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## BACTERIOLOGICAL PROFILE AND SUSCEPTIBILITY PATTERN OF SEPTICEMIC NICU AND PICU CHILDREN OF A TERTIARY CARE RURAL HOSPITAL IN AMRITSAR, INDIA. AND PICU CHILDREN OF A TERTIARY CARE RURAL HOSPITAL IN AMRITSAR, INDIA.

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Septicemia, a common cause of morbidity and mortality among neonates and children is caused by a wide spectrum of constantly changing bacteria. Prompt recognition of the

neonates and children is caused by a wide spectrum of constantly changing bacteria. Prompt recognition of the pathogen and appropriate antimicrobial therapy are the key determinants of positive outcome in this serious pediatric emergency. Bacteriological culture from blood sample of the patient remains the mainstay of diagnosis of septicemia. Blood cultures, collected both by conventional and BacTAlert methods, were performed on 100 NICU and PICU septicemic patients of a tertiary care hospital and their bacteriological profile and antimicrobial susceptibility pattern was studied. Among 41 (41%) culture positive blood samples of neonates, MRSA was the predominant isolate being 11/41 (26.8%), followed by MSSA 7/41 (17.1%), *Acinetobacter* sp, *Enterobacter* sp and CONS each 5/41 (12.2%). *E. coli* were 4/41 (9.8%), *Klebsiella* sp 3/41 (7.3%) and only 1 of 41 (2.4%) was *Citrobacter* sp. Out of 30 (30%) culture positive blood samples from PICU, MRSA isolates were highest, 12/30 (40%), followed by *Acinetobacter* sp being 5/30 (16.7%), *E. coli* 4/30 (13.3%), MSSA & *Enterobacter* sp each 3/30 (10%). 2 *Ps. aeruginosa* (6.7%) and 1 *Klebsiella* sp (3.3%) were also isolated. Most of the gram positive organisms were sensitive to Amikacin, Levofloxacin and Vancomycin. Gram negative isolates were mainly sensitive to Piperacillin-Tazobactam combination followed by Amikacin and Imipenem. There were 8 ESBL and 2 MBL producers. In wake of high incidence and morbidity & mortality due to sepsis in neonates and children, there is need for surveillance at regular intervals to know the changing pathogen profile and their susceptibility patterns so as to formulate policies on rational use of antibiotics and infection control.

### Bacteriological, Profile , Susceptibility, Pattern ,Septicemic NICU ,

to infections leading to septicemia which makes the knowledge of bacteriological profile, its epidemiology in the region or hospital and monitoring of the same essential.<sup>2</sup> The shortcomings of blood culture such as low sensitivity and reporting delay of 24–72 hrs have been overcome in the last decade with the advent of automated continuous blood culture monitoring systems like BacTAlert, ESP System etc.<sup>6</sup> The present study was designed for isolation and identification of the causative bacterial organisms of septicemia in neonatal and pediatric ICU's and selecting their antibiotic susceptibility pattern thus proving helpful in making a proper policy for rational prophylactic & therapeutic use of antibiotics in that hospital.

#### SDHOTMATERIAL AND ME

The present study was conducted in the Department of Microbiology in collaboration with Department of Pediatrics, Sri Guru Ramdas Institute of Medical Sciences and Research, Amritsar after the approval of protocol by the Hospital Ethics Committee. The study design was prospective, observational and included 100 consecutive blood samples of suspected septicemic patients in NICU and PICU each over a period of one and a half year from October 2013 to March 2015. A detailed history was taken and also clinical and laboratory findings were noted.

2-3 ml of blood was withdrawn under strict aseptic precautions from the children preferably before the administration of antibiotic therapy. The samples were collected both in blood culture bottle containing Brain heart infusion broth and automated BacTAlert bottles. The brain heart infusion bottles were incubated overnight at 37°C aerobically and sub cultured on blood and MacConkey agar on day 1, 2, 3, 5, 7 & 10. The BacTAlert bottles were placed in the wells of the fully automated system for incubation and periodic reading was done. Those signalling positive were cultured on Blood and MacConkey's agar. An incubation period of 7 days was allowed for the bottles in the automated system before reporting them negative. Depending upon the observation of Gram's staining, specific biochemical tests were put up for the final identification. The antibiotic susceptibility testing of the isolates was done by Kirby-Bauer disk diffusion method on Mueller Hinton agar as per Clinical Laboratory Standards Institute (CLSI) guidelines. MRSA detection was done by putting Cefoxitin disc (30 µg). Zone of inhibition of ≥22mm was considered susceptible while ≤21mm was labelled as MRSA as per CLSI. ESBL detection (Extended spectrum beta lactamase) was done in all isolates

