Five-year laboratory-based study of *Candida albicans* versus non-*albicans Candida* species at a tertiary pediatric care hospital in Iran

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Original Article

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ABSTRACT

Objective: To determine the distribution of *Candida albicans* and non-*albicans Candida* species in various specimen types of pediatric patients

Design: Retrospective cross-sectional study using laboratory database

Setting: Children's Medical Center, Tehran, Iran from 2012 to 2016

Subjects: All specimens from pediatric patients with positive culture for *Candida* species in microbiological examinations

Intervention: None

Main outcome measures: Frequency of *Candida* species in different specimen types for five years

Results: During the study period, 2755 out of 2,95,525 (0.93%) specimens were positive for *Candida* growth in culture. Of them, 550 (19.96%) were from normally sterile specimens or sites. *C. albicans* was the predominant species (68.9%), significantly isolated from both genders (P = 0.009), and all age groups (P = 0.011). However, in 2014 and 2015 almost 50% of isolates from normally sterile specimens or sites were non-*albicans Candida* species.

Urine samples were the greatest source of isolation (n=1314, 47.7%) followed by throat swabs (n=472, 17.1%) and blood specimens (n=344, 12.5%). Children were the prevailing age group for *Candida* isolation (n=1435, 52.1%) followed by infants (n=1012, 36.7%) and neonates (n=308, 11.2%).

Conclusion: *C. albicans* was the dominant species in Children's Medical Center. However, the proportion of non-*albicans Candida* species was higher in some specimen types and the frequency of *Candida* species was different in various wards. These data could be beneficial in clinical setting.

KEYWORDS: *Candida*, candidemia, candidiasis, infant, Iran

INTRODUCTION

Candida species are among the most important fungi with an opportunistic nature. They can cause a variety of diseases known as candidiasis, ranging from cutaneous involvement to blood stream and systemic infections[1,2]. These fungi are the fourth most prevalent etiology of sepsis in the United States and the third most common cause of healthcare associated sepsis in children[3,4]. Invasive candidiasis is associated with a mortality rate of 10% in children and it results in prolonged hospital stay and economic burden[5].

The genus *Candida* comprises of more than 30 species with clinical importance and *C. albicans* has been reported as the prevailing cause of candidiasis in various patient groups including neonates and children[6,7]. However, an increasing trend in the incidence of non-*albicans Candida* (NAC) species has been observed in recent decades[1,4,8] which is supposed to be associated with the prophylactic use of antifungal drugs[9,10] and it is of great clinical importance. NAC species have less susceptibility to antifungal drugs such as fluconazole in comparison to *C. albicans*[8]. Also, intrinsic resistance to azoles and amphotericin B in some NAC species has been reported[10]. Furthermore, in recent years *Candida auris* has been emerged as a multidrug resistant NAC species which is associated with high mortality rates[11].

The changing role of NAC species in clinical setting implies the need for awareness of epidemiology of *Candida* species[10]. Considering the importance of early initiation of therapy in candidiasis, treatment is usually initiated empirically without the aid of laboratory data[12] which increases the risk of treatment failure in the case of resistant species. Therefore, identification of causal agents as *C. albicans* or NAC species (especially those with intrinsic resistance) helps in appropriate antifungal selection[10,12,13].

Although several studies on different patient groups are available in Iran, few reports have been published on pediatric patients[14-20]. To the best of our knowledge, there is no comprehensive report with large scale study population on pediatrics in Iran. Therefore, the aim of the present study was to retrospectively analyze the prevalence of *C. albicans* and NAC species in a wide variety of specimen types of pediatric patients during a five-year period to provide a general view of the etiology of candidiasis in these patients.

SUBJECTS AND METHODS

Data collection

This retrospective study was carried out for five years from 2012 to 2016 at Children's Medical Center as a tertiary care hospital for pediatrics affiliated to Tehran University of Medical Sciences, Tehran, Iran. The protocol of this study was approved by ethics committee of Tehran University of Medical Sciences. Medical records of 295525 microbiology laboratory specimens from 219486 patients were reviewed in laboratory database and all cases with *Candida* growth in culture were recorded. *Candida* isolation from specimens of blood, central venous catheter, suprapubic urine, abscess drainage, wound discharge, synovial fluid, peritoneal fluid, plural fluid, dialysis fluid and CSF were considered clinically significant.

