



Antimicrobial Susceptibility Pattern of Bacterial Isolates Recovered from Blood Cultures in a Tertiary Care Hospital in Karachi

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Abstract

Objective: The aim of the current study was to find out the frequency of key bacterial isolates causing bloodstream infections and their antimicrobial susceptibility pattern from a tertiary care hospital laboratory.

Methodology: A descriptive cross sectional study was conducted at the Department of Microbiology Ziauddin Hospital Karachi, Pakistan. All 229 blood cultures were collected. The Blood and MacConkey agar plates were incubated aerobically while chocolate agar was incubated in capnophilic atmosphere (5-10°C CO₂). Antibiotic susceptibility of the isolates was determined by Modified Kirby Bauer disc diffusion method on Muller-Hinton-agar according to CLSI M100 30th edition recommendations. Data were entered and analyzed by using Statistical Package for Social Sciences (SPSS) version 20.

Results: The average age of the patients was 55.17±18.33 years with 46.29% male and 53.71% female. The predominant bacterial isolate from blood culture was Coagulase-Negative Staphylococci (CoNS) i.e. 57.64%, followed by E.coli 19.6%. The most resistant antibiotic was Penicillin (86%) for CoNS and S. aureus and Co trimoxazole for S. pneumoniae. E.coli reported resistance for drugs like Ampicillin, Aztreonam, Cefixime and Ceftriaxone. The most resistant antibiotic for K. pneumoniae and A. baumannii was Co trimoxazole whereas for P. aeruginosa was Meropenem. The most effective antibiotics for CoNS and S. aureus were Linezolid, Vancomycin and Teicoplanin. For S. pneumoniae, Ampicillin, Ceftriaxone, Chloramphenicol, Linezolid and Vancomycin were most sensitive. Colistin and Tigecycline were sensitive for E.coli, K. pneumoniae, A. baumannii and P. aeruginosa.

Conclusion: It can be concluded that the efficacy of Linezolid, Vancomycin and Teicoplanin was best for Gram-positive isolates while for Gram negative isolates, Colistin and Tigecycline acted effectively.

Keywords: Antimicrobial resistance, Antimicrobial susceptibility, Bloodstream bacterial isolates

Introduction

Bloodstream infections (BSIs) have been identified as one of the most substantial cause of morbidity and mortality in hospitalized patients. There is a significant increase in incidence day by day. Because of various factors like change in population demographics, flaws in health care delivery system, increased globalization, there is a change in prevalence of bloodstream infection in recent years.¹ Several bacterial species have emerged as particularly important causative agents of bacteremia because the non-susceptibility of their strains to the last line antibiotics usually suggest a multidrug resistant (MDR) nature of bacteria. As such *Staphylococcus aureus* (*S. aureus*), Coagulase negative *Staphylococci* (CoNS), *Streptococcus pneumoniae* (*S. pneumoniae*), *Escherichia coli* (*E. coli*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Acinetobacter baumannii* (*A. baumannii*) are recognized as major threats.² Among these, *E. coli*, *S. aureus*, and *S. pneumoniae* are the most frequent causes of Community acquired bloodstream infections and are responsible for more than one-half of all cases overall.³ In terms of frequency and mortality, key BSI pathogens remain *S. aureus*, *E. coli*, *Klebsiella* but in terms of therapeutic challenges, multidrug-resistant *E. coli* and *Klebsiella* remain most problematic.⁴

Antimicrobial resistance (AMR) is known as one of the major health issue and emerged as incessant threat for both developed and developing countries.⁵ Several intrinsic factors such as point mutation, gene amplification and extrinsic factors like horizontal transfer of resistance genes between bacteria within and across species by transposons, integrons or plasmids are responsible for developing resistance.⁶ One specific issue in everyday clinical practice is the empirical treatment of bacteremia acquired in hospitals caused by multi drug resistant (MDR) organisms.⁷ MDR bacteria complicate therapeutic management and limit treatment options. With the increased use of antibiotics, the resistance developed among bacterial isolates therefore monitoring of drug resistance pattern become critical for appropriate empiric selection of antibiotic therapy.⁸

