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Bacteriological Profile and Antibiogram of Gram Negative Bacteria Isolated from Blood Culture

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ABSTRACT

Introduction: *Blood stream infections are one of the important cause of morbidity and mortality all over the world. Bacteraemia ranges from self-limiting infections to life-threatening septicaemia that requires rapid and aggressive antimicrobial treatment. The mortality rate ranges from 20% to 50% in cases of bacteraemia infections.* ***Aim and Objective:*** *The present study was undertaken to know the profile of gram negative organism causing bacteraemia with their Antibiogram from suspected cases.* ***Material and Method:*** *During a one-year period, 400 blood samples were taken from bacteraemia suspected patients. Blood culture was done by using BacT/Alert 3D system. Further identification of organism was done by different biochemical test. Antimicrobial sensitivity pattern was determined by Kirby Bauer Disc Diffusion method according to CLSI guidelines.* ***Result:*** *Out of 400 samples, the total number of culture positive cases were found to be 131 giving culture positive rate of 32.75%. Gram positive organism were more than gram negative organism, constituting about 75 (57.69%) of total isolates. 56(42.74%) Gram negative organism were isolated in this study. Most frequent pathogen identified among gram negative bacteria were Klebseilla 24(42.8%), followed by E. coli 18(32.14%), Acinetobacter 10(17.85%), Pseudomonas 2(3.57%) and Salmonella 2(3.57%) respectively. Isolated gram negative organism was highly sensitive to Polymyxin B 51(91.07%). After Polymyxin B isolated gram negative bacteria show high sensitivity for Levofloxacin(60.71%), Cefixime (57.78%), Gentamicin, Meropenem, Piperacillin/tazobactum (50%), Cefepime (44.64%) with least sensitivity for Ampicillin/Sulbactum (14.28%).* ***Conclusion:*** *The present study provides information about gram negative pathogens responsible for blood stream infection along with their sensitivity towards commonly used antimicrobial. Antibiotic sensitivity pattern of isolates provides useful guidelines to clinicians in initiating empiric therapy and help in management of blood stream infections.*

Keywords: *Blood stream infections, Blood culture, Antibiotic sensitivity pattern.*

INTRODUCTION

Blood stream infections are one of the important cause of morbidity and mortality all over the world.[1] Blood is normally sterile, but bacteria occur transiently in the blood stream after vigorous chewing or dental surgery or instrumentation of the genitourinary tract or bowel.[2] The incidence of blood stream infection either community acquired or hospital acquired has dramatically increased.[3] If microorganism invade blood stream either continuously, intermittently or transiently it causes serious risk to every organ of body.[4] Microbial incursion to the blood stream resulting from any organism can have serious quick consequences including shock, multiple organ failure, DIC (disseminated intravascular coagulation) and death.[5-7] Bacteria can enter in blood stream through many route but most common sites are genitourinary tract (25% cases), respiratory tract (20% cases) abscesses and miscellaneous sites (20% cases), wound infection (5% cases), uncertain sites (25% cases) and biliary tract (5% cases).[8] Bacteraemia is caused by different types of bacteria which are different from place to place.[9] Among gram negative bacteria *Acinetobacter sp Pseudomonas aeruginosa, E. coli, klebsiella, Haemophilus influenzae, Neisseria meningitidis*

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are responsible for BSI along with *CONS, Staphylococcus aureus, Enterococci and Alpha hemolytic streptococci* among gram positive bacteria.[10-14] Blood culture is the most important procedure for the detection of bloodstream infection.[15] One of the main complication in the treatment of BSI is the increasing resistance of bacteria to antibiotics. Emerging drug resistance among blood stream pathogens limit therapeutic options and complicate patient's management.[16] To check the antibiotic resistance there, is requirement of continuous surveillance of data of clinical isolates along with execution of strict control policies in health care settings.[17-19] Today the only way to reduce mortality due to blood stream infection is early diagnosis and appropriate antimicrobial therapy at the earliest.

The aim of our study was to identify the most frequent gram negative bacteria among patient suspected from blood stream infection along with study of antibiotic sensitivity pattern of isolates thus providing useful guidance to clinicians to modify antibiotic therapy.

METHODS

This study was conducted in the Teerthanker Mahaveer Medical College and Research Centre, Moradabad (UP) India. Study was conducted on 400 patients suspected of blood stream infection attending different departments of Teerthanker Mahaveer Medical College and Research centre (TMMC&RC), Moradabad during March 2016 to January 2017. In this study during blood culture and antibiotic sensitivity test all the standard microbiological methods were followed. Blood sample were collected aseptically for blood culture and sensitivity pattern.