Information including demographic data of patients, hospitalization ward, specimen type and *Candida* species (*C. albicans*/NACspecies) were extracted for all the *Candida* isolates. In the case of age, patients were categorized as neonate (≤28 days), infant (1-12 months) and child (1-18 years).

Laboratory analysis

All specimens were examined based on the routine microbiology laboratory procedures. Culture of specimens of normally sterile body fluids was done in Bactec automated system (Bactec 9120, Becton Dickinson, USA). Culture of other specimen types and sub-culture of positive cases in Bactec were carried out on blood agar (Merck, Darmstadt, Germany), chocolate agar (Merck, Darmstadt, Germany) and MacConkey agar culture media (Merck, Darmstadt, Germany). All culture plates were incubated at 35 °C and checked daily for up to seven days. In order to distinguish between *C. albicans* and NAC species, germ tube test in fresh serum (incubation at 37 °C for up to 3 hours) was performed and all germ tube forming isolates were considered as *C. albicans*.

Statistical analysis

Data were analyzed using chi-square test. Mantel-Haenszel and Extended Mantel-Haenszel chi square were carried out to determine the linear trend over the time. The association between time and annual incidence per

1000 laboratory admissions was studied using Kendall Tau-b. The distribution of *C. albicans* and NAC species in various hospital wards was studied using Pearson's chi-squared test. All analysis was performed in SPSS version 22 and *P* value less than 0.05 was considered as significance level.

RESULTS

During the five-year period of this study, 2755 out of 295525 (0.93%) specimens were positive for *Candida* growth in culture. These isolates were recovered from 1946 out of 219486 (0.89%) patients. In the case of clinical relevance, 550 out of 2755 (19.96%) *Candida* isolates (*C. albicans*, 330 isolates; NAC species, 220 isolates) were recovered from normally sterile sites or specimens (Figures 1A and 1B).

Urine samples were the greatest source of isolation (n=1314, 47.7%) followed by throat swabs (n=472, 17.1%) and blood specimens (n=344, 12.5%). A total of 181 *Candida* isolates were recovered from respiratory tract specimens including sputum, tracheal aspirate and bronchoalveolar lavage. The least common sources of isolation were pleural and synovial fluids (each 1 isolate). Table 1 represents the annual and total number of *C. albicans* and NAC species isolated from various specimen types during the study period.

According to the statistical analysis, the ratio of *C. albicans*/NAC species was significantly increased over time for urine samples (p<0.001). Although the same trend was not observed for other specimen types (Table 1), for pooled data of all the specimens the ratio was changed significantly, as well (p<0.001). The incidence of *Candida* isolation per 1000 microbiology laboratory specimens was changed over time from 4.59 in 2012 to 10.04 in 2016 with a peak at 12.57 in 2014. However, a statistically significant increasing trend was not observed (P=0.327) (Table 1).

Candida species were significantly more isolated from males than females (60.9% vs. 39.1%; *P* = 0.009). Furthermore, *C. albicans* was more frequently isolated than NAC species in general (68.9% vs. 31.1%) and in both genders (female, 72.6% vs. 27.4 %; male, 66.4% vs. 33.6%) which was statistically significant (female, P=0.008; male, P<0.001). The median age of patients was 1 year (range: 1 day to 18 years). Children were the prevailing age group for *Candida* isolation (n=1435, 52.1%) followed by infants (n=1012, 36.7%) and neonates (n=308, 11.2%) (Figure 1-C). The total number of isolates in children and infants in comparison to neonates had a significant increasing trend over the time (P=0.011). Furthermore, in children and infants the ratio of *C. albicans*/NAC species was significantly increased from 2012 to 2016 (children, P<0.001; infant, P=0.036). The same trend was not observed in neonates (P=0.511).

Outpatients constituted the main source of *Candida* isolation (n=454, 16.48%) followed by patients in cardiac intensive care unit (CICU) (n=298, 10.82%). The least number of isolates were recovered from bone marrow transplantation ward with only one isolate during the study period (Figure 2). The frequency of *C. albicans* isolation compared to NAC species was higher throughout the study and the ratio of *C. albicans*/NAC species was significantly different in various hospital wards (P<0.001). Furthermore, the distribution of *C. albicans* and NAC species in various specimen types of different age groups was not similar (Figure 3).