The change in epidemiological and susceptibility patterns of bacterial isolates emphasizes the necessity of constant surveillance of bloodstream infections in tertiary care settings. Prior understanding of infecting organisms and their susceptibility are essential for selection of empirical antimicrobial therapy.⁹ So the aim of the current study was to find out the frequency of key bacterial isolates causing bloodstream infections and their antimicrobial susceptibility pattern from a tertiary care hospital laboratory.

Methodology

A descriptive cross-sectional study was conducted at the Department of Microbiology Ziauddin University

Hospital, Karachi, during the period of 6 months from 1st January 2020 to 30th June 2020. Written approval from the institutional ethical committee and permission from the management of was obtained. Based on results of previous study in a similar setting, prevalence of positive blood culture was 18.2%.¹⁰ To determine the sample size of patients, a prevalence of 18.2% and confidence interval of 95% with 0.05 margin of error was used in the single population proportion formula and the calculated sample size was 229 culture positive cases. Non probability consecutive sampling technique was used. All blood samples for culture and sensitivity from inpatients and outpatients, having age range of 15-60 years were included in the study. Samples showing growth other than bacteria like fungus or yeast were excluded.

All blood cultures were collected from a peripheral vein before starting any antibiotic therapy. The site was cleansed with 70% isopropyl alcohol and allowed to dry. The site was wiped in concentric circles of tincture of iodine moving outwards from the center. The blood culture from pediatric population was collected in pediatric BACTEC bottles and of adult population in standard BACTEC bottles. The blood to broth ratio was 1:10 which was incubated in BACTEC 9240 blood culture instrument at $35.5 \text{ }^\circ\text{C} \pm 1.5 \text{ }^\circ\text{C}$ for 7 days. A fluorescence sensing mechanism was used by BACTEC instrument for the detection of microbial growth. Growth of microorganism detected by instrument alert system was evaluated initially by examining a Gram stained smear of the broth and sub-cultured on 5% Blood agar, Chocolate and MacConkey agar plates and was incubated at 37°C for 18-24 hours for bacterial isolation.

The Blood and MacConkey agar plates were incubated aerobically while chocolate agar was incubated in capnophilic atmosphere ($5\text{-}10^\circ\text{C}$ CO_2). Identification of bacterial isolates was done according to standard microbiological techniques. Antibiotic susceptibility of the isolates was determined by Modified Kirby Bauer disc diffusion method on Muller-Hinton-agar according to CLSI M100 30th edition recommendations. The Muller-Hinton agar plates were incubated aerobically at $35^\circ\text{C} \pm 2$ for 18-24 hours. The control strains that were used as control for disc diffusion testing were *S.aureus* (ATCC-25923), *E. coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853).

Data were entered and analyzed by using Statistical Package for Social Science (SPSS) version 20. Descriptive statistics were used to summarize the categorical variables such as gender and antimicrobial susceptibility was reported as frequencies and percentages. Continuous variables like age were presented as mean and standard deviation.

Results

A total of 229 patients for blood culture and sensitivity were included in the study. The mean age of the pa-

tients was 55.17±18.33 years with 106 (46.29%) males and 123 (53.71%) females. History of prior antibiotic was observed in 19.65% cases. Out of 229 blood cultures, 151(65.9%) were gram positive organism and 78(34.1%) were gram negative organism. The predominant bacterial isolate from blood culture was CoNS i.e. 57.64%, followed by E.coli 19.6%, K. pneumoniae 6.1%, S. aureus 5.6%, P. aeruginosa 5.2%, A. baumannii 3.0% and S.pneumoniae 2.6% as presented in Figure 1.

