ORIGINAL ARTICLE

# Pathogens Causing Blood Stream Infections and their Drug Susceptibility Profile in Immunocompromised Patients

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## ABSTRACT

**Objective:** To determine the types of pathogens causing blood stream infections and their drug susceptibility profile in immunocompromised patients.

**Study Design:** Cross-sectional, observational study.

**Place and Duration of Study:** Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from January to September 2012.

**Methodology:** Blood culture bottles received from immunocompromised patients were dealt by two methods, brain heart infusion (BHI) broth based manual method and automated BACTEC system. The samples yielding positive growth from either of two methods were further analyzed. The identification of isolates was done with the help of biochemical reactions and rapid tests. Antimicrobial susceptibility of the isolates was carried out as per recommendations of Clinical and Laboratory Standards Institute (CLSI).

**Results:** Out of the 938 blood culture specimens received from immunocompromised patients, 188 (20%) yielded positive growth. Out of these, 89 (47.3%) isolates were Gram positive and Gram negative each, while 10 (5.3%) isolates were fungi (*Candida* spp.). In case of Gram positive isolates, 75 (84.3%) were *Staphylococcus* spp. and 51 (67%) were Methicillin resistant. Amongst Gram negative group 49 (55.1%) isolates were of enterobacteriaceae family, while 40 (44.9%) were non-lactose fermenters (NLF). *In vitro* antimicrobial susceptibility of Staphylococci revealed 100% susceptibility to vancomycin and linezolid. The enterobacteriaceae isolates had better susceptibility against amikacin 85.7% compared to tigecycline 61.2% and imipenem 59.2%. For NLF, the *in vitro* efficacy of aminoglycosides was 72.5%.

**Conclusion:** The frequency of Gram positive and Gram negative organisms causing blood stream infections in immunocompromised patients was equal. Vancomycin in case of Gram positive and amikacin for Gram negative organisms revealed better *in vitro* efficacy as compared to other antibiotics.

**Key Words:** *Blood stream infection. Antimicrobial susceptibility. Immunocompromised. Pathogen. In vitro efficacy.*

## INTRODUCTION

In recent years, there has been an increase in number of immunocompromised patients despite advances in medical field.[1] General cellular immunity is reduced in situations such as diabetes, steroid administration, transplant patients, HIV infection, malignancy, radiotherapy and neutropenia induced by chemotherapy. An immunocompromised host is susceptible to different microbial infections apart from being at increased risk for potential complications from the secondary infections.[2] The degree of immunosuppression, the need of additional anti-rejection therapy, and other infections like hepatitis C and cytomegalovirus could also weaken the host defenses. Advances in surgical techniques and immunosuppression therapy have allowed the carrying out of over 24,000 transplants per year, in the USA.[2]

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Nosocomial infections have been reported as a regular complication in recipients of organ transplants, facilitated by the use of invasive procedures, associated diseases and immunosuppression treatment. Bacteremia and septic shock remain important causes of morbidity and mortality in solid organ recipients.[4] The frequency of Gram-positive isolates have been steadily increasing from 29% of single-organism bacteremias in 1970's to 69% in 1990's.[5] In these same trials, the rate of single-agent Gram-negative bacteremias dropped from 71% to 31%.[6] In leukemic and cancer patients, the frequency of Gram positive cocci has been found to be far in excess of Gram negative organisms.[2,6,7]

The changing epidemiology and susceptibility patterns of microorganisms emphasize the necessity of constant surveillance of blood stream infections in haematology and oncology units.[8] Prior understanding of infecting organisms and their susceptibility are essential for selection of empirical antimicrobial therapy. Different studies have been published regarding frequency and pattern of nosocomial infections in tertiary care hospitals and bacterial pathogens causing blood stream infections in intensive care units of these hospitals of our country in the last 10 years.[9-12] However, the study focusing the types of pathogens causing blood stream infections and

their drug susceptibility profile in immunocompromised patients has not been conducted in Pakistan. The rationale of this study was a quest for the right antimicrobials for empirical treatment based upon the antibiogram of pathogens responsible to cause blood stream infections in immunocompromised patients.

The objective of this study was to determine the types of pathogens causing blood stream infections and their antimicrobial susceptibility profile in immunocompromised patients.

## METHODOLOGY

This study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. Sampling technique was non-probability consecutive sampling. Sample size was calculated by using WHO sample size calculator with anticipated population proportion of 12%,[13] absolute precision 5% with 95% confidence level. All patients who were admitted for bone marrow transplant, kidney transplant and diagnosed patients of cancer admitted for treatment were taken as immunocompromised patients. All the pathogens isolated from blood culture specimens of immunocompromised patients were included in the study. No discrimination was made on age and gender basis. In case of organ transplant recipients both pre- and post-transplant patients were included. Contaminated specimens and specimens from immunocompetent patients were excluded from the study.