DISCUSSION

Candida species are among normal commensals of many parts of the body including the skin, mucous membranes, respiratory system, gastrointestinal and genital tracts[21]. Nevertheless, colonization of this organism in any site of the body can cause infection in that area[22,23]. The isolation of *Candida* species from surveillance cultures during hospitalization has been defined as colonization[22,23]. It is evident that colonization is a main marker for potential invasive infection, therefore recovery of *Candida* species from any site of the body should be considered and monitored in hospitalized patients[22-25].

In this study, we analyzed the frequency and distribution of *C. albicans* and NAC species over a 5-year period in a tertiary pediatric care hospital. We observed an absolute predominance of *C. albicans* (68.9%) in general and in both normally sterile (60%) and non-sterile (71.1%) specimens/sites which is in keeping with other studies reporting a high prevalence of this species[4,13,19,20,26-28]. However, the annual isolation ratio of *C. albicans* (*C. albicans*/NAC species ratio) did not follow a homogeneous trend. It was changed from 64.4% in 2012 to 75.1% in 2016 with some fluctuations.

In the present study, urinary tract specimens (urine, urinary catheter, and suprapubic urine samples) consisted the major source of *Candida* isolation (1357 of 2755 isolates, 49.2%) which is due to the higher number of these specimens (more than 50% of annual microbiology laboratory specimens). Generally, *C. albicans* was the most frequent species (64.9%) in urinary tract specimens which is in agreement with other studies[17,19,29]. However, in suprapubic urine samples, NAC species were the prevailing isolated *Candida* (10/17, 58.8 %). The dominance of NAC species is observed in other studies as well[30,31]. Since urinary tract infections represent the most frequent diagnosis of invasive *Candida* infections, candiduria should be considered. However, except for suprapubic urine samples, candiduria could not be directly linked to urinary tract infection and more studies are required for distinguishing between colonization and infection[29].

A total of 181 *Candida* isolates were recovered from sputum, tracheal aspirate and bronchoalveolar lavage. It should be noted that *Candida* pneumonia is a rare infection of the lungs, with the majority of cases occurring secondary to hematological dissemination of *Candida* organisms usually from the gastrointestinal tract or skin[32]. However, critically ill pediatric patients bedridden in ICUs under ventilation or patients with serious underlying conditions such as cystic fibrosis (CF) and cancers because of therapies with broad spectrum antibiotics, oral and inhaled steroids and chemotherapy are more likely to be colonized with *Candida* species in their respiratory tract[33]. *C. albicans* is a common colonizer and potential pathogen found in respiratory specimen cultures of CF patients[34]. As the majority of respiratory specimens in our center were from CF patients, the dominance of *C. albicans* (154/181, 85.1%) was confirmed in this study, as well.

Generally, an increasing trend for NAC species in blood samples has been observed over the time and even frequencies higher than 50% for NAC species have been reported in some pediatric settings[27,28,35,36] Similarly, in present study approximately the lowest overall rate of *C. albicans* isolation (54%) was observed in blood specimens. Furthermore, even the dominance of NAC species was observed in 2013 and 2015 with isolation rates of 53.1% and 61.4%, respectively. These findings are almost in accordance with the previous study carried out on candidemia in this center[20].

C. albicans was five times more frequent than NAC species (15 vs. 3) among cerebrospinal fluid (CSF) specimens. Isolation of *Candida* species from CSF represents an episode of disseminated candidiasis, which most often occurs in neonates. It is strongly associated with the use of the ventricular shunt[37]. It should be highlighted that isolation of *Candida* species from blood and other normally sterile body fluids is of great clinical importance and should be taken into account[24].

In present study, the majority of *Candida* strains (52.1%) were isolated from children (1-18 years old) (Figure 1), while, in previous cross sectional studies carried out in this center, infants (1-12 months) were the most common source of *Candida* isolation[19,20]. Likewise, Oeser *et al*[38] and Nucci *et al*[39] in their studies reported the highest rate of candidemia in infants aged < 1 year. This variation in results could be due to the type of specimens included in each study. In this study, data of all specimen types were included which some of them were dominantly taken from children (Figure 3).

Although *C. albicans* was the dominant species in all age groups throughout the study, the ratio of *C. albicans* to NAC species was not constant (neonate, 2.6; children, 2.5, infant, 1.8). Even higher isolation rate of NAC species was observed for infants in 2013 (51.7%). However, the absolute isolation of *C. albicans* from neonates has been reported by Oeser *et al*[26]. This proportions could change based on specimen types, study populations and geographical origin.