CoNS was isolated in 132 cultures in which the most resistant antibiotic included Penicillin (57.64%) and the most effective antibiotics were Linezolid (100%), Vancomycin (100%) and Teicoplanin (100%). S. aureus was isolated in 13 cultures, among them Linezolid, Vancomycin and Teicoplanin were 100% sensitive whereas the most resistant antibiotic was Penicillin (100%). S. pneumoniae was isolated in 6 cultures out of which the most resistant antibiotic was Co trimoxazole (67%) whereas Ampicillin, Ceftriaxone, Chloramphenicol, Linezolid and Vancomycin were 100% sensitive. The detailed sensitivity pattern of antimicrobials for Gram positive organisms are presented in Table 1.

Looking over the Gram negative organisms, E.coli was isolated in 45 cultures and the most resistant drugs were Ampicillin (93%), Aztreonam (84%), Cefixime (84%) and Ceftriaxone (84%) while Colistin and Tigecycline were 100% sensitive. K. pneumoniae was isolated in 14 cultures, among them the most resistant antibiotic was Co trimoxazole (43%) while Colistin and Tigecycline were 100% sensitive as mentioned in Table 2. P. aeruginosa was isolated in 12 cultures in which Colistin was 100% sensitive. A. baumannii was isolated in 7 cases and the Colistin and Tigecycline were 100% sensitive for this organism reported in Table 3.

Discussion

Despite advances in diagnosis and treatment, curing bloodstream infections (BSI) is still a challenge for medical specialists, and more often they may be life threatening. There is a high rate of morbidity and mortality because of these infections (BSI). The case fatality rate associated with BSIs is between 35-50%.

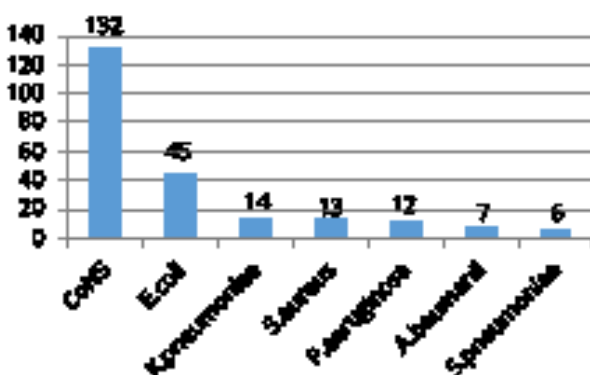


Figure 1: Frequency of key bacterial isolates causing blood stream infection

These infections need immediate diagnosis and antibiotic susceptibility test against involving microorganism, just to timely treat infection by specific antibiotic.¹¹ Traditionally, bloodstream infections, because of resistance against antibiotics, are identified as the root cause of hospital admission specifically in the intensive care units. Although in the past years a list of microorganisms have been identified which have developed resistance against antimicrobial therapy, these include community-onset methicillin-resistant *Staphylococcus aureus* (MRSA) and extended spectrum β lactamase (ESBL) metallo- β -lactamase, and other carbapenemase-producing Enterobacteriaceae.¹²

Current study found that out of 229 blood cultures, 151 (65.9%) were gram positive organisms and 78 (34.1%) gram negative organisms. Similarly other studies also reported higher bloodstream infections caused by gram positive bacteria than gram negative bacteria like studies from Ethiopia found 72.2% vs. 27.8% and 69% vs. 31%.^{10,13} Other studies reported from India showed same higher bloodstream infections caused by gram positive bacteria than gram negative bacteria (53% vs. 39% and 59.3% vs.29.6% respectively)^{14,15,18} but these results are opposed by some of the literature, like study from India¹⁶ and Iran,¹⁷ found that gram negative organisms are superior to gram positive ones in causing bloodstream infections. These difference in results may be due to the geographical variations as some of the microorganism are common in some areas.

