Bacteriological profile and their antimicrobial susceptibility pattern among clinical suspected adult septicemia admitted patients: A study from tertiary care and teaching hospital

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ABSTRACT

Objectives: The objective of this study is to determine the bacteriological profile and their antimicrobial susceptibility pattern among clinically suspected adult septicemia admitted patients and to study the correlation between the clinical presentation and inflammatory biomarkers (C-reactive protein [CRP] and Procalcitonin [PCT]) in the final outcomes of nosocomial sepsis in adult septicemia patients. **Material and Methods:** This is a descriptive cross-sectional study of 1-year duration from November 2022 to November 2023. The study has been approved from the Institutional Ethical Committee. Adult patients with a clinical suspected diagnosis of sepsis were admitted to the medicine ward of the tertiary hospital. Blood samples of adult sepsis suspected patients samples were taken for as a part of septicemia screening. Blood culture and sensitivity were performed according to standard guidelines. The sepsis inflammatory prognostic markers CRP and PCT test were evaluated according to manufactures instruction. **Results:** Out of 300 clinically suspected adult septicemia patients, 93 (31%) patients were isolated culture-positive sepsis while 207 (69%) patients were culture-negative sepsis. Gram-positive organisms were more (69%) as compared to Gram-negative organisms (31%). *Staphylococcus aureus* was predominant among Gram-positive organisms while *Acinetobacter baumanii* complex (9%) followed by *Enterobacter* spp. (8%) isolated in Gram-negative bacilli. Culture-positive sepsis patients have CRP level ≥3.2 mg/dL (80%) and the majority (80%) of these patients have reactive PCT. **Conclusions:** Successful treatment of bloodstream infection depends on early diagnosis and appropriate use of antimicrobial agents. Rapid identification results and antimicrobial susceptibility tests are essential for guiding clinicians in the selection of the most appropriate treatment for patients with bloodstream infections.

Key words: *Adult septicemia, Antimicrobial susceptibility pattern, Bacteriological profile*

Sepsis is defined as a life-threatening organ dysfunction caused by a deregulated host response to infection is increasingly becoming a major health-care problem affecting millions of people each year worldwide [1]. Septicemia indicates systemic symptoms caused by bacteria or toxins in the blood [2]. Despite the availability of broad-spectrum and highly potent antimicrobial agents, life-threatening septicemia remains one of the most important causes of morbidity and mortality worldwide irrespective of the availability of major advances in diagnostic and treatment facilities [3]. Automated blood culture systems are now available. Still, conventional blood culture methods are the dominant approach to isolating bacteria in sepsis patients. The use of early and appropriate antibiotic therapy is essential to improve the survival rates in patients with severe sepsis and septic shock [4]. C-reactive protein (CRP) and procalcitonin (PCT) have been a promising inflammatory biomarker for aiding early diagnosis and treatment in patients with sepsis and septic shock [5].

Approximately 200,000 cases of bloodstream infections occur every year, causing 20–50% mortality worldwide [6]. Respiratory tract, urogenital tract, and intra-abdominal infections are commonly identifiable primary foci of BSIs [7]. Elderly patients and patients with comorbidities are more prone to sepsis, making it more challenging to make an early diagnosis and provide timely therapeutic management [8]. Due to the frequent use of invasive procedures, the increasing trend of Gram-positive isolates as a cause of sepsis has been observed over the period [9]. The sepsis-related organ failure assessment (SOFA) score was used in adult patients only, and the quick SOFA score was introduced for early recognition of organ dysfunction and prognostic assessment of sepsis [10,11].

Access this article online

Received - 14 March 2024
Initial Review - 22 March 2024
Accepted - 31 March 2024

DOI: 10.32677/ejms.v9i1.4530

Quick Response code

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Our aim of the study is to determine the bacteriological profile and antimicrobial susceptibility pattern among clinically suspected adult septicemia-admitted patients in a tertiary care and teaching hospital.

MATERIALS AND METHODS

A descriptive-cross-sectional study was conducted in the Department of Microbiology and Medicine, University College of Medical Sciences and Guru Teg Bahadur, tertiary care hospital for 1-year duration from November 2022 to November 2023 from the day of acceptance of this project study.

Blood samples for culture and antimicrobial susceptibility testing were taken from 300 clinically suspected cases of septicemia admitted to the medicine ward of our hospital. All the adult patients with suspected sepsis of either gender 18 years or above were enrolled in this study and non-septic adult patients who stayed in the ward for <24 h were excluded from the study. Informed consent was taken from all those patients included in this study, and their identity was kept confidential. We used both conventional and automated (according to availability) blood culture methods for identification of microbial pathogens.

