Microbes and Infectious Diseases 2025; 6(4): 6497-6506

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Healthcare-associated infections in pediatric intensive care unit of El-Mahalla general hospital

*Eman G. Abd El Rahman [1], Reda S. Arafah [1], Samar M.H. Emara [*2], Maha M. Osman [3]*

1- *Pediatrics Department, Faculty of Medicine, Benha University, Benha, Egypt*
2- *Pediatrics Department, El-Mahalla General Hospital, Gharbia, Egypt*
3- *Clinical and Chemical Pathology Department, Faculty of Medicine, Helwan University, Egypt*

ARTICLE INFO

Article history:
Received 7 August 2024
Received in revised form 2 September 2024
Accepted 5 September 2024

Keywords:
HAI
PICU
Device-associated infections
Klebsiella

ABSTRACT

Background: Healthcare-associated infections (HAIs) significantly contribute to elevated healthcare expenditures, enhanced morbidity, and increased mortality rates. Patients in the pediatric intensive care unit (PICU) exhibit heightened susceptibility to HAIs. This study aimed to evaluate the prevalence and types of HAIs and their contribution to PICU mortality and morbidity. Also, it aimed to assess possible sources of infection in the environment and health care workers (HCWs). **Methods:** This cross-sectional, observational study was conducted on healthcare-associated infections in all admissions to the Department of PICU of El-Mahala General Hospital during the study period. HAIs were delineated in accordance with the guidelines established by the Centers for Disease Control and Prevention. Standardized microbiological methodologies were employed for their identification. All PICU healthcare workers were subjected to nasal and under-fingernails cultures and inanimate objects. **Results:** A cohort of 211 patients was examined. Among these, 35 patients experienced 41 episodes of bacterial healthcare-associated infections. The incidence rate of HAIs was 19.4 per 100 PICU admissions, with an incidence density of 25.7 per 1000 PICU days. Notably, all identified HAIs were associated with medical devices. Ventilator-associated pneumonia was the predominant type, constituting 43.9% of cases, followed by central line-associated bloodstream infections at 31.7%, bloodstream infections at 14.6%, and catheter-associated urinary tract infections at 9.8%. The most commonly isolated organisms were MDR-Gram-negative bacteria (56.1%), mainly *Klebsiella* (21.9%). **Conclusions:** Healthcare-associated infections constitute a significant problem in PICU, mostly due to multidrug resistant bacteria. It is significantly correlated with morbidity and mortality in PICU.
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Introduction

The predominant complication encountered in the PICU is healthcare-associated infections (HAIs), which are defined as infections that are neither present nor in incubation at the time of admission but manifest after the third day of hospitalization [1].

In intensive care settings, the overall incidence of healthcare-associated infection (HAI) development ranges from 20-40 percent. The infections most frequently documented include

DOI: 10.21608/MID.2024.310453.2139
* *Corresponding author:* Samar M.H. Emara
E-mail address: emarasamar2020@gmail.com

bloodstream infections (BSIs), ventilator-associated pneumonia (VAP), urinary tract infections (URTIs), and surgical site infections (SSIs) [2].

Healthcare-associated BSIs significantly contribute to elevated healthcare expenditures, augmented morbidity, and heightened mortality rates. Predominant pathogens implicated in central line-associated bloodstream infections (CLABSI) include coagulase-negative *Staphylococci* (CONS), Methicillin-resistant *Staphylococcus aureus* (MRSA), various species of Candida, and Gram-negative organisms such as *Klebsiella* and *Enterobacterales* [3].

One known side effect of using invasive mechanical ventilation in critically ill patients is VAP. It ranks as the second most prevalent nosocomial infection within ICU and typically manifests within the first 10 days following endotracheal intubation [4]. The prevention of VAP in ICUs is guided by a variety of strategies, which demonstrate regional variation [5].

Catheter-associated urinary tract infections (CAUTIs) represent a significant health concern in pediatric patients equipped with indwelling urinary catheters, with infection risks escalating daily from three to ten percent as long as the catheter remains [6].

The objective of this study was to assess the prevalence and varieties of HAIs and to analyze their impact on morbidity and mortality within the PICU.

