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MICROBIOLOGICAL PROFILE AND ANTIBIOGRAM OF BLOOD STREAM INFECTIONS IN A TERTIARY CARE HOSPITAL IN SOUTHERN RAJASTHAN

Medical Microbiology

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ABSTRACT

Background: Bloodstream infections are known to cause considerable disability and death among hospitalized patients worldwide. Blood culture is the single most important procedure for bacterial isolation and detection. The aim of the present study was to determine the bacteriological and mycological profile of BSI and their antibiotic susceptibility patterns among clinically suspected cases. **Methods:** A retrospective study was conducted over a period of 2 years from August 2018 to August 2020. Blood culture bottles received from patients of all ages and both sexes with suspected history of fever of unknown origin were put up in BacT/Alert 3D system. The bottles which flagged positive were sub-cultured on Blood agar and MacConkey agar from which identification and antibiotic susceptibility of the isolates was performed using the Vitek-2 system. The reporting of the antibiotic susceptibilities was as per CLSI. **Results:** From a total 7230 blood cultures, growth was observed in 1256 samples (1256/7230 17.37%). Only 681 isolates (681/1256 9.41%) were considered pathogenic, out of which 587 were Gram-negative bacilli (587/681 86.19%), 74 were Gram-positive cocci (74/681 10.86%) and the remaining 20 were fungal isolates (20/681 2.93%). *Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* were the main pathogens isolated. A MDR pattern was observed primarily in *K. pneumoniae*, *Acinetobacter* spp. and *Enterococcus* spp. with other Gram-positive cocci susceptible to vancomycin and linezolid and the Gram-negative bacilli showed sensitivity to imipenem followed by amikacin. **Conclusion:** Our findings underscore the need of periodic surveillance of blood culture isolates for etiologic agents, their antibacterial and antifungal susceptibility patterns that will influence appropriate empirical treatment.

KEYWORDS

bloodstream infections, bacteremia, septicemia, VITEK-2

INTRODUCTION

Bloodstream infections (BSIs) include bacteraemia and fungemia.[1] Although blood is normally a sterile environment,[2] the mere presence of pathogens in the blood is not a life-threatening condition. However, a dysregulated host response to BSI can lead to sepsis.[3,4,5] The diagnosis of non-septicaemic ailments by recovering bacteria from blood make blood cultures very useful tools for diagnosing several infections.[6,7] BSI are the leading causes of fatality world over and are amongst the most common health care associated infections.[8] Isolating the offending pathogens and identifying their sensitivity patterns remain the main stay of definitive diagnosis and management of BSI.

MATERIALS AND METHODS

This study was carried out in the Department of Microbiology for 2 years between August 2018 and September 2020 retrospectively. Blood received from patients of all ages, both sexes attending the OPDs and admitted inpatients with a history of fever of unknown origin (FUO) were processed. Single blood samples from each patient were obtained. Samples collected from intravenous or central lines were rejected.

Using strict aseptic precautions, 5 ml of blood was collected from each adult patient and inoculated immediately into 50 ml of Brain Heart Infusion (BHI) broth blood bottle containing 0.025% of sodium polyanethol sulphonate as anticoagulant (obtained from bioMerieux, Marcy-l'Étoile, France). In paediatric patients, 2 ml of blood was inoculated in 10 ml of paediatric BHI broth blood bottle. Blood culture bottles were put up in BacT/Alert 3D automated system (bioMerieux, Marcy-l'Étoile, France).

A positively flagged bottle was subsequently sub-cultured on to Blood agar and MacConkey agar plates. Final identification and antibiotic susceptibility of the organisms was performed using the VITEK-2 automated system (bioMerieux, Marcy-l'Étoile, France). ID and AST cards of respective isolates obtained from bioMerieux were used. For Gram-positive isolates: ID- 21342, AST- P628 with Ref-414534 was used. For fungal isolates: YST ID- 21343, AFST- YS08 with Ref-420739 was used. For Gram-negative isolates: ID-21341, AST for lactose fermenters- N280 with Ref-414531 and for AST of non-lactose fermenters- N281 with Ref-414532 was used. Reporting of the antibiotic susceptibility was according to CLSI guidelines.