A total of 163 isolates were required for the study, however, 188 culture positive isolates were included in this study. All blood samples were collected aseptically in Brain Heart Infusion (BHI) broth before the start of antimicrobial therapy. Details like hospital identity number, age, gender of the patients, type and place of submission of specimen were recorded on a formated proforma. Blood culture specimens from immunocompromised patients admitted in adult and Peads Oncology Departments of Combined Military Hospital (CMH), Rawalpindi, Military Hospital (MH) Rawalpindi, Armed Forces Bone Marrow Transplant Centre (AFBMTC) Rawalpindi and Armed Forces Institute of Urology (AFIU), Rawalpindi, were considered for the study.

Blood culture bottles were dealt by two methods brain heart infusion (BHI) broth based manual method and automated BACTEC system. The bottles containing BHI broth were incubated with or without agitation and inspected macroscopically for evidence of turbidity indicative of microbial growth once daily and were subcultured after 24 hours, 48 hours and 96 hours on blood and MacConkey agars. The BACTEC 9050 continuous monitoring blood culture systems (CMBCSs) was used in some cases to get indication of culture positive cases. The BACTEC system features a $CO_2$ sensor at the base of each culture bottle, the BACTEC instrument uses a fluorescence sensing mechanism to detect the growth of microorganisms.[14] Positive blood culture bottles were evaluated initially by examining a Gram-stained smear of the broth. Subculture from the positive bottle was carried out and further identification of organism was made with the help of biochemical reactions and rapid tests like catalase, oxidase, coagulase, analytical profile index (API) 20 E and API NE.

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).[15] 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.[15] The results were interpreted as frequencies and percentages.

## RESULTS

Out of the 938 blood culture specimens from immunocompromised patients received, 188 (20%) yielded the growth of bacterial/fungal microorganism. Majority of the patients were males (70.7%). The age range varied from 5 months to 90 years, 65 of them were below the age of 12 years, with mean age of 24.38 ± 19.59 years.

Out of 188 blood culture positive isolates, 89 (47.34%) were Gram positive organisms and similar number i.e. 89 (47.34%) were Gram negatives while 10 (5.32%) isolates were fungi (*Candida* spp.). *Staphylococcus* spp. predominated the list of Gram positive isolates, while *E. coli* and *Pseudomonas* spp. were the leading pathogens amongst Gram negative isolates. Out of 75

**Table I:** Antibiotic susceptibility pattern of Gram positive isolates (n=89).

| Antibiotics | *Staphylococcus* spp. (n = 75) | | Other gram positive organisms (n = 14) | |
|---|---|---|---|---|
| | Number of sensitive isolates | Percentage (%) | Number of sensitive isolates | Percentage (%) |
| Penicillin | 4 | 5.33 | 3 | 21.42 |
| Cloxacillin | 20 | 26.67 | -- | -- |
| Cephradine | 20 | 26.67 | -- | -- |
| Chloramphenicol | 60 | 80.0 | 9 | 64.29 |
| Gentamicin | 48 | 64.0 | 3 | 21.42 |
| Amikacin | 70 | 93.33 | 3 | 21.42 |
| Cotrimoxazole | 37 | 49.33 | -- | -- |
| Doxycycline | 60 | 80.0 | 9 | 64.29 |
| Tigecycline | 56 | 74.67 | 2 | 14.29 |
| Erythromycin | 20 | 26.67 | -- | -- |
| Clindamycin | 37 | 49.33 | -- | -- |
| Ciprofloxacin | 36 | 48 | 2 | 14.29 |
| Fusidic acid | 31 | 41.33 | -- | -- |
| Imipenem | -- | -- | 4 | 28.57 |
| Teicoplanin | -- | -- | 5 | 35.71 |
| Vancomycin | 75 | 100 | 14 | 100 |
| Linezolid | 75 | 100 | 14 | 100 |

*Others include 8 Enterococcus* spp., *3 Streptococcus* spp. *and 3 Corynebacterium* spp.

Staphylococci, 60 (80%) isolates were coagulase negative and 51 (67%) were Methicillin resistant. The detailed microbiology of pathogens isolated and the antimicrobial susceptibility of Gram positive and Gram negative organisms causing blood stream infections in immunocompromised patients is shown in Table I and II respectively.

**Table II:** Antibiotic susceptibility pattern of Gram negative isolates (n=89).

| Antibiotics | Enterobacteriaceae family (n = 49) | | Non-fermenters (NF) (n = 40) | |
|---|---|---|---|---|
| | Number of sensitive isolates | Percentage (%) | Number of sensitive isolates | Percentage (%) |
| Ampicillin | 3 | 6.12 | 2 | 5.0 |
| Ceftriaxone | 11 | 22.44 | 6 | 15.0 |
| Ceftazidime | -- | -- | 24 | 60.0 |
| Cefipime | -- | -- | 21 | 52.5 |
| Aztreonam | -- | -- | 15 | 37.5 |
| Imipenem | 29 | 59.2 | 25 | 62.5 |
| Amoxicillin+ clavulanate | 2 | 4.1 | 5 | 12.5 |
| Tazobactam+ piperacillin | 17 | 34.70 | 28 | 70 |
| Sulbactam+ piperacillin | 12 | 24.49 | 24 | 60 |
| Sulbactam+ cefoperazone | 24 | 49 | 25 | 62.5 |
| Gentamicin | 16 | 32.7 | 29 | 72.5 |
| Amikacin | 42 | 85.71 | 29 | 72.5 |
| Cotrimoxazole | 13 | 26.53 | 7 | 17.5 |
| Doxycycline | 9 | 18.37 | 10 | 25.0 |
| Tigecycline | 30 | 61.22 | 4 | 10.0 |
| Ciprofloxacin | 13 | 26.53 | 28 | 70.0 |