Patients and methods

This observational, cross-sectional investigation encompassed all patients who were admitted to the PICU of El-Mahala General Hospital for at least 48 hours between May 2022 and October 2022.

Ethical consideration

An approval from the Research Ethics Committee in Benha Faculty of Medicine was obtained (code number: MS17-3-2022). Ethical permission from the parents was obtained before doing the study.

Inclusion criteria: Age from one month to 18 years, infections were identified as occurring 48 hours or more following admission to the PICU, and infection control instructions are strictly followed such as hand hygiene, spacing between cases, proper sterilization/disinfection of various medical item, aseptic precautions whenever any invasive procedures are being carried out and use of personal protective equipment.

Exclusion criteria: Age less than one month, patient with PICU stay less than 3 days, patient with history of acquired or congenital immunodeficiency, as well as scenarios where an infection was either present or in the incubation phase at the point of admission.

Methods

All patients were subjected to the following: Detailed medical history data collection and assessment of infection risk factors such as using central venous catheterization, mechanical ventilation, and urinary catheterization. Additionally pediatric risk of mortality (PRISM) score is predicated on several parameters including cardiovascular and neurologic vital signs, acid-base and blood gas metrics (acidosis, pH, pCO2, total CO2, and paO2), blood chemistry tests (glucose, potassium, creatinine, and urea), and hematology tests (white blood cell count, platelet count, with prothrombin time (PT) and partial thromboplastin time (PTT) collectively counted as one measure), outcomes related to HAIs, such as length of stay (LOS) and mortality rates, were meticulously documented. This was complemented by a thorough clinical examination, routine laboratory tests, and disease-specific investigations, which encompassed radiological assessments (e.g., chest X-rays) and bacteriological cultures derived from urine, blood, or sputum samples.

Microbiologic methods and healthcare-associated infections definitions

Definitions of HAIs were established in alignment with the Centers for Disease Control and Prevention and National Health Surveillance case definitions. Catheter associated urinary tract infection (CAUTI) is classified as a urinary tract infection where an indwelling urinary catheter (UC) was in place for over two calendar days, with the catheter present either on the day of the event or the preceding day. Central line-associated blood stream infection (CLABSI) involves a laboratory-confirmed bloodstream infection occurring when a central line (CL) was maintained for more than two calendar days, and the line was present on the day of the event or the day prior. Ventilator associated pneumonia (VAP) describes pneumonia instances in patients mechanically ventilated for over two days, with the ventilator in place at the time of the event or the day before. Identification of VAP utilizes a

synthesis of radiological, clinical, and laboratory criteria [7,8]. During the study, all PICU healthcare workers underwent routine bimonthly nasal and under-fingernail swab cultures, alongside similar assessments of inanimate objects within the environment.

Upon admission, bacteriological cultures were systematically obtained from various potential infection sites including blood, urine, sputum, bronchoalveolar lavage, and cerebrospinal fluid as required. Subsequent cultures were initiated based on clinical suspicion of infection at respective sites. The identification and antimicrobial susceptibility of each isolate were determined using standard microbiological techniques employing the Kirby–Bauer disc diffusion method to detect bacterial resistance or susceptibility to various antibiotics, in accordance with the clinical and laboratory standards institute (CLSI) guidelines [9,10].

Statistical analysis

Data collected were systematically coded, inputted, and analyzed using IBM SPSS 23.0 for Windows, a sophisticated database software. Representations of qualitative data were articulated through frequencies and percentages. The association among disparate qualitative variables was examined using the Chi-square (X^2) test. In the analysis of quantitative variables, both the mean ± SD and the median alongside the range were derived. The independent t-test was applied for assessing differences across quantitative metrics, whereas the Mann-Whitney U test was utilized for evaluating nonparametric datasets. Statistical significance was adjudicated based on a P value threshold, where values greater than 0.05 were considered non-significant and those less than 0.05 as significant. Logistic regression analysis was employed to forecast the likelihood of an outcome, contingent on various independent variables, suitable for cases where the dependent variable is qualitative (categorical).

