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PATHOGENS CAUSING BLOOD STREAM INFECTIONS;

IN CARDIAC PATIENTS & THEIR SUSCEPTIBILITY PATTERN FROM A TERTIARY CARE HOSPITAL

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ABSTRACT... Blood stream infections (BSI) remain a major cause of debility and death around the world. BSI accounts for 10-20% of all Nosocomial infections. Empirical antimicrobials are based on the susceptibility pattern of the pathogens isolated in a specific institute from time to time. We have conducted this study only on cardiac Patients over two & half years of study duration. **Study design:** Cross sectional study. **Settings:** Microbiology Department, Allama Iqbal Medical College/Jinnah Hospital, Lahore. **Study Period:** January 2013 to July 2015. **Materials & Methods:** A total of 5411 blood culture specimens were collected from cardiac patients including patients admitted to cardiology ward, coronary care unit (CCU), pre-operative and post-operative cardiac surgery patients. The bottles containing BHI broth were incubated and were subcultured after 24 hours, 72 hours, 120 hours, and 168 hours on blood and MacConkey agars. Isolates were further identified with the help of Gram staining, biochemical reactions and rapid tests like catalase, oxidase, coagulase, Analytical Profile Index (API) 20E and API 20NE. Antimicrobial susceptibility of the isolate was carried out on Mueller-Hinton agar by Modified Kirby Bauer disc diffusion technique according to the isolate as per recommendations of Clinical and Laboratory Standards Institute (CLSI) guidelines 2013. **Results:** Out of total 5411 patients, 3958(73.14%) were male, 1453(26.85%) were females. Out of total 5411 Specimens, only 486 (8.98%) were positive for bacterial growth. Out of total 486 positive blood cultures, 261 (53.71%) were Gram positive isolates and 225 (46.29%) were Gram negative isolates. Out 486 positive blood cultures, 96 (19.75%) were from cardiology ward, 67 (13.78%) were from CCU, 113 (23.25%) were from pre operative cardiac surgery ward, 210 (43.20%) were from post operative cardiac surgery ward. Among Gram positives, Staphylococcus Species were most common organism isolated from 246 (50.61%) blood culture specimens. Among Gram negatives, *Pseudomonas aeruginosa* and *E.coli* were predominant organisms, isolated from 81(16.66%) and 72(14.81%) blood culture specimens respectively. **Conclusion:** Gram Positive isolates were more common as compared to Gram negative isolates. Vancomycin and Linezolid were the most effective drugs among Gram positive isolates. Piperacillin-Tazobactam was most potent antimicrobial against *Pseudomonas aeruginosa*. For coli forms Tigecycline was most effective drug.

Key Words: Blood Stream Infections, CCU, *Staphylococcus aurous*, *Pseudomonas aeruginosa*

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Novelty Statement

On blood stream infection, there is lot work done but on specifically cardiac patients no study has been done from Pakistan. So regarding cardiology patients our study is unique and first of its kind.

INTRODUCTION

Blood stream infections (BSI) remain a major cause of debility and death around the world and are responsible for worsening of living conditions of millions of people. Approximately 200,000 cases of bacteremia occur annually with mortality rates ranging from 20-50% worldwide.[1] BSI accounts for 10-20% of all Nosocomial infections.[2] Many blood stream infectionsin post operative cardiac patients are caused by wide variety of microorganisms (bacteria, fungi, parasites and viruses) but bacterial infections are the most common cause. And this pattern depends on

geographical location.[3-4] Antimicrobial agents are used empirically to limit the episode of illness. Empirical antimicrobials are based on the susceptibility pattern of the pathogens isolated in a specific institute from time to time.

Different foci within the body like respiratory Tract, intra-abdominal and genitourinary tract, serve as a nidus for these infections.[5-6] Incidence of these infections has considerably increased due to the use of indwelling medical devices, changing antibiotic resistance pattern of microorganisms and failure to follow infection control techniques by medical personnel.[7-9]

Antibiotics resistance is a growing problem around the globe. This is especially true in developmental countries like Pakistan, where antibiotics are widely used, unregulated over the counter sale of antimicrobials, mainly for self treatment of suspected infections without prescription, would certainly lead to emergence and rapid dissemination of resistance.[10] Multi-drug resistance was defined as organisms resistant to three or more drugs of the following classes; Beta lactams (Ceftriaxone, Cefpirome), carbapenems (Imipenem, meropenem), aminoglycosides (amikacin, gentamicin) and fluoroquinolones (ciprofloxacin).

