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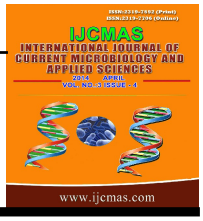

Original Research Article

Study of prevalence and antimicrobial susceptibility pattern in blood isolates from a tertiary care hospital in North Kerala, India

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Keywords

Blood stream infections
Antibiotic resistance
ESBL
MRSA

A B S T R A C T

Blood stream infections vary from minor infections to life-threatening sepsis and cause a significant public health problem. Treatment of Blood stream infections is becoming difficult due to the increasing trend of antibiotic resistance. Rational and correct use of antibiotics requires identifying of microbial pathogens and its drug resistance patterns in a community A retrospective study was carried to identify the microbial profile in the blood culture isolates and their antibiotic susceptibility patterns in a tertiary care teaching hospital from June 2013 to December 2013 according to standard protocol. The interpretation of data was done by using WHO net surveillance software. Out of 1196 blood samples processed 113 (9.44%) were culture positive. Majority of patients 43 (38.3%) were more than sixty years of age. Out of total 113 Gram positive cocci were 60 (53.57%) followed by Gram negative bacilli 53 (46.4%). Among Gram positive organisms S.aureus was highest 40 *(*67%) followed by *coagulase negative Staphylococcus* 17 (28%). In *S.aureus* 13(32.5%) were MRSA and were highly sensitive to vancomycin, Linezolid. In Gram negative bacteria *E. coli* was highest 17 (32.6%) and ESBL production was found to be highest in *Acinetobacter baumannii*. 83.37% followed by *Klebsiella pneumoniae* 53.85%. and *Escherichia coli* (12.5%). All isolates were sensitive to Imipenem and meropenem.

Introduction

Bacterial blood stream infections (BSI) constitute a significant public health problem and are an important cause of morbidity and mortality in hospitalized patients. Around 200,000 cases of bacteraemia occur annually with mortality rates ranging from 20 - 50% worldwide (Bailey and Scott's 2002). In India and developing countries septicemia is an important cause of illness and death among hospitalized patients (Sharma et al 1997, Diekma et al 2003).

Bacteraemia is a state in which bacteria circulate through vascular system. Septicaemia is a life threatening condition when bacteria multiply at a rate that outdoes their removal by phagocytes. The symptoms are produced by microbial toxins and cytokines produced by

inflammatory cells (Koneman's diagnostic microbiology, 6th ed.). Blood stream infection may results from an infection in an organ or tissue. However, the primary site is not often evident (Koneman's diagnostic microbiology, 6th ed)

Both Gram positive and Gram negative bacteria have been found to cause septicaemia, it can be confirmed by blood culture (Daniel et al, 2006, Manjula et al 2005). It is therefore necessary to document results obtained from analysis of blood culture for preparing the antibiotic policy for effective management of septicaemia. Timely administration of drugs in patients with septicaemia drastically reduces mortality rate (Warren et al 2001). However there is an increasing rate of drug resistance among bacterial pathogens (Diekema et al 1999).so it is necessary to determine the profile and antibiotic sensitivity pattern of bacterial isolates from blood samples so this study was done in a tertiary care centre to know the resistance pattern of isolates from septicaemia patients. The timely detection and identification of pathogens and antibiotic susceptibility pattern can have a great diagnostic and prognostic significance.

Materials and Methods

A retrospective cross sectional study was conducted after getting approval from Institutional ethics committee. Based on review of records of 1196 patients for whom blood culture were processed in Microbiology laboratory from June 2013 to December 2013. Data on sociodemographic variables such as age, gender, blood culture results, antibiotic susceptibility pattern were collected manually by using a pre-prepared data abstraction format from the medical records

Two blood samples were collected aseptically from patients for routine blood culture before taking any antibiotics. The vein puncture site was disinfected with 70% alcohol and 2% tincture of iodine before collecting blood for culture. All the samples of blood, which were collected under strict aseptic precautions constitute the study material and were analyzed. In adults minimum of 10 ml and in infants and children 5 ml of blood was collected. Minimum of two blood samples with a gap of one hour was collected. All BACTEC positive samples were subjected to Gram stain followed by inoculation on Blood and macconkey agar and incubated at 37°C for 48 hours. The bacteria were identified based on the colony morphology, colony gram stain and biochemical reactions. Biochemical test was undertaken to classify bacteria at species level such as catalase, coagulase, novobiocin and optochin disk for gram positive and oxidase, indole, citrate, urea, triple sugar iron, Lysine decarboxylase, Arginine and ornithine and motility test for Gram negative bacteria following standard procedures.