RESULTS

From a total 7230 blood cultures, growth was observed in 1256 samples (17.37%) and only 681 isolates (9.41%) were considered pathogenic organisms. Among the bacterial isolates, Gram-negative bacilli (GNB) made up the majority of 587 isolates (86.19%). Seventy four (10.86%) pathogenic Gram-positive cocci (GPC) were isolated. *Staphylococcus aureus* was the main Gram-positive cocci (75.67%) followed by *Enterococcus* spp. (20.27%) and lastly the *Streptococcus* spp. (4.05%). The fungal isolates constituted only 20 in numbers (2.93%) of which 19 isolates belonged to *Candida* spp. and a single isolate of *Trichosporon asahii* was obtained.

The distribution of isolated GNB is depicted in Figure 1 and the Candida spp. is recorded in Figure 2.

Figure 1: Distribution of isolated GNB. (n=587)

The others refer to 1 isolate each of *Citrobacter freundii, Moragnella morganii and Achromobacter denitrifications.*

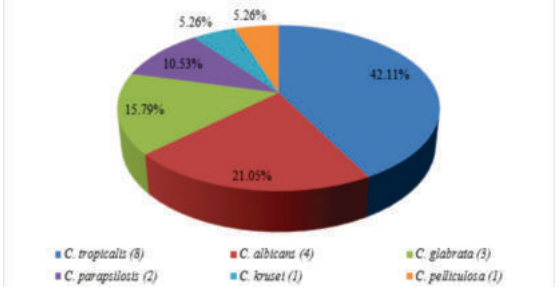

Figure 2: Distribution of *Candida* isolates. (n=19)

Our study portrays a predilection towards adult patients (85.8%) when compared to BSI in the paediatric age group (14.2%) as tabulated in Table 1.

Table 1: Comparison of predominant organisms isolated from adults and children.

Predominant organisms among adult population	Predominant organisms among paediatric age group

Organism	Percentage	Organism	Percentage
Escherichia coli	33.71%	*Klebsiella pneumoniae*	44.33%
Klebsiella pneumoniae	17.29%	*Enterobacter* species	17.53%
Pseudomonas aeruginosa	11.3%	*Candida* species	11.34%
Other organisms	38.7%	Other organisms	26.8%

Only 27 (3.96%) patients walking in to the OPD were diagnosed with BSI compared to 654 (96.03%) inpatients. A male predominance was observed (448/681 65.78%) over the female population (233/681 34.21%).

Antibiotic sensitivities of S. *aureus* and GNB are denoted in Table 2 and Table 3 respectively.

Table 2: Antibiotic sensitivity patterns of *S. aureus.* (n=56)

Antibiotics	*S. aureus*
Gentamicin	36.6%
Cefoxitin	28.7%
Ciprofloxacin	32.3%
Erythromycin	35.3%
Clindamycin	36.3%
Vancomycin	98.7%
Linezolid	99.2%
Teicoplanin	98.6%
Tigecycline*	98.4%

Table 3: Antibiotic sensitivity pattern of GNB (only percentages of sensitive isolates are depicted).

Antibiotics	*Klebsiella pneumoniae* (n=144)	*Escherichia coli* (n=193)	*Pseudomonas aeruginosa* (n=63)	*Enterobacter species* (n=41)	*Acinetobacter species* (n=57)
Amoxicillin+ Clavulanate	44 (30.6%)	69 (35.8%)	-	15 (36.6%)	-
Piperacillin+ Tazobactam	46 (31.9%)	78 (40.4%)	-	17 (41.5%)	-
Ceftriaxone	53 (36.8%)	82 (42.5%)	-	18 (43.9%)	-
Cefepime	54 (37.5%)	83 (43%)	28 (44.4%)	19 (46.3%)	22 (38.6%)
Cefoperazone + Sulbactam	55 (38.2%)	83 (43%)	-	19 (46.3%)	-
Imipenem	104 (72.2%)	145 (75.1%)	52 (82.5%)	32 (78%)	38 (66.6%)
Meropenem	107 (74.3%)	148 (76.7%)	53 (84.1%)	32 (78%)	38 (66.6%)
Amikacin	89 (61.8%)	126 (65.3%)	43 (68.3%)	29 (70.7%)	32 (56.1%)
Ciprofloxacin	46 (31.9%)	68 (35.2%)	29 (46%)	16 (39%)	16 (28.1%)
Tigecycline	136 (94.4%)	187 (96.9%)	-	40 (97.6%)	53 (92.9%)
Colistin	142 (98.6%)	192 (99.5%)	62 (98.4%)	41 (100%)	54 (94.7%)
Doripenem	-	-	53 (84.1%)	-	37 (64.9%)
Minocycline	-	-	-	-	-
Ceftazidime	-	-	28 (44.4%)	-	19 (33.3%)
Co-trimoxazole	-	-	-	-	-
Levofloxacin	-	-	30 (47.6%)	-	17 (29.8%)

