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ABSTRACT

Objective: Identification and resistotyping of aerobic and facultative anaerobic bacteria and fungus causing sepsis. Methods: The study was conducted at a tertiary care hospital in Eastern India from 1st August 2014 to 31st January 2015with clinically suspected sepsis patients. Neonatal sepsis screening was done as per standard clinical guidelines. Specimens were collected, transported and processed for microbiological work up as per specified scheme using BACT ALERT 3D & VITEK-2 AES. Data were analysed according to standard statistical methods. Results: Total 96 samples for blood culture were received from clinically suspected patients. Of these, 25 were culture positive (26.04%). Isolated organisms included 13 Gram positive cocci, 8 Gram negative bacilli, 4 non albicans Candida. Gram positive cocci were more frequently isolated than Gram negative bacilli (52%). 5 out of 11 samples from Neonatal Intensive Care Unit (NICU) were found to be culture positive(45.4%). Isolated organisms from NICU included 2 Candida spp., 2 Escherichia coli &1 Methicliin resistant Staphylococcus aureus MRSA. Majority of the isolated Gram positive Coccci were Staphylococcus aureus (69.23%). Most of the isolated Staphylococcus were Methicillin resistant (7 out of 13 i.e. negative isolates, 53.84%). Among Gram Non-Enterobacteriaceae were predominant (62.5%), whereas, all three isolated Enterobacteriaceae were Escherichia coli. Half of the Gram negative isolates were Carbapenemase producer and 6 out of Gram negative isolates were resistant 8 (75%) to Fluoroquinolones. One Acinetobacter isolate was susceptible to Imipenem but resistant to Meropenem. Conclusion: On the verge of emergence of multidrug resistant pathogens, decision regarding empirical treatment of septicaemia must be based on knowledge of distribution of pathogens and their resistotype.

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Introduction

Sepsis constitutes to be one of the most serious situations in infectious diseases. Sepsis is a medical disaster and rapid succession keeps health personnel on their toes in a bid to confine the situation. Neonatal septicaemia is a major cause of neonatal mortality which is elevated by occurrence of multidrug resistance (MDR) Gram negative bacteria.¹ Sepsis has been described at least since the time of Hippocrates.² In the developed world about 0.2 to 3 per 1000 people gets sepsis yearly or about a million cases per year in the United States. Estimates suggest sepsis affects millions of people a year.³

Common signs and symptoms include fever, increased heart rate, increased breathing rate, and confusion.⁴ There may also be symptoms related to a specific infection such as a cough with pneumonia or painful urination, with a kidney infection. In the very young, old, and people with a weakened immune system, there may be no symptoms of a specific infection and the body temperature may be low or normal rather than high.⁵ Severe sepsis is sepsis causing poor organ function or insufficient blood flow. Insufficient blood flow may be evident by low blood pressure, high blood lactate, or low urine output. Septic shock is low blood pressure due to sepsis that does not improve after reasonable amounts of intravenous fluids are given.²

Sepsis is caused by an immune response triggered by an infection.^{5,6} The infection is most commonly by bacteria, but can also be by fungi, viruses, or parasites.⁵ Common locations for the primary infection include: lungs, brain, urinary tract, skin, and abdominal organs. Risk factors include young or old age, a weakened immune system from conditions such as cancer or diabetes, and major trauma or burns.⁴ Diagnosis is based on meeting at least two systemic inflammatory response syndrome (SIRS) criteria with a presumed infection. Blood cultures are recommended preferably before antibiotics are started; however, infection of the blood is not required for the diagnosis.⁴ Medical imaging should be done looking for the possible location of infection.² Other potential causes of similar signs and symptoms include: anaphylaxis, adrenal insufficiency, low blood volume, heart failure, and pulmonary embolism among few others.⁵

Sepsis is usually treated with intravenous fluids and antibiotics. This is often done in an intensive care unit. If fluid replacement is not enough to maintain blood pressure, medications that raise blood pressure can be used. Mechanical ventilation and dialysis may be needed to support the function of the lungs and kidnevs. respectively.⁴ To guide treatment, a central venous catheter and an arterial catheter may be placed. Other measurements such as cardiac output and superior vena cava oxygen saturation may also be used. People with sepsis need preventive measures for deep vein thrombosis, stress ulcers and pressure ulcers, unless other conditions prevent such interventions. Some might benefit from tight control of blood sugar with insulin.² The use levels of corticosteroids is controversial.⁷ Activated drotrecogin alfa, originally marketed for severe sepsis, has not been found to be helpful, and was withdrawn from sale in 2011.8

Disease severity partly determines the outcome with the risk of death from sepsis being as high as 30%, severe sepsis as high as 50%, and septic shock as high as 80%.⁹ The total number of cases worldwide is unknown as there is little data from the developing world.⁹ Estimates suggest sepsis affects millions of people a year.² In the developed world about 0.2 to 3 per 1000 people gets sepsis yearly or about a million cases per year in the United States.^{9,10} Rates of disease have been increasing.²



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Materials and Methods

The study was conducted at a tertiary care hospital in Eastern India from 1st August 2014 to 31st January 2015.

