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Original Research Article

Microbial profile and antimicrobial susceptibility pattern in paediatric intensive care unit of a tertiary care hospital, North East India

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ABSTRACT

Introduction: Health care associated infections commonly encountered in paediatric intensive care units (PICU) are respiratory tract infections, and bloodstream infections. Monitoring of prevalence rates and antimicrobial susceptibility of different pathogens is necessary for proper management of PICU infections.
Aim & Objective: This study aimed to determine the microbial profile causing infections in patients admitted to PICU and their antimicrobial susceptibility pattern.
Materials and Methods: A cross sectional study was conducted involving all paediatric patients admitted in PICU during a 19 months period. Specimen viz. blood culture, urine, and tracheal aspirate were sent for culture and sensitivity and results were observed and their antibiotic susceptibility pattern was noted.
Results: Out of total 372 patients, 72 (19.35%) were found culture positive. The isolation rate was very high in tracheal aspirates (82.14%) as compared to blood (14.29%) and urine (14.21%). Gram negative bacilli (76.3%) were the most common pathogen group isolated, *Klebsiella pneumoniae* (30.5%) commonest followed by *Acinetobacter* species (19.4%). *Enterococcus* species (13.8%) was the most common gram positive isolate followed by Methicillin resistant *Staphylococcus aureus* (MRSA) (6.94%). Higher susceptibility was observed to Tigecycline & Meropenem among gram negative isolates while Linezolid and Vancomycin were most susceptible to gram positive cocci.
Conclusion: Gram negative bacteria were the predominant pathogens mostly *Klebsiella* species while *Enterococcus* species were the most common among Gram positive bacteria. Isolates showed multiple drug resistance to commonly used antimicrobials- Cephalosporins and Fluoroquinolones etc.

1. Introduction

Increased isolation of antibiotic-resistant microorganisms has become one of the most vital threats to the existing healthcare sector.[1] Healthcare associated infection (HAI) in paediatric intensive care units (PICU) and neonatal intensive care units (NICU) witness more notorious incidence rate in hospital.[2] The reasons behind this are - prolonged hospital stay, the severity of diseases in PICU patients, excessive use of antimicrobials, exposure to various medical interventions like peripheral intravenous or central venous lines, urinary catheterization, mechanical ventilation etc.[2,3] The most important nosocomial infections in ICUs are bloodstream infections (BSIs), ventilator-associated pneumonia (VAP), and urinary tract infections (UTIs).[3] Patients admitted in PICU with infection might have acquired it in the community level, indoor ward, or from peripheral hospitals. Moreover, infections caused by multidrug resistant bacteria have resulted in increased mortality and morbidity of the patients. Also profile of microbial agents isolated might

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be different from different clinical specimen. Therefore monitoring of prevalence rates of different pathogens along with their antimicrobial susceptibility patterns is necessary for proper management of infections in PICU patients, in order to develop or modify the hospital antibiotic policy.[4] Widely available and convenient measurement of an institution's pathogens and susceptibilities is an Antibiogram.[5] There is paucity of published literature on microbial profile and antimicrobial susceptibility pattern of infections in PICU patients in this region.

Therefore this study was undertaken with the aim to determine the microbial profile causing infections in patients admitted to PICU and their antimicrobial susceptibility pattern in a tertiary care hospital of North East India.

2. Materials and Methods

2.1. Study design and settings

A cross sectional study was conducted in PICU of a tertiary care hospital in Assam from December 2018 to August 2020.

All paediatric patients admitted in PICU during the study period who gave their assent with informed consent by their guardians or care-givers were included in the study. While all other paediatric patients not admitted in PICU were excluded from the study.

2.2. Data collection

Demographic data and clinical information such as age, gender, admission date, clinical diagnosis, and specimen collection date were recorded. The specimen viz. blood culture, urine, and tracheal aspirate were sent for culture and sensitivity from PICU during the study period. The microbiological culture and susceptibility result of all patients admitted in PICU during the period of study was observed.The total number of positive cultures in different specimens, culture isolates, and their antibiotic susceptibility and resistance pattern was noted.