Septicemia is a common cause of morbidity and mortality in neonates and children despite advanced measures for early diagnosis and treatment, introduction of new antimicrobial agents, considerable progress in hygiene, and aggressive enteral feeding.<sup>1</sup> Neonatal sepsis is defined as a clinical syndrome of septicemia presenting with systemic signs and symptoms of infection in the first four weeks of life.<sup>2</sup> On the basis of time or postnatal age of onset, neonatal sepsis can be classified into Early onset neonatal sepsis (within 72 hours to 7 days of birth) and Late onset sepsis (7th day to 1 month of birth).<sup>3</sup> Early onset infections are thought to be commonly transmitted vertically from mother to child. Late onset neonatal infections are primarily caused by bacteria thriving in external environment i.e. they are either Community or Hospital acquired, mostly acquired from the hands of the care takers but may be acquired during delivery by vertical transmission from the mother.<sup>3,4</sup> Septicemia in children in PICU is a common cause of morbidity and mortality. Most of the infections in PICU are nosocomial. The longer the stay, greater is the contact of patient with the healthcare personnel, higher are the chances of infection through exposure to environmental organisms and invasive procedures, which may end up in septicemia. Risk factors for sepsis in PICU are prolonged central venous catheter use, receipt of extracorporeal membrane oxygenation, mechanical ventilation, dialysis and total parenteral nutrition. A wide variety of bacteria can cause septicemia in neonates and children and this bacteriological profile is constantly under change. The common gram positive organisms are *Staphylococcus aureus* (MSSA) and Methicillin resistant *Staphylococcus aureus* (MRSA), *Coagulase negative Staphylococci* (CoNS). Gram negative organisms *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter* sp, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Listeria* sp, *Acinetobacter* sp, *Enterobacter* sp, *Elk* membranes or after their rupture.<sup>2,5</sup> *Staphylococcus aureus* CoNS and *Citrobacter* sp, *Pseudomonas* sp., both Methicillin sensitive and resistant; are more common causes of Late onset septicemia. Bacteriological culture of the offending pathogen from blood sample of the patient remains the mainstay of diagnosis of septicemia. Both neonates and children are susceptible

*taphylococcus* Schildren in PICU with 6.7% as shown in Table 1. All strains in the current study were susceptible to Vancomycin *aureus* followed closely by Piperacillin–tazobactam. Gram negative isolates showed maximum susceptibility to Imipenem (72.8%) and Piperacillin-tazobactam (69.7%) followed by Amikacin (63.7%). combined showed a high *bsiella sp elK* and *E.coli* (Table 2 & 3) resistance of 75% each to Third generation cephalosporins, of 62.5% *E* to fluoroquinolones and 87.5% to aminoglycosides. 3 of 4 (75%) isolated from NICU and PICU were ESBL producers, 2 of 3 *coli*, all from NICU were positive for the same. *sp*(66.7%) of *Klebsiella* MBL production, however was demonstrated in only 1 of 5 *Acinetobacter sp* strains from NICU.

able no 1 showing distribution of bacterial isolates in NICU & T

PICU N=30	NICU N=41	NAME OF THE ISOLATE
12(40%)	11(26.8%)	MRSA
3(10%)	7(17.1%)	MSSA
3(10%)	5(12.2%)	Enterobacter sp
5(16.7%)	5(12.2%)	Acinetobacter sp
(NOT included)	5(12.2%)	CONS
4(13.3%)	4(9.8%)	Escherichia coli
2(6.7%)	0	Pseudomonas aeruginosa
1(3.3%)	3(7.3%)	Klebsiella sp
0	1(2.4%)	Citrobacter sp