Patients with clinical suspicion of blood stream infection and fever of unknown origin were included in the study. Primarily secondarily immunoor compromised patients were excluded from the study. Screening criteria for neonatal septicaemia was selected as per standard guidelines.¹¹ clinical Specimens were collected, transported and processed for microbiological work up as per specified scheme (Figure-1, Figure-2a, Figure-2b, and Figure-2c).¹²

Finally isolated and identified non fastidious aerobic and facultative anaerobic bacteria or fungi were enlisted and data were analysed according to standard statistical methods.

Results

During the six months of study period from 1st August 2014 to 31st January 2015, total 96 samples for blood culture were received from clinically suspected patients. Of these, 25 were culture positive (26.04%). Isolated organisms included 13 Gram positive cocci, 8 Gram negative bacilli, 4 non albicans Candida. Gram positive cocci were more frequently isolated than Gram negative bacilli (52%) (Figure-3). 11 samples were received from NICU and 5 were found to be culture positive (45.4%). Isolated organisms from NICU included 2 Candida Escherichia SDD., 2 coli &1Methicillin resistant Staphylococcus aureus (Figure-4).

Majority of the isolated Gram positive Coccci was *Staphylococcus aureus* (69.23%) (Figure-5). Most of the isolated *Staphylococcus spp.* were Methicillin resistant (7 out of 13 i.e, 53.84%) (Figure-6). Among Gram negative isolates, bacilli

other than Enterobacteriaceae were predominant 5 out of 8 i.e. (62.5%), whereas, all three isolated Enterobacteriaceae were *Escherichia coli* (Figure-7). 25% (2 out of 8) and 50% (4 out of 8 samples) of the Gram negative isolates were Extended spectrum β lactamase (ESBL) producer & Carbapenemase producer respectively (Figure-8). 6 out of 8 (75%) Gram negative isolates were resistant to Fluoroquinolone antibiotics (Figure-9). One Acinetobacter isolate was susceptible to Imipenem but resistant to Meropenem. All of the isolated Gram positive isolates were sensitive to Vancomvcin and Linezolid whereas all Gram negative isolates were sensitive to Colistin, Polymyxin-B and Tigecycline.

Discussion

There is paucity of data regarding sepsis in south-east Asia. However, case selection criteria and implication of specific identification system may cause variation of findings in different studies. In this present study, 26.04% of received blood samples were found to be culture positive where as Savita *et al* found 52.01% positivity.¹ In this present study, majority of the isolates were Gram positive isolates on the contrary, in the study of Savita et al, majority of the isolates were gram negative ones.¹ In the study of Savita et al, Klebsiella pneumoniae was the leading cause of sepsis followed by Acinetobacter whereas in this study, Acinetobacter is the prime cause among the Gram negative isolates.¹ In this present study, non-albicans Candida were found to be an important pathogenic group similar to the study of Savita et al.1 However, emergence of multidrug resistance among different isolates corroborates with the findings of other workers from Tanzania, Uganda and Nigeria¹. Differential susceptibility to Imipenem and Meropenem as observed in one *Acinetobacter* spp. may be due to presence of a specific efflux pump,



In this present study, All of the isolated Gram positive isolates were sensitive to Vancomycin and Linezolid whereas all Gram negative isolates were sensitive to Colistin, Polymyxin-B and Tigecycline.

Conclusion

The expeditious detection and identification of blood borne pathogens for diagnosis of sepsis is one of the most functions important of microbiology laboratory. Decision regarding empirical treatment of septicaemia and antimicrobial stewardship must be based on knowledge of distribution of pathogens and their resistance this perception. pattern. In close collaboration between clinicians and microbiologists should produce significant outcome to fight against blood borne infection and overcome the threat of emergence of multidrug resistance.

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Figure 2a. Blood culture bottles for BACTALERT 3D



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Figure 2c. Vitek-2AES Identification card for Gram positive bacteria