Blood samples were collected from each patient before the beginning of empirical antibiotic treatment. Samples were transferred into BacT/Alert culture bottles and labelled with details of patients. Culture bottles were incubated in BacT/Alert for blood culture that showed signs of microbial growth, subculture were made. Bacterial growth on the subculture was identified by their characteristic of bacterial colony, gram reaction and other specific biochemical reactions.

The antibiotic sensitivity of blood culture isolates was determined by Kirby Bauer disc diffusion method. The antibiotic tested included Amikacin, Chloramphenicol, Ampicillin/Sulbactum, Co-trimoxazole, Amoxycillin, Cefuroxime, Cefoperazone, Ceftriaxone, Ciprofloxacin, Ofloxacin, Gentamicin, Levofloxacin, Imipenem, Meropenem, Polymyxin B, Cefoperazone/Sulbactum, Piperacillin/tazobactum, Cefixime, Cefepime.

RESULTS

Analysis was conducted on 400 patients suspected of blood stream infection. The total number of culture positive cases was found to be 131 giving culture positive rate of 32.75%. Among positive culture, males were 58(44.61%) and females were 73(55.72%), giving male and female ratio 1:1.25 (Table 1). The culture positive patients age ranged from one day to 80 years. Among positive culture, 52(40%) isolates were from 1-20 years' patients ,39(30%) isolates from 21-40 age group, 29 (22%) from 41-60 age group, 10 (7.69%) from 61-80 age group patients respectively. Gram positive organism were more than gram negative organism, constituting about 75 (57.69%) of total isolates. 56 (42.74%) Gram negative organism were isolated in this study (Table 2, figure 1). Most frequent pathogen identified among gram negative bacteria were *Klebseilla* 24 (42.8%), followed by *E. coli* 18 (32.14%), *Acinetobacter*10 (17.85%), *Pseudomonas* 2(3.57%) and *Salmonella* 2 (3.57%) respectively (Table 3, Figure 2).

Isolated gram negative organism was highly sensitive to Polymyxin B 51(91.07%). After Polymyxin B isolated gram negative bacteria show high sensitivity for Levofloxacin (60.71%), Cefixime (57.78%), Gentamicin, Meropenem, Piperacillin/tazobactum (50%), Cefepime (44.64%) with least sensitivity for Ampicillin/Sulbactum (14.28%) (Table 4), Figure 3)

Table 1: Sex wise distribution of culture positive and culture negative samples

Blood culture	Male	Female	Total
Culture positive	59(45.03%)	72(54.96%)	131(32.75%)
Culture negative	117(66.85%)	152(67.55%)	269(67.25%)
Total	176	224	400

Table 2: Type of Organism isolated in Blood Culture

Culture positive organism	Number
Gram positive organism	75(57.25%)
Gram negative organism	56(42.74%)

Table 3. Showing number of gram negative bacteria according to sex

Organism	Male (28)	Female (28)	Total (56)
E. coli	8(28.57%)	10(35.71%)	18(32.14%)
Klebseilla	9(32.14%)	15(53.57%)	24(42.85%)
Acinetobacter	7(25%)	3(10.71%)	10(17.85%)
Pseudomonas	2(7.14%)	0	2(3.57%)
Salmonella	2(7.14%)	0	2(3.57%)

DISCUSSION

Blood stream infections are wide range of disorder that can differ from bacteraemia to fatal septicaemia. If bacteria present in blood is multiply in blood stream this condition is referred as septicaemia. Septicaemia is one of the important causes of mortality all around the world. With delay in the diagnosis and treatment of septicaemia there is a chance of increase in the mortality rate up to 50%. In recent years' number of septicaemia cases has been increasing significantly. Gold standard to know the etiological agent of blood stream infection is blood culture.[19]