Results

In this study, 211 patients who were hospitalized in the PICU for more than 48 hours were included. Of these, 35 patients developed a total of 41 episodes of bacterial HAIs. The incidence rate of HAIs was established at 19.4 per 100 PICU admissions, with an incidence density of 25.7 per 1000 PICU days. All identified HAIs were associated with medical devices. The breakdown of these infections included ventilator-associated pneumonia, which constituted 43.9%, followed by central line (CL)-associated bloodstream infections at 31.7%, catheter-associated bloodstream infections at 14.6%, and CAUTIs at 9.8%. Device-associated infection (DAI) rates per 1000 device days were calculated as follows: 14.2 for ventilator-associated pneumonia per 1000 ventilator days, 6.4 for CL-associated bloodstream infections per 1000 CL days, and 0.78 for CAUTIs per 1000 urinary catheter days. Gram-negative bacteria emerged as the predominant pathogens, accounting for 56.1% (n=23/41) of all isolates.

Table 1 shows that patients age ranged from 1 to 156 months with median of 45.6, predominantly males (70.7%). As regards their weight it ranged from 4 to 35 kg with a median of 10 kg. The predominant infection type was ventilator-associated pneumonia, comprising 43.9% of cases.

Table 2 shows that the most isolated pathogen among VAP was *pseudomonas aeruginosa* (17.1%). While the most isolated pathogen among CLABSI was *Staphylococcus aureus* (24.4%). While the most isolated pathogen among blood stream infections was coagulase negative *Staphylococci* among (9.8%) of the patients. While the most isolated pathogen among CAUTI was *Escherichia coli* (4.9%).

Table 3 shows a statistically significant difference between different types of health-care infections as regard PRISM score, length of stay, inotropes and vasopressors and outcome especially elevated in VAP with significant difference from other types.

Figure 1 shows antibiotic resistance among isolated organisms from HAIs in the PICU. A total of ten (24.4%) isolated organisms showed extended-spectrum beta-lactamase resistance, eight isolates (19.5%) showed multidrug resistance, eleven isolates (26.8%) were resistant to methicillin-resistant *Staphylococcus aureus*, and five (12%) were carbapenem-resistant *Enterobacteriaceae*.

Table 4 reveals a statistically significant correlation between patient outcomes and the PRISM III score, most of the patients who died (41.7%) had PRISM scores ranging from 15 to 19, while most of the patients who survived (69%) had PRISM scores ranging from 0 to 4 (p<0.001). Also, when the PRISM score increases, the rate of observed mortality increases.

Table 5 showed that results of cultures from health care workers and inanimate items revealed that coagulase-negative staphylococci was the most frequently isolated organism from under fingernail (60%), nasal swabs (40%), and inanimate objects (30%). Whereas coagulase-negative *Staphylococci* are a part of normal skin flora. There are 4 isolated methicillin-resistant coagulase-ve *Staphylococci* organisms from healthcare workers under fingernail swab and nasal swab and 1 isolated methicillin-resistant staph organism from inanimate object that may act as a potential source of infection.

Table 6 shows that after applying logistic regression analysis for predictors of healthcare-acquired infection; CVC insertion, endotracheal tube insertion, urine catheter insertion, antibiotics used, mechanical ventilation, positive inotropes use, PRISM score, and length of stay can be used as independent factors for predicting healthcare-acquired infections.

Table 1. Demographic data and types of hospital-acquired infections in the pediatric intensive care unit among the studied group.

Variables		**(n=41)**
Age (month)	Median (IQR)	45.6 (15.5)
	Range	1– 156
Sex (n. %)	Male	29 (70.7%)
	Female	12 (29.3%)
Weight (Kg)	Median (IQR)	10 (9)
	Range	(4 – 35)
Height (Cm)	Median (IQR)	75 (27)
	Range	(56 – 150)
Head circumference (Cm)	Mean ± SD	46 (7)
	Range	(37 – 57)
Types of hospital-acquired infections	VAP	18 (43.9%)
	Central line-associated blood stream infections	13 (31.7%)
	Blood stream infections	6 (14.6%)
	Catheter associated urine tract infection	4 (9.8%)

Data is presented as Mean ± SD, Median (IQR), Range, or frequency (percentage). IQR: Interquartile range.

Table 2. Distribution of healthcare-associated infection (HAI) pathogens.

