Original Research Paper

Microbiology

ETIOLOGY OF BLOOD CULTURE FROM SEPTICEMIA CASES AND THEIR ANTIBIOGRAM AT A TERTIARY CARE HOSPITAL OF MANIPUR.

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ABSTRACT This study was conducted in the Department of Microbiology, RIMS, Hospital from suspected septicaemic outpatients and inpatients departments during the period from 1st Nov.2013 - 31st Oct. 2015. A total of 644 blood culture samples were processed, of which 80(12.4%) were culture positive. Among the culture positive 72(90%) were isolated from inpatients and only 8 isolates (10%) were from out-patients. The most predominant isolates is *S.aureus* 33 (41.25%) followed by *E.coli* 18 (22.5%), CoNS 9 (11.25%), *Enterococcus species* 7(8.75%), *Salmonella Species* 5(6.25%), *Klebsiella species* 3(3.75%), Candida species 3(3.75%), *Pseudomonas species* 2(2.5%). Imipenem was the most effective antimicrobial against gram negative bacilli whereas Vancomycin was the most effective antimicrobial against gram positive cocci.

KEYWORDS :

Introduction

Blood stream infections (BSIs) cause significant morbidity and mortality worldwide and are one of the common healthcare associated infections. They remain endemic in critical care wards and lead to epidemic out breaks. In the ICU, the accumulation of a number of immune-compromised patients and their nursing and invasive procedures provide a favourable environment to the growth and transmission of nosocomial infections.[1,2.]

BSIs range from self-limiting infections to life-threatening sepsis that requires rapid and aggressive anti-microbial treatment. The onset of blood infections needs immediate attention with rapid and accurate antimicrobial treatment. This needs the correct and earliest identification of etiological agents. Bacteria belonging to groups of *Staphylococci, Streptococci, Enterococci* or *Enterobacteriaceae* are the most common pathogens associated with blood infections.[3]Further, it has been observed that morbidity and mortality of blood infections is higher in case of Gram negative bacilli as compared to Gram positive species.[4] Situation get further complicated with greater septic shock and mortality rate in case of high grade bacteremia or poly-microbial infections.[5]

Identification of various organisms in a patient's blood is of immense diagnostic and prognostic importance. Blood cultures are essential in the diagnosis and treatment of the etiologic agents or sepsis. It has been observed that inadequate therapy is the common reason for antimicrobial resistance.[6]

Antimicrobial therapy is initiated empirically before the results of blood cultures are available due to the high mortality and morbidity associated with septicaemia.therefore, identification of blood infections pathogens and their antimicrobial susceptibility is of utmost importance for the selection of empirical therapy.[7-8]

Throughout the world multi-drug resistant nosocomial infections are one of the leading causes of death and morbidity amongst hospitalized patients, accounting a major burden on the patients and public health system of any country.[9]

Further, it is also important epidemiologically to collect data on etiology and antimicrobial surveillance as same changes geographically. Such epidemiological data help clinicians to select right antibiotics according to common pathogen and their antibiogram prevailing in given area.[10-11]

Therefore the present study was undertaken to analyse the various organisms causing septicaemia and their antibiotic resistance patterns as it would be useful for initiating the empiric antibiotic therapy.

Aims and objectives

- To study the microbiological profile and antibiotic susceptibility pattern of blood culture isolates in RIMS hospital.
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Materials and methods.

This study was conducted in the Department of Microbiology, RIMS, Hospital from various outpatient and inpatient departments during the period from 1st Nov.2013 - 31st Oct. 2015. Blood was collected from clinically suspected septicemia cases following strict aseptic precautions. Blood was inoculated aseptically into brain heart infusion broth (1:10 dilution). The culture bottles were incubated at 37°C aerobically and periodic subcultures were done onto MacConkey's agar, blood agar and chocolate agar after overnight incubation on day 1, day 3, day 5 and on day 7 for isolation. The growth obtained was identified by conventional biochemical test. The bacteria were identified by colony morphology, Gram staining and biochemical tests. Antibiotic susceptibility tests were performed by Kirby Bauer's disk diffusion method in Mueller Hinton agar and interpreted as per CLSI guidelines. [12]

Results:

A total of 644 blood culture samples were processed out of which 80(12.4%) were culture positive. Among the culture 72(90%) were isolated from inpatients i.e. Medicine (32.5%), Surgery (8.5%), Paediatric(26.2%), Radiotherapy(8.7%), Orthopaedic(3.7%), ICU(10%) and only 8 isolates (10%) were from out-patients.

