Distribution of the invasive pathogenic isolates in blood culture with their antimicrobial susceptibility pattern in a diagnostic laboratory in Karachi

Ambreen Fatima,[1] Hareem Gohar,[2] Kamran Dawood,[3] Mehwish Sajjad,[4] Fatima Fasih,[5] Zeb Hussain[6]

Abstract

Objective: To determine the frequent bacterial pathogens causing blood stream infections in various age brackets, and to discover their antibiotic susceptibility pattern.

Method: The retrospective, descriptive, observational, cross-sectional study was conducted at the microbiology laboratory of Patel Hospital, Karachi, and comprised positive blood culture bacterial isolates analysed between July 1, 2018, and June 30, 2019. Standard microbiological techniques were employed for the identification and antimicrobial susceptibility testing. Data was analysed using SPSS 20.

Results: Of the 3450 specimen, 1243(36%) were positive; 668(53.7%) from male and 575(46.3%) from female subjects, and 771(62%) were gram-positive whereas 472(38%).were gram-negative. Salmonella typhi was the most common pathogen 139(11.1) among gram-negative organisms, followed by Acinetobacter species 103(8.2%), Escherichia coli 96(7.7%) and Klebsiella species 42(3.4%). Among gram-positive bacteria, the predominant isolates were staphylococcus epidermidis 650(52%), staphylococcus aureus 67(5.4%) and enterococci 28(2.3%). Linezolid (99.8%), vancomycin (99%) and chloramphenicol (69%) were found to be the most sensitive antibiotics among gram-positive cocci. Meropenem (60%), amikacin (46%) and gentamicin (40%) were the most sensitive antibiotics for multidrug resistant gram-negative bacteria.

Conclusion: The identification of frequent bacterial pathogens in blood cultures of patients may guide clinicians in proper empirical selection of antibiotics in patients with bacteraemia.

Keywords: Blood stream infection, BSI, Antibiotic susceptibility, AST, Bacteriological profile, Multi-drug resistance, MDR. (JPMA 72: 2443; 2022)

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Introduction

Blood stream infection (BSI) is a life-threatening condition despite the availability of effective antimicrobial therapy and enhancement in substantive care. Early diagnosis and appropriate treatment can reduce the complications associated with BSIs, otherwise it would lead to extended hospitalisation, increased medical expenses and higher mortality rate.[1] The frequency of microorganism isolated and its antimicrobial susceptibility pattern vary in different geographical areas due to variation in antimicrobial prescribing and consumption practices.[2] Therefore, awareness about epidemiological trends and antimicrobial susceptibility patterns of common pathogens in a given region helps in empirical selection of antibiotics.[3] Antibiotic resistance among bacteria causing BSIs is alarmingly increasing, as a result of irrational antibiotic use, making common infections more challenging to treat, and ultimately resulting in catastrophic outcomes for patients. Therefore,

1,4,5Department of Pathology, Dow International Medical College, Dow University of Health Sciences, Karachi, 2,6Department of Microbiology, Dow University of Health Sciences, Karachi, 3Patel Hospital, Karachi, Pakistan.
Correspondence: Ambreen Fatima. Email: ambreen.fatima@duhs.edu.pk

continuous surveillance on blood culture isolates is required to monitor their susceptibility pattern and to overcome the growing incidence of resistance to conventional drugs.

Both gram-positive and gram-negative bacteria can cause serious infections, but BSIs due to gram-negative bacilli are more lethal and cause more serious consequences due to the prevalence of multidrug resistant (MDR) strains among them.[4] It is reported that gram-negative bacteria are responsible for 25% of nosocomial and 45% of community-acquired (CA) BSIs.[5] Out of the gram-negative isolates, salmonella species, escherichia (E.) coli, klebsiella (K.) species, and pseudomonas (P.) species are the most commonly identified organisms. Moreover, the incidence of gram-positive organism is also increasing due to device-related infections. Coagulase negative staphylococcus (S.) and S. aureus constitute the most common causal agents of BSIs among gram-positive organisms.[6]

There is a need to monitor the progress of antimicrobial stewardship programmes, that may assist in making policies to control antimicrobial resistance in a regulated way.[7] Antimicrobial agents administered empirically based on hospital antibiogram can help lessen the spread

of multidrug resistance as well as antibiotic abuse.[8]

The current study was planned to determine the frequent bacterial pathogens causing BSIs in various age brackets, and to discover their antibiotic susceptibility patterns in order to develop an empirical treatment strategy for septicemic patients.