2.3. Bacterial isolation and identification

The clinical specimens were processed and analysed following standard microbiological protocol. Blood cultures were processed using paired aerobic (REDOX 1) bottles in VersaTREK automated culture system for blood and body fluids (TREK Diagnostic Systems, Cleveland, OH).[6] Bacterial isolates were identified and speciated using both conventional identification method and Vitek–2 Compact (BioMerieux Diagnostics) system which uses a fluorogenic methodology for organism identification and a turbidometric method for susceptibility.[7] Antimicrobial susceptibility testing (AST) was performed by modified Kirby Bauer disc diffusion method against antimicrobial discs recommended by Clinical Laboratory Standard Institute (CLSI) and the automated minimum inhibitory concentration (MIC) method using Vitek2 compact system (BioMerieux Diagnostics), while Vancomycin MIC was tested using broth microdilution method.[8] AST interpretation was based on Clinical Laboratory Standard Institute (CLSI) criteria.[8] The quality control strains used for disc diffusion tests were *Escherichia coli* ATCC 25922, *Enterococcusfaecalis* ATCC 29212 and for Vitek 2 compact MIC method *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213 (procured from Himedia Labs Pvt. Ltd, Mumbai).

2.4. Statistical analysis

Statistical analysis was done and data are presented as numbers, mean and their percentages.

3. Results

A total of 372 patients admitted in the PICU who comprised the study population were analysed. Males (n= 207, 55.64%) were more than females (n=165, 44.36%) with mean age 31.39 ± 37.0 months. (Table 1) The samples of these patients were sent for microbiological culture and susceptibility testing. The age group of more than 12 months (n=137, 21.9 %) revealed highest culture positivity while gender wise culture positivity was higher in males (n=42, 20.29%).

There were 72 (19.35%) patients who were found culture positive. Acute Encephalitic Syndrome (AES) (n=19, 26.38%) was the most common primary disease of the culture positive PICU patients followed by pneumonia (n=15, 20.83%) and sepsis (n=11, 15.27%). Meningitis (n=6, 8.33%), severe acute malnutrition (SAM) (n=4.5, 55%), head injury, hydrocephalus (n=3 each, 4.1%) are other less common primary disease or condition of the culture positive PICU patients. (Table 2)

The isolation rate was very high in tracheal aspirates (n=23 of 28, 82.14%) as compared to blood (n= 23 of 161, 14.29%) and urine (26 of 183, 14.21%). Gram negative bacilli (GNB) (55 of 72; 76.3%) was the most common pathogen group isolated followed by Gram positive cocci (GPC) (16 of 72; 22.2%)) and only one Candida species comprised the rest. *Klebsiella pneumoniae* (n= 22, 30.5%) was the most common isolate among the GNB isolates followed by *Acinetobacter* species (n=14, 19.4%), *Escherichia coli* (n= 7, 9.7%), *Citrobacter species* (n=6, 8.3%), *Pseudomonas aeruginosa* (n=4, 5.5%), and two isolates of *Enterobacter cloacae*. *Enterococcus* species (n= 10 of 72, 13.8%) was the most common GPC isolate followed by five isolates (n=5, 6.94%) of Methicillin resistant *Staphylococcus aureus* (MRSA). Sample wise distribution of culture isolates is shown in (Table 3).

Table 1: Age and Gender wise distribution of subjects and culture positive cases

	Groups	Total cases (n=372)	Percentage	Total Culture Positive (n=72)	Culture Positivity Rate (%)
Age (months)	0-6	159	42.74%	29	18.24%
	>6 to 12	76	20.43%	13	17.11%
	>12	137	36.82%	30	21.90%
Gender	Female	165	44.35%	30	18.18%
	Male	207	55.64%	42	20.29%

Table 2: Distribution of primary diseases of culture positive cases

Primary diseases	Culture positive			
	Blood	Urine	Tracheal aspirate	Total (n with %)
AES	1	5	13	19(26.38%)
Pneumonia	6	3	6	15(20.83%)
Sepsis	3	4	4	11(15.27%)
Meningitis	4	2	0	6(8.33%)
SAM	2	2	0	4(5.55%)
Hydrocephalus	2	1	0	3(4.16%)
Head injury	2	1	0	3(4.16%)
Seizure	0	1	0	1(1.38%)
AGE	1	0	0	1(1.38%)
ALL	0	1	0	1(1.38%)
ARI	0	1	0	1(1.38%)
Diabetes	0	2	0	2(2.77%)
Empyema	1	0	0	1(1.38%)
Nephrotic syndrome	0	3	0	3(4.16%)
Abdominal Koch	0	1	0	1(1.38%)
Total	22	27	23	72