Variables (n. %)	**Culture +ve**
VAP(n=18)	
Pseudomonas aeruginosa	7 (17.1%)
Klebsiella spp.	6(14.6%)
Coagulase -ve Staphylococci	2 (4.9%)
Acinetobacter spp.	1 (2.4%)
E-coli	1(2.4%)
Staphylococcus aureus	1 (2.4%)
CLABSI (n= 13)	
Staphylococcus aureus.	10 (24.4%)
Klebsiella spp.	2 (4.9%)
Staphylococcus epidermidis.	1 (2.4%)
BSI (n=6)	
Coagulase-ve Staphylococci	4(9.8%)
Pseudomonas spp.	1(2.4%)
Enterobacter spp.	1(2.4%)
CAUTI (n=4)	
E-coli	2 (4.9%)
Klebsiella spp.	1 (2.4%)
Proteus spp.	1 (2.4%)

Data is presented as frequency (percentage). VAP: Ventilator associated pneumonia, CLABSI: Central line-associated bloodstream infections, CAUTI: Catheter associated urine tract infection.

Table 3. Comparison of morbidity and mortality in different types of health-care infections.

Variables *(n. %)*		**VAP (n=18)**	**CLABSI (n=13)**	**BSI (n=6)**	**CAUTI (n=4)**	***P* value**
PRISM score	0 – 4	3 (16.7%)	10 (76.9%)	4 (66.7%)	3 (75%)	0.03
	5 – 9	2 (11.1%)	2 (15.4%)	1 (16.7%)	1 (25%)	
	10 – 14	6 (33.3%)	0 (0%)	0 (0%)	0 (0%)	
	15 – 19	4 (22.2%)	1 (7.7%)	1 (16.7%)	0 (0%)	
	≥ 20	3 (16.7%)	0 (0%)	0 (0%)	0 (0%)	
Length of stay	9 – 15 days	7 (38.9%)	9 (69.2%)	6 (100%)	3 (75%)	0.04
	16 – 33 days	11 (61.1%)	4 (30.8%)	0 (0%)	1 (25%)	
Inotropes and vasopressors	No	2 (11.1%)	7 (53.8%)	6 (100%)	2 (50%)	0.002
	Dobutrex	9 (50%)	1 (7.7%)	0 (0%)	1 (25%)	
	Dobutrex & noradrenaline	0 (0%)	1 (7.7%)	0 (0%)	0 (0%)	
	Dopamine & Dobutrex	7 (38.9%)	3 (23.1%)	0 (0%)	1 (25%)	
	Dopamine & Dobutrex & adrenaline	0 (0%)	1 (7.7%)	0 (0%)	0 (0%)	
Outcome	Alive	8 (44.4%)	11 (84.6%)	6 (100%)	4 (100%)	0.01
	Died	10 (55.6%)	2 (15.4%)	0 (0%)	0 (0%)	

*Fisher exact test, Non-significant: *P >0.05*, Significant*: P ≤0.05* Data is presented as frequency (percentage).

Table 4. Relation between PRISM score and outcome among the studied patients.

Variables (n. %)	**Alive (n=29)**	**Died (n=12)**	**Total**	**Observed mortality (%)**	***P* value**
0 – 4	20 (69%)	0 (0%)	20	0 %	<0.001
5 – 9	6 (20.7%)	0 (0%)	6	0 %	
10 – 14	2 (6.9%)	4 (33.3%)	6	66.7 %	
15 – 19	1 (3.4%)	5 (41.7%)	6	83.3 %	
≥ 20	0 (0%)	3 (25%)	3	100 %	

Data is presented as frequency (percentage). *Fisher exact test, Non-significant: *P* >0.05, Significant: *P* ≤0.05

Table 5. Culture results among health care workers.