Emergence of resistance to antimicrobial is a major challenge to infectious disease medicine. Because it is the major cause of treatment failure in BSI.[11] Antimicrobial resistance results in increased morbidity, mortality, and cost of health care. The rate of resistance varies in different studies. There was a high rate of nosocomial multi-drug resistant (MDR) organisms isolated from different specimens. During the last decade, an increase in the rates of antimicrobial resistance has been recognized worldwide and an increased frequency of MDR isolates in the clinical setting has been demonstrated.

Identification of various organisms in a patient's blood is of massive diagnostic and prognostic importance. Blood culture is gold standard techniques in the diagnosis of blood stream infections. It will help isolate the bacterial pathogens and determine their antibiotic sensitivities, which later helps in the formation of bacteriological profile and antibiotic resistance pattern of these pathogens which, subsequently, serves as a guide for the selection of appropriate treatment for these infections. The timely and appropriate use of antibiotics is currently the only way to treat bacteremia BSI. So early diagnosis and initiation of treatment for blood stream infections significantly reduces the morbidity and mortality associated with these infections. Therefore, blood culture is the mainstay of diagnosis and treatment of blood stream infections.

In most cases, for the benefit of patient, empirical antibiotic therapy frequently initiated to treat blood stream infections without exact knowledge of the causative organism and its antimicrobial susceptibility.[12] This requires the knowledge of common bacterial pathogens prevalent in that area based on blood culture results, to help clinician choose the right antibiotic therapy. Therefore, this study is carried out to analyse the frequency of various bacterial pathogens that are responsible for blood stream infections as identified by blood culture which would serve as a useful guide for clinicians in deciding upon empirical antibiotic therapy for these infections.

MATERIALS AND METHODS

It is a cross sectional study conducted at Microbiology Department, Allama Iqbal Medical College/Jinnah Hospital, Lahore from January 2013 to July 2015. Sampling technique was nonprobability consecutive sampling. A total of 5411 blood culture specimens were collected from Cardiac patients including patients admitted to cardiology ward, coronary care unit (CCU), pre-operative and post-operative cardiac surgery patients. A duplicate sample from same patient during same episode of illness was excluded from study. Details like hospital identity number, age, gender of the patients, type and place of submission of specimen were recorded on a formatted performa. All blood culture specimens were collected following thorough cleaning of the venous site with 70% alcohol and subsequently followed by pyodiene. The rubber cap of each

of the culture broths bottles was immediately cleaned with 70% alcohol, the used needle replaced with a newer needle and the venous blood injected into Brain Heart Infusion (BHI) and Sodium thioglycolate broths in the ratio of one part of blood to ten parts of the broth. Blood culture bottles were dealt by manual method. The bottles containing BHI broth were incubated without agitation and inspected macroscopically for evidence of turbidity indicative of microbial growth once daily and were subcultured after 24 hours, 72 hours, 120 hours, and 168 hours on blood and MacConkey agars. Positive blood culture bottles were evaluated initially by examining a Gram-stained smear of the broth. Subculture from the positive bottle were further identified with the help of Gram staining, biochemical reactions and rapid tests like catalase, oxidase, coagulase, Analytical Profile Index (API) 20E and API 20NE.[13] Antimicrobial susceptibility of the isolate was carried out on Mueller-Hinton agar by Modified Kirby Bauer disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines 2013.[14] The plates were incubated aerobically at 35°C ± 2 for 18 – 24 hours. Zone of inhibition around the discs were interpreted as per CLSI guidelines 2013.[14] The results were interpreted as frequencies and percentages.

RESULTS

Total 5411 blood specimens were collected from cardiac patients of Jinnah Hospital, Lahore, during study duration. Out of total 5411 patients, 3958(73.14%) were male, 1453(26.85%) were females. Out of total 5411 Specimens, only 486 (8.98%) were positive for bacterial growth. Out of total 486 positive blood cultures, 261 (53.71%) were Gram positive isolates and 225 (46.29%) Gram negative isolates.

