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BACTERIAL PROFILE AND RESISTANCE PATTERN OF BACTERIAL ISOLATES FROM BLOOD CULTURE - A FIVE YEAR STUDY IN TERTIARY CARE TEACHING HOSPITAL.

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ABSTRACT

Background: Blood stream infection (BSI) remains one of the most important causes of morbidity and mortality all over the world. The data about the bacterial profile of BSI and their resistance pattern in a particular hospital set up can help to decide the empirical line of treatment in these patients. **Aims and Objectives:** To determine the bacterial profile of BSI and their antimicrobial resistance patterns in our hospital. **Materials and Methods:** A retrospective analysis of the bacterial isolates from blood culture specimens collected from patients admitted in tertiary care hospital was conducted over the period of five years; from 2010 to 2014. **Results:** Blood samples from 673 clinically diagnosed cases of septicemia were processed. Growth was obtained in only 6.98% cases. Out of these isolates 51.06% were gram positive cocci and 48.94% were gram negative bacilli. *Staphylococcus aureus* (27.66%) was the predominant organisms followed by *P. aeruginosa (*19.15%). Amongst gram positive organisms maximum resistance was seen to Penicillin, Ampicillin and Erythromycin while in gram negative bacilli maximum resistance was observed to Ampicillin, Cefuroxime. The Vancomycin and Linezolid are highly active against gram positive organisms whereas Amikacin and Imipenem are highly active against gram negative organisms causing blood stream infections. **Conclusion:** Blood stream infections in our hospital are caused predominantly by *Staphylococcus aureus.* Antimicrobial resistance surveillance reports on regular basis which can provide valuable insight into resistance trends at a particular medical facility to assist in guidance in the appropriate choice of empiric therapy.

KEYWORDS: Blood stream infections, bacterial profile, resistance pattern, blood culture.

INTRODUCTION

Blood stream infection (BSI) remains one of the most important causes of morbidity and mortality all over the world. About 200,000 cases of bacteremia occur annually with mortality rates ranging from 20-50% worldwide. [1] In developing countries like India septicemia is an important cause of illness and death among hospitalized patients. BSI along with increased mortality rate also prolongs patient stay in the hospital and leads to increased health care costs. [2, 3] In many studies a wide range of bacteria has been described in febrile patients with gram negative bacteria such as *Escherichia coli, Pseudomonas aeroginosa, Klebsiella species, Neisseria meningitidis, Haemophilus influenzae* and gram positive such as *Coagulase negative Staphylococci (CONS), Staphylococcus aureus, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus agalactiae, and Enterococcus spp.* [4-8] These infections can be diagnosed by blood culture, which is available in few hospitals in developing countries.[9]

Today the only way to reduce mortality caused by BSI is to diagnose and start antimicrobial therapy at the earliest. Epidemiology of bacterial pathogens causing BSI changes with geography, the population concerned and seasonal variation. Emergence of multi drug resistant pathogens limits the therapeutic options available. This underscores the need of regular monitoring of pathogens and their resistance pattern in hospitals and to formulate the antibiotic policy. The present study was aimed to determine the bacterial agents associated with BSI and their antimicrobial resistance patterns in our hospital.

MATERIALS AND METHODS

A retrospective analysis of the aerobic bacterial isolates from blood culture was conducted after institutional ethical committee approval. Blood culture specimens collected over the period of five years; from year 2010 to 2014 were included in the study.

Socio-demographic data such as age, gender, blood culture results, antibiotic resistance pattern were collected manually from the Department of

Microbiology. Procedure for processing of blood culture was as per the WHO standard operative procedures. Briefly about 5 ml of venous blood was collected aseptically using blood sample collection sets and antiseptics (70% alcohol and 2% tincture iodine) and transferred into a bottle containing sterile Brain heart infusion (BHI) broth [Himedia, India]. A minimum blood-to-broth proportion of 1 in 10 was maintained. Blood culture broths were incubated at 37^0C and checked for sign of bacterial growth every day up to 7 days, and bottles with positive signs of growth were further processed for gram stain and sub-cultured was made onto blood agar, Mac Conkey agar and incubated at 37^oC for 24 hours. [10]