AES- Acute Encephalitic Syndrome
SAM- Severe Acute Halnutrition
AGE- Acute Gastroenteritis
ALL- Acute Lymphoblastic Leukaemia
ARI- Acute Respiratory Infection

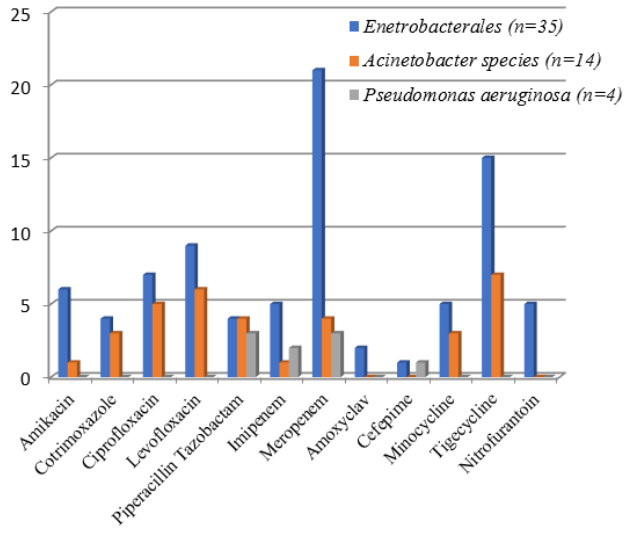

Figure 1: Antibiotic susceptibility pattern of Gram negative bacilli isolates of all samples

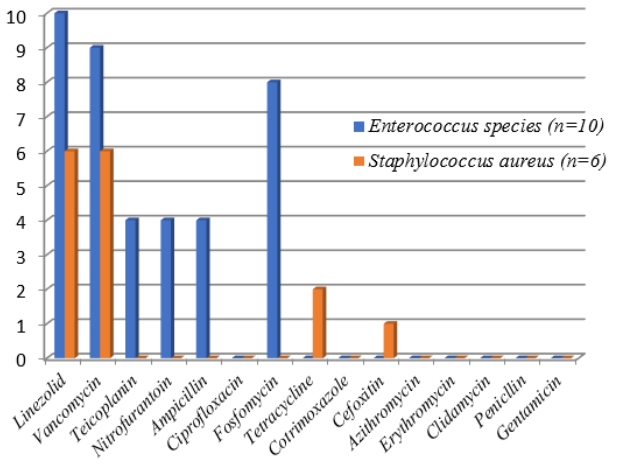

Figure 2: Antibiotic susceptibility pattern of gram positive cocci isolates of all samples

Table 3: Distribution of organisms isolated from different specimen

Specimen	Gram negative (N= 55, 76.3%)						Gram positive (N= 16, 22.2%)				
	Klebsilapneumoniae (n)	*Escherichia coli*(n)	*Citrobacter species* (n)	*Enterobacter cloacae*(n)	*Acinetobacter species* (n)	*Pseudomonas aeruginosa* (n)	*Enterococcus species* (n)	*Staphylococus aureus*(n)	MRSA (n)	*Candida species* (n)	Total isolates = n (%)
Blood (n=161)	7	0	1	2	6	0	2	1	4	0	23 (14.29%)
Tracheal aspirate (n=28)	10	1	2	0	6	4	0	0	0	0	23 (82.14%)
Urine (n=183)	5	6	3	0	2	0	8	0	1	1	26 (14.21%)
Total (n= 372)	22	7	6	2	14	4	10	1	5	1	72 (19.35%)

Table 4: Antibiotic susceptibility frequency of gram negative bacilli isolates

Antimicrobial agent	Blood Isolate		Tracheal aspirate isolate			Urine Isolate	
	Enterobacterales (n=10)	*Acinetobacter species* (n=6)	*Enterobacterales* (n=13)	*Acinetobacter species* (n=6)	*P aeruginosa* (n=4)	*Enterobacterales* (n=12)	*Acinetobacter* species (n=2)
Amikacin	4	1	0	0	0	2	1
Cotrimoxazole	3	NT	3	0	0	1	0
Ciprofloxacin	3	4	1	1	0	3	0
Levofloxacin	2	4	4	2	0	3	1
Piperacillin Tazobactam	2	2	1	2	3	1	0
Imipenem	2	1	3	0	2	0	0
Meropenem	2	3	9	1	3	10	2
Amoxicillin & Clavulanic acid	1	NT	NT	0	0	1	1
Cefepime	1	0	0	0	1	0	0
Cefotaxime	0	0	0	0	0	0	0
Minocycline	3	1	0	2	0	2	0
Tigecycline	9	6	4	1	0	2	0
Nitrofurantoin	NT	NT	NT	NT	NT	5	NT

