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Rare microorganisms in blood culture samples from intensive care unit patients in a tertiary care hospital in Eastern India – A dangerous threat

Sharmila Gupta[1], Soma Bose[2]

[1]Senior Resident, [2]Assistant Professor, Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata, West Bengal, India

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ABSTRACT

Background: Sepsis is a potentially life-threatening condition with significant mortality and morbidity. An estimated 48.9 million cases and 11 million deaths occur due to sepsis worldwide. The gold standard for the diagnosis of suspected sepsis patients is blood culture. **Aims and Objectives:** The aims and objectives of the study are to evaluate the growth of rare microorganisms in blood cultures of intensive care unit (ICU) patients, to determine their antimicrobial susceptibility patterns, and also to assess the associated risk factors in those patients. **Materials and Methods:** This study was conducted for 1 year (August 2023–July 2024) in our department including blood culture samples from ICU patients and relatively rare microorganisms isolated were included in the study accounting for 40 cases. Blood specimens were cultured in automated BD BACTEC™ Fx40 blood culture system and identification of microorganisms and their antimicrobial susceptibility pattern were detected by Vitek2 system. **Results:** Female patients were more in number than male patients and patients of 0–20 years' age group were most commonly affected. *Staphylococcus hominis* was most commonly isolated out of all rarely isolated microorganisms. The different parameters for the assessment of sepsis severity in our study were C-reactive protein (CRP), Neutrophil count, D-dimer, Procalcitonin, and quick sequential organ failure assessment (qSOFA) score. Out of all, CRP was most commonly raised among sepsis patients. The most common risk factor involved was prolonged ICU stay. **Conclusion:** Appropriate collection and processing of blood culture samples can reduce sepsis-related morbidity and mortality manifold. Combination of biomarkers study and qSOFA scoring system can improve diagnosis and monitoring of sepsis patients.

Key words: Sepsis; Vitek2; BD BACTEC™; Fx40; Biomarkers; Intensive care unit

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INTRODUCTION

Sepsis is the systemic host response to an infection characterized by concurrent activation and inhibition of several inflammatory systems, commonly associated with tissue injury and organ failure and initiated by an originally confined infectious source.[1] It is a clinical syndrome that complicates severe infection and is characterized by inflammation, including increased microvascular permeability, vasodilatation, and end-organ damage.[1] Sepsis is one of the most important causes of morbidity and mortality with an estimated 48.9 million cases and 11 million deaths worldwide.[2] Blood culture is the gold standard for detecting bloodstream infections and is invaluable for diagnosis and treatment.[2] It is the best approach for optimal care of patients in sepsis involving isolation of clinically relevant microorganisms from blood cultures and their appropriate identification as well as timely reporting of antimicrobial susceptibility testing results. The growth of rare microorganisms should be correlated

Address for Correspondence:
Dr. Sharmila Gupta, Senior Resident, Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata - 700014, West Bengal, India. **Mobile:** +91-9330420016. **E-mail:** sharmilagupta28091994@gmail.com

clinically as blood culture contamination has a huge impact on patient care services.[3] All blood culture samples should be collected with utmost care and aseptically to prevent the growth of contaminants.[3] With the development of automated blood culture systems, the time to report blood culture isolate identification and antibiotic susceptibility patterns have markedly reduced.[3] No growth for blood culture specimens now can be declared after 5 days of incubation.[4] There are certain critical factors for the optimal recovery of isolates from blood cultures such as proper and adequate skin disinfection, volume of blood collected for culture, number of blood samples collected, and timing of blood collection, i.e., before or after starting antimicrobial agents, blood culture bottles/media types, dilution of blood, use of anticoagulants, subcultures, duration of incubation and monitoring.[5] The latest model used is BD BACTEC™ Fx40 blood culture system.[5] There are few markers for sepsis such as C-reactive protein (CRP), Procalcitonin, D-dimer, and rise in Neutrophil counts which can indirectly predict treatment response and sepsis outcomes in patients.[6] A scoring system called quick sequential organ failure assessment (qSOFA) can be calculated bedside and it includes respiratory rate, Glasgow coma scale, and systolic blood pressure.[7] A qSOFA score of equal or more than two indicates enhanced severity with prolonged intensive care unit (ICU) stay and increased mortality.[8] As there are surprisingly lesser studies in this field, so we aimed to evaluate the growth of rare microorganisms in blood cultures of ICU patients, to determine their antimicrobial susceptibility patterns, and also, to assess the associated risk factors in those patients. This study was conducted in the Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata.