Materials and Methods

The retrospective, descriptive, observational, cross-sectional study was conducted at the microbiology laboratory of Patel Hospital, Karachi, and comprised positive blood culture bacterial isolates analysed between July 1, 2018, and June 30, 2019. After approval from the institutional ethics review committee, the sample was raised using non-probability consecutive sampling technique. All positive blood cultures of both inpatients and outpatients with suspected bacteraemia and history of fever were included. No patient age restrictions were imposed although single first positive sample taken from one patient were excluded. The data was gathered using Oracle software.

All samples had been withdrawn after stringent aseptic measures by thorough cleaning of the venous site with 70% alcohol, followed by povidone iodine and finally with alcohol (Triple Swab technique) from two different locations (20 min apart) and before the initiation of antibiotics.[9] The collected blood samples about 1 ml per site were immediately inoculated into commercially supplied BacT Alert bottles.

All BacT Alert positive samples were subjected to gram staining followed by inoculation on chocolate agar, 5% sheep blood agar (SBA), and MacConkey agar plates and incubated at 37 degrees C for 18-24 hours. Identification of isolates was done in accordance with the standard bacteriological technique, and were distinguished based on gram stain, colony morphology and biochemical tests, such as catalase, coagulase, bile esculin (BE), optochin (OP) Bacitracin (BC) discs for gram-positive isolates, and oxidase, urease, citrate, indole, triple sugar iron tests for gram-negative isolates with serological identification for salmonella species.[10] Analytical Profile Index-20E (API-20E) was further used to distinguish gram-negative rods (GNRs). Antibiotic susceptibility testing (AST) was performed on Muller-Hinton agar by Kirby-Bauer Disk Diffusion method in accordance with the Clinical Laboratory Standard Institute (CLSI) guidelines.[11] For gram-positive bacteria, ciprofloxacin (CIP) (5µg), erythromycin (E) (15µg), penicillin (P) (10U), ampicillin (30ug), chloramphenicol (30ug), vancomycin (VA) (30µg), gentamicin (CN) (10µg), linezolid (LZD) (30µg), oxacillin (1µg), sulphamethoxazole/trimethoprim (SXT) (25µg),

and clindamycin (DA) were used. For gram-negative bacteria, ampicillin (AMP) (10µg), CIP (5µg), ceftriaxone (CRO) (30µg), ceftazidime (CAZ) (30µg), CN (10µg), tobramycin (10µg), amikacin (30µg), SXT (25µg), azithromycin (AZT), amoxicillin-clavulanic acid (AMC) (30/10µg), tazobactum-piperacillin (TZP) (100/10µg), and meropenem (30µg) were used.[12] The susceptibility breakpoints were interpreted according to CLSI guidelines 2020.[11] E. coli (ATCC25922), S. aureus (ATCC25923) and P. aeruginosa (ATCC27853) were used as quality control strains for culture and susceptibility testing.

Data was analysed using SPSS 20. For variables like microorganism, gender and antibiotic susceptibility, frequencies and percentages were calculated. For quantitative variable age, mean and standard deviation (SD) was considered.

Results

Of the 3450 specimen, 1243(36%) were positive; 668(53.7%) from male and 575(46.3%) from female subjects. The overall age range was 1-99 years and the distribution of bacteria in different age groups was noted (Figure). There were 771(62%) gram-positive and 472(38%) gram-negative isolates.

Salmonella typhi was the most common pathogen 139(11.1) among gram-negative rods (GNRs), followed by acinetobacter (A.) species 103(8.2%), E. coli 96(7.7%) and K. species 42(3.4%).

