 (21.5%) were positive for *Candida* infections. These results were in contrast to the previously published studies of Rahbar *et al.* from Iran [30], Motoa *et al.* from Colombia [31], Kumar *et al.* from India [32], Alimehr *et al.* from Iran [33] and Comert *et al.* from Turkey [34]. These studies recorded frequencies of *Candida* species in the ICU ward of 12%, 5.7%, 57.3%, 12%, and 6.6%, respectively. In the current study, *C. glabrata* (25/44; 56.8%) was the most frequent *Candida* species isolated from rectal swab samples. The frequency rates of *C. glabrata* and *C. albicans* in hospitalized children were 12.2% and 9.3%, respectively. These findings are in agreement with the studies conducted by Rahbar *et al.* and Alimehr *et al.* from Iran [30,33]. However, in the studies carried out by Ardehali *et al.* [7], Ruan *et al.* [35] and Comert *et al.* [34], *C. albicans* was the most commonly isolated *Candida* species in positive samples. Moreover, Motoa *et al.* and Kumar *et al.* stated that *Candida tropicalis* had the highest frequency in ICU [31,32]. According to the national surveillance studies, the rate of nosocomial systemic *Candida* infections varied greatly between different types of ICUs and hospitals. In recent years, a shift from *C. albicans* to non-albicans *Candida* species among ICU patients has been reported [36,37]. Among *Candida* species, *C. glabrata* is the second most frequent causative agent of nosocomial systemic infections. Significantly, *C. glabrata* is more resistant to antifungal agents, and is harder to eradicate than other *Candida* species [36].

Therefore, this species is probably a problematic challenge to clinicians in health-care settings [30]. In total, the subgroup analysis of the present research in different age groups indicated that both *Candida* species and *Enterococcus* species had the highest proportion in the <1 year and 1–3 year age groups, respectively. It could be concluded that children, and more importantly neonates, are susceptible to pathogens such as *Candida* and *Enterococcus* species becauseof their defective and immature immune systems [38]. Therefore, it is critical to implement a protective policy against such agents in the high-risk age groups mentioned. The rates of co-colonization of *Candida* species with *Enterococcus* species and VRE isolates in patients were 23/205 (11.2%) and 11/205 (5.4%), respectively. Making use of a nematode infection model, a study interestingly revealed that the co-infection of *Enterococcus* and *Candida* species was linked to less pathology and less mortality [39]. A switch from yeast-form growth to a hyphal, invasive and pathogenic state is a critical step in *Candida* pathogenesis. In co-infections, the hyphal morphogenesis of *Candida* species was inhibited by bacteria-derived products or molecules [30,39]. However, the exact mechanism of this process is not fully understood. As illustrated by the results, 95% of patients had a record of antibiotic therapy, and in most cases, the duration of antibiotic usage was 2–5 days. Out of 205 patients included in the present study, only three (1.5%) and five (2.4%) had used antifungal and antiviral drugs. In recent decades, the decrease in antifungal therapeutic choice and widespread use of wide-spectrum antibacterial and antifungal drugs has led to the condition that the most pathogenic microorganisms have acquired an incredible capability to mutate and acquire resistance. Therefore, a number of pathogens in health-care settings could develop resistance to frequently used antimicrobial drugs [40–42].

In conclusion, the current study revealed that VRE colonization was very high in hospitals and the main mechanism of resistance to vancomycin was the presence of the *vanA* gene. VRE isolates could transmit the *vanA* gene to other enterococci and Gram-positive bacteria. According to the results, it is necessary that the susceptibility of *Enterococcus* isolates to vancomycin be identified before prescription.

Data availability statement

All data generated or analysed during this study are included in this published article.

Conflicts of interest

The authors declare that they have no competing interests.

Funding

This research did not receive any specific grant from funding agencies in public, commercial or non-profit organizations.

Authors' contributions

FS, AB, NA, FF and MM conceived the study; FS, AB, FF, NE, MS and MM performed the data curation, formal analysis; and FS, NA and FF contributed to the methodology and to project administration. All the authors contributed to writing the original draft and FF, NE and MS reviewed and edited it. FS and FF performed the language editing.

Acknowledgement

We would like to thank the Paediatric Infectious Diseases Research Centre, Shahid Beheshti University of Medical Sciences, Tehran, Iran, for their kind cooperation.

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