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Study of multi drug resistance pattern in the bacterial samples isolated from neonatal blood samples

Kaiser Ahmad Wani[1], Abida Malik[1], Irfan Mohiudin[1], Sumira Tyub[2]

[1]G B Pant Children Hospital, Srinagar, Jammu & Kashmir, India
[2]Centre of Research for Development, University of Kashmir, Srinagar, J&K, India

***Corresponding author**: kaisserahmed@yahoo.co.in

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ABSTRACT

Blood cultures provide essential information for the evaluation of a variety of diseases particularly, in patients with suspected sepsis. Pathogens causing neonatal infections and their antibiotic susceptibility patterns may change over time and differ between countries. The present study is an attempt to study the multidrug resistance in the neonatal blood samples. For this purpose 3031 blood culture samples were studied in department of microbiology G B Pant children hospital for a period of two years. Out of 3031 samples received 21.54% samples tested positive for bacterial growth by Bact/Alert 3D system yielding both gram positive and negative bacteria like Cocci spp and Bacilli Spp. as well as yeasts were also obtained. Out of total organism cultured, 58.03% were gram positive while 26.95% accounted gram negative bacilli, 14.29% fungi and 0.78% others. After identification the isolates were subjected to antimicrobial susceptibility testing. Among gram positive Cocci, vancomycin, linezolid, netilamicin, teicloplanin, tigecycline showed 100% sensitivity followed by gentamicin and amikacin. Among Gram negative Bacilli, tigecycline showed 100% sensitivity followed by imipenem, pipercillin plus tazobactum. The prevalence of Methicillin resistant Staphylococcus aureus was 50 %, Extended Spectrum Beta-Lactamases was 20% (Klebsiella spp, E. Coli, Salmonella typhii) and Carbapenem resistance of 17.61% (Klebsiella spp, Acinetobacter Spp, Pseudomonas Spp). So this study highlights the emergence of gram positive organism as predominant cause of sepsis in paedriatic patients. Various gram positive and gram negative isolates are mostly susceptible to vancomycin, tigecycline antibiotics which are high end antibiotics used at the centre but the appearance of resistance to carbapenems, cephalosporins, aminoglycosides is of serious concern.

Key words: Sepsis, Neonatal, Multidrug resistance, Blood cultures, Bacteria, antibiotics.

INTRODUCTION

Neonatal sepsis refers to the presence of microbes or their toxins in blood. It is documented by positive blood culture in the first four weeks of life and is one the leading cause of neonatal mortality in India (Bhatt et al. 2012). It encompasses systemic infections of the newborn. The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries (Raina et al. 2016). To prevent deaths and disability at the time of birth, there has been intervention of facility based caring (Thakur et al. 2016) and the focus should be

particularly on neonatal sepsis or meningitis which accounted for 16% of neonatal deaths in 2013 (Liu et al. 2105). Early identification of the organism and prompt antibiotic treatment are essential to reduce the escalating rates of morbidity and mortality. Many underlying disease and conditions should however be considered along with possible sources of infection, invasive procedures including central intravenous catheters, empiric therapy and defense systems of the host while calculating morbidity and mortality. Personal hygiene of the staff in neonatal intensive care unit and newborn babies, skin and umbilical stump care are very important in preventing neonatal infections which could go a long way in reducing neonatal septicemia (Raina et al., 2016). Increasing antimicrobial resistance is a worldwide concern. The infection caused by Multi drug resistant organisms is more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics. (Kalposh et al. 2104). Increased prevalence of Extended spectrum beta-lactamases (ESBLs) and methicillin-resistant Staphylococcus aureus (MRSA) and multidrug resistant strains is a concern in Neonatal Intensive Care Units (NICU) worldwide. The knowledge of bacteriological profile and its antibiotic sensitivity patterns is of immense help in saving lives of neonates with septicemia (Kalposh et al. 2104).

So, the present study is conducted to determine the susceptibility pattern of pathogens causing neonatal sepsis and to provide resistance pattern of these isolates to pediatricians for better patient care.