Blood culture broth with no bacterial growth after 7 days were sub-cultured before being reported as a negative. The bacterial isolates were identified with colony morphology; gram staining reaction, biochemical and serological tests. Antimicrobial susceptibility testing was performed for the bacterial isolates according to Clinical Laboratory Standards Institute (CLSI) guide lines. [11]

The antibiotic discs and their concentrations used were: Penicillin G (P, 10IU), Amoxicillin (AMC, 20µg), Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 5µg), Trimethoprim+Sulphamethazole (SXT, 25µg) Gentamicin (GM 10µg), Ceftriaxone (CTX, 30µg), Erythromycin (ER, 10µg), Oxacillin (OX, 5µg) and Nalidixic acid (NA, 30µg). All the antimicrobials used for the study were obtained from Himedia, India. The criteria used to select the antimicrobial agents tested were based on the availability and frequency of prescription for the management of bacterial infections in the hospital set up. The reference strains used as control were *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923). In this study multi-drug resistance was defined as simultaneous resistance to two or more antimicrobial agents.

STATISTICS

The antibacterial resistance was explained in terms of percentage and recorded in tabular form. The chi-square test was used to assess the association between variables with the help of Graph pad prism version 5.01software. A p-value of less than 0.05 was considered as statistically significant.

RESULTS

Total 673 blood culture specimens from patients with suspected bacteremia were processed over the period of five years from January 2010 to December 2014. Of these patients 330(49.03%) were females and 343 (50.97%) were males. The mean age of patients was 26.45 ± 20.06 years. 474 were adults and 199 were of pediatric age group.

The overall prevalence of bacteria isolated from blood culture of bacteremia suspected patients was 47/673 (6.98%). Of these culture positive samples 17 (36.17%) were females and 30 (63.82%) were males whereas 16 belonged to pediatric age group and 31 were adults (Table 1). The infection rate in males was significantly higher in comparison to females. All infections were monomicrobial. Microorganisms recovered from blood cultures included 24 (51.06%) gram positive cocci and 23 (48.94%) gram negative bacilli. *Candida albicans* was isolated from four blood cultures and non albicans *Candida* was grown in one specimen. The blood cultures specimens with fungal growth were excluded from final analysis.

Table 1. Age and gender distribution of the patients enrolled in the study.

		Culture result		Total	X^2 value	P value
		No growth	growth			
Sex	Female	326	17	343	4.426	0.035(<0.05)
	Male	300	30	330		
Age group	< 1 yr	71	8	79	4.031	0.26 (>0.05)
	1-14 yr	112	8	120		
	15-50yr	356	21	377		
	>50 yr	87	10	97		

The four most frequently isolated sepsis-causing bacteria were as follows: *Staphylococcus aureus*, 27.66%; *P. aeruginosa*, 19.15%; CONS, 17.02% and *Escherichia coli*, 8.51 %.(Table 2)Among the total 24 gram positive isolates recovered, *Staphylococcus aureus* was 13*(54.17*%) followed by *CONs- 8*(33.33%), *Streptococcus pneumonia, Enterococcus species* 2(8.33%) and *Streptococcus viridians 1(4.16%).*Among the total 23 gram negative bacilli isolated *Pseudomonas aeruginosa* were 9 (39.13%), *Escherichia coli* constituted 4(17.9%) followed by *Klebsiella pneumoniae* 3 (13.04%), *Salmonella typhi* 3(13.04%), *Acinetobacter baumannii* 2 (8.07%), *Citrobacter species* and *Enterobacter* species 1 (4.35%) each.

Table 2: Frequency of occurrence of bacteria isolated from blood cultures of hospitalized patients from year 2010 to 2014.