The antibiotics mentioned as those reported in VITEK 2. Manual AST was not performed for any of the isolates.

DISCUSSION

Blood stream infections are generally secondary to genitourinary tract infections (25%), respiratory tract infections (20%), abscesses (10%), surgical wound infections (5%) and miscellaneous infections of uncertain sites (25%).[9] However, when the primary site becomes undiscernible, isolating pathogenic microorganism from the blood stream constitutes BSI.

From various parts of the country,[10,11,12,13,14,15,16,17] blood culture positive percentages have been derived to be between 7-44%. Our study percentage falls on the lower numbers (17.37%) of this range. No matter the percentage, the most common organisms to be isolated from positive blood cultures tend to be the GNB world over as captured by Qureshi *et al*[12] and Wariso *et al*.[18] Recorded as the most common causes of hospital-acquired infections of the urinary tract and pneumonia, GNB often spill from these sites into the blood stream leading to bacteraemia and sepsis. However, studies of Sharma *et al*[19] and Jhajhria *et al*[10] contradict these findings. Instead of GNB, they have isolated larger numbers of GPC from patients of BSIs.

We identified hospitalized in-patients to be more burdened (96.03%) with blood stream infections when compared to walk-in patients attending the Out Patient Departments (3.96%). The incidence of hospital-acquired BSI has been reported to correlate with increasing use of central venous catheters, patient illness (e.g., carcinoma, trauma) and other predisposing factors like intensive care unit stay, stay in high-risk nursery and hand washing practices of medical staff.[20] Another finding was a strong male inclination (65.78%) supported by studies of Rani *et al*[21] and Kaur *et al*.[22] Disagreeing with this notion is the study by Zenebe *et al*,[23] who observed higher frequency of BSI to occur among females.

Common organisms isolated from infected blood include *Staphylococcus aureus, Escherichia coli,* Coagulase negative staphylococci (CoNS), *Enterococcus* spp., *Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Proteus* spp. and β-haemolytic streptococci, not in any particular order. BSI pathogens isolated in multiple studies[11,12,24,25,26] including the present study overlap with this pathogen list. The predominant isolate among adults of the current study were *Escherichia coli* (32.71%) followed by *Klebsiella pneumoniae* (17.29%) whereas, among the paediatric age group, *Klebsiella pneumoniae* predominated (44.33%). Wariso *et al*[18] who conducted their study in Nigeria, echo similar findings among the paediatric population presenting with BSI of their region. As we could not obtain paired samples, the pathogenic potential of many CoNS isolates could not be confirmed. Also, the establishment of the MDR isolates to be actual BSI pathogens or mere hospital environmental contaminants could not be made as a direct consequence of single samples received in the laboratory.

GNB are known to be susceptible to aminoglycosides, cephalosporins, β-lactam+β-lactamase inhibitor combinations, fluroquinolones and carbapenams unless the strains are enzyme producing or phenotypically manifesting properties of chromosomal/ plasmid resistance genes that can inhibit the activity of these antimicrobial agents. Our study observed that 63.6% of GNB isolates were susceptible to aminoglycosides and 75.5% to carbapenems. The highest susceptibility recorded was to the toxic drugs tigecycline (95.3%) and colistin (98.3%). The sensitivities to the other groups of drugs were only moderate (Table 3).

