RESEARCH ARTICLE

Patterns of Antimicrobial Resistance in a Pediatric Cardiac Intensive Care Unit: Five Years' Experience

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ABSTRACT

Objectives: The identification of antimicrobial resistance of isolates present in pediatric cardiac intensive care unit (PCICU) is important to prevent further spread, because this department limited choice of antibiotic. The aim of the present study is to report the antibiotic resistance rate of most frequently pathogens in PCICU during a five-year period.

Methods: A prospective study was performed on 4228 clinical samples (bloodstream, wound samples, respiratory tract, tracheobronchial tree, and central venous catheter) from patients in PCICU during the period 2012-2016. Identification of isolates and antibiotic susceptibility testing were performed by Vitek 2 automated system.

Results: The percentages of most frequently isolated microorganisms in our PCICU were as follows: *Klebsiella pneumoniae* 8.9%, *Pseudomonas aeruginosa* 7.5%, *Staphylococcus aureus* 6.9%, *Coagulase-Negative Staphylococci* 5.3%, and *Candida spp.* 3.4%. During study period there is tendency increasing the percentage of detection *P. aeruginosa* from 2.6% to 10.8% (p=0.018), *K. pneumoniae* from 2.6% to 10.5% (p=0.023), and *Candida spp.* from 1.6% to 5.9% (p=0.033). These isolates showed tendency of significant increasing resistance to 3rd generation cephalosporins and carbapenems.

Conclusion: The present study reported that most frequent isolates in our PCICU were *P. aeruginosa* and *K. pneumoniae*. Reporting of dramatically increasing resistance rates of these isolates necessitates a well-designed hospital infection control strategy, including good hygiene, microbiological monitoring; all of this will greatly reduce the risk of nosocomial infection. *J Microbiol Infect Dis 2017; 7(3):132-138*

Keywords: microbiologic monitoring, antibiotic resistance, pediatric cardiac intensive care unit

INTRODUCTION

Pneumonia, septicemia and postoperative wound are most frequent types of infections in patients requiring intensive care treatment. Postoperative infections are important and main cause for the increasing morbidity, such as the widespread use of antibiotics, reoperations, prolonged stay in intensive care unit (ICU), and all of this increases cost of treatment and the use of resources [1]. Moreover, postoperative infections are a major factor for increasing mortality [2-5].

Postoperative surgical site infections are a major cause of postoperative morbidity and mortality in cardiac surgery. While surgical site infection in adult cardiac surgery has been well characterized and studied, in pediatric cardiac surgery, the classification, prevention and management is less well studied and significant practice variation exist [6-8].

As is known, children have a relatively underdeveloped immune system, because of this is at high risk for nosocomial infection, especially when they need prolonged hospital stay, major surgery, or invasive procedures [9-11].

In pediatric cardiac surgery bacterial infections are basic cause of morbidity and mortality [12]. Pediatric patients are more susceptible for infection than adult particularly in the first two years of life. The identification of antimicrobial resistance of isolates present in pediatric cardiac intensive care unit (PCICU) is important to prevent further spread, because this department

limited choice of antibiotic. An important factor in the development of hospital infections is antibiotic therapy. Long and not always well-founded use of reserve antibiotics as empirical therapy leads to the selection of virulent nosocomial strains. The common organisms isolated from blood stream infection are *Klebsiella pneumoniae*, *Coagulase-negative Staphylococci (CNS)*, and *Pseudomonas aeruginosa* respectively. Common nosocomial pathogens identified from lower respiratory tract infections are *P. aeruginosa* followed by *S. aureus* [13].

All these findings support the need of strict adherence to monitoring antibiotic resistance of strains in pediatric intensive care unit (PICU), together with the control measures to prevent the spread of multi-drug resistant microorganism. The objective of the present study is to report the antibiotic resistance rate of most frequently pathogens in PCICU during a five-year period, from 2012 to 2016.

METHODS

Study design

A prospective study of microbial landscape and antibiotic resistance rates of strains from patients in PCICU was conducted, during the study period 2012-2016.

Data collection

Data were collected from newborns and children of the first three years hospitalized in pediatric cardiac ICU after cardiac surgery (surgery on the heart and major blood vessels). All patients in the PCICU were monitored for bacterial infection at body sites for a period at least one month. Strains were analyzed by infection site and pathogen type.