Organism	Year					TOTAL (%)
	2010	2011	2012	2013	2014	
S.aureus	1	5	3	3	1	13(27.66)
Pseudomonas	0	1	4	1	3	9(19.15)
CONs	0	3	3	2	0	8(17.02)
E. Coli	0	0	1	2	1	4(8.51)
Klebshiella	0	1	1	1	0	3(6.38)
Salmononella	0	2	1	0	0	3(6.38)
Enterococcus	0	0	1	0	1	2(4.25)
Acinetobacter	0	1	1	0	0	2(4.25)

Overall the range of resistance for gram positives and for gram negative was from 0% to 100%.
The *in vitro* susceptibility profiles of the most prevalent gram-positive bacteria to ten antimicrobial agents are summarized in Table 3.

Table 3: In vitro antimicrobial susceptibility of Gram-positive blood culture isolates in percentage

Antimicrobial agents	*S. aureus*	*CONS*	*Enterococcus*
Penicillin	53.85	25	0
Ampicillin	69.23	37.5	0
Cefuroxime	92.31	50	50
Cefoperazone	92.31	50	50
Cefotaxime	92.31	50	50
Cefrtriaxone	92.31	62.5	50
Amikacin	92.31	75	50
Gentamicin	84.62	75	50
Ciprofloxacin	76.92	37.5	0
Vancomycin	100	100	100
Linezolid	100	100	100
Tetracycline	92.31	75	50
Erythromycin	76.92	25	50
Clindamycin	84.62	50	50
Co trimoxazole	76.92	75	0

All gram positive organisms were uniformly susceptible to Vancomycin and Linezolid. Maximum resistance was found against Penicillin, Ampicillin, Erythromycin and Ciprofloxacin in descending order. *Staphylococcus aureus* was the commonest gram positive organism which showed resistance ranging from 0 to 46%.No MRSA was isolated. Resistance amongst CONS and *Enterococcus spp.* ranged from 0 to 75% and 0 to 100% respectively.

The antimicrobial susceptibility profiles of the most prevalent gram-negative bacteria are shown in Table 4. Amongst gram negative bacilli, resistance in *Salmonella* was minimum (0-66%) and was maximum in Acinetobacter (50-100%). All gram negative bacteria showed 0% resistance against Imipenem with exception of *Acintobacter spp.* which showed 50% resistance.

Table 4: Antimicrobial susceptibility of Gram-negative bacteria in percentage.

Antimicrobial agents	*Pseudomonas*	*E. coli*	*Klebsiella*	*Salmonella*	*Acinetobacter*
Ampicillin	0	0	0	66.66	50
Cefuroxime	22.22	0	33.33	66.66	0
Cefoperazone	44.44	0	66.66	66.66	50
Cefotaxime	88.88	0	66.66	100	50
Ceftazidim	66.66	0	66.66	100	0
Cefrtriaxone	88.88	0	66.66	100	0
Amikacin	100	50	100	100	50
Gentamicin	77.77	50	100	66.66	0

Ciprofloxacin	77.77	0	66.66	33.33	0
Imipenem	100	100	100	100	50
Meropenum	88.88	25	100	66.66	50
Co trimoxazole	55.55	25	66.66	66.66	0

Pseudomonas, the commonest gram negative bacteria amongst the isolates, were resistant to Ampicillin (100%), Cefuroxime (78%), Ceftazidim (44%), Trimethoprim- Sulphamethoxazole (44%), Gentamicin (23%) and Ciprofloxacin (23%). and Ceftriaxone (12%), Cefotaxime (12%), Amikacin (0%) and Imipenem (0%).

Beta lactamase production was seen in *E.coli* (50%), *Klebsiella* (0%). *Klebsiella spp* were resistant to Ampicillin (100%), Trimethoprim-sulphamethoxazole (33.33%), Cefuroxime (66.66%), Cefotaxime (33.33%), Ceftazidime (33.33%) ceftriaxone (33.33%), amikacin(0%) Gentamicin (0%) and Ciprofloxacine (100%). *E. coli* were resistant to Ampicillin (100%), Trimethoprim-sulphamethoxazole (75%), Cefuroxime (100 %), Cefotaxime (100%), Ceftazidim (100%) and Ceftriaxone (100%), Amikacin (50%) Gentamicin (0%) and Ciprofloxacin (100%).