Aims and objectives

1. To evaluate the growth of rare microorganisms in blood culture among ICU patients
2. To determine their antimicrobial susceptibility patterns
3. To assess the associated risk factors.

MATERIALS AND METHODS

A cross-sectional study was conducted for 1 year (August 2023–July 2024) in the Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata, including blood culture samples from ICU patients and relatively rare microorganisms isolated were included in the study accounting for 40 cases. The blood cultures were performed by BD BACTEC™ Fx40 system and they were subcultured on Blood agar and MacConkey agar once the bottles flagged positive. Their identification and antimicrobial susceptibility testing were done by Vitek2 system as per standard protocol.[9] The bottles which flagged no growth up to 5 days of incubation were reported as no growth. Relevant history was taken from patients relatives.

Inclusion criteria

All relatively rare microorganisms such as *Enterobacter cloacae*, *Sphingomonas paucimobilis*, *Candida albicans*, *Candida auris*, *Burkholderia cepacia*, *Aeromonas* species, *Pseudomonas stutzeri*, *Klebsiella oxytoca*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, *Staphylococcus sciuri*, *Staphylococcus saprophyticus*, *Pseudomonas luteola*, *Pantoea agglomerans,* and *Kocuria* species isolated were included in the study.

Exclusion criteria

Commonly isolated microorganisms isolated from blood culture samples were excluded.

Ethical clearance

For the present study, the ethical approval was taken from the Institutional Ethics Committee, Calcutta National Medical College and Hospital, Kolkata.

RESULTS

All rarely isolated microorganisms were included in the study from August 2023 to July 2024 in the Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata. Table 1 shows the distribution of age group and gender. Table 2 shows different rare microorganisms isolated and their antimicrobial susceptibility testing patterns. Table 3 shows different parameters for the assessment of sepsis severity. Figure 1 shows sepsis patients with variable qSOFA score. Table 4 shows different associated risk factors in sepsis patients.

Table1 shows distribution of age and gender among sepsis patients.

Table 3 shows number of sepsis patients with raised levels of laboratory (Biomarkers) and clinical parameters.

Figure 1 shows sepsis patients with variable qSOFA score.

Table 1: Distribution of age group and gender among patients with sepsis with rare microorganisms

Age range	Male	Female
0–20	11	10
21–40	2	6
41–60	3	3
61–80	2	2
81 and above	1	0
Total	19	21

Table 2: Different rare microorganisms isolated from blood culture samples of ICU patients and their antimicrobial susceptibility patterns

Microorganisms	No. of cases	Average time to positivity (days)	Sensitive antimicrobial agents (No. of drug sensitive cases)
Enterobacter cloacae	3	2	AK (2), DO (1), LE (2), MRP (1), AT (1)
Sphingomonas paucimobilis	5	1	CPM (2), CFS (3), CIP (3), COT (4), GEN (4), IPM (3), MRP (3), PTZ (2), AK (1), CAZ (3), LE (2), MIN (3)
Candida albicans	1	2	AMB (1), NYT (1)
Candida auris	1	3	AMB (1), CAS (1)
Burkholderia cepacia	4	2	CAZ (3), CFS (3), MRP (3), MIN (1), COT (1)
Aeromonas species	1	2	AK (1), TOB (1), IPM (1), LE (1)
Pseudomonas stutzeri	1	2	AK (1), CAZ (1), CIP (1), GEN (1), PTZ (1)
Klebsiella oxytoca	1	1	GEN (1), TGC (1), MRP (1)
Staphylococcus haemolyticus	7	2	COT (5), TET (7), TGC (5), VA (7), TEI (5), AMP (1), CD (4), LZ (7), DAP (4)
Staphylococcus hominis	10	3	CIP (4), COT (5), DAP (1), LZ (6), LE (7), TEI (8), TGC (4), TET (7), VA (8), CD (5), DAP (6), AMP (1), AMC (1), E (1), TGC (2)
Staphylococcus sciuri	1	3	CD (1), DAP (1), LZ (1), VA (1), TEI (1), TGC (1), TET (1)
Staphylococcus saprophyticus	1	4	CIP (1), DAP (1), LZ (1), TGC (1), TET (1), TEI (1), VA (1)
Pseudomonas luteola	1	3	AK (1), CPM (1), CFS (1), CIP (1), IPM (1), MRP (1), PTZ (1), GEN (1)
Pantoea agglomerans	1	2	CFS (1), IPM (1), TGC (1), MIN (1)
Kocuria species	2	3	AK (1), VA (2), CD (1), LZ (2), TEI (2), MRP (1)
Total	40		