Among gram-positive cocci (GPC), the predominant isolates were staphylococcus epidermidis 650(52%), S. aureus 67(5.4%) and enterococci 28(2.3%). Linezolid (99.8%), vancomycin (99%) and chloramphenicol (69%) were found to be the most sensitive antibiotics among gram-positive cocci. Meropenem (60%), amikacin (46%)
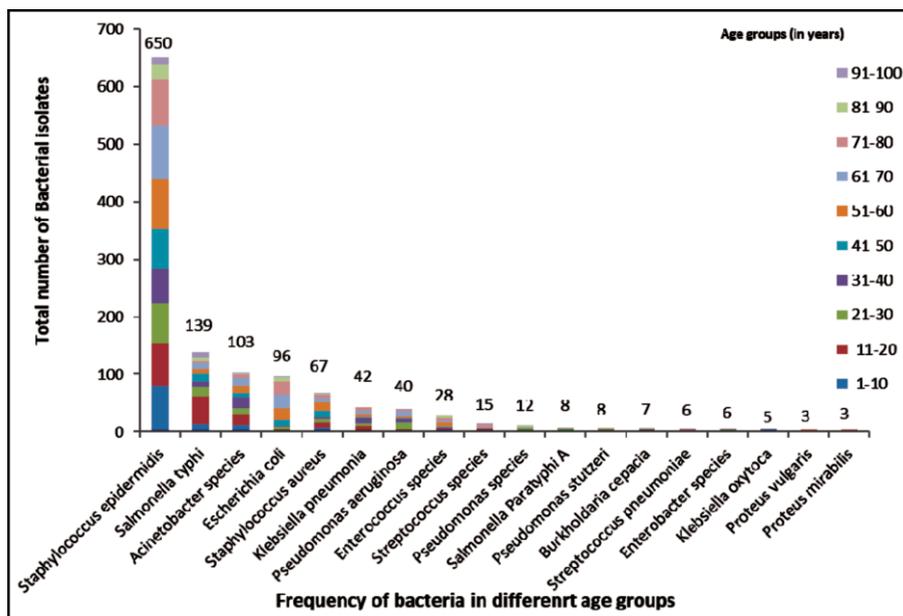
Table-1: Antibiotic sensitivity pattern of gram-positive cocci (GPC) isolated from blood samples.

Antibiotics tested	Staphylococcus epidermidis n=650 (%)	Staphylococcus aureus n=67 (%)	Enterococci n=28 (%)
Penicillin	76 (11.6%)	8 (12%)	5 (18%)
Ampicillin	NT	NT	5 (18%)
Cefoxitin	177 (27%)	22 (33%)	NT
Gentamicin	454 (70%)	41(61%)	NT
Erythromycin	55 (8.5%)	11 (16%)	0
Clindamycin	271 (42%)	26 (39%)	NT
Ciprofloxacin	231 (35.5%)	17 (25%)	3 (11%)
Cotrimoxazole	115 (18%)	11 (16%)	NT
Vancomycin	100%	100%	21 (75%)
Linezolid	100%	66 (98.5%)	100%
Chloramphenicol	458 (70%)	45 (67%)	12 (43%)

Table-2: Antibiotic sensitivity pattern of gram-negative rods (GNRs) isolated from blood samples.

Antibiotic tested	Salmonella species n=139 (%)	Acinetobacter species n=103 (%)	E. coli n=96 (%)	Klebsiella pneumoniae n=42 (%)	Pseudomonas aeruginosa n=40 (%)
AMP	15 (11%)	NT	2 (2%)	NT	NT
AMC	NT	NT	43 (45%)	6 (14%)	NT
TZP	NT	13 (12.6%)	52 (54%)	9 (2%)	17 (42%)
CRO	29 (21%)	5 (5%)	7 (7%)	2 (4%)	NT
CAZ	NT	7 (6.7%)	NT	NT	23 (58%)
CFM	27 (19%)	4 (3.8%)	1 (1%)	0	0
CFP	NT	7 (6.7)	11 (11.4%)	3 (7%)	12 (30%)
AK	NT	23 (22%)	71 (74%)	15 (36%)	19 (48%)
CN	NT	23 (22%)	52 (54%)	11 (26%)	20 (50%)
MEM	100%	15 (15%)	77 (80%)	12 (29%)	13 (33%)
CIP	3 (2%)	11 (11%)	17 (18%)	9 (21%)	21 (53%)
C	16 (11.5%)	NT	57 (59%)	15 (38%)	NT
SXT	16(11.5%)	8 (8%)	18 (19%)	6 (14%)	NT
AZT	100%	NT	NT	NT	NT