MATERIAL AND METHODS

The present study has been conducted in the department of Microbiology, G B Pant Children Hospital, Srinagar for a period of two years from January 2014. The study has been carried out on the samples received in microbiology laboratory on routine basis. Blood from 3031 neonates admitted in Neonatal and Paedriatic Intensive Care Units (NPICU) with clinical suspicion of septicemia were received in Microbiology laboratory for culture and sensitivity. Blood was collected after aseptic precautions in ready to use Bact/Alert PF plus culture bottles (yellow color) for paedriatic use (bioMerieux). These bottles were labeled with patient information and transported immediately to Microbiology lab. These culture bottles were loaded into the instruments after scanning the bar code of the bottle and incubated in Bact/Alert 3D (bioMerieux) a fully automated blood culture system for detection of growth in blood culture. Positive and Negative culture bottles were determined by Bact/Alert Microbial detection system. No action was taken until the Bact/Alert instrument signaled a culture positive or negative. Blood culture was considered negative only after five days of incubation.

In this study two automated systems namely BacT/Alert system and Vitek 2 compact were used for early detection of organisms and their antibiotic sensitivity pattern. The Vitek ID and Antimicrobial Susceptibility Testing (AST) cards were chosen according to the result of the Gram staining of the growths in blood cultures. For gram negative bacteria, 145µL of 0.5-McFarland bacterial suspensions, were pipette out into 0.45% sodium chloride. For identification of Gram-negative bacteria, the GN 341 cards (bioMerieux) were used and AST-280, AST-281 for AST according to the manufacturer's instructions and for identification of Gram-positive bacteria, the GP 342 cards (BioMerieux) were used and AST-628, for AST according to the manufacturer's instructions. The Vitek-2 ID and AST cards were logged and loaded into the Vitek-2 Compact system. The Minimum Inhibitory Concentrations (MIC) obtained were resolved into the 3 clinical categories susceptible, intermediate and resistant according to the interpretative criteria provided under the guidelines of automated systems recommendations.

RESULTS

Of a total of 3031 blood culture samples studied 21.54 % (653) were found to be positive for bacterial growth while 78.45% (2378) yielded no growth (Table1). Gram positive organism were predominant with 58.03% (379) while as only 27.1% (177) were found to be gram negative organism. Some fungi were also found comprising 14.29% (94) and other unidentified organisms were 0.78% (5). Among gram positive organisms, the predominant isolates were Coagulase negative *Staphylococcus* 35% (132) followed by *Staphylococcus aureus* 22% (84), *Enterococous* 6% (24), group of gram positive organism of varying clinical significance 46, 12 unidentified organisms and 75 as contaminants (Table 2). *Staphylococcus aureus* showed 50% MRSA and 50% MSSA strains while CONS showed 65% MR-CONS and all Staphylococcus showed least resistance to vancomycin, linezolid, tigycycline and teicloplanin. Coagulase negative Staphylococcus showed least resistance to vancomycin, linezolid, tigecycline and teicloplanin (fig1). Of group of gram positive isolates, only one isolate of *Streptococcus pyogenes*, 4 isolates of *Listeria monocytogenes* and one isolate of *Listeria innocua* were of clinical significance while rest were of no or little clinical significance (Table 2). Twelve gram positive isolates could not be identified while 75 were considered as contaminants.

Among gram negative isolates, the predominant isolate were *Acinetobacter* spp. and *Klebsella* Spp. with 77 and 42 isolates respectively. These were followed by *E.coli* with 26 isolates,

Burkholderia capaciae with 10 isolates, Pseudomonas Spp. with 10 isolates, Enterobacter with 7 isolates and finally *Salmonella* Spp with 5 isolates (Table 2). Enterobacteriacae showed Multidrug resistance to pencillin, cephalosporins, carbapenems and aminoglycosides (fig.2).20% were ESBL producers among 177 gram negative isolates while 17.61% were Carbapenemase resistant Enterobacteriacea (CRE).

