International Journal of Medical and Health Research
ISSN: 2454-9142
www.medicalsciencejournal.com
Volume 4; Issue 9; September 2018; Page No. 01-04

Bacteriological profile and antibiogram of blood culture isolates of septicemic patients from neonatal and pediatric intensive care units

Dr. Sanjeev Kumar[1*], Dr. Vivek Parasher[2], Sheetal Sharma[3]
[1, 3] Department of Microbiology, PIMS, Udaipur, Rajasthan, India
[2] Department of Pediatrics, PIMS, Udaipur, Rajasthan, India

Abstract
Septicemia is one of the important causes of mortality and morbidity in neonates and children. Aim of this study was to assess the frequency and antibiogram of microorganisms isolated from blood cultures. The study was conducted on blood cultures obtained from NICU & PICU during the period of January 2016 to October 2017. Isolation, species identification and antibiotic susceptibilities were done by automated Bact Alert and Vitek 2C methods. A total of 182 samples were received during the study period out of which 19 (10.44%) were culture positive. Among 19 culture positive samples, children were 10 and neonates were 09. The commonly isolated organism was Coagulase Negative Staphylococci (CONS) (57%), followed by Candida albicans (21%), Acinetobacter spp. (10.5%), Escherchia coli (5.2%) and Klebsiellae pneumonia (5.2%). The present study contributes information on the prevalence of microorganisms in blood stream infections and also indicates the presence of fungemia, which highlights the need for periodic surveillance of etiologic agents.

Keywords: blood culture, automated methods, candida, PICU, NICU

Introduction
Blood is normally a sterile environment, so the detection of bacteria in the blood is always signifies the abnormal. Blood cultures provide essential information for the find a various diseases like endocarditis, pneumonia, pyrexia of unknown origin and particularly in patients suspected sepsis [1]. Septicemia is one of the important causes of mortality and morbidity in neonates and children. In last few decades, increasing antimicrobial resistance is a problematic for throughout the world. The prevalence of drug resistance of blood borne pathogens is increasing and it also varies in accordance with geographical and regional location [2-5]. Infection rates in intensive care units (ICUs) have been found to be the highest among all hospital acquired infections in various multicenter studies carried out in the United States of America (USA) and Europe [6, 7]. This can be occurs due to the following reasons viz., use of large numbers of invasive monitoring devices, endotracheal and tracheostomy tube along with patient factors including age, immunocompromise state, malnutrition and severe underlying diseases [8, 9].
Sepsis is a common and threatening infection in the ICUs, where up to 35% of patients develop such a condition at some situation during their stay. The associated mortality is 27% but exceeds 50% in cases of septic shock [10]. The Blood Stream Infections (BSI) caused by multi drug resistant (MDR) organisms areresponsible for prolonged hospital stay, increase the risk of death and require treatment with more costly antibiotics.
Blood culture is a valuable tool for the diagnosis of septicemia, especially in pediatric wards. However blood culture detects bacteremia in only 50% of patients clinically suspected of having sepsis [11], with an even lower rate of positivity when patient is on antibiotic therapy [12]. Blood culture contamination is a major confusing problem that may happen at various stages of collecting blood and culture [13]. In most instances, the source of the contaminants probably is the existing normal flora on the patient's skin [14].
In most of the cases, antimicrobial therapy is started empirically before the results of blood culture reports. Think of that the high mortality and morbidity associated with septicemia, right choice of empiric therapy is of importance [15] as blood culture is routinely taken at the time of admission to ICU for all patients suspected to have infection, even though it may positive only in a few patients. We attempted to determine the incidence of bacteremia in clinically suspected cases and to describe the bacteriological profile and antimicrobial resistance of blood culture isolates from NICU and PICU of our hospital, so that the study can provide guidelines for choosing an effective antibiotic therapy in cases of septicemia.