DISCUSSION

In the present study 47/673 (6.98%) out of 673 bloodstream samples which were obtained from BSI suspected patients were positive. Many Indian studies have reported Blood stream infection rates ranging from 8.39% to 44%.[12, 13] This result was consistent with other Indian study by Vanitha RN et al who reported 8.39% of blood stream infection, but unlike other Indian studies conducted in Delhi by Mehta M et al which shows more than 20% positivity.[6] The blood culture positivity depends upon numerous factors such as the number and amount of blood cultures taken, the system and type of blood culture medium used for bacterial detection. In addition to this most of the patients already receive some kind of antibiotics before they come to the tertiary care hospital and self medication is very common because of the off the counter availability of medicines.

The study revealed males have significantly higher culture positivity than females. Cohen et al in their study also reported significantly higher incidence of BSI in males than females. The exact mechanisms of this gender wise difference in the infection risk are uncertain, but could possibly be related to differences in skin colonization or unidentified anatomical differences between men and women.[14]

In our study, 53.5% of infections were caused by gram-positive and 46.4% by gram negative bacteria. Similar type of finding is also reported by Sumita et al who isolated 53.57% gram positive cocci and 46.4% gram negative bacilli.[15] The predominant bacteria in our study were *Staphylococcus aureus*, 27.66%; followed by *P. aeruginosa*, 19.15%; CONS, 17.02% and *Escherichia coli*, 8.51%. Many Indian and some others (Dagnew et al) studies have reported *Staphylococcus aureus* as the most common bacterial organism causing blood stream infections.[12, 15, 16] The diversity in the spectrum and frequency of occurrence can be justified due differences in the study plan, geological location, and disparity of the etiological agents, seasonal variation and the difference in blood culture system.

The resistance range for both gram positive and negative organisms was 0-100%. *S.aureus* was 100% sensitive to Vancomycin, Linezolid which was comparable with other studies.[7, 18] MRSA ranging from 14% to 59% have been reported by several Indian authors. [13, 19]

B lactamase producing E coli were 50% but none of the other gram negative bacteria were ESBL producers. Amikacin was found effective against all enterobacteriacae spp. except E.coli. Salmonella strains isolated from blood cultures were quite sensitive to third generation Cephalosporins, Amikacin and Imepenem. Pseudomonas was found highly sensitive to Amikacin and Imepenem with 12-44% resistance to third generation Cephalosporins. Emergence of multidrug resistance (MDR) strains due to indiscriminate use of antimicrobial agents. Several studies in India and globally have already reported the alarming rise in MDR strains.[20]

One of the important outcomes of the present study was to decide upon the possible empirical treatment for the patients with BSI in our hospital. Resistance analysis of bacterial isolates specify that Vancomycin and Linezolid were highly active against gram positive organisms whereas Amikacin and Imipenem were highly active against gram negative organisms causing blood stream infections in our hospital set up.

Early commencement of appropriate antimicrobial treatment plays a vital role in reducing morbidity and mortality in blood stream infections. This early empirical treatment is based on the information about the likely pathogen and its antibiotic resistance pattern. Present study provided much needed information on the prevalence of bacterial pathogens in blood stream infections and their antibiotic sensitivity patterns. The analysis of drug resistance will help in formulating antibiotic policy and to decide the vacation period for any antibiotic in particular if required. The data will also help in limiting the indiscriminate use of antibiotics.

CONCLUSION

To conclude, the blood stream infections in our hospital are caused predominantly by *Staphylococcus aureus*. The resistance range of both gram positive and gram negatives organisms causing BSI was alarmingly elevated. Antimicrobial resistance surveillance reports on

regular basis which can provide valuable insight into resistance trends at a particular medical facility to assist in guidance in the appropriate choice of empiric therapy. Execution of antibiotic policy by antibiotic restriction, combination therapy and antibiotic recycling may help to decrease or prevent the emergence of resistance strains.