Out 486 positive blood cultures, 96 (19.75%) were from cardiology ward, 67 (13.78%) were from coronary care unit (CCU), 113 (23.25%) were from pre operative cardiac surgery ward, 210 (43.20%) were from post operative cardiac surgery ward.

Among Gram positives, Staphylococcus Species were most common organism isolated from 246 (50.61%) blood culture specimens. Among Gram negatives, *Pseudomonas aeruginosa* and *E.coli* were predominant organisms, isolated from 81(16.66%) and 72(14.81%) blood culture specimens respectively.

The detailed antimicrobial susceptibility of Gram positive and Gram negative organisms causing blood stream infections in cardiac patients is shown in Table-II and III respectively.

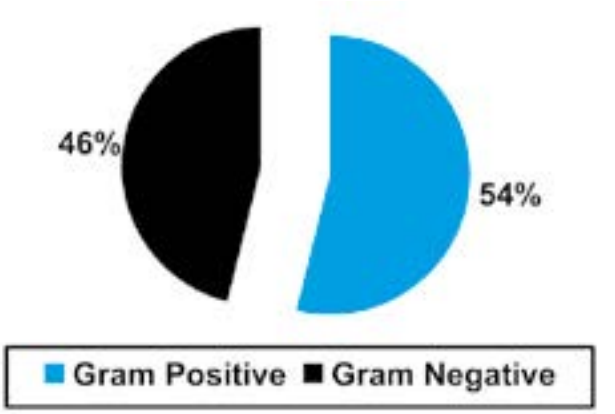

Figure-1. Distribution of Gram Positive & Gram Negative Isolates

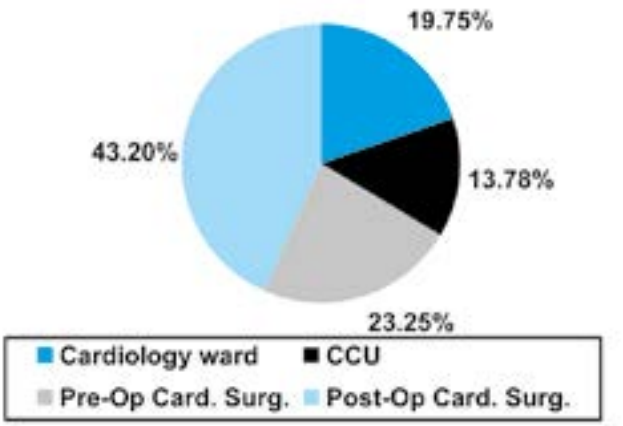

Figure-2. Distribution of Positive Blood Cultures

Isolates	Number n=486	%

Methicillin sensitive *Staphylococcus aureus* (MSSA)	150	30.86%
Coagulase Negative Staphylococcus (CoNS)	48	9.87%
Methicillin Resistant *Staphylococcus aureus* (MRSA)	48	9.87%
Streptococcus species	15	3.08%
Pseudomonas aeruginosa	81	16.66%
Escherichia coli	72	14.81%
Enterobacter species	42	8.64%
Klebsiella Species	21	4.32%
Salmonella Species	9	1.85%

Table-I. Frequencies of Different Isolates

Antibiotics	Staphylococcus Spp. n = 246	Streptococcus Spp. n= 15
Penicillin	4 (1.56%)	12 (80.00%)
Methicillin	198 (80.48%)	12 (80.00%)
Co-amoxiclav	198 (80.48%)	14 (93.33%)
Ciprofloxacin	155 (63.00%)	13(86.67%)
Moxifloxacin	158 (64.22%)	14(93.33%)
Clindamycin	74 (30.08%)	---
Erythromycin	74 (30.08%)	12 (80.00%)
Amikacin	175 (71.13%)	---
Gentamicin	162 (65,85%)	---
Vancomycin	246 (100%)	15 (100%)
Linezolid	246 (100%)	15 (100%)
Ceftriaxone	----	13(86.67%)

Table-II. Antibiotic Susceptibility Pattern of Gram Positive Isolates (n= 261)