In current study, among gram positive isolates, CoNS was commonly found in the cases of bacteremia, likewise in other studies.^{19,20} The justification for the result is that the CoNS are usually present over the skin that's why when blood sample is collected it contaminates the sample. Another reason can be the extensive use of intravascular catheters and indwelling prosthetic devices which increased the incidence rate of nosocomial infections and the causative organism identified is CoNS which usually infect the medical devices. Looking over the Gram negative bacteria, E. coli was identified in majority of cases and finding is also favored by one of the study done in China.¹⁹ E. coli is associated with infections during surgery specifically the surgeries involving gastrointestinal tract or urinary system which introduces the E.coli into the blood and is spread throughout the body.

In developing countries, the steep increase in septicemia cases is a serious health issue and is a major challenge for physicians to select appropriate antibiotics but if there is resistance against antibiotic then can lead to a serious complication.¹³ Literature also revealed that a list of bacteria including both Gram negative and Gram positive bacteria, are responsible for these serious complications because of their resistance.¹⁴ So it is very important to timely detect, identify and testing antimicrobial susceptibility pattern of bloodstream microorganism.

Table 1. Sensitivity pattern of Antimicrobial for Gram Positive Organisms

| Drugs | CoNS n=132 | | S.aureus n=13 | | S.pneumoniae n=6 | |
|-----------------|---------------|-----------|------------------|-----------|---------------------|-----------|
| Drugs | Sensitive | Resistant | Sensitive | Resistant | Sensitive | Resistant |
| Penicillin | 14% | 86% | 0 % | 100% | NT | NT |
| Cephalexin | 37% | 63% | 38% | 62% | NT | NT |
| Cloxacillin | 37% | 63% | 38 % | 62% | NT | NT |
| Ampicillin | NT | NT | NT | NT | 100% | 0% |
| Ceftriaxone | NT | NT | NT | NT | 100% | 0% |
| Erythromycin | 23% | 77% | 62% | 38% | 67% | 33% |
| Clindamycin | 67% | 33% | 77% | 23% | 67% | 33% |
| Chloramphenicol | NT | NT | NT | NT | 100% | 0% |
| Co trimoxazole | 45% | 55% | 69% | 31% | 33% | 67% |
| Levofloxacin | 46% | 54% | 31% | 69% | 83% | 17% |
| Ciprofloxacin | 46% | 54% | 31% | 69% | NT | NT |
| Gentamicin | 73% | 27% | 69% | 31% | NT | NT |
| Linezolid | 100% | 0% | 100% | 0% | 100% | 0% |
| Vancomycin | 100% | 0% | 100% | 0% | 100% | 0% |
| Teicoplanin | 100% | 0% | 100% | 0% | NT | NT |

NT= Not Tested

Table 2. Sensitivity pattern of Antimicrobial For Gram Negative Organisms

| Drugs | E.coli n=45 | | K.pneumoniae n=14 | |
|-------------------------|----------------|-----------|----------------------|-----------|
| Drugs | Sensitive | Resistant | Sensitive | Resistant |
| Ampicillin | 7% | 93% | NT | NT |
| Cefixime | 16% | 84% | 64% | 36% |
| Ceftriaxone | 16% | 84% | 64% | 36% |
| Aztreonam | 16% | 84% | 64% | 36% |
| Meropenem | 89% | 11% | 79% | 21% |
| Co amoxiclav | 44% | 56% | 79% | 21% |
| Piperacillin Tazobactam | 80% | 20% | 86% | 14% |
| Co trimoxazole | 29% | 71% | 57% | 43% |
| Ciprofloxacin | 33% | 67% | 71% | 29% |
| Gentamicin | 69% | 31% | 86% | 14% |
| Amikacin | 93% | 7% | 86% | 14% |
| Colistin | 100% | 0% | 100% | 0% |
| Tigecycline | 100% | 0% | 100% | 0% |