Globally, the distribution of *Candida* species differs according to the type of the patients, their underlying diseases and risk factors. Subsequently, the distribution differs in various hospital wards[39]. In the present study, the number of *C. albicans* recovered from all hospital wards was dramatically higher than NAC species (Figure 2). The majority of *Candida* isolates (N = 930, 33.8% of total isolates) were recovered from the ICU wards. This finding is in agreement with the statement that ICU staying is an independent risk factor for invasive candidiasis development[3]. However, it is claimed that the incidence of candidemia has increased over the last decade in internal medical wards (IMWs) and the burden of candidemia is shifting to general departments[34,35]. Also It is reported that candidemia in IMWs have own unique characteristics and high mortality rate[40]. Accordingly, *Candida* species should be considered in all hospital wards.

Collectively, the present study provided a general view of frequency and distribution of *C. albicans* and NAC species based on the specimen types, age groups and hospital wards. It could be considered as the main advantage of this study because there were no comprehensive data in pediatric patients in Iran.

However, NAC species did not identify to the species level which is a disadvantage for this study. Taken together, it seems that *Candida* isolation have an increased trend over the time, however we could not find a significant increase throughout the study period. Accordingly, further studies are needed to prove this claim. Also, identification of *Candida* isolates to the species level and determination of their antifungal susceptibility patterns could be so beneficial for appropriate treatment of patients.

CONCLUSION

C. albicans was the dominant species in Children's Medical Center. However, the proportion of NAC species was higher in some specimen types and the frequency of *Candida* species was different in various wards. It indicates that clinicians should be aware about the distribution of *Candida* species in their hospital and even in various wards. It provides a basic information for appropriate treatment of patients.

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Table 1. The annual and total number of *Candida albicans* and non-*albicans Candida* species isolated from various specimen types in a pediatric tertiary care hospital in Iran

Specimen	Candida species	Year					Total N_i	Total N (%)	chi-square test
		2012	2013	2014	2015	2016			
Urine	CA[a]	98	164	256	178	156	852	1314 (47.7)	p<0.001
	NAC[b]	72	172	70	81	67	462		
Throat swab	CA	16	48	110	134	91	399	472 (17.1)	P=0.496
	NAC	0	13	15	27	18	73		
Blood	CA	36	30	43	22	56	187	344 (12.5)	P=0.561
	NAC	21	34	40	35	27	157		
Stool	CA	0	35	47	34	7	123	185 (6.7)	P=0.217
	NAC	0	13	22	24	3	62		
Sputum	CA	3	1	24	25	38	91	97 (3.5)	P=0.937
	NAC	0	0	1	4	1	6		
Dialysis fluid	CA	3	17	8	9	16	53	64 (2.3)	P=0.075
	NAC	2	3	5	0	1	11		
Wound discharge	CA	14	3	0	9	7	33	48 (1.7)	P=0.084
	NAC	2	2	1	4	6	15		
BAL[c]	CA	0	0	10	11	11	32	46 (1.7)	P=0.538
	NAC	0	1	5	3	5	14		
CVC[d]	CA	4	1	4	1	13	23	43 (1.6)	P=0.359
	NAC	1	2	8	6	3	20		
Tracheal aspirate	CA	5	3	7	7	9	31	38 (1.4)	P=0.760
	NAC	1	1	1	1	3	7		
Urinary catheter	CA	1	1	6	5	8	21	26 (0.9)	P=0.278
	NAC	1	0	2	1	1	5		
Ocular specimens	CA	3	3	5	2	2	15	22 (0.8)	P=0.925
	NAC	2	1	0	4	0	7		
CSF[e]	CA	2	2	2	2	7	15	18 (0.7)	P=0.730
	NAC	0	1	0	0	2	3		
Suprapubic urine	CA	0	0	4	3	0	7	17 (0.6)	P=0.628
	NAC	0	0	6	2	2	10		
Peritoneal fluid	CA	1	3	2	2	1	9	10 (0.4)	P=0.493
	NAC	0	1	0	0	0	1		
Abscess drainage	CA	0	0	0	0	2	2	4	P=0.317