** Enterobacterales includes Klebsiella pneumoniae, Escherichia coli, Enterobacter species, Citrobacter species*

NT: Not tested

Table 5: Antibiotic susceptibility frequency of gram positive cocci isolates

Antimicrobial agent	Blood Isolate		Urine Isolate	
	Enterococcus species (n=2)	*Staphylococcus aureus* (n=5)	*Enterococcus* species (n=8)	*Staphylococcus aureus* (n=1)
Linezolid	2	5	8	1
Vancomycin	2	5	7	1
Teicoplanin	0	NT	4	NT
Nitrofurantoin	NT	NT	4	0
Ampicillin	0	0	4	0
Ciprofloxacin	0	0	0	0
Fosfomycin	NT	NT	8	NT
Gentamicin 120 mcg	0	NT	0	NT
Tetracycline	NT	2	NT	0
Cotrimoxazole	NT	0	NT	0
Cefoxitin	NT	1	NT	0
Azithromycin	NT	0	NT	NT
Erythromycin	NT	0	NT	NT
Clindamycin	NT	0	NT	NT
Penicillin	NT	0	NT	0
Gentamicin	NT	0	NT	0

NT: Not tested

3.1. Antimicrobial susceptibility pattern

The antibiotic susceptibility testing showed a varied result. *Enterobacterales* isolates from Blood culture (n=10) showed highest susceptibility to Tigecycline (90%) followed by Amikacin (40%) while those from tracheal aspirate (n=13) and urine (n=12) showed highest susceptibility to Meropenem (69% and 83% respectively). Nitrofurantoin (41.6%) was the second most susceptible antimicrobial agent among *Enterobacterales* urine isolates. All the *Acinetobacter* species isolates from Blood culture (n=6) were susceptible to Tigecycline while *Pseudomonas aeruginosa* tracheal isolates were equally susceptible to Piperacillin Tazobactam (75%) and Meropenem (75%). (Table 4) Among the Gram positive isolates, all Enterococci and Staphylococci isolates were susceptible to Linezolid (100%) and Vancomycin (93.7%). One isolate of *Enterococcus* was found to be Vancomycin resistant. Fosfomycin (n=8) was susceptible to all the Enterococci urine isolates. (Table 5)

4. Discussion

As our institute is the only tertiary care hospital in the most eastern part of Assam catering almost six districts of the state as well as neighbouring state of Arunachal Pradesh, patients are mostly referred from primary and secondary health care govt. hospitals and private hospitals of the region.

During this study period a total of 372 PICU patients were taken as our study subjects and only 19.35% had positive culture result. The mean age of the study subjects was 31.39 ± 37.0 months with males (55.64%) more than female (44.35%). Most of the patients admitted in PICU were of infectious diseases viz. Acute encephalitic syndrome, bacterial sepsis, respiratory diseases like viral or bacterial pneumonia. Diseases of central nervous system, malnutrition and malignancy are the other categories of patients admitted in PICU. Of all these, Acute Encephalitic Syndrome (26.38%) was the leading primary disease in our study.

In a previous study by Camilla et. al. (2012) from São Paulo, Brazil reported respiratory tract infection as the dominant primary disease, male (54.73%) predominance.[9] Our study revealed 82.14% culture positivity from tracheal secretions, blood (14.29%); and urine (14.21%). Avcu et.al.(2021) in their study reported the most frequently detected HAI types as BSI (50%), VAP (40.9%) and UTI (9.1%), respectively.[3] Kumar et al.(2021) in their study reported overall 10.8% culture positivity with highest isolation rate from tracheal secretions (35.6%) as compared to blood (4.8%) and urine (9.7%) which is similar to our study findings.[10] Gupta A et al. (2011) reported 61% healthcare-associated pneumonia, 27% bloodstream infections and 9% urinary tract infections among PICU

admitted patients in New Delhi.[11] The predominant organism group isolated in our study was Gram negative bacteria (76.3%). Frequency of Gram negative bacteria (GNB) isolates was 69.56% from blood, 100% from tracheal aspirate, and 61.53% from urine specimen in our study. Other studies around the world reported GNB as the predominant pathogen of blood stream infection.[12–14]