REFERENCES

1. Bailey and Scott's Diagnostic microbiology: A textbook for isolation and identification of pathogenic microorganisms. In 11th edition Edited by Forbes BA, Sahm DF, Weissfeld AS. St. Louis: The Mosby Company, 2002: 378–422.
2. Tziamabos AO, Kasper DL: Principle and practice of infectious diseases. Frank Polizano J., 2005; 26: 2810–16.
3. Madsen KH, Sorensen HT: Secular trends in incidence and mortality of bacteremia in Danish country. APMIS, 1999; 107: 346–52.
4. Asrat D, Amanuel Y: Prevalence and antibiotic susceptibility pattern of bacterial isolates from blood culture in Tikur Anbessa hospital, Addis Ababa. Ethiopia. Ethiop Med J., 2001; 39(2): 97–104.
5. James AK, Mark EJ, Deborah CD, Clyde T, Daniel FS and Gregory AV: Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States in 2002. Ann Clin Microbio Antimicrobi, 2004; 3(7): 1–8.
6. Manjula M, Pyria D, Varsha G: Antimicrobial susceptibility pattern of blood isolates from a teaching Hospital in north India. Japan J Infec Dis., 2005; 58: 174–76.
7. Daniel RK, Scott AF, James MB, Sanjay S: Brief Report: Incidence, Etiology, Risk Factors, and Outcome of Hospital acquired Fever. J Gen Intern Med., 2006; 21: 1184–87.
8. Rina K, Nadeem SR, Kee PN, Parasakthi N: Etiology of blood culture isolates among patients in a multidisciplinary teaching hospital in Kuala Lumpur. J Microbiol Immunol Infect., 2007; 40: 432–37.
9. Becker JU, Theodosis C, Jacob ST, Wira CR, Groce NE: Surviving sepsis in low-income and middle-income countries: new directions for care and research. Lancet Infect Dis., 2009; 9(9): 577–82.
10. World Health Organization. Manual for the laboratory identification and antimicrobial susceptibility testing of bacterial pathogens of public health concern in the developing world. WHO/CDS/CSR/RMD/, 2003, 6. Available on line at http://www.who.int/csr/resources/publications/drugresist/WHO_CDS_CSR_RMD_2003_6/en/. Accessed on April, 2010.
11. Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement M100-S20. Wayne, PA, USA: CLSI; 2010.
12. Vanitha RN, Kannan G, Venkata NM, Vishwkanth D, Nagesh VD, Yogitha M et al. A retrospective study on blood stream infection and antibiotic susceptibility pattern in tertiary care teaching hospital. Int J. Pharma Pharma Sci, 2012; 4543-48.
13. Khanal, B,. Harish BN, Sethuraman KR and Srinivasan S. Infective endocarditis: Report of prospective study in an Indian Hospital. Trop Doct., 2002; 32: 83-85.
14. Cohen B, Choi YJ, RN, Hyman S, Furuya EY, Neidel M and Larson E. Gender Differences in Risk of Bloodstream and Surgical Site Infections. J Gen Intern Med, 2013; 28(10): 1318–25.
15. Rajeevan S, Ahmad SM and Jasmin PT. Study of prevalence and antimicrobial susceptibility pattern in blood isolates from a tertiary care hospital in North Kerala, India. Int. J. Curr. Microbiol. App. Sci, 2014; 3(4): 655-62.
16. Jain A, Agarwal A, Verma RK, Awasthi S & Singh KP. Intravenous device associated blood stream staphylococcal infection in paediatric patients. Indian J Med Res., 2011; 134: 193-99.
17. Dagnew et al. Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia .BMC Research Notes, 2013; 6: 283.
18. Rathod SD, Bhatia PV, Patel PH, Pethani JD, Patel LR, Chauhan B .Bacteriological analysis and resistance Pattern among various culture isolates from Neonatal septicemia at tertiary care hospital Of Ahmadabad. National journal of medical research print issn, 2249- 4995.
19. Tak V, Mathur P, Lalwani S, Mishra MC. Staphylococcal blood stream infections: Epidemiology, resistance pattern and outcome at a level 1 Indian trauma care center, 2013; 1(5): 46-50.
20. Nathisuwan S, Burgess DS, Lewis II JS. Extended-Spectrum β-Lactamases: epidemiology, detection, and treatment. Pharmacotherapy, 2001; 21: 920–28.