Bloodstream strains were collected from patient with either two or more positive blood cultures. Respiratory tract specimens included nasopharyngeal swabs and sputum. Other types of specimens obtained from the patients were: swabs from cardiac surgical wounds, Broncho-alveolar lavage, central venous catheter (CVC), aspiration catheter, tracheostomy, pleural cavity, peritoneal fluid. All specimens were collected at the bed site, transported to the Laboratory of Microbiology and were inoculated on proper culture media within two hours according to the guidelines [14].

Samples cultivation

Clinical specimens were inoculated onto 5% sheep blood agar, Mannitol salt agar, Endo agar, Sabouraund dextrose agar (Himedia, India). Plates were incubated at 37°C for 18-24 hours.

Identification of isolates

Methods used for confirmation of identification included test of colonial morphology, haemolytic activity on appropriate agar media, Gram strain, rapid tests (coagulase, oxidase, catalase, indole) and use of automated identification system Vitek 2-Compact (bioMerieux, Marcy l'Etoile, France).

Antibiotic susceptibility testing

The following antibiotics were used for antimicrobial susceptibility testing: ticarcillin/clavulanic acid, amoxicillin/clavulanic acid, ceftazidime, ceftriaxone, cefepime, amikacin, meropenem, gentamicin, ciprofloxacin, levofloxacin. Susceptibility tests were performed with broth microdilution method (Vitek 2 – Compact (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's guideline recommendations. Colonies from 18-24 hours culture were used to inoculate the microdilution cards.

All data were analyzed using Microsoft Access and Excel. Trends over time of antibiotic resistance rates were determined by linear regression with the yearly data. A p value of <0.05 was considered to be statistically significant.

RESULTS

During study period from January 2012 to December 2016 3901 isolates from 4228 clinical samples (bloodstream, wound samples, respiratory tract, tracheobronchial tree, and central venous catheter) were included to the study. Respiratory tract were most frequent isolates 81.2% (3168), followed by tracheobronchial tree 11.9% (468), bloodstream infections (BSI) 2.4% (95), wound samples 1.7 % (67) and CVC 1.1% (45). The percentage of Gram-positive cocci was 66.4% (2711), Gram-negative bacilli 27% (1057) and fungi 3.4% (133).

The percentages of most frequently isolated microorganisms in our PCICU were as follows: *Streptococcus spp.* 49.9%, *K. pneumoniae* 8.9%, *P. aeruginosa* 7.5%, and *S. aureus* 6.9%. During study period the rate of positive hemocultures was increased from 7.5% to 18.2% (p <0.05), in the mean of 16.6±1.3. The most frequent pathogens were *K. pneumoniae* (29.4%) and *Candida species* (18.9%) (Table 1).

In tracheobronchial tree site *P. aeruginosa* (20.1%), was the most commonly pathogen, which was followed by *K. pneumoniae* (15.8%). Wound and CVC infections were mostly caused by coagulase-negative staphylococci (31.3% and 28.9% respectively); fifty nine percent reported isolates from respiratory tract were Gram-positive cocci.

During study period there is tendency increasing the percentage of detection *P. aeruginosa* from 2.6% to 10.8%, *K. pneumoniae* from 2.6% to 10.5% *Stenotrophomonas maltophilia* from 1.6% to 5%, *Candida spp.* from 1.6% to 5.9%. At the same time there is increasing percentage of *Streptococcus spp.* from 56.6% to 33% (Table 2).

Gram-negative bacilli are frequently associated with nosocomial infections in ICU patients. *P. aeruginosa* showed high proportion and increasing of resistance against to cephalosporins (ceftazidime from 8.3% to 66.7% p=0.018, cefepime from 23.1% to 71.7% p=0.019), to aminoglycosides (gentamicin from 7.7% to 69.5% p=0.007, amikacin from 16.7% to 84.7% p=0.039) and to carbapenems (meropenem from 8.3% to 83.1% p=0.004) (Table 3).

K. pneumoniae isolates showed tendency of increasing resistance to 3rd generation cephalosporins (ceftriaxone from 38.4% to 85.7% and carbapenems (meropenem from 0% to 7.1%) (Table 4).