Variables (n. %)	**Culture + ve**	**MRSA**	**MRCONS**	**MSCONS**
Culture from under fingernail swab (n=15)				
Coagulase – ve *Staphylococci*	9 (60%)	-	1	8
Staphylococcus aureus	3 (20%)	0	-	-
No growth	3 (20%)			
Culture from nasal swab (n=15)				
Coagulase – ve *Staphylococci*	6 (40%)	-	3	4
Staphylococcus aureus	3 (20%)	0	-	-
Beta hemolytic *Streptococci*	2 (13.3%)			
No growth	4 (26.7%)			
Swap from inanimate object (n=10)				
Coagulase –ve *Staphylococci*	3 (30%)	-	0	3
Staphylococcus aureus	2 (20%)	1	-	-
Candida albicans	1 (10%)			
No growth	4 (40%)			

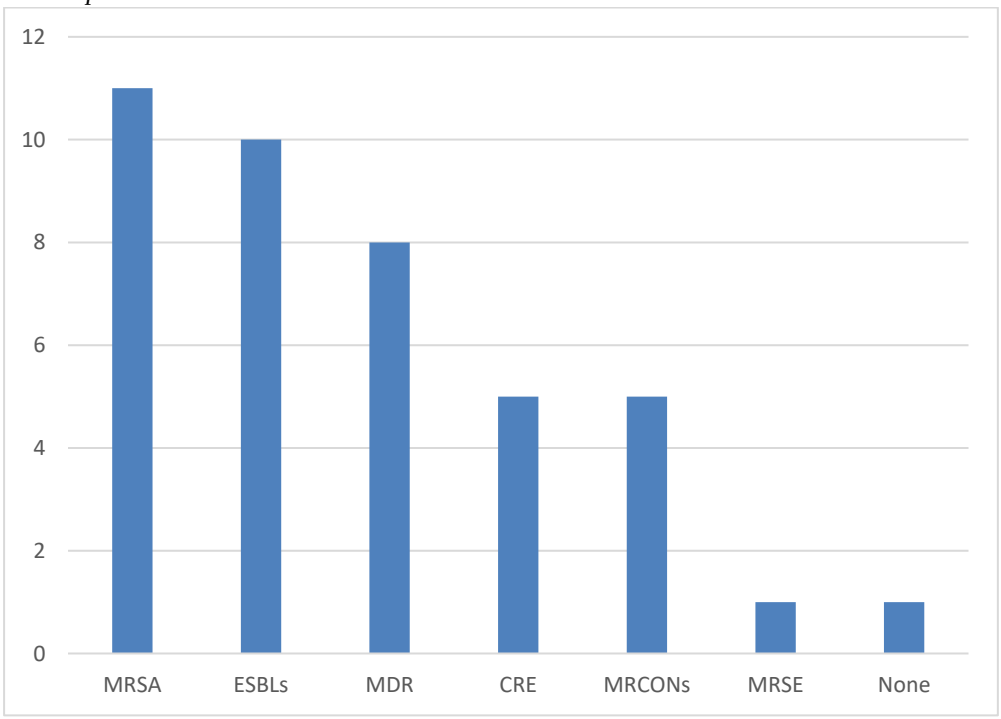
Data is presented as frequency (percentage). MRSA: Methicillin resistant *Staphylococcus aureus*, MRCONS: Methicillin resistant coagulase -ve *Staphylococci*, MSCONS: Methicillin sensitive coagulase -ve *Staphylococci*

Table 6. Logistic regression analysis for predictors of healthcare acquired infections.

Variables	Univariate analysis		Multivariate analysis	
	***P* value**	**Odds (CI 95%)**	***P* value**	**Odds (CI 95%)**
Age	0.006	1.44 (1.02 – 1.89)	0.19	3.08 (0.58 – 16.3)
Sex	0.41	0.53 (0.12 – 2.43)	-	-
CVC	0.03	9.4 (4.3 – 22.3)	0.001	0.68 (1.02 – 1.75)
Endotracheal tube	0.005	21.15 (2.45 – 182.3)	0.02	0.43 (1.03 – 1.40)
Urine catheter	0.004	13 (3.5 – 48.2)	0.03	1.65 (1.78 – 3.17)
Antibiotic used	0.01	1.82 (1.32 – 2.49)	0.02	2.93 (1.64 – 8.51)
Mechanical ventilation	0.03	11.74 (1.42 – 122.2)	0.04	2.84 (1.21 – 3.92)
Inotropes use	0.005	21.15 (2.45 – 182.3)	0.02	13.18 (1.43 – 121.5)
WBCs	0.45	0.88 (0.6 – 1.21)	-	-
Hemoglobin	0.32	1.35 (0.75 – 2.4)	-	-
Platelets	0.33	1.003 (0.99 – 1.01)	-	-
CRP on admission	0.89	1.17 (0.11 – 12.48)	-	-
PRISM score	0.001	1.69 (1.23 – 2.34)	0.005	1.7 (1.17 – 2.48)
Length of stay	0.003	1.22 (1.07 – 1.39)	0.004	1.21 (1.06 – 1.38)