Materials and methods
This study was carried out from January 2016 to October 2017 in a tertiary care institute. Consecutive blood samples received from NICU and PICU were included, clinical details of patients were noted.
The specimens were collected by nurses and sent to the microbiology laboratory. Following skin preparation with alcohol and then 10% povidone-iodine solution, the skin was allowed to dry for 1 min prior to venipuncture [16]. A blood sample of up to 4 ml was collected with a syringe and then transferred immediately into BACT/ALERT PFpediatric aerobic blood culture bottles (Biomerieux, USA). BACT/ALERT PF bottle consists of 20 ml peptone-enriched Tryptic soy broth (TSB), supplemented with Brain Heart Infusion (BHI) solids and activated charcoal (8.5%w/v) and

0.025% sodium polyethanol esulfonate. The atmosphere of the BacT/Alert PF bottle is oxygen and nitrogen, thus eliminating the need to vent the bottle [17-19]. After receiving, the bottle was visually inspected for growth, proper inoculation and appropriate patient information. The bottles were incubated in a BACT/ALERT 3D machine for 5 days at 37°C. Bottles were monitored for the presence of bacterial growth every 10 min. by the instrument. Any growth-positive bottle was processed promptly after detection, while the corresponding bottle was not processed until the bottle was recorded as positive by BacT/Alert instrument. Subcultures and direct smears were performed from positive growth bottle. Cultures that were initially indicated to be positive but did not contain bacterial growth were re-incubated and monitored for the remainder of the incubation period [17]. All growth positive isolates primarily identified by Gram staining, Colony morphology on Blood agar, MacConkey agar medium and performed basic biochemical test like, Catalase, Oxidase. After primary identification, we selected the Vitek2C cards of GP-ID for Gram Positive Cocci (GPC) and GN-ID for Gram Negative Bacilli (GNB) respectively. For antibiotic susceptibility test, we used AST–P628 for GPC, AST- N281 for Non-fermentative GNB (NFGNB) and AST- N280 for Lactose fermentative GNB. The antimicrobial agents tested were Benzylpenicillin, Oxacillin, Gentamicin, Ciprofloxacin, Levofloxacin, Erythromycin, Clindamycin, Daptomycin, Teicoplanin, Vancomycin, Tetracycline, Rifampicin, Trimethoprim/Sulfamethoxazole for catalase positive GPC. Ticarcillin/Clavulanic acid, Piperacillin/Tazobactam, Ceftazidime, Cefoperazone/Sulbactam, Cefepime, oripenem, Imipenem, Meropenem, Gentamicin, Ciprofloxacin, vofloxacin, Minocycline, Tigecycline, Colistin, Trimethoprim/ Sulfamethoxazole for NFGNB and Ampicillin Amoxicillin/ Clavulanic acid, Piperacillin/Tazobactam, Cefuroxime, Cefuroxime axetil, Ceftriaxone, Cefoperazone/Sulbactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Trimethoprim/ Sulfamethoxazole for Lactose fermenting GNB.

Result& discussion

A total of 182 samples were received during the study period out of which 19 (10.44%) were culture positive. Incidence and distribution of Blood culture positive and negative among male and female neonates & childrenwere shown in Table 1. Among 19culture positive samples, children were 10 and neonates were 09, out of 10 children 4 males and 6 females. Whereas out of09 neonates 07 males and 02 females.

A total of 9 different organisms were isolated. The commonest organism was Coagulase Negative Staphylococci (CONS) (57%), Candida albicans (21%), Acinetobacter spp. (10.5%), Escherchia coli and Klebsiellae pneumonia each one isolate as shown in Table 2.Mostly CONS were isolated from children, we considered as skin contaminants because they were not grown in repeated samples.

In Klebsiellae pneumonia and Acinetobacter species Colistin was found to sensitive and remaining all drugs were found resistant including Carbapenems. In Eshcherichia coli Colisitin, Carbapenems and Fluoroquinolones found to be sensitive and remaining all drugs were resistant. In Candida albicans 2 isolates were resistant to all antifungal agents, whereas 2 isolates were showed sensitive to Nystatin, Amphotericin B and resistant to remaining antifungal.

In our study, blood cultures sent from patients hospitalized in the NICU and PICU were evaluated prospectively. In the present study the growth was found in 10.4% of blood cultures. In the study conducted by Ergul *et al.* [20] in which blood cultures in a children's hospital were evaluated, the growth of any microorganism in blood cultures were reported with a rate of 7.6%. In our study, positive growth in blood cultures was found with a rate similar to the literature.