(a) Conventional blood culture – About 5–10 mL of blood from adult patients were collected aseptically in a conventional method. Blood was collected as soon as possible before administration of antibiotics into Brain–Heart Infusion broth. Blood culture bottles were incubated at 37°C and subcultures after 48h onto blood and MacConkey's agar. Identification of isolate was done by Gram's stain, catalase, coagulase (slide and tube), oxidase, and other biochemical tests. If no growth observed until 7th day, the sample was reported as sterile [12].

(b) Automated blood culture – About 10 mL of blood was drawn from two different peripheral sites under strict aseptic conditions in every patient. If the patient had a central venous line in place, two samples were drawn from that line. Samples were then immediately put into designated blood culture bottles and sent to the microbiology department, then placed in their respective automated system. Positive samples once flagged positive were then subcultured on 5% sheep blood agar and MacConkey agar. Identification of isolate was done by Gram's stain, catalase, coagulase (slide and tube), oxidase, and biochemical tests. If there was no flag in the bottle after 5 days, then reported as sterile. For all the tests, positive and negative controls were kept [12].

This study has been approved by the Institutional Ethical Committee (IEC No: GTBHEC 2022/P-177). A repeat sample of the same patient was not considered in the present study.

(c) Antimicrobial susceptibility testing – This method was carried out on Mueller–Hinton agar plates under the guidelines outlined in the Clinical and Laboratory Standards Institute (CLSI 100th edition). Susceptibility testing was carried out by the standard Kirby–Bauer disc diffusion method as per the latest CLSI guidelines. It is worth noting that not all antibiotics were tested for every microorganism, and as part of the quality control process, *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) control strains were used in the Kirby–Bauer disc diffusion method [13,14]. Every batch of Mueller–Hinton agar and antibiotic disc was tested using ATCC control strains.

(d) Inflammatory markers – The sepsis inflammatory prognostic markers quantitative CRP based on turbidimetry method and PCT semi-quantitative test based on lateral flow assay was also performed according to manufacturer instruction.

Data Management and Statistical Analysis

The data accrued on all adult sepsis were analyzed using the Statistical Package for the Social Sciences's latest version. The Chi-square test was used in assessing the associations between categorical variables. A p=0.05 or less was considered statistically significant.

RESULTS

Out of 300 clinically suspected adult septicemia patients, 93 (31%) patients were isolated culture-positive sepsis while 207 (69%) patients were culture-negative sepsis (Fig. 1). This is statistically significant among culture-positive and culture-negative bloodstream infection patients (p<0.03, Chi-square test).

In the present study, males were predominated than females (M: F=1.5:1). The age group (21–30) years of age was the most predominated group followed by 31–40 years of the clinically suspected patients. About 24% of patients were belonging to age more than 60 years. This is not statistically significant (p>0.06, paired t-test) (Fig. 2).

Overall culture-positive 31% (93 cases) of sepsis patients, Gram-positive organisms were more (69%) as compared to Gram-negative organisms (31%). *S. aureus* was predominant among Gram-positive organisms while *Acinetobacter baumanii* complex (9%) was followed by *Enterobacter* spp. (8%) isolated in Gram-negative bacilli (Fig. 3).

Out of 35 isolates of *S. aureus*, 29% (10 isolates) were methicillin resistance *S. aureus* while 71% (25 isolates) were

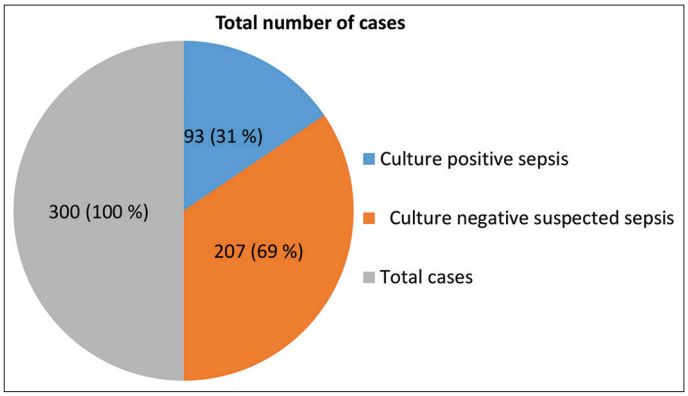

Figure 1: Distribution of clinical suspected adult septicemia patients in the study group. (n=300)