Klebsiella pneumoniae and *Acinetobacter* spp. were the most multidrug resistant isolates among the isolated GNB. These isolates demonstrated resistance to most of the commonly used first-line antibiotics as well as decreased sensitivity to aminoglycosides and carbapenems when compared to the other Gram-negative isolates. Similar observations were made by Sharma *et al*[19] in regard to Gram-negative isolates of their study. Their susceptibility patterns established that GNB isolates are most susceptible to only colistin and tigecycline antibiotics.

Among the GPC, *Staphylococcus aureus* isolates showed high level of resistance to antibiotics such as cephalosporins and macrolides owing to the fact that 71.3% of our isolates were methicillin-resistant *Staphylococcus aureus* (MRSA). This is attributable to the fact that MRSA isolates are multidrug resistant. Additionally, as *Enterococcus* spp. are intrinsically resistant to large numbers of antibiotics, low sensitivity was seen towards first- and second-line antibiotics used for treatment of Gram-positive cocci. For these reasons, the Gram-positive isolates of this study showed very high sensitivity only to the last line of antibiotics i.e., linezolid (98.9%) and glycopeptide antibiotics (97.9%). Authors Jhajhria *et al*,[10] Mehta M *et al*,[11] Sharma M *et al*,[27] Atul G *et al*[28] and Mustafa M *et al*[29] reverberate these findings.

Resistance to cephalosporins and quinolones was observed commonly in both Gram-positive and Gram-negative isolates of this study. Also, none of the last line antibiotics used for both Gram-positive and Gram-negative isolates showed 100% sensitivity. This implies that there

were a few isolates in this study which were manifesting resistance to the last line of antibiotics as well. Sadly, the number of such isolates will steadily increase world over if not identified and their spread controlled.

As the number of fungal isolates identified during the study period was very less (n=20), their antifungal susceptibility patterns do not hold any meaning of consequence. However, we would like to impress that excellent sensitivities were demonstrated by all the fungal isolates to the commonly used antifungal agents, the azoles.

CONCLUSION

We conclude that majority of pathogens isolated from blood are GNB demonstrating resistance to multiple currently available antibiotics in both adult and paediatric patients. A paired sample will help include rare and opportunistic pathogens commonly missed in single blood cultures.