Table 1: Number of microorganisms isolated from blood culture of neonatal samples

S. No		Microorganism	Number (%age)
1	Total samples studied 3031	Culture positive	653 (21.54%)
2		Culture negative	2378 (78.45%)
3	Culture positive 653	Gram positive organisms	379 (58.03%)
4		Gram negative organisms	177 (27.1%)
5		Fungi	94 (14.29%)
6		Others	5 (0.78%)

Table 2: Gram positive and Gram negative organisms isolated from the neonatal blood samples

S. No.	Gram positive			Gram negative	
	organism		Number (%age)	organism	Number (%age)
1	Coagulase negative staphylococcus		132 (35)	Acinetobacter spp	77 (44%)
2	Staphylococcus aureus		84 (22)	Klebsiella spp	42 (24%)
3	Contaminants		75 (20)	E .coli	26 (14%)
4	Clinically insignificant organisms		46 (12)	Pseudomonas spp	10 (5%)
5	Enterococcus		24 (6)	Burkholderia capaciae	10 (5%)
6	Unidentified organisms		12 (3.16)	Enterobacter	7 (4%)
7	Streptococcus pyogenes		01 (0.2)	Salmonella spp.	5 (3%)
8	*Listeria monocytogenes*	4	05 (1)		
	Listeria innocus	1			
	Total		**379**	**Total**	**177**

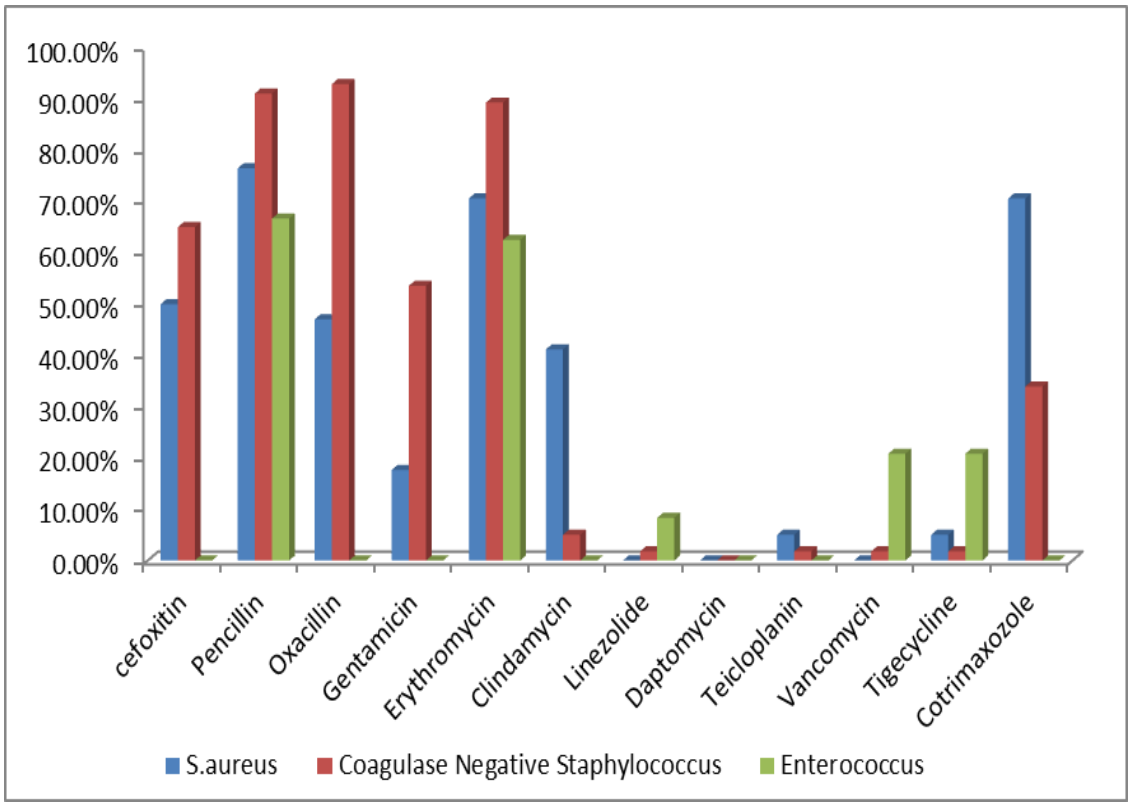

Fig. 1. Resistance among gram positive isolates.