Kumar et al.(2021) reported a predominant isolation of GNB (86.4%) in their study viz.from blood (20.8%), tracheal aspirate (48.2%), and urine (8.6%).[10] In another study by Singhi et al. on nosocomial bloodstream infection in PICU found predominance of Gram negative bacteria; *Klebsiella pneumoniae* (20.1%), *Enterobacter* spp. (16.6%), and *Acinetobacter* spp. (8.6%) being commonest.[15] The present study revealed isolation of *Klebsiella pneumoniae* (30.5%), *Acinetobacter* species(19.4%), *Escherichia coli* (9.7%), *Citrobacter species* (8.3%) and *Pseudomonas aeruginosa* (5.5%)while *Enterococcus* species (13.8%) from all specimen. In a meta-analysis on burden of health care associated infections in Southeast Asia, Ling et al. reported *Pseudomonas aeruginosa*, *Klebsiella s*pecies, and *Acinetobacter baumannii* as the most common organisms.[16]. Kumar et al.(2021) also reported higher isolation of GNB (86.4%) in PICU viz. *Escherichia coli* (17.4%), *Klebsiella pneumoniae* (16.2%), *Pseudomonas aeruginosa* (12.2%) and *Staphylococcus aureus* (9.5%).[10] Rehman et.al. also reported 93.65% culture positive in endotracheal (ET) secretion, mostly by *Klebsiella pneumoniae* (41.93%).[17]

Microorganism are mostly acquired from environment and it depends upon some factors like geographical distribution, hospital environmental conditions, colonization of ET tube lumen starts as early as 12 hours with peak at 96 hours.[18] In patients specially using mechanical ventilation, GNB tend to survive within a biofilm.[19] Antibiotics susceptibility pattern of GNB isolates in our study were multidrug resistant to three or more groups of antimicrobial agents; most of the isolates were resistant to penicillin, cephalosporin, fluoroquinolones, tetracycline, and sulfa groups. This may be due to unnecessary, inappropriate or suboptimal antimicrobial agents used or prescribed from community or referred healthcare facility.

WHO (2014) reported more than 50% resistance to third generation Cephalosporin and Fluoroquinolones in hospitals of most of the regions of the globe.[20]

Gram positive bacteria isolates in our study had highest susceptibility to Linezolid and Vancomycin. Some earlier studies e.g. Sarangi et.al and Singh et.al. reported Linezolid and Vancomycin to be highest susceptible in NICU setting.[21,22] High isolation of MDR bacteria in our study may be due to that fact that our hospital is the only tertiary care hospital which caters health services not only in upper Assam but also covers some states of North East India. Moreover patients admitted in our setting are mostly referred cases from other hospitals, who were either already treated with antibiotics or due to over the counter use in improper dosage.

5. Limitations

There were some limitations to the study like inadequate specimen, isolation of contaminant bacteria in some specimen, and unable to analyse clinical outcome in terms of recovery or mortality. More clinico-epidemiological studies will be needed to validate our findings. Secondly, we could not analyse the device associated infections in the study population, which would have enabled us to analyse various types of HAIs.

6. Conclusions

Gram negative bacteria were the predominant pathogens in all PICU specimen. *Klebsiella* species and *Acinetobacter* species were the most common pathogens isolated while *Enterococcus* species were the common among Gram positive bacteria. Although isolates showed multiple drug resistance to commonly used antimicrobials but a good susceptibility pattern was observed for Amikacin, Cefoperazone-sulbactam, Meropenem, Linezolid, and Vancomycin. Due to advent of carbapenem resistant Gram negative bacteria, use of carbapenems in PICU infection may lead to failure of antibiotic therapy. Routine microbial culture and antimicrobial susceptibility testing in PICU specimen and periodic review of hospital antibiotic policy should be mandatorily practised to prevent morbidity and mortality.

7. Source of Funding

None.

8. Conflict of Interest

None.

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