NT= Not Tested

Table 3. Sensitivity pattern of Antimicrobial For Gram Negative Organisms

| Drugs | P.aeruginosa n=12 | | A.baumanii n=7 | |
|-------------------------|----------------------|-----------|-------------------|-----------|
| | Sensitive | Resistant | Sensitive | Resistant |
| Ceftriaxone | NT | NT | 12% | 88% |
| Ceftazidime | 84% | 16% | NT | NT |
| Aztreonam | 83% | 17% | NT | NT |
| Meropenem | 78% | 22% | 15% | 85% |
| Piperacillin Tazobactam | 85% | 15% | 11% | 89% |
| Co trimoxazole | NT | NT | 10% | 90% |
| Ciprofloxacin | 80% | 20% | 10% | 90% |
| Gentamicin | 79% | 21% | 11% | 89% |
| Amikacin | 88% | 12% | 12% | 88% |
| Colistin | 100% | 0% | 100% | 0% |
| Tigecycline | NT | NT | 100% | 0% |

NT= Not Tested

A study was carried out in Ethiopia in 2012 found that majority of antibiotics tested was resistant so not responded well to kill bloodstream infections. Resistance against Gram positive and Gram negative were compared and the results noted were 23.5% versus 58.8%, and 20% versus 100% respectively.¹⁰ The overall antimicrobial susceptibility pattern of Gram-positive bacteria in current study found 86% of resistance against Penicillin whereas antibiotics like Linezolid, Vancomycin and Teicoplanin having lower resistance rate but higher sensitivity rate in comparison to many other drugs which were tested but the results were slightly varied from the other studies done globally.^{15,21,22}

Focusing the antimicrobial susceptibility pattern of Gram-negative bacteria, it has been found that resistance against ampicillin is 93% which is very high as compared to other drugs like Co-trimoxazole. On the other hand Colistin was reported with higher sensitivity rate which is same as reported by previous studies conducted throughout the world^{16,17} Molecular epidemiology studies have identified a globally distributed clone, ST131, and defined the importance of international travel in the acquisition and spread of these infections.²³ Within the ST131 clone, most of the fluoroquinolone resistant and ESBL producing strains have been identified within a highly pathogenic H30 sub-clone.²⁴ Health care-associated BSI due to ESBL producing E. coli has been recognized as a significant complication following trans-rectal prostate biopsy.²⁵

In current study, Linezolid, Vancomycin and Teicoplanin were having the high efficacy against gram positive isolates in comparison to other drugs. The current findings favored by multiple studies from different areas of world.^{17,18} Current study has clearly reported

the drugs which showed resistance and those which were effective, which can be helpful for physicians to prescribe appropriate antibiotic and reduce the further expansion of resistance. Besides that, there is a need of awareness regarding antimicrobial resistance just to stop self-medication.

Conclusion

It can be concluded that the Gram-positive isolates were predominant as compared to the Gram-negative ones. Among Gram positive isolates CoNS was predominant followed by S.aureus and S.pneumoniae in which Linezolid, Vancomycin and Teicoplanin were the most effective drugs. Among Gram negative isolates E. coli was predominant followed by K.pneumoniae, P.aeruginosa and Acinetobacter baumannii in which Colistin and Tigecycline were the most effective drugs. Current results also found the presence of high rates of resistance to the most commonly used antibiotics due to their easy availability, low cost and self-medication. The antibiotics that were highly sensitive in the current study were the ones that are rarely prescribed by physicians, so people do not take those drugs for self-medication.

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Authors' Contribution Statement

HZS contributed to the conception, design, acquisition, analysis, interpretation of data, drafting of the manuscript, critical review of the manuscript, and final approval of the version to be published. FIA contributed to the design, acquisition, analysis, and interpretation of data. HG contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. AF contributed to the acquisition, analysis, interpretation of data, and drafting of the manuscript. SS contributed to the acquisition, analysis, and interpretation of data. MS contributed to the analysis, interpretation of data, and drafting of the manuscript. All authors are accountable for their work and ensure the accuracy and integrity of the study.

Conflict of Interest

Authors declared no conflict of interest

Grant Support and Financial Disclosure

None

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.