Table 4: Showing antibiogram of gram negative isolates

ANTIBIOTIC	E. coli 18 (N%)	Klebseilla 24 (N%)	Acinetobacter 10 (N%)	Pseudomonas 2 (N%)	Salmonella 2 (N%)	Total 56(N%)
Amikacin	5 (27.77%)	7(38.88%)	1(10%)	0	1(50%)	14(25%)
Chloramphenicol	7 (38.88%)	8(33.33%)	2(20%)	0	0	17(30.35%)
Ampicillin/ Sulbactum	2 (11.11%)	4(16.66%)	1(10%)	0	1(50%)	8(14.28%)
Co-trimoxazole	5 (27.77%)	7(38.88%)	4(40%)	1(50%)	2(100%)	19(33.92%)
Amoxycillin	8 (44.44%)	3 (12.5%)	1 (10%)	0	0	12(21.42%)
Cefuroxime	9(50%)	12(50%)	2(20%)	0	2(100%)	25(44.64%)
Cefoperazone	9(50%)	9(37.5%)	3(30%)	0	2(100%)	23(41.07%)
Ceftriaxone	9(50%)	11(45.83%)	2(20%)	0	2(100%)	24(42.85%)
Ciprofloxacin	3 (16.66%)	7(38.88%)	1(10%)	1(50%)	1(50%)	13(23.21%)
Ofloxacin	7 (38.88%)	15(62.5%)	1(10%)	1(50%)	2(100%)	26(46.42%)
Gentamicin	10 (55.55%)	13(54.16%)	3(30%)	1(50%)	1(50%)	28(50%)
Levofloxacin	12 (66.66%)	16(66.66%)	4(40%)	0	2(100%)	34(60.71%)
Imipenem	9(50%)	10(41.66%)	6(60%)	1(50%)	0	26(46.42%
Meropenem	9(50%)	12(50%)	6(60%)	1(50%)	0	28(50%)
Polymyxin B	17 (94.44%)	20(83.33%)	10(100%)	2(100%)	2(100%)	51(91.07%)
Cefoperazone/ Sulbactum	10 (55.55%)	8(33.33%)	3(30%)	0	1(50%)	22(39.28%)
Piperacillin/tazo bactum	11 (61.11%)	10(41.66%)	4(40%)	2(100%)	1(50%)	28(50%)
Cefixime	12 (66.66%)	11(45.83%)	5(50%)	0	1(50%)	29(51.78%)
Cefepime	10(55.55%	8(33.33%)	5(50%)	0	2(100%)	25(44.64%)