The greatest percentage of Candida spp. strains were isolated from BSI and resistance rate to antibiotics was to amphotericin B 22.2% (95%CI 3.9-59.8), to fluconazole 100% (95%CI 62.9-100), to intraconazole 88.9% (95%CI 50.7-99.4), to ketoconazole 11.1% (95%CI 0.6-49.3).

Table 1. Isolates reported from pediatric cardiac intensive care unit according to the site of infection.

Microorganism	Bloodstream, n (%)	Tracheo-bronchial tree, n (%)	Respiratory tract, n (%)	Surgical site, n (%)	CVC, n (%)	Others[1], n (%)	Total, n (%)
Staphylococcus aureus	3 (33.1)	20 (4.2)	222 (7)	12 (17.9)	4 (8.8)	11 (18.9)	272 (6.9)
CNS	7 (7.3)	69 (14.7)	95 (3)	21 (31.3)	13 (28.9)	5 (8.6)	210 (5.3)
Streptococcus spp.	2 (2.1)	56 (12)	1873 (59.1)	5 (7.4)	2 (4.4)	10 (17.2)	1948 (49.9)
Enterococcus spp.	5 (5.2)	24 (5.1)	230 (7.2)	8 (11.9)	4 (8.8)	10 (17.2)	281 (7.2)
Escherichia coli	1 (1)	17 (3.6)	64 (2)	3 (4.4)	1 (2.2)	5 (8.6)	91 (2.3)
Klebsiella pneumoniae	28 (29.4)	74 (15.8)	224 (7)	5 (7.4)	9 (20)	9 (15.5)	349 (8.9)
Enterobacter spp.	5 (5.2)	23 (4.9)	124 (3.9)	5 (7.4)	4 (8.8)	2 (3.4)	163 (4.1)
Acinetobacter baumannii	1 (1)	28 (5.9)	57 (1.8)	2 (2.9)	2 (4.4)	2 (3.4)	92 (2.3)
Pseudomonas aeruginosa	7 (7.3)	94 (20.1)	180 (5.6)	5 (7.4)	5 (11.1)	4 (6.8)	295 (7.5)
Burkholderia cepacia	15 (15.7)	4 (0.8)	1 (0.03)	1 (1.4)	1 (2.2)	0	22 (0.5)
Stenotrophomonas maltophilia	3 (3.1)	31 (6.6)	11 (0.3)	0	0	0	42 (1.1)
Candida spp.	18 (18.9)	28 (5.9)	87 (2.7)	0	0	0	133 (3.4)
Total	95	468	3168	67	45	58	3901 (100.0)

CVC= Central venous catheter, CNS= Coagulase-negative *Staphylococci,* [1]Aspiration catheter, tracheostomy, pleural cavity, peritoneal fluid

Table 2. The changes in detection rate of isolates by years (2012-2016) reported from pediatric cardiac intensive care unit.

Microorganism	2012, n (%)	2013, n (%)	2014, n (%)	2015, n (%)	2016, n (%)	p-value[1]
Staphylococcus aureus	15 (5)	31 (6.5)	123 (9.3)	78 (6.2)	25 (4.5)	0.881
CNS	28 (9.3)	26 (5.4)	44 (3.3)	58 (4.6)	54 (9.8)	0.973
Streptococcus spp.	170 (56.6)	294 (61.8)	682 (51.7)	620 (49.3)	182 (33)	0.056
Enterococcus spp.	30 (10)	37 (7.7)	96 (7.2)	75 (5.9)	43 (7.8)	0.244
Escherichia coli	8 (2.6)	12 (2.5)	30 (2.2)	32 (2.5)	9 (1.6)	0.124
Klebsiella pneumoniae	8 (2.6)	21 (4.4)	110 (8.3)	152 (12.1)	58 (10.5)	0.023
Enterobacter spp.	16 (5.3)	21 (4.4)	76 (5.7)	28 (2.2)	22 (3.9)	0.309
Acinetobacter baumannii	6 (2)	4 (0.8)	38 (2.8)	21 (1.6)	23 (4.1)	0.255
Pseudomonas aeruginosa	8 (2.6)	12 (2.5)	76 (5.7)	139 (11)	60 (10.8)	0.018
Burkholderia cepacia	1 (0.3)	1 (0.2)	0	6 (0.4)	14 (2.5)	0.181
Stenotrophomonas maltophilia	5 (1.6)	3 (0.6)	3 (0.2)	6 (0.4)	28 (5)	0.366
Candida spp.	5 (1.6)	13 (2.7)	41 (3.1)	41 (3.2)	33 (5.9)	0.033
Total	300	475	1319	1256	551	