	NAC	0	0	1	0	1	2	(0.1)	
Ear discharge	CA	0	0	1	0	0	1	3	P=0.480
	NAC	0	1	1	0	0	2	(0.1)	
Nasal discharge	CA	0	0	0	0	2	2	2	-
	NAC	0	0	0	0	0	0	(0.07)	
Synovial fluid	CA	0	1	0	0	0	1	1	-
	NAC	0	0	0	0	0	0	(0.015)	
Pleural fluid	CA	0	0	0	0	0	0	1	-
	NAC	0	0	0	0	1	1	(0.015)	
Total	CA	186	312	529	444	426	1897	2755	p<0.001
	NAC	102	245	178	192	141	858	(100)	
Total admission		62749	60146	56253	59921	56456		295525	
Incidence per 1000		4.59	9.26	12.57	10.61	10.04		9.32	P=0.327

a. *Candida albicans*, b. Non-*albicans Candida* species, c. bronchoalveolar lavage, d. central venous catheter, e. cerebrospinal fluid

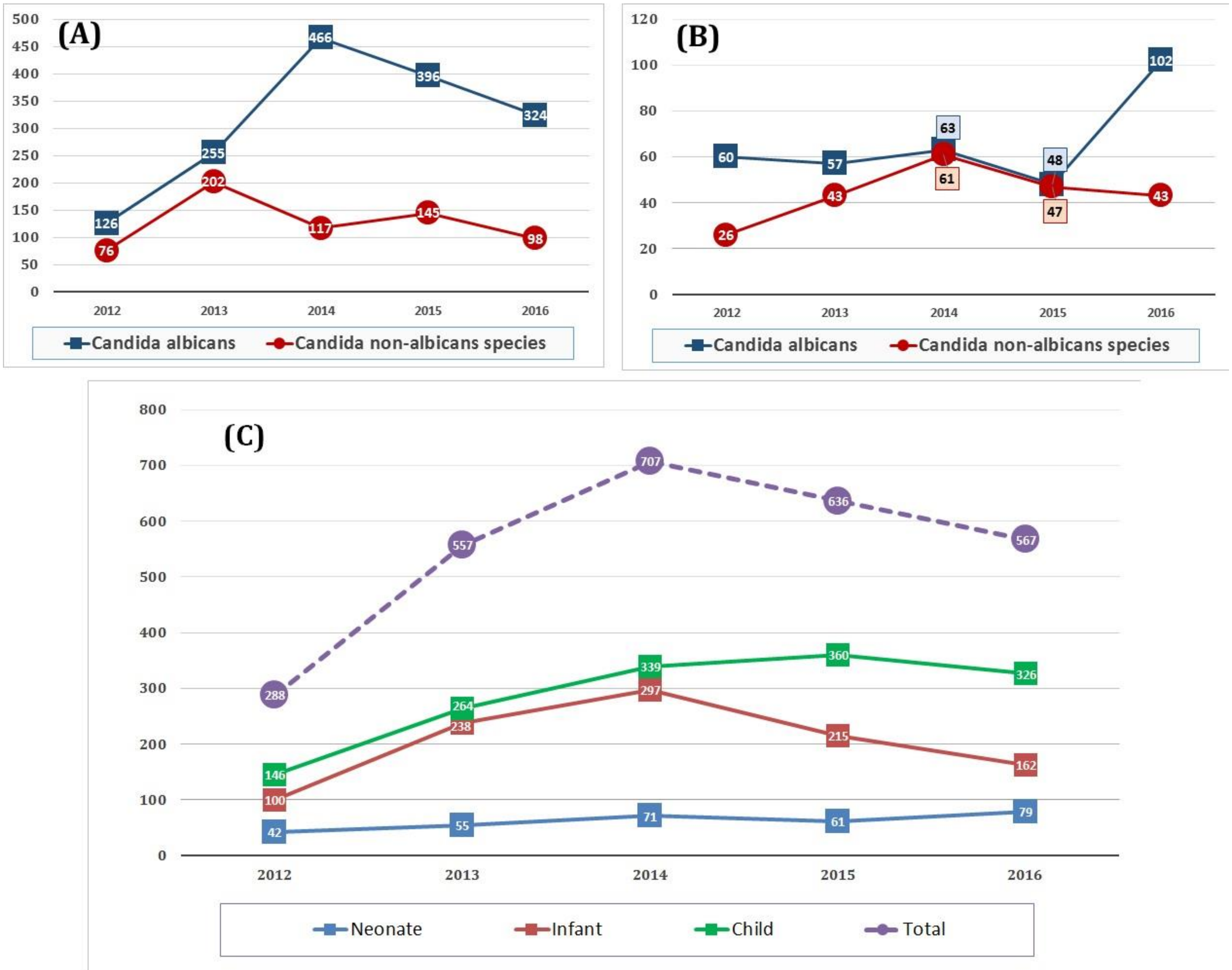

Figure 1. The annual frequency of *Candida albicans* and non-*albicans Candida* species isolated from normally non-sterile body specimens/sites (A) and normally sterile body specimens/sites (B) and the annual frequency of *Candida* species in three age groups of patients (C) in a pediatric tertiary care hospital in Iran.