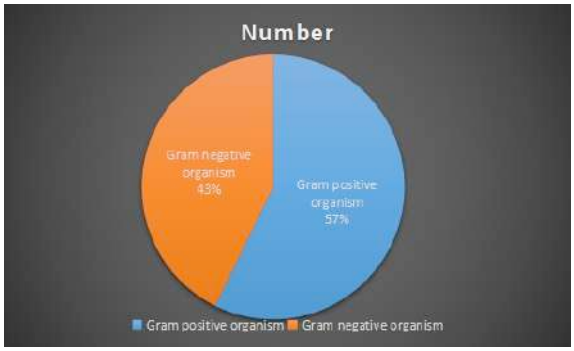

Fig 1: Showing type of organism

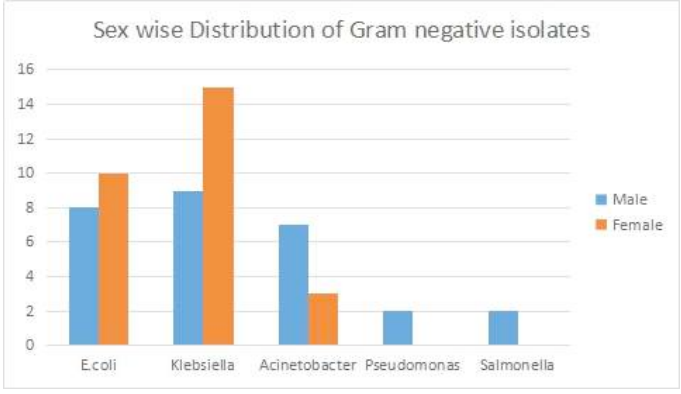

Fig 2. Showing number of gram negative bacteria according to sex

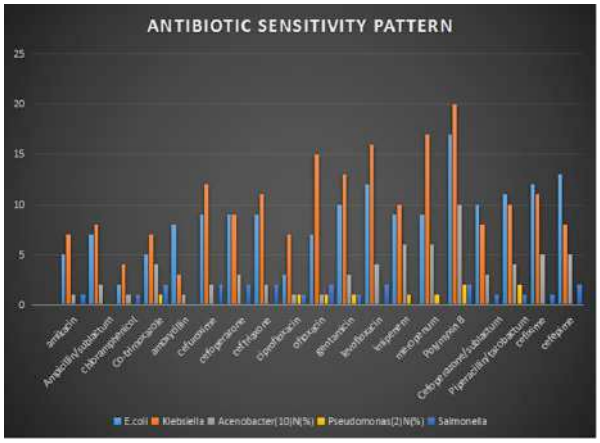

Fig: 3 Showing antibiotic sensitivity pattern of gram negative isolates

The present study gives information about bacterial isolates causing bloodstream infection. It also provides information about antibiotic sensitivity pattern that plays an important role in management of septicaemia cases. In our study total 400 samples were taken, out of which 131 showed growth i.e positivity rate was 32% whereas in 68% of cases there was no growth. Positivity rate in our study is similar to the study of Sharma et al[20] where 33.9% samples showed growth, In the study of Sultana et al (2016)[21] culture positivity rate was 49.28% which is higher to our study. Our finding was little different from the study of Gulrez M et al[22] Alam et al[23], Arora et al[24] ,Roy et al[25] and Gohel K et al[26] in which 12.2%, 20.9%, 20.02%, 16.4%, 9.2% isolates were obtained respectively.

The variation in the positivity rate among studies may be due to most of the patient are given antibiotics before they come to the hospital or may be due to self-medication which is more common.[27] Difference in positivity rate from place to place is also due to different blood culture systems used in laboratories, amount and number of blood culture taken, the study design, and difference in the infection control policies between countries. Gram positive bacteria was isolated in maximum number (57.28%) as compared to gram negative bacteria (42.74%) which is similar to study of Kingsley OC (2013)[28], Chimese SM (2012)(29), Gohel K et al (2014) . Our result is also similar to the study of Sumita rajeevan et al (2013) in which gram positive were 53.7% and gram negative were 46.7%, Sultana et al. (2016) also found similar results in their study. In the study of Gohel K et al gram-positive bacteria accounted for 58.3% of cases with staph. aureus dominance and gram negative bacteria accounted for 40.2% with Enterobacteriaceae dominance. This study also showed similarity with the study of S Moyo et al. Among which Gram positive bacteria (82.1%) being significantly more in number than gram negative (17.9%). Studies show that a variety of microorganism are capable of causing septicemia, which depends on different factors such as different geographical location, epidemiological variations along with other factors such as patient's population, limited sample size and duration of study (Debananda Sahoo, 2016). In our study, antibiotic sensitivity range for gram negative bacteria was 0-100%. All gram-negative isolate showed highest sensitivity towards polymyxin B with 91.07% sensitivity rate and least sensitivity for ampicillin/Sulbactum with 14.28% rate. Sensitivity of gram negative isolates for gentamicin (50%), Piperacillin-tazobactum (50%) was similar to the study of Jyoti P. Sonawane et al in which gram negative isolates show 53.13% sensitivity for gentamicin and 52.34% sensitivity for Piperacillin-tazobactum.

CONCLUSION

In our study, both gram positive and gram negative bacteria were isolated from suspected patients of BSI. Gram positive bacteria were isolated in high number (57.69%) as compared to gram negative bacteria (42.74%).

In our study Klebseilla (24) was the most common gram negative organism followed by E. coli (18), Acenobacter (10), Pseudomonas (2), Salmonella (2) identified as causative organism for blood stream infection. Male and female both are prone to blood stream infection but in our study females (54.96%) are more infected with BSI as compared to males (45.03%) The reason behind this is not so clear.

Antibiotic sensitivity pattern showed that all gram-negative bacteria had highest sensitive to Polymyxin B (91.07%) and least sensitive to Ampicillin/Sulbactum (14.28%). E. coli showed highest sensitivity for Polymyxin B (94.44%) least sensitive for Ampicillin/Sulbactum (11.11%), Klebseilla showed 83.33% sensitivity for Polymyxin B minimum sensitivity for Amoxycillin (12.5%), Acenobacter was 100% sensitive for Polymyxin B and 10% sensitive amikacin. Pseudomonas and Salmonella were 100% sensitive for Polymyxin B.

Our study showed that, there is increases in the resistance among gram negative bacteria for different group of antibiotics, which create problem in management of blood stream infection.

The report on the current knowledge of bacterial sensitivity profile of patient, which is provide by microbiology lab from time to time is necessary for early diagnosis and treatment. This information helps clinicians to make right decisions for better treatment.

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