AMP: Ampicillin, AMC: Amoxicillin-Clavulanic acid, TZP: Tazobactum-Piperacillin, CRO: Ceftriaxone, CAZ: Ceftazidime, CFM: Cefixime, CFP: Cefepime AK: Amikacin, CN: Gentamicin, MEM: Meropenem, CIP: Ciprofloxacin, C: Chloramphenicol, SXT: Sulphamethoxazole, DA: Clindamycin, AZT: Azithromicin.

Figure: Distribution of bacteria in different age groups.

and gentamicin (40%) were the most sensitive antibiotics for multidrug resistant gram-negative bacteria.

The antibiotic sensitivity patterns of GPC (Table-1) and GNRs (Table-2) were noted separately.

Discussion

Periodic surveillance of blood culture isolates with their antimicrobial susceptibility pattern is needed to assess the prevalence of bacterial pathogens in blood culture. This enables the clinicians to be aware of developing resistant strains, and to implement effective empirical therapy.[13] Furthermore, results from blood culture surveillance can be used effectively in infection prevention and control.[14] Among developing countries, like Pakistan, where high-tech diagnostic facilities are sparse, newer mutant bacterial strains are also emerging, making it more difficult to control the infections.[15] Moreover, physicians prescribe antimicrobial agents more frequently and all classes of antibiotics are readily available over the counter (OTC) which further contributes to the emergence of resistant bacteria.[16]

The current study showed the occurrence of BSI, and evaluated the sensitivity profile to main antibiotics used to treat such infections. The positivity rate was 36% which is comparable to a study conducted at the Armed Forces Institute of Pathology, Rawalpindi (27%).[17] Another study in Pakistan reported BSIs as the second most frequent health-associated infection (21.5%).[18] Culture positivity is slightly higher in this part of world compared to studies done globally, reporting 31.2% and 11.2%.[1,19] Being a third world country having poor healthcare strategies Are valid reasons, but different countries also have different blood culture systems, research populations, study designs, geographical locations, aetiological agents and antibiotic stewardship policies.[12]

In the current study, GPC rate (62%) was higher than GNR (38%). Conversely, some studies have revealed a greater occurrence of GNR than GPC.[6]

In the current study, men had higher levels of cultural positivity (53.7%) than women (46.3%). These results were consistent with earlier findings.[20]

Among the gram-positive isolates (766), the predominant isolates were S. epidermidis (650; 52%), S. aureus (67; 5.4%) and enterococci (28; 2.3%). Over the past two decades, S. species (coagulase-negative staphylococci), the normal skin flora are progressively being considered bloodstream pathogens in select settings. Similar observations were found in most studies.[5,21]

Among the gram-negative bacteria (477), the major isolates were salmonella species (139; 11%), followed by A. species (103; 8.2%), E. coli (96; 7.7%), K. species (42; 3.4%) and P. aeruginosa (40; 3.2%) These findings were compatible with earlier studies.[6]

The age group of 61-70 years yielded the greatest number of positivity in blood culture, with S. epidermidis being the most common pathogen, followed by E. coli, which is in line with a study in Bangladesh.[16]

Methicillin-resistant S. aureus (MRSA) and MDR against both gram-positive and gram-negative bacteria were found to be excessive in the current study, which is consistent with earlier findings.[12]

Majority of S. aureus and coagulase-negative staphylococci isolates were resistant to cefoxitin (67% and 73%respectively) (Table-2). Cefoxitin is preferred to oxacillin as it is a better inducer for mecA gene (MRSA). This is consistent with the findings reported in Ethiopia in 2018 which reported MRSA to be 72%.[12] Another study in India in 2018-2019 reported MRSA to be 97.5%.[9] MRSA BSIs remain associated with worst clinical outcomes as they cause metastatic infections, such as osteomyelitis, infective endocarditis and sepsis. Prevalence of vancomycin-resistant enterococci (VRE) in the current study is estimated to be 25%. This is higher than that reported in various regional studies.[17,22] The management of VRE infections poses a real challenge for the clinicians, as the therapeutic option is often limited to linezolid.