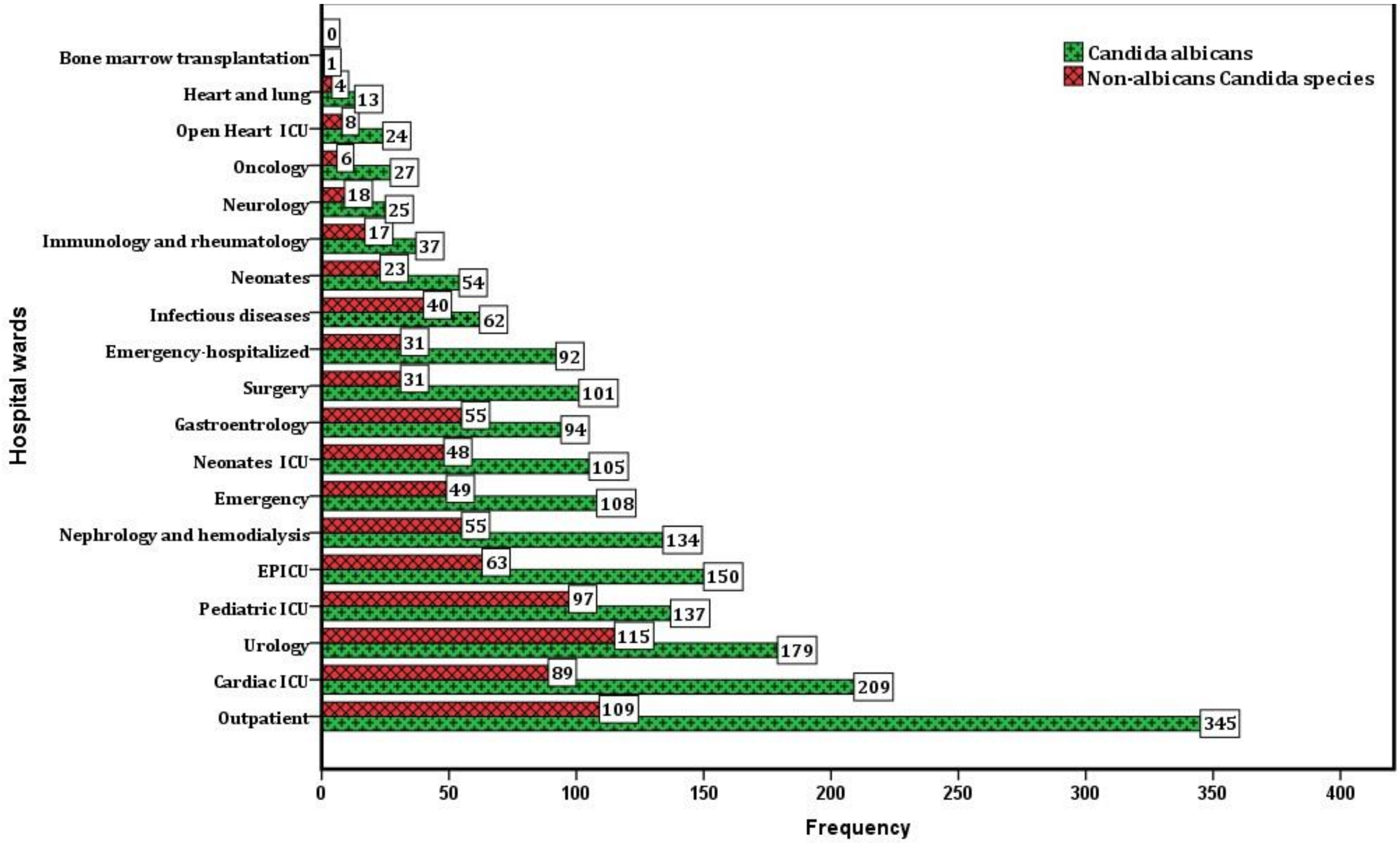

Figure 2. The frequency of *Candida* isolates recovered from pediatric patients based on their hospitalization wards