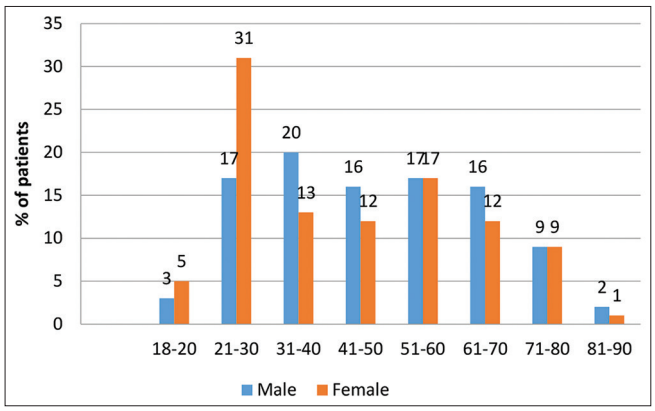

Figure 2: Gender and age-wise suspected adult septicemia patients in the study group. (n=300)

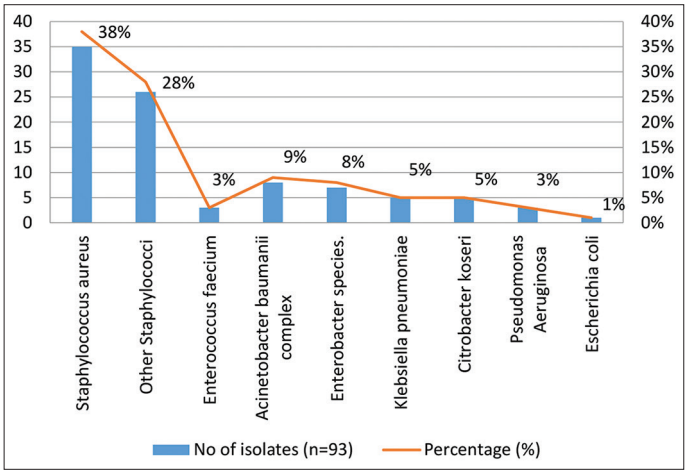

Figure 3: Analysis of microorganisms in clinically suspected adult septicemia patients (n=93)

methicillin-sensitive *S. aureus* in adult clinical suspected septicemia patients. Coagulase-negative Staphylococci/other Staphylococci were not further speciated so not considered for antimicrobial susceptibility testing. The antimicrobial susceptibility tests were carried out as per the latest standard CLSI guidelines. Teicoplanin (MIC breakpoint from 0.124 mg/L to 4 mg/L) and vancomycin (MIC breakpoint from 0.5 to 2 µg/mL) disk diffusion breakpoints were not recommended according to CLSI standards so the minimal inhibitory concentration determination from E-Strip method was done. Teicoplanin and vancomycin were tested from the E-strip method and MIC breakpoints were taken (Tables 1 and 2).

Among clinically suspected adult septicemia patients in this study, culture-positive sepsis patients have CRP levels ≥3.2 mg/dL (80%) and the majority (80%) of these patients have reactive procalcitonin (Fig. 4).

In the present study, among adult septicemia patients, Gram-positive bacteria have more prevalence (69%) than Gram-negative organisms. 7% (34 patients) have involved the respiratory system as a primary source of infections. Diabetes mellitus Type 2 (56%) has maximum comorbidity, followed by hypertension (42%) and renal failure (39%) as a risk factor in adult septicemia patients (Table 3).

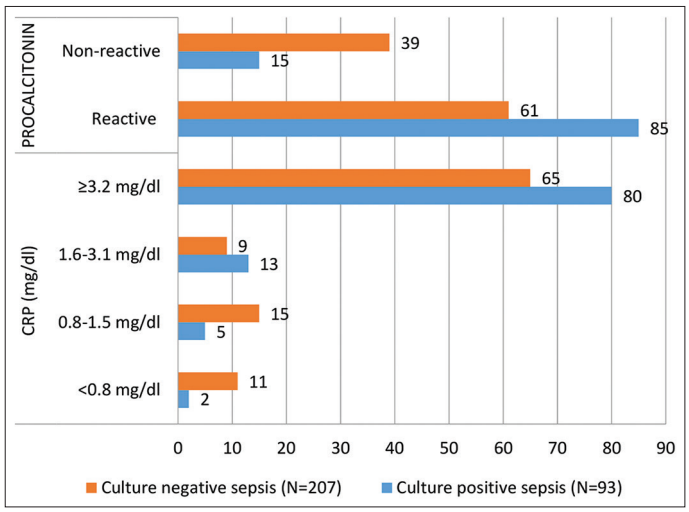

Figure 4: Correlation of C-reactive protein and procalcitonin among clinically suspected adult septicemia patients (n=300)