Distribution of Isolates in Blood culture:

Organisms	No. of isolates(%)
Gram positive isolates	**49 (61.25%)**
S.aureus	33 (41.25%)
CoNS	9 (11.25%)
Enterococcus spp.	7 (8.75%)
Gram Negative	**28 (35%)**
E.coli	18 (22.5%)
S.Typhi/Paratyphi	5 (6.25%)
Klebsiella spp.	3 (3.75%)
Pseudomonas spp.	2 (2.5%)
Fungal isolates	**3 (3.75%)**
Candida spp.	3 (3.75%)

Antibiotic Resistance pattern of Gram positive isolates.

Antibiotics	S.aureus(%) n=33	CoNS(%) n=9	Enterococcus spp.(%) n=7
Vancomycin(30µg)	0(0%)	0(0%)	0%
Linezolid(30µg)	0(0%)	0(0%)	0%
Gentamicin(10µg)	0(0%)	1(11%)	-
High level Gentamicin(120µg)	-	-	1(14.2%)
Cefoxitin(30µg)	**19(60.6%)**	**7(77.7%)**	6(85.7%)
Ciprofloxacin(5µg)	15(46.6%)	5(55%)	6(85.7%)
Erythromycin(15µg)	7(21.4%)	6(62.5%)	7(100%)
Clindamycin(2µg)	8(23.5%)	4(37.5%)	-
Penicillin	-	-	7(100%)
Cotrimoxazole(1.25/23.75µg)	17(52.1%)	-	-

In *S.aureus* 60.6% were Methicillin-resistant *S.aureus* (MRSA) and were highly sensitive to Vancomycin, Linezolid, Gentamicin.

In CoNS 77.7% were MRCoNS and highly sensitive to Vancomycin, Linezolid, Gentamicin.

Enterococcus spp. were highly sensitive to Vancomycin, Linezolid and resistant to Erythromycin, Penicillin.

Antibiotic Resistance pattern of Gram Negative isolates.

Antibiotics	E.coli(%) n=18	Klebsiella spp.(%) n=3	Salmonella (%) n=3	Pseudomonas spp.(%) n=2
Imipenem(10µg)	5.5%	0%	0%	50%
Ceftazidime(30µg)	83.3%	100%	-	50%
Ceftazidime+Clavulanic acid	77.7%	100%	-	50%
Ciprofloxacin(5µg)	26.6%	0%	100%	100%
Piperacillin+Tazobactam(100/10µg)	36.3%	0%	25%	50%
Cotrimoxazole(1.25/23.75µg)	30%	33.3%	0%	-
Gentamicin(10µg)	20%	33.3%	-	50%
Amikacin(30µg)	20%	-	-	-
Ceftriaxone(30µg)	100%	50%	0%	-
Colistin	-	-	-	0%

In Gram negative organisms *E.coli* was highest 18(22.5%) followed by *S.Typhi/Paratyphi* 5(6.25%), *Klebsiella spp.* 3(3.75%) and *Pseudomonas spp.* 2(2.5%). Extended-spectrum beta lactamase (ESBL) production was found to be 25%.

E.coli was highly sensitive to Imipenem, Gentamicin, Piperacillin + tazobactam and resistant to cephalosporins. *Klebsiella spp. we*re highly sensitive to Imipenem, Ciprofloxacin and resistant to cephalosporins.*Pseudomonas spp.* were sensitive to Imipenem, Colistin and highly resistant to Ciprofloxacin. *S.Typhi/Paratyphi* were sensitive to Imipenem, cephalosporins and resistant to Ciprofloxacin.

Discussion:

Blood culture is gold standard to identify organisms and antibiotic susceptibility since early and appropriate antimicrobial treatment result in decreased mortality and morbidity among blood infection patients. However, to initiate such therapy, knowledge about pathogen and their antimicrobial susceptibility profile is required.[7-8]Thus, in the era of antimicrobial resistance, defining the pathogen distribution and drugs resistance provides the basis for empirical therapy. Therefore, in the present study, the etiological and antimicrobial profile of blood culture isolates was ascertained in a health care hospital.