Current findings showed linezolid, vancomycin and chloramphenicol as the most sensitive antibiotics among gram-positive bacteria. The outcomes were in line with earlier studies.[6,19]

The high occurrence of antibiotic resistance in GNRs linked to BSIs is concerning. This occurrence is due to production of extended spectrum beta lactamases (ESBLs) and carbapenemase resistant enterobacteriaceae (CRE) by gram-negative bacteria. These enzymes hydrolyze beta lactam antimicrobials, making them inefficient as well as causing cross-resistance to other antimicrobials. Meropenem, amikacin, and gentamicin appear to be the last-resort antimicrobials among gram-negative bacteria. This is consistent with earlier findings.[6,8] It is important to consider the importance of carbapenem resistance as it is the main contributing factor for MDR and usually the definitive step before pandrug resistance (PDR). In the current study, the highest meropenem resistance was found in A. species (85%), followed by K. species (71%) and P. aeroginosa (67%). E. coli consistently had the lowest rate of meropenem resistance (20%). Carbapenem resistance been reported earlier as well.[23]

Compared to previous studies cited above, the antibiotic sensitivity profile of various organisms isolated in the current study was diverse. This could be because the sensitivity of organisms to antibiotics varies depending on the prevalence of strains, antibiotic use, and resistance profile in a specific region.

In the light of the findings, appropriate analysis of positive blood culture pathogens and surveillance of their antibiotic susceptibility profile are recommended for reducing the frequency of BSIs. Rational antibiotic prescribing policies and strategies to enhance infection control practices must be implemented. The contribution of physicians in antibiotic administration workshops can also help decrease such resistance.

Conclusion
The identification of frequent bacterial pathogens in blood cultures of patients may guide clinicians in proper empirical selection of antibiotics in patients with bacteraemia. There was a high rate of MDR bacterial pathogens.

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References
1. Opintan JA, Newman MJ. Prevalence of antimicrobial resistant pathogens from blood cultures: results from a laboratory based nationwide surveillance in Ghana. Antimicrob Resist Infect Control 2017;6:64. doi: 10.1186/s13756-017-0221-0..
2. Rani DR, Chaitanya BS, Rajappa JS, Kumar RB, Prabhakar KK, MVT KM, et al. Retrospective analysis of blood stream infections and antibiotic susceptibility pattern of Gram negative bacteria in a tertiary care cancer hospital. Int J Med Res Health Sci 2017;6:19-26.
3. Keihanian F, Saeidinia A, Abbasi K, Keihanian F. Epidemiology of

antibiotic resistance of blood culture in educational hospitals in Rasht, North of Iran. Infect Drug Resist 2018;11:1723-8. doi: 10.2147/IDR.S169176.

4. Vaghela HG, Duttaroy B, Prajapati KC. Bacteriological profile and antibiogram of blood culture isolatesfrom paediatric patients with special reference to ESBL and MRSAin a tertiary care centre. Indian J Microbiol Res 2019;6:261-5. Doi; 10.18231/j.ijmr.2019.057.

5. Mia AR, Zerin T. Antibiogram of blood culture isolates of patients from a hospital in Dhaka, Bangladesh. Matrix Sci Med 2020;4:1-5. DOI: 10.4103/MTSM.MTSM_4_19.

6. Fasih F, Baig S, Zameer S, Naseem S, Sharafat S. Bacteriological profile and drug resistance patterns of blood culture isolates: A five year audit from tertiary care hospital. Isra Med J 2019;11: 449-53.

7. Septimus EJ. Antimicrobial Resistance: An Antimicrobial/Diagnostic Stewardship and Infection Prevention Approach. Med Clin North Am 2018;102:819-29. doi: 10.1016/j.mcna.2018.04.005.

8. Ejaz A, Khawaja A, Arshad F, Tauseef A, Ullah R, Ahmad I. Etiological Profile and Antimicrobial Patterns in Blood Culture Specimens in a Tertiary Care Setting. Cureus 2020;12:e11000. doi: 10.7759/cureus.11000.