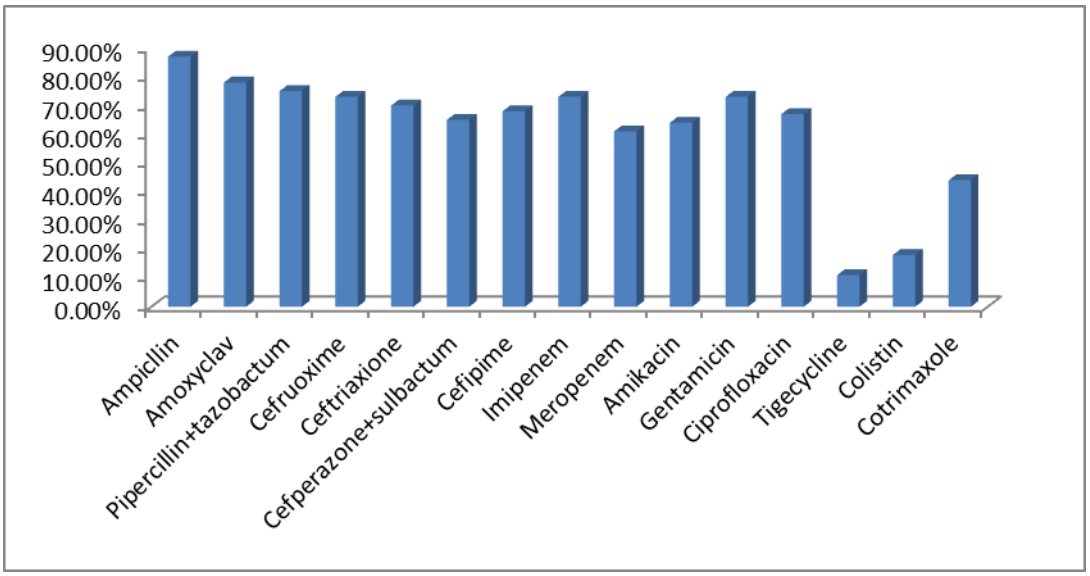

Fig. 2. Resistance pattern of gram negative isolates.

DISCUSSION

Estimates indicate that 56524 neonates die each year from resistance attributed to neonatal sepsis deaths caused by bacteria resistant to first line antibiotics in India (Laxminarayan et al. 2016). The study of bacteriological profile with their antibiotic sensitivity pattern in a particular geographical area plays significant role in effective management of neonatal septicemia (Raina et al. 2016).The emergence of resistant bacteria in NICCU setting leads to failure in the treatment of neonatal septicemia. To supplement the management of septicemia in neonates we need to do longitudinal surveillance of the NICCU and formulate periodic guidelines for empiric treatment (Kalposh et al. 2014). The present study is an attempt to study the neonatal blood samples. In this study, blood culture positivity was seen in 653 of 3031 (21.54%) blood culture samples which is quite similar to (Iregbu et al. 2006; Shah et al. 2012) but is lower as compared to other studies (Bhatt et al. 2012; Chelliah et al. 2014; Kalposh et al. 2014). and at same time is slightly higher than study by (Kaistha et al. 2010).The lower incidence may be due to reasons that majority of patients are referred from periphery hospitals or by physicians and have already received high end antibiotics before reaching our hospital. It also has been observed that over dilution of little blood from neonates in broth bottles may also hinder some growths. (Iregbu et al. 2006; Agarwal and Bhat, 2015). The blood culture technique or sepsis due to anaerobeic, viral or fungal pathogens also adds to low blood culture isolation rate (Galhotra et al. 2015; Raina et al. 2016).