DISCUSSION

Bloodstream infections are a major challenge in medicine that causes substantial morbidity and mortality [15]. Changing patterns of the isolates, increasing rates of antimicrobial resistance, and wide application of new medical technologies like the usage of indwelling devices may change the epidemiology and outcome of BSIs [16]. Hence, it is important to continually review and update the epidemiology of BSIs mainly for the antibiotic susceptibility pattern of the common pathogens, so that it would be useful for the treatment of patients [17]. This study documents the prevalence, patient demographics, microbial profile, and outcome of sepsis with organ dysfunction in suspected adult septicemic patients of the medicine ward population in a tertiary care hospital.

In our study, culture positivity was found to be 31%. The rate of culture positivity in septicemia cases nearly similar to our study was reported in the study of Wasihun *et al.* [18] (28%) while not in concordance with the study by Khara and Lakhani [3] (49.03%), Kante *et al.* (17%) [19], and Gupta *et al.* (16.5%) [7]. In this study, the male and female ratio is (1.5:1). Similar to the present study, there was a preponderance of male patients in the studies conducted by Shah *et al.* [12], Kumar P *et al.*, [20] Jain *et al.* [21], and Nobandegani and Motamedifar *et al.* [22] Among adult patients, most common age group involved is 21–20 years followed by 31–40 years. The majority of isolates in our study were Gram-positive organisms (69%) as compared to Gram-negative organisms (31%). This finding is in agreement with the study done by Shah *et al.* [12], who reported a majority of Gram-positive organisms (73%) than Gram-negative organisms (26.9%). These findings can be correlated with the study conducted by Kabi *et al.* [23] and Dagnew *et al.* [6] in which they reported 73% and 69% of Gram-positive organisms, respectively.

In this study, Gram-positive organisms are 69% and Gram-negative organisms are 31% similar to a study done by Dagnew *et al.* [6] Among Gram-positive organisms, most common is *S. aureus* (38%) followed by Coagulase-negative *staphylococcus*

Table 1: Antimicrobial susceptibility patterns of Gram-positive organisms in the clinical suspected adult septicemia patients (*n*=38)

Antibiotics	*Staphylococcus aureus* n=35 (%)		*Enterococcus faecium* 03 (%)	
	S (%)	R (%)	S (%)	R (%)
Erythromycin (15µg)	09 (26)	26 (74)	-	03 (100)
Gentamicin (10 µg)	28 (80)	07 (20)	NR	NR
Teicoplanin (MIC)	35 (100)	-	03(100)	-
Tetracycline (10 µg)	30 (86)	05 (14)	-	03 (100)
Ciprofloxacin (5 µg)	09 (26)	26 (74)	-	03 (100)
Clindamycin (2 µg)	21 (60)	14 (40)	NR	NR
Chloramphenicol (30 µg)	30 (86)	05 (14)	NR	NR
Vancomycin (MIC)	35 (100)	-	03(100)	-
Linezolid (30 µg)	35 (100)	-	NR	NR
High level gentamicin (120 µg)	NR	NR	02 (67)	01 (33)

NR: Not recommended, S: Susceptible, R: Resistance

Table 2: Antimicrobial susceptibility patterns of Gram-negative organisms in the clinical suspected adult septicemia patients (n=26)