CVC: Central venous catheter, WBCs: White blood cells, CRP: C-reactive protein , PRISM score **:** Pediatric Risk of Mortality

Figure 1. Antibiotic resistance of isolated organisms of hospital acquired infections in the PICU (n = 41). CRE= Carbapenem-resistant *Enterobacterales*, ESBLS=extended spectrum Beta lactams, MDR=multidrug resistance, MRSA=Methicillin resistant *Staphylococcus aureus*, MRCONS=Methicillin resistant coagulase –ve *Staphylococci*, MSCONS=Methicillin sensitive coagulase – ve *Staphylococci*, MRSE=Methicillin resistant *Staphylococcus epidermidis*



Discussion

Our research demonstrated an HAI incidence rate of 16.9 per 100 PICU admissions, with an incidence density of 25.7 per 1000 PICU days. These findings align closely with the HAI rates reported in developing countries, which range between 15.1% and 22.1% [11]. **Atici *et al.*** [12] identified a PICU HAI rate of 22.24%.

Our study showed that the majority of the studied patients were males, ages ranging from 1 to 156 months with a median of 45.6. **Akinkugbe *et al.*** [13] studied 1930 critically ill children and determined the effect on their disease course, of which 44% were female. The median age was 38 months (10–118).

Avcu *et al.* [14] conducted a study to determine the occurrence and distribution of HAIs,

as well as antibiotic susceptibility trends in a PICU. The patients' average age was 81.9 months, with a range of 3 to 190 months. Among the patients who developed HAIs, half (n=11, 50%) were female.

In this study, VAP was the most prevalent form, accounting for 43.9% of the cases. Central line (CL)-associated bloodstream infections, bloodstream infections, and CAUTIs accounted for 31.7%, 14.6%, and 9.8% of the cases, respectively. Numerous studies that were comparable to ours found that VAP was the most prevalent infection [15,16]. However, according to **Hacımustafaoğlu *et al.*** [17], bloodstream infections were identified as the predominant cause of HAIs in the PICU.

Our study showed that VAP, the most common serious HAI, showed increased morbidity and mortality, as (16.7%) of VAP had PRISM scores≥ 20. In contrast, other types of HAIs had PRISM scores < 20, length of stay most of the patients with VAP stayed from 16 to 33 days, As regards inotropes and vasopressors, (38.9%) of patients with VAP used dopamine and dobutrx in comparison to (23.1%) of patients with CLABSI and (25%) of patients with CAUTI. Also, (55.6%) of patients with VAP died in comparison to (15.4%) of patients with CLABSI and none of the patients with BSI or CAUTI. The reason for this is that VAP is commonly caused by aggressive organisms.

As regards the most commonly isolated organisms , Our study showed that , 56.1% were Gram-negative bacteria (GNB) and 43.9% were Gram-positive bacteria. This distribution is consistent with earlier research in both adults and children, which found that the majority of HAIs in ICUs are caused by GNB, with *Enterobacteriaceae* accounting for 25-30% of all isolates [18,19]. *Pseudomonus* spp. was the most frequently isolated organism in VAP in the current investigation. This is consistent with earlier research [20]. However, many investigations have identified i spp. as the most common bacteria related with VAP [18,21].

In the National Nosocomial Infection Surveillance System (NNISS) survey of PICU patients, coagulase-negative *Staphylococcus* spp. were identified as the major bacterium associated with CLABSI, accounting for 37.8% of cases that were reported [22]. In our study, we found staphylococcus aureus, the predominant organism causing CLABSI. Also, the most isolated organisms in BSI and CAUTI were coagulase-ve *Staphylococci* and *Escherichia coli* respectively.