Antibiotics	Pseudomonas Spp. n = 81	Escherichia coli n = 72	Enterobacter Spp. n = 42	Klebsiella Spp. n = 21	Salmonella Spp. n = 9
Ampicillin	---	4(5.55%)	3(7.14%)	0(0.0%)	4 (44.44%)
Co-amoxiclav	---	13(18.05%)	21 (50.00%)	3(14.28%)	6(66.66%)
Ciprofloxacin	65(80.24%)	48 (66.66%)	24(57.14%)	15(71.42%)	3(33.33%)
Moxifloxacin	66(81.48%)	49 (68.05%)	26(61.90%)	15(71.42%)	3(33.33%)
Amikacin	36(44.44%)	60(83.33%)	27(64.28%)	12(57.14%)	---
Gentamicin	12(14.81%)	27(37.5%)	12(28.57%)	6(28.57%)	---
Ceftriaxone	---	24(33.33%)	9(21.42%)	6(28.57%)	9(100%)
Ceftazidime	30(37.03%)	24(33.33%)	9(21.42%)	6(28.57%)	9(100%)
Cefoperazone	30(37.03%)	24(33.33%)	9(21.42%)	6(28.57%)	9(100%)
Aztreonam	35(43.20%)	26(36.11%)	27(64.28%)	6(28.57%)	9(100%)
Piperacillin-Tazobactam	74(91.35%)	60(83.33%)	39 (92.85%)	18(85.71%)	9(100%)
Cefoperazone-Sulbactam	72(88.89%)	66(91.66%)	33(78.57%)	18(85.71%)	9(100%)
Meropenem	63(77.78%)	60(83.33%)	27(64.28%)	18(85.71%)	9(100%)
Tigecycline	---	71(98.61%)	42(100%)	20(95.23%)	9(100%)

Table-III. Antibiotic Susceptibility Pattern of Gram Negative Isolates (n= 225)

DISCUSSION

Out of total 5411 patients, 3958(73.14%) were male, 1453(26.85%) were females. Only 486 (8.98%) were positive for bacterial growth. Out of total 486 positive blood cultures, Gram positive isolates were more prevalent 261 (53.71%) as compared to Gram negative isolates 225 (46.29%). Blood cultures were collected from four different units of cardiac patients. Among 486 positive blood cultures, post operative cardiac surgery specimens yielded the most positive 210 (43.20%) blood cultures. At second number, 113 (23.25%) positive cultures were yielded from pre operative cardiac surgery ward blood cultures specimens. At third place, 96 (19.75%) positive cultures were yielded from cardiology ward specimens. At last place 67 (13.78%) positive cultures were yielded from CCU specimens.

Over all and among Gram positive isolates,

Staphylococcus Species were most common organism isolated from 246 (50.61%) blood culture specimens out which 48(9.87%) were MRSA. Followed by Streptococcus spp. 15(3.08%) among Gram positive isolates. *Pseudomonas aeruginosa* 81(16.66%) was the most yielded isolate among Gram negatives organisms, followed by *E.coli* 72 (14.81%), Enterobacter species 42(8.64%), Klebsiella Species 21 (4.32%), Salmonella Species 9 (1.85%).

Vancomycin and Linezolid were most potent antimicrobials, susceptible to 100% Gram positive isolates. Penicillin was least effective only 4 (1.56%) isolates of Staphylococcus spp. were susceptible to it.

Among Gram negative Isolates Piperacillin-Tazobactam was most potent, susceptible to 74(91.35%) isolates of *Pseudomonas aeruginosa*. Gentamicin was laest effective, susceptible to only 12(14.81%) isolates of *Pseudomonas aeruginosa*. For *E.coli* isolates Tigecycline was most effective, susceptible to 71(98.61%) isolates, Ampicillin was least effective, susceptible to only 4(5.55%) isolates. For Enterobacter spp. isolates, Tigecycline was most effective, susceptible to 42(100%) isolates, Ampicillin was least effective, susceptible to only 3(7.14%) isolates. For Klebsiella spp. isolates, Tigecycline was most effective, susceptible to 20(95.23%) isolates, Ampicillin was least effective, was not susceptible to any isolates. For Salmonella spp. isolates, Tigecycline was most effective, susceptible to 9(100%) isolates, Ampicillin was least effective, was susceptible to 4 (44.44%) isolates.