REFERENCES

1. Viscoli C. Bloodstream Infections: The peak of the iceberg. Virulence 2016;7(3):248-251.
2. Ochei J, Kolhatkar A. Medical Laboratory Science, Theory and Practices. New York: Tata McGraw-Hill; 2008. Pus, Abscess and Wound Drain; pp. 311-347.
3. Jain A. Roy. I, Gupta MK, Kumar M, Agarwal SK. Prevalence of extended spectrum beta lactamase producing gram negative bacteria in septicemic neonates in tertiary care hospital. J Med Microbiol 2008;52(5):421-425.
4. Siefert H, Wisplinghoff H. Topley and Wilson's Microbiology and Microbial Infections. 10th Ed. Vol. 1. London: Edward Arnold; 2005. Bloodstream infection and endocarditis; pp. 509–26.
5. Mulholland EK and Adegbola RA. Bacterial infections: a major cause of death among children in Africa. N Engl J Med 2005;352(1):75-77.
6. Collee JG, Miles RS and Watt B. Mackie & McCartney Practical Medical Microbiology. 14th Ed. New York: Churchill Livingstone; 1996. Tests for the Identification of Bacteria; pp. 131-151.
7. Cheesbrough M. District Laboratory Practices in Tropical Countries. 2nd Ed. Vol 2. Cambridge: Cambridge University Press; 2000. Antimicrobial susceptibility testing; pp. 132-143.
8. Diekma DJ, Beekman SE, Chapkin KD, Morel KA, Munson E, Doem GV, et al. Epidemiology and outcome of nosocomial and community onset blood stream infection. J Clin Microbiol 2003; 41(8):3655-60.
9. Tille PM. Bailey & Scott's Diagnostic Microbiology. 13th Ed. Edinburgh: Mosby; 2013. Bloodstream infections; pp. 778-797.
10. Jhajhria A, Yadav AK, Parihar G, Gupta PS. Bacteriological profile and antimicrobial susceptibility of blood culture in a tertiary care hospital, Ajmer. Int J Med Health Res 2018;4(6):07-11.
11. Mehta M, Priya D, Varsha G. Antimicrobial susceptibility pattern of blood isolates from a teaching Hospital in North India. Japan J Infec Dis 2005;58(3):174-176.
12. Qureshi M, Aziz F. Prevalence of microbial isolates in blood culture and their antimicrobial susceptibility profile. Biomed 2011;27:136-39.
13. Devi AV, Sahoo B, Damrolien S, Praveen SH, Lungran P, Devi MK. A Study on the Bacterial Profile of Bloodstream Infections in Rims Hospital. J Dent Med Sci 2015;14(1):18-23.
14. Khanal B, Harish BN, Sethuraman KR, Srinivasan S. Infective Endocarditis: Report of a Prospective Study in an Indian Hospital. Trop Doct 2002;32(2):83-85.
15. Sharma PP, Halder D, Dutta AK. Bacteriological profile of neonatal septicemia. Indian Pediatr 1987;24(11):1011-1017.
16. Anbumani N, Kalyani J, Mallika M. Distribution and antimicrobial susceptibility of bacteria isolated from blood cultures of hospitalized patients in a tertiary care hospital. Indian J Pract Doct 2008;5(2).
17. Arora U, Devi P. Bacterial profile of blood stream infections and antibiotic resistance pattern of isolates. J K Sci 2007;9:186-190.
18. Wariso KT, Alex-Wele MA, Obiagwu CS, Bob-Manuel M, Igunma AJ, Awopeju ATO, Jonah AA, Atemie KJJ. Bacteriological Profile and Resistance Pattern of Blood Culture Isolates in a Tertiary Hospital in Port-Harcourt, Nigeria. IJTDH [Internet]. 27Apr.2018 [cited 21Sep.2020];30(2):1-8.
19. Sharma R, Sharma R, Gupta S. Bacteriological Analysis of Blood Culture Isolates with their Antibiogram from a Tertiary Care Hospital. Int J Pharm Sci Res 2015;6(11):4847-51.
20. Karlowsky JA, Jones ME, Draghi DC, Thornsberry C, Sahm DF, Volturo GA. Prevalence and antimicrobial susceptibilities of bacteria isolated from blood cultures of hospitalized patients in the United States. Clin Microbiol Antimicrob 2004;10:3-7.
21. Rani NV, Kannan G, Narendra MV, Vishwakanth D, Nagesh VRD, Yogitha M, et al. A retrospective study on blood stream infections and antibiotic susceptibility patterns in a tertiary care teaching hospital. Int J Pharm Pharm Sci 2012;4(1):543-8.
22. Kaur A, Singh V. Bacterial isolates and their antibiotic sensitivity pattern in clinically suspected cases of fever of unknown origin. JK Sci 2014;16(3):105-109.
23. Zenebe T, Kannan S, Yilma D, Beyene G. Invasive bacterial pathogens and their antibiotic susceptibility patterns in Jimma University Specialized Hospital, Jimma, Southwest Ethiopia. Ethiop J Health Sci 2011;21(1):1-8.
24. Nwadioha SI, Nwokedi EOP, Kashibu E, Odimayoand MS, Okwori EE. A review of bacterial isolates in blood cultures of children with suspected septicemia in a Nigerian tertiary Hospital. Afr J Microbiol Res 2010;4(4):222-225.
25. Arora U, Devi P. Bacterial Profile of Blood Stream Infections and Antibiotic Resistance Pattern of Isolates. JK Sci 2007;9(4):186-189.
26. Barati M, Taher MT, Abasi R, Zadeh MM, Shamshri AR. Bacterial profile and antimicrobial susceptibility of blood culture isolates. Iran J Med Sci 2010;35(2):109-115.
27. Sharma M, Goel N, Chaudhary U, Aggarwal R, Arora DR. Bacteraemia in children. Indian J Pediatr 2002;69(12):1029-32.
28. Garg A, Anupurba S, Garg J, Goyal RK, Sen MR. Bacteriological profile and antimicrobial resistance of blood culture isolates from a university hospital. J Indian Acad Clin Med 2007;8(2):139-43.
29. Mustafa M, Ahmed SL. Bacteriological profile and antibiotic susceptibility patterns in neonatal septicaemia in view of emerging drug resistance. J Med Allied Sci 2014;4(1):2-8.