We showed antibiotic resistance among isolated organisms from HAIs in the PICU. A total of ten (24.4%) isolated organisms showed extended-spectrum beta-lactamase resistance, eight isolates (19.5%) showed multidrug resistance, eleven isolates (26.8%) were resistant to methicillin-resistant *Staphylococcus aureus*, and five (12%) were carbapenem-resistant *Enterobacteriaceae*.

Almazeedi *et al.* [23] showed antibiotic resistance among isolated organisms from HAIs in the PICU. A total of 44 (30.3%) samples showed extended-spectrum beta-lactamase resistance, 37 (25.5%) showed multidrug resistance, eight (5.5%) were methicillin-resistant *Staphylococcus aureus*, and three (2.1%) were carbapenem-resistant *Enterobacteriaceae*. A total of 53 (36.6%) had no resistance to antibiotics.

In our study, most of the patients who died (41.7%) had PRISM scores ranging from 15 to 19, while most of the patients who survived (69%) had PRISM score ranging from 0 to 4. Also, when the PRISM score increases, the rate of observed mortality increases.

Our investigation demonstrated that coagulase-negative staphylococci, as a part of The skin normal flora, emerged as the most prevalently isolated organisms, detected in 60% of samples collected from beneath fingernails, 40% of nasal swabs, and 30% of inanimate objects. Conversely, Staphylococcus aureus was less frequently identified, with isolation rates of 20% from fingernail swabs, 20% from nasal swabs, and 20% from inanimate object samples. Beta hemolytic *Streptococci* was isolated from nasal swabs in very limited culture (13.3%) and candida albicans were also isolated in very limited culture from an inanimate object (10%). There are 4 isolated methicillin-resistant coagulases-ve *Staphylococci* organisms from healthcare worker's underfinger nail and nasal swab and 1 isolated methicillin-resistant staph organism from an inanimate object that may act as a potential source of infection. In our study, we found Gram-negative bacilli ,the main pathogens in our ICU, not identified from HCW hands nor from the environment. This shows the success of the Infection Control Program implemented by our hospital.

In another study, cultures obtained from beneath the fingernails and throats of HCWs indicated a high recovery rate of coagulase-negative *Staphylococci*, ranging from 80% to 100%, and *Staphylococcus aureus*, ranging from 20% to

33.3%. These organisms are predominantly harbored by colonized children in the ICU, from whom they are transmitted to others via hospital personnel [24].

After applying logistic regression analysis for predictors of healthcare-acquired infection; CVC insertion, endotracheal tube insertion, urine catheter insertion, antibiotics used, mechanical ventilation, positive inotropes use, PRISM score, and length of stay can be used as independent factors for predicting healthcare-acquired infections.

Moustafa *et al.* [15] used logistic regression and found that LOS, Pediatric Logistic Organ Dysfunction (PELOD), and MV were significantly associated with HAIs. Significant predictors of HAIs were LOS and MV. While univariate analysis revealed a significant correlation between the PELOD score and PRISM score HAIs.

The principal strength of this study lies in the implementation of a standardized protocol across all participating PICUs. Patients who developed HAIs exhibited higher illness severity, extended PICU LOS, and elevated rates of resistant pathogens, although mortality was significantly impacted. This research provides a comprehensive overview of PICU-HAI epidemiology and ecology in Egypt, potentially serving as a benchmark and identifying areas for improvement in pediatric care within the country. The most significant limitation of our study was the relatively low number of enrolled patients.

Conclusions

- Heathcare-associated infection (HAIS) constitute significant problem approximately 19.4 per 100 PICU admissions, mostly caused by resistant bacteria.
- Patient with HAIs had significantly longer LOS, higher mortality, increased inotropic requirements and higher PRISM score especially elevated in VAP.
- Mechanical ventilation (MV), CVC, UC, length of stay and PRISM score were significantly predictors of HAIs.

Acknowledgments

None to be declared.

Authors' contributions

All authors contributed to the study conception and design. Material preparation was performed by Reda S. Arafah. Data collection and analysis were performed by Samar M.H. Emara. The first draft of the manuscript was written by Eman G. Abd El Rahman and Maha M. Osman all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding

None

Competing interest

No competing interests to declare.

Data availability

All data generated or analyzed during this study are included in this puplished article.

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