In this study of 644 blood culture samples 80(12.4%) were culture positive. Among the culture 72(90%) were isolated from inpatients and only 8 isolates (10%) were from out-patients which is consistent with the study of Lavanya Jagdish *et* al. [13] in which 55.7% inpatients showed positive blood culture and only 14.7% outpatients were positive for blood culture. Among Gram positive cocci, *S. aureus* was most common isolates followed by CoNS. While 29.4% ICU patients were

positive for blood culture but in our study only 10% culture positive in ICU this difference may be due to less ICU beds in our centre. Fungal septicemia caused by *Candia albicans* isolated is 3.5% in this study unlike the finding of Manjusha Pandey et al [14] in the similar study conducted in Lucknow.

A similar study was also conducted by *A.Vijaya et al* [15] in this centre during the year 2010 to 2012. The most predominant isolates is S.typhi (30%)followed by CONS (28%), *S*.aureus (19%), *E*.coli (11%) but in this study, *E*.coli (22.5%)is the most predominant isolates among the gram negative bacilli and followed by Salmonella (6.5%) . This variation in culture may be due to time variation and emergence of multidrugs resistant *E*.coli. In this studies, Gram positive isolates showed MRSA (60.6%), MRCoNS(70.7%), HLGR Enterococcus (14.2%) and Gram negative isolates showed ESBL (25%) whereas in previous study of A.Vijaya *et al* MRCoNS were 38.5% and 66.8% respectively. The present study observed that vancomycin and linezolid appeared to maintain activity against gram positive isolates in 100% of the cases. A high sensitivity to vancomycin (100%) was observed by A.Vijaya *et al.* In a study conducted by Damrolien Shan et al[16] in this same centre HLGR Enterococcus isolated from various clinical samples   was 38.8 % but in this study HLGR Enterococcus is 14.2% only.

In our study among the gram negative bacilli, *E.coli* was highest 18(22.5%) followed by *S.Typhi/Paratyphi* 5(6.25%), *Klebsiella spp.* 3(3.75%) and *Pseudomonas spp.* 2(2.5%).   Extended-spectrum beta lactamase (ESBL) production was found to be 25%. *E.coli* was highly sensitive to Imipenem, Gentamicin, Piperacillin + tazobactam   and resistant to cephalosporins. *Klebsiella spp. w*ere highly sensitive to Imipenem, Ciprofloxacin and resistant to cephalosporins. *Pseudomonas spp.* were sensitive to Imipenem, Colistin and highly resistant to Ciprofloxacin. *S.Typhi/Paratyphi* were sensitive to Imipenem, cephalosporins and resistant to Ciprofloxacin.

M. Saleem *et al*[17] conducted a study in Pudicherry tertiary hospital, the study results almost consistent with our study as majority of the isolates among Gram negative bacilli   is *Escherichia coli* and among the gram positive isolates *Staphylococcus aureus.* Imipenem was the most effective antimicrobial against gram negative bacilli whereas Vancomycin was the most effective antimicrobial against gram positive cocci. Similar finding is also observed by Debananda Sahoo et al[18] in Tertiary Care Hospital of Eastern India.

Conclusion:

Blood infections or septicemia remains one of the most important causes of morbidity and mortality especially in developing countries. Symptoms based diagnosis has limited specificity in identifying exact causes. Cephalosporin is one of the most commonly used antibiotic This study shows GNB is highly resistant to Cephalosporin thus, the greatest thread with GNB MDR is that the infection are usually untreatable due to limited options of antibiotic availability resulting into use of preserve drugs like colistin by clinicians could soon lead to pan drugs resistance. Due to irrational used of antibiotics, it has been observed that drug resistance is emerging at alarming level among blood infection isolates and hence the epidemiological data collected will proved to be a useful guide for physicians to select and start correct empirical therapy.

The successful treatment of sepsis cases generally relies on early diagnosis and appropriate antimicrobial therapy. It has been concluded that the empirical treatment should be based on prevalence of bacterial isolates and their antibiotic profiles Therefore, in the present study, the etiological and antimicrobial profile of blood culture isolates was ascertained in a health care hospital.

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