2 AST culture isolates in

N	N	N	N	N	4(18)	Vancomycin
T	T	T	T	T	80(100)	
N	N	N	N	N	4(7)	Cefotaxime
T	T	T	T	T	80(3.9)	
0	0	0	2(40)	0	4(7)	Cefpodoxime
					80(3.9)	
0	0	1(25)	0	0	N	Ceftriaxone
					T	
0	0	1(25)	0	0	N	Ceftazidime
					T	
0	0	1(25)	0	0	N	Cefoperazone
					T	
0	0	1(25)	0	0	N	Cefepime
					T	
1(10)	0	1(25)	4(80)	0	2(12)	Levofloxacin
					6(7)	
						Piperacillin
0	1(33)	3(75)	3(60)	2(40)	3(16)	+Tazobactam
					8(9)	
1(10)	1(33)	2(50)	4(80)	2(40)	N	Imipenem
					T	

able no 3 AST of culture isolates in PICU: T

PICU. (NT- NOT TESTED)	Citrobacter	Klebsiella sp N (%)	E.coli N (%)	Acinetobacter sp N (%)	Enterobacter sp N (%)	CONS	Staph aureus	Organism
								Antibiotics
NT	NT	NT	NT	NT	1(20)	3(16.7)		Ampicillin
1(100)	0	2(50)	2(40)	1(20)	3(60)	9(50)		Amikacin
NT	NT	NT	NT	NT	2(40)	5(27.8)		Amoxyclav
0	0	1(25)	1(20)	0	1(20)	6(33.3)		Gentamicin
								Erythromycin
NT	NT	NT	NT	NT	1(20)	12(66.7)		
NT	NT	NT	NT	NT	2(40)	10(55.6)		Clindamycin

statistically to obtain valid conclusions.

### SNOITAVERSOB

Out of the total 200 consecutive blood samples of suspected septicemia patients from NICU and PICU, 71 (35.5%) patients had septicemia confirmed by positive bacterial cultures. NICU showed a higher number of isolates i.e. 41 (41%) followed by PICU, 30 (30%). In NICU, LOS (66%) was more common than EOS (34%). Males were more commonly affected than females. The mean gestational age of septicemic neonates was  $34.07 \pm 3.13$  weeks (mean  $\pm$  SD) in NICU. Preterm neonates had a statistically significant higher rate of septicemia (82%) than the term neonates (17%). In PICU, septicemia was more frequently reported in children under 1 year (36.7%) however, NICU age had no influence over culture positivity here. Mean weight of septicemic neonates in this study was  $1.78 \pm 0.43$  kg (mean  $\pm$  SD) although it didn't influence culture positivity statistically. The most important risk factor of suspected septicemia in neonates in our study population was respiratory distress (41.5%), followed by premature rupture of membranes (34%) & meconium stained liquor (24.5%) as against CNS disease in was the predominant gram positive *taphylococcus aureus* SPICU. pathogen both in NICU (43.9%) and PICU (50%). There was a high prevalence of MRSA (61%) due to colonization of neonates hospitalized in NICU, thus being the most common cause of LOS (44%) in NICU and also a notorious pathogen causing septicemia in PICU. (12.2% each) followed *inetobacter spAc* and *Enterobacter sp* (40%). (2.4%) were *sp Citrobacter* (7.3%) and *sp bsiella elK* (9.8%), *E. coli* by the predominant gram negative isolates In NICU. On the other hand, *inetobacter sp* AcPICU had a predominance of *bsiella elK* (10%) and *Enterobacter sp* (13.3%), *E. coli* (16.7%), was isolated only in the blood of septicemic *Pseudomonas*, (3.3%). *sp*

Ps aerugi	Klebsiella				
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were methicillin- resistant. This reflects the high prevalence of MRSA due to colonization of neonates hospitalized in NICU. 7 In our study as well, it was the most common cause of LOS. (44%) in NICU and was also the notorious pathogen causing septicemia in PICU (40%). MRSA has been reported by Foglia as one of the leading causes of nosocomial pneumonias especially ventilator associated in PICUs, as also found in our study. 18

of the current study was totally susceptible to *taphylococcus aureus* S Vancomycin i.e.100% both in NICU and PICU followed closely by Piperacillin-tazobactam. This was in concordance with study by Sharma and Srinivasa et al.19,20 Routinely used antibiotic like Amikacin showed a better sensitivity in PICU (73.3%) than NICU (50%).