9. Chaturvedi P, Lamba M, Sharma D, Mamoria VP. Bloodstream infections and antibiotic sensitivity pattern in intensive care unit. Trop Doct 2021;51:44-8. doi: 10.1177/0049475520977043.

10. Habyarimana T, Murenzi D, Musoni E, Yadufashije C, N Niyonzima F. Bacteriological Profile and Antimicrobial Susceptibility Patterns of Bloodstream Infection at Kigali University Teaching Hospital. Infect Drug Resist 2021;14:699-707. doi: 10.2147/IDR.S299520..

11. Wayne PA. Clinical And Laboratory Standards Institute. Performance Standards For Antimicrobial Susceptibility Testing. Inform Suppl 2011;31:100-121.

12. Kitila KT, Taddese BD, Hailu TK, Sori LM, Geleto SE, Mengistu GZ, et al. Assessment of Bacterial Profile and Antimicrobial Resistance Patternof Bacterial Isolates from Blood Culture in Addis Ababa Regional Laboratory, Addis Ababa, Ethiopia. Clin Microbiol 2018;7:2-6. doi:10.4172/2327-5073.1000312

13. Mariyah Y;Tarana S, Dalip KK. Evaluation of Bacteriological Profile and their Antimicrobial Susceptibility Pattern in Blood Stream Infections in a Multispeciality Hospital. J Clin Diagnostic Res 2020;14:13-6

14. Santella B, Folliero V, Pirofalo GM, Serretiello E, Zannella C, Moccia G, et al. Sepsis-A Retrospective Cohort Study of Bloodstream Infections. Antibiotics (Basel) 2020;9:851. doi: 10.3390/antibiotics9120851.

15. Ghafoor T, Hussanain A, Qureshi MM, Mehmood T, Ali L. Antimicrobial susceptibility pattern of blood culture isolates from patientswith suspected neonatal sepsis in a tertiary care hospital in Pakistan. Sri Lankan J Infect Dis 2020;10:30-7. DOI: 10.4038/sljid.v10i1.8244.

16. Iqbal H, Mahboob N, Ahmed M, Mamun KZ, Rahim A, Azad KAK. Bacteriological profile and antimicrobialsusceptibility patterns of blood cultureisolates among febrile patients in atertiary care hospital, bangladesh. J Dhaka Med Coll 2018;27:114-22. Doi; 10.3329/jdmc.v27i2.45820

17. Sana F, Satti L, Zaman G, Gardezi A, Imtiaz A, Khadim T. Pattern of Blood Stream Infections and their antibiotic susceptibility profile in a Neonatal intensive care unit of a tertiary care hospital; a current perspective. J Pak Med Assoc 2019;69:1668-72. doi: 10.5455/JPMA.298528.

18. Saleem Z, Hassali MA, Godman B, Hashmi FK, Saleem F. A multicenter point prevalence survey of healthcare-associated infections in Pakistan: Findings and implications. Am J Infect Control 2019;47:421-4. doi: 10.1016/j.ajic.2018.09.025.

19. Vasudeva N, Nirwan PS, Shrivastava P. Bloodstream infections and antimicrobial sensitivity patterns in a tertiary care hospital of India. Ther Adv Infect Dis 2016;3:119-27. doi: 10.1177/2049936116666983.

20. Kaur P, Kumari S. Identification and senstivity patteren of blood culture isolates in a tertiary care hospital. J. emerg. technol. innov. res. 2019;6:660-4.

21. Gupta S, Kashyap B. Bacteriological profile and antibiogram of blood culture isolates from a tertiary care hospital of North India. Trop J Med Res 2016;19:94.

22. Roy MP, Bhatt M, Maurya V, Arya S, Gaind R, Chellani HK. Changing trend in bacterial etiology and antibiotic resistance in sepsis of intramural neonates at a tertiary care hospital. J Postgrad Med 2017;63:162-8. doi: 10.4103/0022-3859.201425.

23. Cai B, Echols R, Magee G, Arjona Ferreira JC, Morgan G, Ariyasu M, et al. Prevalence of Carbapenem-Resistant Gram-Negative Infections in the United States Predominated by Acinetobacter baumannii and Pseudomonas aeruginosa. Open Forum Infect Dis 2017;4:ofx176. doi: 10.1093/ofid/ofx176.