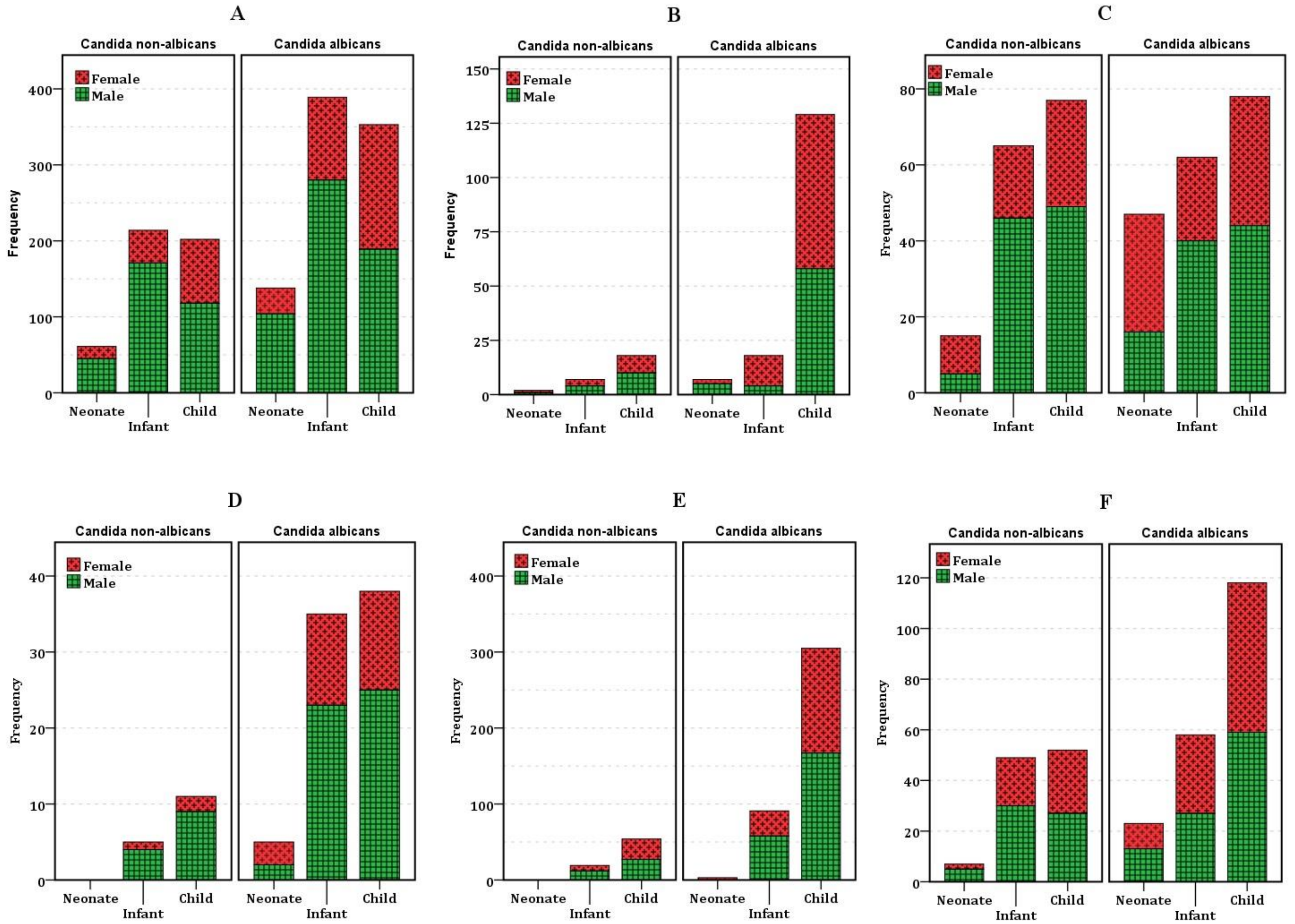

Figure 3. The frequency of *C. albicans* and non-*albicans Candida* species based on age group and gender for urinary tract specimens (A: urine, suprapubic urine and urinary catheter), respiratory system specimens (B: sputum, bronchoalveolar lavage, tracheal aspirate), blood specimens (C), other body fluids (D: pleural, peritoneal, synovial, dialysis and cerebrospinal fluids), throat swabs (E) and other specimen types (F: central venous catheter, wound discharge, abscess drainage, stool, and specimens from eyes, nose and ear).