[1] Linear regression, CNS= Coagulase-negative *Staphylococci*

Table 3. Antibiotic resistance of Pseudomonas aeruginosa isolated from infections in pediatric cardiac intensive care unit

Antibiotic	2012, n (%)	2013, n (%)	2014, n (%)	2015, n (%)	2016, n (%)	p-value*
Ticarcillin/clavulanic acid	2 (16.6)	4 (25)	19 (25)	31 (22.2)	37 (61.4)	0.130
Ceftazidime	1 (8.3)	1 (8.3)	31 (40.8)	98 (70.7)	40 (66.7)	0.018
Cefepime	3 (23.1)	4 (25)	22 (28.9)	77 (55.3)	43 (71.7)	0.019
Meropenem	1 (8.3)	1 (8.3)	28 (36.8)	78 (55.6)	50 (83.1)	0.004
Amikacin	2 (16.7)	1 (8.3)	14 (18.3)	88 (63.2)	51 (84.7)	0.039
Gentamicin	1 (8.3)7.7	1 (8.3)	19 (25)	74 (52.3)	42 (69.5)	0.007
Ciprofloxacin	2 (16.6)	0	8 (10.4)	23 (16.2)	38 (63.3)	0.128
Levofloxacin	0	2 (16.6)	22 (8.9)	19 (13.9)	38 (63.3)	0.079

* Linear regression

Table 4. Antibiotic resistance of *Klebsiella pneumoniae* isolated from infections in pediatric cardiac intensive care unit

Antibiotic	2012, n (%)	2013, n (%)	2014, n (%)	2015. n (%)	2016. n (%)	p-value*
Amoxicillin/clavulanic acid	1 (12.5)	7 (33.3)	48 (43.9)	50 (32.3)	55 (94.7)	0.088
Ceftazidime	3 (41.1)	10 (47.6)	86 (78.1)	109 (71.7)	51 (87.5)	0.022
Ceftriaxone	3 (38.4)	9 (42.9)	89 (80.3)	115 (75.9)	50 (85.7)	0.033
Cefepime	3 (38.4)	10 (47.6)	95 (86.4)	106 (69.9)	45 (77.2)	0.141
Meropenem	0	0	2 (1.8)	13 (8.4)	4 (7.1)	0.045
Amikacin	1 (12.5)	2 (9.5)	1 (0.9)	10 (6.9)	2 (3.8)	0.447
Gentamicin	2 (25)22.8	10 (47.6)	85 (77.7)	94 (61.4)	38 (65.5)	0.155
Ciprofloxacin	1 (12.5)	2 (9.5)	20 (18.1)	16 (10.2)	13 (22.8)	0.285
Levofloxacin	1 (12.5)	2 (9.5)	6 (5.4)	9 (5.9)	7 (12.3)	0.316

* Linear regression

DISCUSSION

According to systems involved infection in PICU our study results showed the most common systems affected were respiratory tract and bloodstream. In most of other studies are the commonest infection affected by respiratory tract followed urinary tract infection [15-16].

Organisms that affect PICU in general, were variable among hospitals. In same researches, CNS is the most common pathogens in PICU, followed by *K. pneumoniae*, *P. aeruginosa* and *S. aureus* [17-18]. Bo-Tao Ning et al. reported that the most common pathogens were *Acinetobacter baumannii* (25.6%), *Escherichia coli* (20.2%), *S. maltophilia* (20.2%), *K. pneumoniae* (16.2%) and *P. aeruginosa* (9.4%) [19]. Our study showed *K. pneumoniae* the commonest, followed by *P. aeruginosa* then *Candida spp.* Furthermore, during study period there is tendency of increasing percentage of *P. aeruginosa* from 2.6% to 10.8% (p=0.018), *K. pneumoniae* from 2.6% to 10.5% (p=0.023), *Candida spp.* from 1.6% to 5.9% (p=0.033).