Gram negative isolates showed maximum susceptibility to Imipenem (72.8%) and Piperacillin-tazobactam (69.7%) followed by Amikacin (63.7%). This was in coherence with Zaidi et al, where the WHO recommended Ampicillin and Gentamicin for the treatment of neonatal sepsis may no longer be effective as the sensitivity of Klebsiella and E coli to these drugs was low. E.coli and Klebsiella sp showed a high resistance of 75% to Third generation cephalosporins, of 62.5% to fluoroquinolones and 87.5% to aminoglycosides. The resistance of gram negatives to third generation cephalosporins (80.9%) was high in agreement with Movahedian, Aurangzeb et al.15,21 with 81% and 71.6% respectively. The high resistance of these organisms to third generation cephalosporins can be attributed to the frequent production of ESBL by these organisms. Different rates of ESBL production have been reported by various authors.22,23

Organism	Sensitivity (%)						Antibiotics
	Sp N (%)	Ac N (%)	Ec N (%)	St N (%)	Sh N (%)	Other N (%)	
Amikacin	NT	NT	NT	NT	NT	2(13.3)	Amikacin
Ampicillin	1(100)	1(100)	4(100)	5(100)	3(100)	11(73.3)	Ampicillin
Amoxycylav	NT	NT	NT	NT	NT	3(20)	Amoxycylav
Gentamicin	0	100	0	0	2(66.7)	5(33.3)	Gentamicin
Erythromycin	NT	NT	NT	NT	NT	7(4.7)	Erythromycin
Clindamycin	NT	NT	NT	NT	NT	6(40)	Clindamycin
Vancomycin	NT	NT	NT	NT	NT	15(100)	Vancomycin
Cefotaxime	NT	NT	NT	NT	NT	3(20)	Cefotaxime
Cefpodoxime	0	1(100)	2(50)	1(20)	2(66.7)	3(20)	Cefpodoxime
Ceftriaxone	0	1(100)	1(25)	1(20)	1(33.3)	NT	Ceftriaxone
Ceftazidime	0	1(100)	1(25)	1(20)	1(33.3)	NT	Ceftazidime
Cefoperazone	0	1(100)	1(25)	1(20)	1(33.3)	NT	Cefoperazone
Cefepime	0	1(100)	1(25)	1(20)	1(33.3)	NT	Cefepime
Levofloxacin	2(100)	1(100)	2(50)	4(80)	3(100)	7(4.7)	Levofloxacin
Piperacillin+Tazobactam	2(100)	1(100)	4(100)	4(80)	3(100)	15(100)	Piperacillin+Tazobactam
Imipenem	2(100)	1(100)	4(100)	4(80)	3(100)	NT	Imipenem

## استنتاج

Pediatric septicemia can be prevented to a large extent by strict adherence by healthcare workers to hygienic guidelines especially hand washing. Screening of healthcare providers for MRSA carriage should be done and those found to be colonized should be treated.

An antibiotic policy with involvement of microbiologists should be implemented in the hospital to prevent the indiscriminate use of antibiotics thereby improving clinical outcome decreasing economic burden on the patient's family, the hospital and the country.

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## NOISSISCUD

Septicemia, a life threatening emergency, is an important cause of morbidity and mortality in children especially neonates. It poses a great challenge to pediatricians all over the world especially in developing countries like India. In our study the incidence of microbiologically detected septicemia by BacT/alert and conventional methods in clinically suspected patients in NICU was 41% (41/100). This was consistent with study by Mustafa and Ahmed in NICU in Hyderabad with a similar 44.2%, Shrestha R et al (41.3%) in Nepal, in Tanzania by Kayange et al (39%), in Korea by Shim et al (43.2%). Repeated subcultures increase the chances of isolation of the organisms from blood cultures, as was seen in this study. 7, 8, 9, 10

The incidence of septicemia in PICU in our study was 30%. Similar result was obtained by Tsering et al in Sikkim with 36% and Becerra et al (a developing country) with 20%. Naher et al in Dhaka with 53%. 11,12

The blood culture yield by using the BacT/alert system in the current study was 34 % which was similar to Bhat Y and Ahmed et al with 29% and 26% respectively. 13,14

The 66% blood culture positivity in neonates with Late Onset Sepsis may be due to the relatively high mortality in early onset cases where delay in arrival to a tertiary care centre like ours due to patients mostly being from low socioeconomic status and rural backgrounds , might lead to death of the neonate prior to admission / treatment.7,13,15

Sundaram V and Shrestha et al. reported an increase in the incidence (43.3% & 56.8% resp.) and a *aureus* . Sof neonatal sepsis caused by decrease in the incidence of same caused by gram- negative bacilli. Similar findings were obtained in our study. 16, 17

strains (11/18) i.e.61% *taphylococcus aureus* S A higher number of

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