Antibiotic susceptibility testing was performed on Muller Hinton agar using agar disc diffusion method. The antimicrobials for disc diffusion testing was amoxicillin+clavulanic acid (20 + 10 μg), ceftriaxone (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), erythromycin (15 μg), gentamycin (10 μg), penicillin ((10 IU), trimethoprim-sulphamethoxazole (25 μg) and tetracycline (30 μg). The resistance and susceptibility were interpreted according to the (CLSI guidelines). *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeroginosa* (ATCC 27853) were used as reference strains for culture and

susceptibility testing. All the BACTEC negative samples which didn't show growth after five days were reported as negative. WHO antibiotics susceptibility surveillance software was used for analysis.

Results and Discussion

Out of 1196 blood sample taken up for the study, 113 (9.44%) were positive for aerobic bacterial growth. Of these 113 culture positive samples 60 (53.09%) were females and 53 (46.9%) were males with age ranging from 1 day to 90 year. Among them majority of patients 43 (38.3%) were more than sixty years of age. All infections were monomicrobial and microorganisms which were recovered from blood cultures included Gram positive cocci 60 (53.57%), Gram negative bacilli 53 (46.4%).

Among the total gram positive isolates recovered, *Staphylococcus aureus* was 40 *(*67%) followed by *coagulase negative Staphylococcus* 17 (28%), *Streptococcus pneumonia, Streptococcus viridians and Enterococcus species*1 each (1.6%) as shown in Figure no.1

Among the total 52 (46.4%) Gram negative bacilli isolated *Escherichia coli* constituted17 (32.6%) followed by *Klebsiella pneumoniae* 13 (25%), *Citrobacter species* 9 (17.3%), *Acinetobacter baumannii* 7(13%), *Psedomonas aeruginosa* 5(9%), *Salmonella typhi* 2(4%) as shown in Figure No. 2

In the study *Staphylococcus aureus* was isolated from 28 adults and 5 each from neonates and paediatric patients. Of the total 17 *coagulase negative staphylococcus* 16 were isolated from adults and 1 from paediatric patient. Distribution of gram positive isolates among various age groups is shown in Figure 3

In the study *Escherichia coli* was isolated from 15 adults and 1 each from neonate and paediatric patients. *Klebsiella pneumoniae* was isolated from 13 patients of whom 6 were adults and 6 neonates. Age category wise distribution of gram negative isolates are given in Figure 4

Among the gram negative isolates *Escherichia coli* was seen in 13 (76.4%) females and 4 (23.5%) male patients and *Klebsiella pneumoniae* was seen in 7 male and 6 female patients. Gender wise distribution of gram negative isolate are shown in Figure 5

Antimicrobial resistance patterns of different Gram positive and Gram negative isolates were as follows: among *S aureus* 32.5% were MRSA and 88.2% were *MRCONS.* All *MRSA* and *MRCONS exhibited* 0% resistance to linezolid, teichoplanin and vancomycin. The *Streptococcus pneumonia* and *Enterococcus species* in our study were sensitive to all antibiotics. Resistance pattern of Gram-positive isolate is shown in Table no. 1

Among the antibiotics used for gram negative bacteria, it showed 0% resistance against Imipenem and Meropenem. The percentage of beta lactamase producing organisms were as follows: *E.coli* (12.5%), *Klebsiella pneumoniae* (53.8%), *Acinetobacter baumannii* (83.8%), *Citrobacter spp*.(68.5%). *Salmonella typhi* isolated were sensitive to all antibiotics.