Studies of surveillance of nosocomial infections may help to decrease the frequency incidence of infections and reducing long ICU stays and hence the costs. Infections with Candida spp. are one of the most leading causes of nosocomial bloodstream infections [20]. PICU patients with candidemia are at highest risk of death [21]. In same studies (Becerra M R, 2010) the most common isolate in BSI in pediatric patients was *Candida spp.* (41%), followed CNS. In same studies (Singhi S et.al 2008) candidemia developed in 30.2% cases [6,7].

Risk factors for candidemia are previous colonization, long ICU stays, presence of CVC, parenteral nutrition, illness severity and prolonged use of antibiotics [22]. Candidemia is particularly important, because it is associated with high mortality, both in adults [23] and children, especially at lower ages and in those with comorbidities [24].

The same authors in the study of BSI in PICU identified that the most frequent pathogens were CNS (24%), *K. pneumoniae* (16%), *Candida spp.* (15%), *P. aeruginosa* (7%) and *S. aureus* (6%) [17].

Our findings are consistent with results of other authors [25-26]. In our study BSI was increased from 7.5% to 18.2% (p<0.05), the most frequent pathogens were *K. pneumoniae* (29.4%) and *Candida spp.* (18.9%). It is likely that the most important cause of our high prevalence of Candida spp. is the extensive use of broad spectrum antibiotics. Furthermore, in our study resistance rates of *Candida spp.* isolates to amphotericin B was 22.2% (95%CI 3.9-59.8), to fluconazole 100% (95%CI 52.9-100).

The tracheobronchial tree and oropharynx of patients on mechanical ventilation are frequently contaminated by microorganisms [27]. The relation between this colonization and pulmonary infection, however, is not yet clear. Johanson et al. [28] showed that 23% of patients colonized by bacteria later developed pulmonary infection. Gram-negatives, mainly *P. aeruginosa* the most common organism in tracheobronchial tree [29], as in our study, and with K.pneumoniae, both caused 35.9% of infection.

Clinical studies report increasing resistance rates of *P. aeruginosa* in PICU, which is a factor of nosocomial infection [30-31]. Wang LG. et al. reported that out of 126 *P. aeruginosa* isolates more than 50% were resistance to capbaphenems, 33.3% to ceftazidime [32]. In our study, examining the five-year average antimicrobial resistance distributions of *P. aeruginosa* strains, the highest resistance observed for carbapenems, aminoglycosides and antipseudomonal cephalosporins. In particular, resistance to meropenem dramatically increased from 8.3% to 83.1% (p=0.004), to ceftazidime from 8.3% to 66.7% (p=0.018), and to gentamicin from 7.7% to 69.5% (p=0.007). *K. pneumoniae* isolates showed tendency of increasing resistance to 3rd generation cephalosporins and carbapenems (to ceftriaxone from 38.4% in 2012 to 85.7% in 2016 p=0.033, to ceftazidime from 41.1% to 87.5% p=0.022, to meropenem from 0% to 7.1% p=0.045 respectively.

As is known, developing drug resistance can severely limit the therapeutic options for treatment of serious infections. In our opinion, there are possible reasons for the high rate of resistant isolates in our hospital include more critically ill patients which admitted to the new PICU; more patients being referred from local

hospitals; and the spread of resistant strains from adult wards. However, further evaluation of the reasons for the high rate of resistance of *P. aeruginosa* in the PICU is warranted. We should improve testing technique, early and appropriate empirical antibiotics therapy is crucial according to clinical experience and antibiotic sensitivity. The effective treatment of frequently isolates in ICU is paramount to prevent multidrug resistance.

In conclusion, the present study reported that most frequent isolates in our PCICU were *P. aeruginosa* and *K. pneumoniae*. Reporting of dramatically increasing resistance rates of these isolates necessitates a well-designed hospital infection control strategy, including good hygiene, microbiological monitoring, and nosocomial control; all of this will greatly reduce the risk of nosocomial infection.

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