AK: Amikacin, DO: Doxycycline, LE: Levofloxacin, MRP: Meropenem, AT: Aztreonam, CPM: Cefepime, CFS: Cefoperazone+Sulbactam, CIP: Ciprofloxacin, COT: Cotrimoxazole, GEN: Gentamicin, IPM: Imipenem, PTZ: Piperacillin+Tazobactam, CAZ: Ceftazidime, MIN: Minocycline, AMB: Amphotericin B, NYT: Nystatin, CAS: Caspofungin, TOB: Tobramycin, TGC: Tigecycline, TET: Tetracycline, VA: Vancomycin, TEI: Teicoplanin, AMP: Ampicillin, CD: Clindamycin, DAP: Daptomycin, LZ: Linezolid, AMC: Amoxicillin+Clavulanate, E: Erythromycin, ICU: Intensive care unit

Table 3: Different parameters for assessment of sepsis severity

Parameters	No. of patients where raised (percentage)
CRP	38/40 (95)
Neutrophil count	30/40 (75)
D-dimer	10/40 (25)
Procalcitonin	28/40 (70)
qSOFA score (more than or equal to 2)	21/40 (52.5)

CRP: C-reactive protein, qSOFA: Quick sequential organ failure assessment

Table 4: Different associated risk factors in sepsis patients developing septicemia with relatively rare microorganisms

Risk factors	No. of patients (percentage)
Prolonged ICU stay	21 (52.5)
Diabetes mellitus	14 (35)
Chronic kidney disease	2 (5)
Cerebrovascular accident	3 (7.5)
Malignancy patients	3 (7.5)
Post-transplant patients	2 (5)
Elderly (more than 60 years age)	5 (12.5)
Underlying primary infections-	
Pneumonia	5 (12.5)
UTI	4 (10)
Meningitis	1 (2.5)
Endocarditis	2 (5)

ICU: Intensive care unit, UTI: Urinary tract infection

Table 4 shows different associated risk factors in sepsis patients developing septicemia with relatively rare microorganisms.

Statistical analysis

The data obtained were analyzed with the statistical tool R. The different percentages were calculated. Fisher's exact test/One-way Chi-square test was used for comparative analysis. The tests were evaluated at a confidence level of 95% and $P<0.05$ was considered statistically significant. In our study, all risk factors were found to be statistically not significant with P-values more than 0.05, but these risk factors may be contributory.

DISCUSSION

The present study was conducted in the Department of Microbiology, Calcutta National Medical College and Hospital, Kolkata, with objectives of evaluating the growth of rare microorganisms in blood cultures of ICU patients, to determine their antimicrobial susceptibility patterns and also to assess the associated risk factors in those patients. The key findings of our study were as follows: Female patients were more in number than male patients and patients of 0–20 years age group were most commonly affected. *S. hominis* was most commonly isolated out of all rarely isolated microorganisms. The different parameters for the assessment of sepsis severity in our study were CRP, Neutrophil count, D-dimer, Procalcitonin, and qSOFA score. Out of all, CRP was most commonly raised among

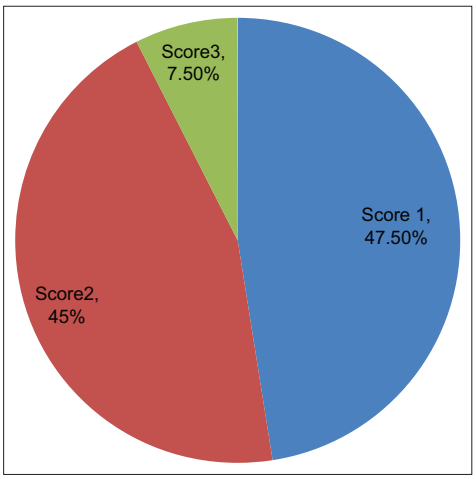

Figure 1: Sepsis patients with variable quick sequential organ failure assessment score

sepsis patients. The most common risk factor involved was prolonged ICU stay.