A different group of organisms are responsible for blood stream infections (BSI's) and same has been reported by various researchers. The most common cause in nosocomial blood stream infections is Gram-positive bacteria, followed by Gram negative bacteria and fungi [8, 21]. When microorganisms isolated from blood cultures were assessed, it was observed that Gram-positive bacteria generally constitute the majority, because they include Coagulase Negative Staphylococci (CoNS), which may arise from skin flora and are mostly considered contamination. In study conducted by Jyoti *et al.* [8], CoNS were isolated with a rate of 25.9% in blood cultures. In our study, all of the Gram positive bacteria isolated from blood culture were CoNS (57.8%), similar to study conducted by Ergul *et al*. [20]. Although, on the contrary many studies from India and other developing countries have reported GNB predominance in hospitalized patients: studies in India by Gupta and Kashyap [22] with 58.3%GNB and 41.65% GPB, Singh *et al.* [23] with 51.82% GNB and 46.56% GPB. In a study conducted by Katyal *et al.* [21], the A. baumanii 52.3% was the most common GN isolate followed by E.coli 27.2%, K. pneumoniae 14.3% and P. aeruginosa 5.5%. In the study by Ergul *et al*., in which blood cultures in a children's hospital were evaluated, the most common Gram-negative bacteria was reported as Enterobacteriaceae and a gradual increase in non-fermentative Gram-negative bacteria was reported to have been observed the years. In the present study, we have isolated equal number of isolates belongs to Enterobacteriaceae family and non-fermentative Gram negative bacilli. In recent years, Candida species have been isolated with a significantly increased rate in blood cultures because of an increase in the frequency of neutropenia, premature delivery, surgical procedures and intravascular catheter use. Ergul *et al*. reported that the fungi isolated in their study constituted 6.8% and 10.8% of all microorganisms. In our study, fungi were found to be grown with a rate of 21%. In ICUs, C. albicans has generally been reported to be the most commonly isolated species among fungal agents [24]. In the present study among Candida species, we have also isolated only C.albicans 100%. Whereas Jyoti *et al*., the most commonly isolated species among fungal species were Non-candida albicans with a rate of 94.8%, followed by C. albicans with a rate of 5.2%.

Hence, the main purpose of the present study was to assess the antibiogram of the major pathogens among the clinically diagnosed cases of sepsis. In our study we found Klebsiellae pneumonia was resistant to all the antibiotics except Colistin, whereas E. coli was sensitive to Carbapenems and Fluoroquinolones only. Acinetobacter spp. with multiple antibiotic resistances and it is important because it leads to infections especially in patients with immunosuppression and serious underlying morbidities and receiving treatment with

broad-spectrum antibiotics [22]. In the study by Bayram *et al*. [24], Imipenem resistance was reported with a rate of 63.5%, Amikacin resistance was observed 71.6% and Gentamicin resistance was 85.1% in Acinetobacter spp. In our study, Colistin was sensitive and remaining all drugs were resistant including Carbapenems, Aminoglycosides and Fluoroquinolones. Several other studies also reported high frequency of Gram-negative isolates as MDR [25, 26, 27]. Indiscriminate use of antibiotics and lack of appropriate antibiotic policy in the hospital could be the main reasons for this existing problem.

Tables

Table 1: Incidence and distribution of Blood culture positive and negative among male and female neonates & children

	Female (77)		Male (105)		
	Neonates	**Children**	**Neonates**	**Children**	**Total (%)**
Blood culture positive	02	06	07	04	19 (10.44%)
Blood culture negative	19	50	38	56	163 (89.56%)
Total no. of blood cultures	21	56	45	60	182 (100%)

Table 2: Comparison of isolates between Neonates and Children

Organisms	Neonates (09)	Children (10)
Micrococci (1)	-	1
Staphylococcus epidermidis (6)	-	6
Staphylococcus hominis (2)	-	2
Staphylococcus haemolyticus (2)	2	-
Escherichia coli (1)	1	-
Klebsiella pnemoniae (1)	-	1
Acinetobacter lwoffii (1)	-	1
Acinetobacter baumanii (1)	-	1
Candida albicans (4)	4	-

Conclusion

In conclusion, Knowledge of the causative agents of septicemia is useful in the selection of antimicrobials for empiric therapy. As Staphylococcus spp. was the most common organism isolated, it suggests that good hygiene practices by the patient, their attendants and the health care providers is very crucial especially in neonatal and pediatric ICUs. Also fairly high rate of isolating Candida albicans needs attention. A continuous monitoring of blood cultures in pediatric age group is necessary to understand bacteriological profile and their antibiogram in different age groups to provide better patient care.

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