A study from Lahore in 2006 yielded 27.9% positive blood cultures as compared to our study 8.98%. Sample size of that study was 1814, much less than our study 5411. Secondly our study was conducted only on cardiac patients. But in that study samples from all types of patients were included in the study. *Staphylococcus aureus* was isolated in 24.1% positive samples in that study while in our study it is isolated from 30.86% of positive cultures. Out of total yielded *Staphylococcus aureus*, 31.25% were MRSA in that study, while in our study 24.24% were MRSA out of total *Staphylococcus aureus*. In that study 93.7% of enterobacteriaceae isolates were resistant to third generation cephalosporins while in our study 66.66% were resistant to third generation cephalosporins. In our study 21.34 % Gram negative isolates were resistant to carbapenems as compared to 6.49% isolates of that study. In our study 11.11% Gram negative isolates were resistant to Piperacillin-Tazobactam and 12.0% Gram negative isolates were resistant to Cefoperazone-Sulbactam, these combinations were not tested in that study. Tigecycline has very good susceptibility pattern in our study, 98.6% enterobacteriaceae isolates were susceptible to it. It was not tested in that study.[15]

In 2012, a study from Rawalpindi was conducted on 938 blood cultures specimens collected from immunocompromised patients. They yielded 20% positive blood cultures as compared to 8.98% of our study. In that study 47.3% isolates were Gram positives as compared to 53.71% of our study. In that study MRSA prevalence was quite high, up to 67% of *Staphylococcus aurous* were MRSA, as compared to 24.24% MRSA of our study. Carbapenem resistance rate among Gram negative Rods was 40.8% was more than our study i.e.; 21.33%. Tigecycline has much better susceptibility 98.6% in our study as compared to 61.22% of that study. In our study 11.11% Gram negative isolates were resistant to Piperacillin-Tazobactam as compared to 49.44% of that study. In our study 12.0 % Gram negative isolates were resistant to Cefoperazone-Sulbactam, as compared to 44.94% of that study. Because patient was immunocompromised in that study, we can say that isolates were more resistant to antimicrobials in that study.[13]

A study was conducted in 2013 in Lahore on blood culture specimens taken from cancer patients. It yielded 56% Gram positive bacteria and 44% Gram negative bacteria as compared to 53.71%Gram positive Isolates and 46.29% Gram negative isolates of our study. Among enterobacteriaceae, amazingly only 10% strains were resistant to ceftriaxone and no resistance

strains were found against Cefoperazone in that study as compared to 66.66% resistance against Ceftriaxone and Cefoperazone of our study. In *P. aeruginosa* resistance against Cefoperazone was 0% in that study and it is 62.97% in our study. Main difference between was that sample size was too small in this study. Only 50 isolates were included as compared to 486 isolates of our study. Secondly Minimum inhibitory concentrations were determined by broth dilution method but we determined antimicrobial susceptibility by Modified Karby Baur disc diffusion method.[16]

A study from India published in 2011. Positive blood culture rate was 8.3% almost equal to our study 8.98%. Gram negative isolates were 71.6% and Gram Positive isolates were 28.4% as compared to 44% and 56% respectively of our study. The resistance among Gram negative isolate was quite high. Gentamicin 84.4%, Amikacin 65.6% and Cefotaxime 81.3% resistant to Gram negative isolates in that study as compared to 54.66% isolates resistant to 3rd generation cephalosporin, 37.5% isolates resistant to Amikacin. Main difference between two studies was study population, in Indian study neonates were being studied and in our study only cardiac patients were studied.[17]

Antimicrobial resistance is on rise but carbapenems and beta-lactam beta lactamase inhibitor combinations are still effective.

CONCLUSION

Gram positive isolates were more common as compared to Gram negative isolates. Vancomycin and Linezolid were the most effective drugs among Gram positive isolates. Piperacillin-Tazobactam was most potent antimicrobial against *Pseudomonas aeruginosa*. For coliforms Tigecycline was most effective drug.

Conflict of Interest Statement:

All the authors of this manuscript declare no conflict of interest to any individual or any organization.

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3	Prof. Dr. Fouzia Ashraf	Review of the Manuscript	
4	Dr. Shagufta Iram	Review of the Manuscript	
5	Shahida Hussain	Statistical analysis of data	