Figure.1

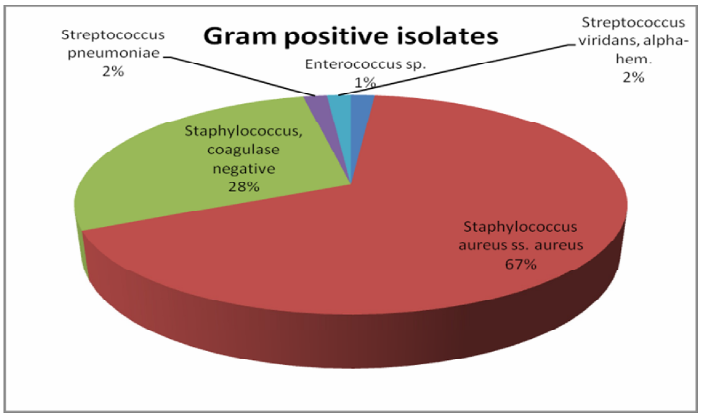

Figure.2

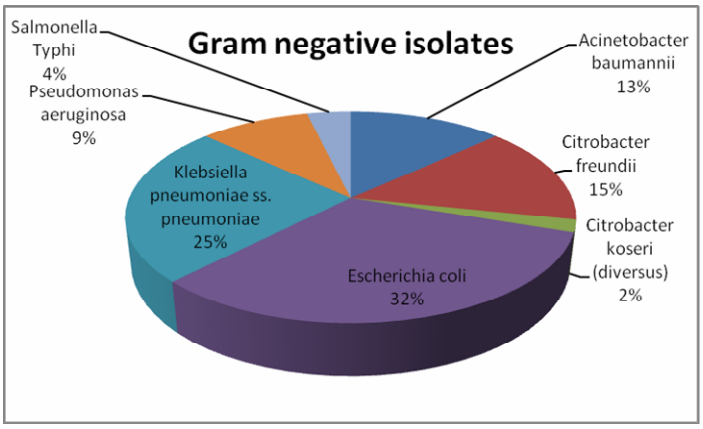

Figure.3

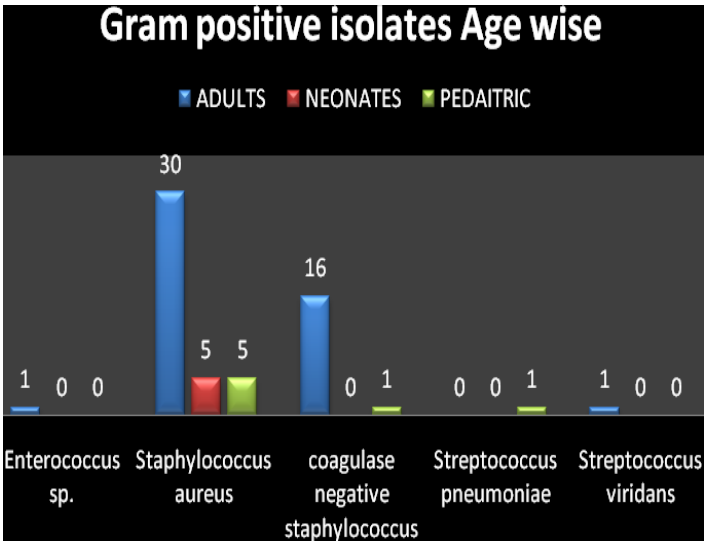

Figure.4

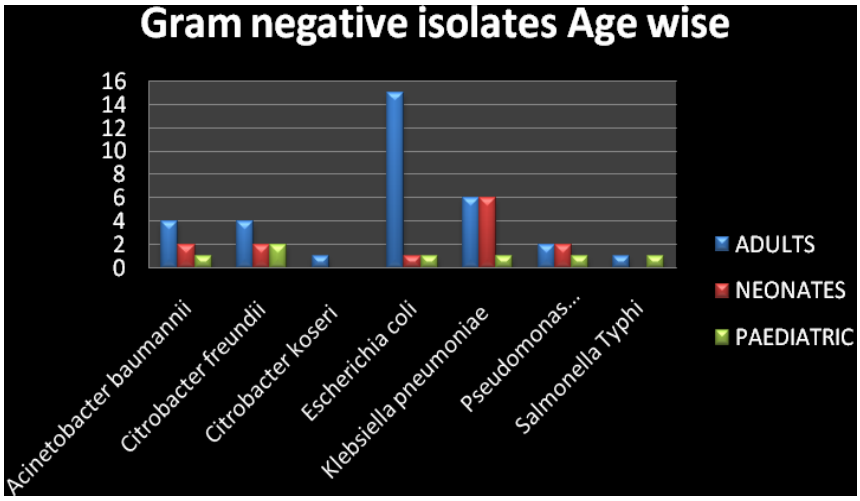

Figure.5

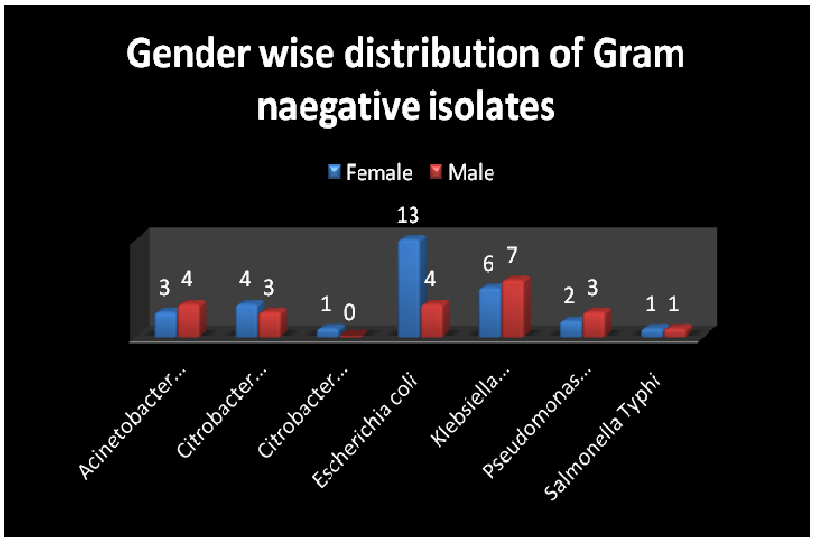

Table.1 Resistance pattern of Gram positive isolates

Antibiotics	Resistance among *S. aureus* %	Resistance among *CONS* %
Amoxcillin clavulanate	45.5	88.2
Cefoxitin	32.5	88.2
Clindamycin	22.5	41.2
Erythromycin	38.5	64.7
Gentamycin	43.6	88.2
vancomycin	0	0
Teichoplanin	0	0
Linezolid	0	0
Levofloxacin	32.5	70.6

Table.2 Antibiotic Resistance pattern of predominant Gram negative micro-organisms isolated from patients

Antibiotics	Organism isolated			
	E.coli	***Klebsciellae***	***Acinetobacter***	***Pseudomonas***
Amoxicillin+Clavulanic	73.3%	90.9%		
Ceftazidime	75%	76.9%	85.7%	40%
Cefotaxime	75%	76.9%	-	-
Ceftriaxone	85.7%	68.5%	69.2%	60%
Cefoxitin	12.5%	53.8%	83.37%	-
Amikacin	11.8%	66.7%	57.1%	O%
Gentamycin	37.5%	61.5%	42.9%	20%
Ciprofloxacin	75%	61.5%	57.8%	20%
Levofloxacin	75%	61.5%	57.8%	20%
Piperacillin+Tazobactam	75%	50%	66.7%	0%
Imepenem	0%	0%	0%	0%

In the face of increasing antibacterial resistance, it is important in defining the species distribution and resistance patterns of microbial pathogens causing BSIs, and thus providing the basis for appropriate empirical therapy. Mortality rates doubled, from 30% to 60%, when inappropriate empirical antibiotic therapy was given to ICU patients with BSIs (Ibrahim et al 2000)

This study revealed that 113 (9.44%) out of 1196 bloodstream samples which were obtained from BSI suspected patients were positive. This result was consistent with other Indian study who reported 8.39% (Vanitha RN et al 2012) and 11.2% (Shalini S et al 2010) of blood stream infection, and another Iran based study which reported 10.8%. (Hamed Ghadiri et al 2012) of blood stream infection, but unlike other Indian studies conducted in Delhi which shows more than 20% positivity(Mehta M et al 2005).