In our study, female patients (52.5%) were more commonly affected as compared to male patients (47.5%) and patients of 0–20 years age group were most commonly affected. In another study by Lakbar et al., sepsis and septic shock were found to be more prevalent in males than female patients.[10] Also as per the WHO, older and very young have the highest risk for sepsis.[2] These discrepancies may be due to difference in demographic patterns. In our study among different rare microorganisms isolated, *S. hominis* was most common. Out of all Gram-negative bacilli, meropenem was mostly sensitive; out of *Candida* species, Amphotericin B was mostly sensitive while out of all Gram-positive cocci, vancomycin and linezolid were most commonly sensitive. As per several reports, coagulase-negative *Staphylococcus* species (CONS) may be skin commensal contaminating blood cultures but if we correlate clinically and patient is found to be symptomatic with raised biomarkers then CONS can be considered as potential pathogens.[6] Somewhat difference in drug susceptibilities was noted in another study by Khan et al.,[11] These discrepancies may be due to difference in antibiogram patterns in two different geographical regions. In our study, one case of *C. auris*, one case of *P. agglomerans,* and 2 cases of *Kocuria* species were detected which are among the emerging pathogens at the present time. As per a study by Briano et al., *C. auris* candidemia was noted in critically ill, colonized patients.[12] In another study by Masoud et al., *P. agglomerans* which is a rare human pathogen was found for the 1st time in Tanzania in 2024.[13] A nationwide outbreak of septicemia was also noted due to intravenous fluid contaminated with this bacteria.[6] As per a study by Ziogou et al., infection with *Kocuria* species, a rare human pathogen was found in immunocompromised patients, patients with severe underlying disease, long-term indwelling devices, and malignancy patients.[14] In our study also, *Kocuria* infections were found one in a malignancy patient and the other patient had endocarditis. As per another study by Robles-Marhuenda et al., *Kocuria* species may rarely cause native valve endocarditis.[15] In our study, CRP was the most sensitive marker and was found to be raised in 95% sepsis patients. As per another study by Ahuja et al., biomarkers may be helpful in early diagnosis, prognosis of patients, and monitoring of treatment response in sepsis patients.[16] In our study, 47.5% patients had qSOFA score of 1, 45% patients have qSOFA score 2, and 7.5% patients have qSOFA score 3. As per various data, qSOFA score may be <2 in patients with early sepsis and sepsis should not be excluded in such cases.[7] In our study, prolonged ICU stay was the most common risk factor among ICU patients for developing sepsis with rare microorganisms. Similar results were found in a study by Kabi et al.[17]

Strength and limitations of the study

It was an extensive study. We have done the isolation of rare microorganisms from blood cultures in our laboratory. Furthermore, their antimicrobial susceptibility patterns, biomarkers, and associated risk factors were studied. Although it was an extensive study, the molecular methods for gene detection in drug-resistant isolates and genotyping could not be done due to lack of facility.

CONCLUSION

If blood culture collection and processing can be optimized, then it can lead to higher rates of detection of clinically relevant microorganisms including the detection of rare isolates. This will help in overall reduction in hospital stay of patients with septicemia including ICU stay. Biomarkers and qSOFA score are good in assessing patient's clinical condition and blood culture samples should be sent if sepsis is suspected for early diagnosis and better management of patient.

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SG- Definition of intellectual content, literature survey, prepared first draft of manuscript, implement of study protocol, data collection, data analysis, manuscript preparation, submission of article, concept, design of study, statistical analysis, and interpretation; **SB-** Editing and manuscript revision.

Work attributed to:
Calcutta National Medical College and Hospital, Kolkata, West Bengal, India.

Orcid ID:
Dr. Sharmila Gupta - https://orcid.org/0009-0005-6982-3907
Dr. Soma Bose - https://orcid.org/0009-0003-6449-5225

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