*NLF includes 25 Pseudomonas* spp.*, 13 Acinetobacter* spp., *one Alcaligenes* spp. *and one Stenotrophomonas maltophilia.*

## DISCUSSION

Bacterial resistance to antimicrobial agents is an ongoing serious problem in the treatment of blood stream infections (BSIs). BSIs caused by bacterial pathogens are often due to strains that are resistant to a broader range of antimicrobial agents.[16] This study highlights the high rate of antimicrobial resistance among bacterial pathogens isolated from BSIs and confirms that Rawalpindi area is no exception to progressive antimicrobial resistance against major bacterial pathogens.

Over the last few decades, there has been a shift from Gram negative to Gram positive organisms especially coagulase negative Staphylococci (CoNS) and *Staphylococcus aureus* as causative agents in causing BSIs in immunocompromised patients.[8] Similar trend were noted in the studies in India as both Gram positive and negative microorganisms were isolated in equal proportion. A study carried out in Saudi Arabia in 2006 revealed that Gram positive microorganisms predominately *Staphylococcus aureus* and coagulase negative Staphylococci accounted for about 57% of BSIs in immunocompromised patients. Among Gram negative organisms, majority of isolates were *E. coli* and *Pseudomonas* spp.[17] The present results are almost in concordance with these findings.

Another study carried out in Turkey, focusing BSIs in cancer patients revealed that *Staphylococcus* spp. and *E. coli* were the most common pathogens causing BSIs among both groups of organisms.[8] These results are also in conformity to this study. Similar, studies carried out in Iran and Bangladesh have similar microbiological profile as ours as regards pathogens causing BSIs in immunocompromised patients.[18,19] However, a recent study carried out in India on neutropenic patients revealed that Gram negative pathogens outnumbered the Gram positives as causative agents of bacteremia in this group.[5]

The antimicrobial susceptibility pattern revealed a high level of resistance to routinely used antimicrobials including third generation cephalosporins and fluoroquinolones. Similar results were noted in studies carried out in India.[5,20] Antibiogram of pathogens causing BSIs in immunocompromised patients of the region revealed that amikacin had good *in vitro* activity against both Gram positive and Gram negative organisms. Vancomycin and linezolid has revealed excellent *in vitro* activity against Gram positive organisms as 100% of the present isolates were susceptible to these compounds. This is a significant finding in the backdrop of formulating empirical therapy for immunocompromised patients in our population. In addition, doxycycline and chloramphenicol has also revealed encouraging *in vitro* efficacy against Gram positive organisms.

As regards third generation cephalosporins, quinolones and β-lactam/β-lactamase inhibitor combinations, the *in vitro* efficacy against members belonging to family enterobacteriaceae revealed poor results. These antibiotics have been used and abused to a significant extent in our healthcare settings, thus, paving the way for the development of bacterial resistance. The studies done in other parts of the world also revealed that Gram negative rods are developing significant resistance to these group of antibiotics.[13,20,21] Amikacin, however, revealed encouraging results against members of family enterobacteriaceae followed by tigecycline and carbapenems in this study. In case of non-lactose fermenters isolated from blood culture of immunocompromised patients, amikacin and ciprofloxacin revealed better *in vitro* efficacy. Similarly, β-lactam/β-lactamase inhibitor combinations comprising of sulbactam/piperacillin, tazobactam/piperacillin and sulbactam/cefoperazone as well as carbapenems has revealed good results against non-lactose fermenters. These results are in conformity to work done at other centres.[13,20] The *in vitro* susceptibility of carbapenems against all groups of Gram negative organisms in the local setup is also lower than what is reported in India.[22] With rapid development of carbapenem resistance enterobacteriaceae (CRE)

around the globe, it is imperative that carbapenems should be used with caution, justification and according to susceptibility result in our hospitals.

The rapid emergence of antibiotic resistance and their implications for formulating empirical therapy warrants that institutions caring for immunocompromised patients should have active ongoing microbiological surveillance. Such intent of monitoring infections due to antibiotic-resistant isolates would definitely improve the current antimicrobial regimens especially in a resource limited country like ours. Further studies are definitely required to know the broader spectrum antimicrobial susceptibility of pathogens causing BSIs in immunocompromised patients.

## CONCLUSION

The frequency of Gram positive and Gram negative organisms causing blood stream infections in immunocompromised patients of the studied setup are equal. Methicillin resistance in *Staphylococcus* spp. and third generation cephalosporins resistance in Gram negative organisms is alarmingly high. Vancomycin and linezolid showed excellent *in vitro* activity against Gram positive isolates. Amikacin revealed better *in vitro* efficacy against both Gram positive and Gram negative isolates. Both lactose fermenters as well as non-lactose fermenters showed increasing carbapenems resistance.

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