The bacteriological profile has changed worldwide from predominant gram negative to predominant gram positive bacteria isolation. Many recent studies have reported the emergence of some new emerging organisms such as Coagulase Negative Staphylococcus aureus (CONS), Nonfermentaive group of organism (NFGO) and Candida spp. as a cause of neonatal septicemia (Liu et al., 2015). Our study showed predominance of gram positive organisms 58.03% as compared with gram negative organisms 27.1%, which is comparable to studies conducted by Thakur et al (2016) and Galhotra et al (2015). Bacterial infection was found to be higher 85.14% in neonates followed by fungal infection 14.29%. These results are in consensus with the reports by Raina et al (2016) and Agarwal & Bhat (2015). Among gram positive organisms Coagulase Negative Staphylococcus 132 (35%) with *S. epidermidis* was the most predominant organism isolated which had also been obtained by Bhatt et al. (2012), Galhotra et al. (2015) and Kumaravel & Ramesh (2016). Out of 84 Staphylococcus isolated 50% were MRSA while rest were MSSA in our study which is much higher than study by Kaistha et al. (2010) but almost near to study by Chelliah (2014). So screening for MRSA in every *Staphylococus aureus* isolated will be of immense value for providing efficient patient care. All MRSA strains were sensitive to Vancomycin. The colonization of the skin and nasopharynx by CONS and *S. aureus* in health care workers, overcrowding in nurseries and NICU, and improper hand washing techniques may lead to transmission of gram positive organisms in neonates horizontally .There is isolation of gram positive bacilli for example *Listeria* in our study like other studies in the north India. The reason could be the overcrowding in

Nurseries /NICU and lack of knowledge about infection control measures among health care providers (Kalposh et al. 2014). Twelve (3.16%) gram positive strains were not identified. This might be because the blood components such as blood cells and fibers could not be separated completely from the bacterial pellets, which may have affected the biochemical reaction involved in the vitek2 identification process (Ling et al. 2003).
For *Staphylococcus aureus* 100% sensitivity was seen for vancomycin, linezolid, daptomycin, and teicoplanin in accordance with Mehta et al. (2014).Linezolid, daptomycin, teicloplanin, vancomycin, tigecycline were highly effective against gram positive organisms. Majority of gram positive isolates were resistant to pencillin , cotrimaxozole, erythromycin, clindamycin, gentamicin, oxacillin and cefoxitin (Kalposh et al. 2014; Shah et al. 2012; Agarwal et al. 2015; Galhotra et al. 2015). CONS were the most common pathogen that caused neonatal septicemia in our study as seen in Wu et al (2009) and Kumaravel et al. (2016).The main problem was distinguishing true blood culture infections from contamination .The wide spread use of vancomycin in NICU has resulted in VRE with 20%resistance to vancomycin in *Enterococous* spp.(16). Among gram negative isolates 27.7% isolates were Acinetobacter, *Klebsiella*, E.*coli*, *Pseudomonas*, etc. which showed concordance with studies by Bhatt et al. (2012), Raina et al. (2016) and Thakur et al (2016). Of these 20% were ESBL producers while 17.6% were carbapenemase producers with almost similar finding in study Shah et al (2012) but when compared with study (Ahmed et al. 2010) done at another tertiary hospital in this region it confirmed the spread of ESBL to neonatal unit and evolving emergence of carbapenem resistance. These organisms cause common-source outbreaks because they can live in multi-use medication vials, soap and inadequately processed equipment (Raina et al. 2016). Simple hygienic measures, such as hand washing practices, the use of sterile equipment patient cohorting and screening of attending staff for MRSA, ESBL,VRE and CRE can help prevent the further spread of these resistant strains. The study stresses that multidrug resistance is serious problem and need for surveillance and promotion of correct and restrictive antibiotic policies including specific antibiotic therapy after studying sensitivity pattern.

Ethical Clearance

Since the study has been conducted on the blood samples sent to laboratory on routine basis so no ethical clearance is required.

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