Of the 112 patients, 59 (52.67%) were females and 53 (47.32%) were males. The diversity of microorganisms that invade the bloodstream has been systematically studied by several researchers. In our study, 53.5% of infections were caused by Gram-positive and 46.4 % by Gram-negative bacteria. The predominant bacteria in our study were *S.aureus* and *Escherichia coli*. It conforms to other studies were *Staphylococcus aureus* and *E.coli* was the most common bacterial organism causing blood stream infections (Vanitha et al 2012).

In the present study *Staphylococcus aureus* (35.75) was most frequently found which correlates with other Indian study (Amita Jain et al 2011). *Escherichia coli* (15.1%) and *Coagulase negative staphylococcus* (15.1%) were the second commonest bacterial isolate followed by, *Klebsciellae pneumonia* (11.6%), *Citrobacter species (Citrobacter freundii* and *Citrobacter koseri*) (8%), *Acinetobacter baumannii* (6.2%) *Pseudomonas aeruginosa* (4.4%) *Salmonella typhi* 2 (1.7%). These findings were comparable to the observation of a study conducted in 12 ICU's in seven Indian cities showed *Enterobacteriaceae* (46%), *Acinetobacter* spp. (6%) (Mehta A et al 2007). However there are other

studies were the distribution of various bacteria are different (Vanitha RN et al 2012). The probable justification for these differences could be the study design, geographical location, and difference of the etiological agents, seasonal variation and the difference in blood culture system.

In the present study 13 (32.5%) of *S.aureus* were *MRSA*. There are other Indian studies with 26.7% and 59 % of *MRSA* in BSIs respectively (Parameswaran *et al* 2011, Vibhor Tak et al 2013). S.aureus was 100% sensitive to vancomycin, linezolid which was comparable with other studies (Pattanayak et al 2013). The beta lactamase producing *E.coli iand Klebsciellae pneumonia* in our study was 12.5% and 53.85 respectively which is compareable with other studies.In our study (Kim YK et al 2002), beta lactamase production was quite high in *Acinetobacter spp*. 83.37%, but all our isolates were sensitive to imipenem and meropenem unlike other studies (Uma Karthika R. et al 2009)

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