Antibiotics	*Acinetobacter baumannii* 7 (27%)		*Enterobacter species* 6 (23%)		*Klebsiella Pneumoniae* 5 (19%)		*Citrobacter koseri 4* (15%)		*Escherichia coli* 1 (4%)		*Pseudomonas aeruginosa* 3 (12%)	
	S	R	S	R	S	R	S	R	S	R	S	R
Ciprofloxacin (5 µg)	3 (43)	4 (57)	3 (50)	3 (50)	2 (40)	3 (60)	0	4 (100)	1 (100)	0	1 (33)	2 (67)
Ceftriaxone (30 µg)	4 (57)	3 (43)	2 (33)	4 (67)	3 (60)	2 (40)	2 (50)	2 (50)	1 (100)	0	2 (67)	1 (33)
Ceftazidime (30 µg)	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	1 (33)	2 (67)
Imipenem (10 µg)	5 (71)	2 (29)	1 (17)	5 (83)	2 (40)	3 (60)	1 (25)	3 (75)	0	1 (100)	2 (67)	1 (33)
Piperacillin+tazobactam (100/10 µg)	4 (57)	3 (43)	3 (50)	3 (50)	1 (20)	4 (80)	1 (25)	3 (75)	0	1 (100)	2 (67)	1 (33)
Aztreonam (30 µg)	6 (86)	1 (14)	2 (33)	4 (67)	4 (80)	1 (20)	3 (75)	1 (25)	0	1 (100)	1 (33)	2 (67)
Tobramycin (10 µg)	1 (14)	6 (86)	3(50)	3 (50)	2 (40)	3 (60)	2 (50)	2 (50)	1 (100)	0	2 (67)	1 (33)
Polymyxin B	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	3 (100)	0

NR: Not Recommended; S: Susceptible; R: Resistance

Table 3: Frequency of isolates, the primary source of infection, risk factor, and comorbidities among culture-proven cases (n=93)

Variable	Source of infection (%)	Comorbidities (more than 1) (%)
Gram-positive bacteria 64 (69%)	Respiratory system 34 (37)	Diabetes mellitus-II 52 (56)
Gram-negative bacteria 29 (31%)	Urinary tract 14 (15)	Hypertension 39 (42)
	Gastrointestinal tract 16 (17)	Renal failure 36 (39)
	Multiple site 11 (12)	COPD 32 (34)
	Skin & soft tissues 7 (8)	Hepatic failure 25 (27)
	Nervous system 6 (6)	Hematological malignancy 19 (20)
	Cardiovascular system 3 (3)	
	Unknown 2 (2)	
Variables		**Frequency (300)**
Clinical diagnosis	Sepsis	234 (78)
	Septic shock	66 (22)
Outcome	Death	84 (28)
	Discharge	216 (72)

(28%) and *Enterococcus faecium* (3%). This is in accordance with a study carried out earlier by Oral *et al*., [24] Ayobola *et al*., [25], and Mehta *et al*. [26] while in Gram-negative organisms, the most common is *Acinetobacter* spp. (9%) followed by *Enterobacter* spp. (8%) and *Klebsiella* spp. (5%), *Citrobacter* spp. (5%), *Pseudomonas* (3%), and *E. coli* (1%). Approximately the same prevalence was seen in a study conducted by Vendemiato *et al*. [27]. *S. aureus* is 100% susceptible to vancomycin, linezolid, and teicoplanin, while Gram-negative organisms are mostly susceptible to ciprofloxacin and tobramycin and resistant to imipenem and piperacillin + tazobactam in the present study.

It was observed that a large number of patients whose blood samples were positive showed a high prevalence of other comorbidities, like diabetes mellitus (56%), hypertension (42%), chronic kidney disease (39%), bronchial asthma/Chronic obstructive disease (34%), and Chronic renal failure (27%). The comparable results were also documented in another study done by Zahra *et al*. [28] and Mayr *et al*., [29] which showed that severe sepsis/septic shock is more likely in patients with a history of uncontrolled diabetes mellitus, chronic renal disease, and chronic liver disease.

Biomarkers are expected to provide better information about the presence of a relevant bacterial infection, its severity, and treatment response, with early and rapid recognition to provide high diagnostic accuracy. CRP and PCT as biomarkers fit many of these criteria and have depicted high diagnostic

accuracy for septic conditions of the patients [30]. Assessment of patients with sepsis must include proper use of CRP and PCT for early and specific diagnosis and treatment of patients [31]. The present study used a semi-automated system for the detection of CRP and immunochromatographic test for the semi-quantitative detection of PCT while most other studies used immunoluminometric method and were able to achieve high sensitivity and modest specificity. In this study among culture-positive sepsis patients, 80% of patients have CRP value >3.2 mg/dL and 85% have shown reactive PCT. Our study finding is comparable with the other studies done in different parts of India [32].

CONCLUSIONS

The Indian scenario of BSIs is very complicated and responsible for a higher rate of mortality. Etiology ranges from Gram-positive and Gram-negative spectrum of bacteria to candida species and shows varying drug resistance patterns. The combined efforts of infection control practitioners, microbiologists, and public health professionals are needed to limit the spread of MDR organisms. The selection of appropriate antibiotics for the treatment should be individualized to improve outcomes. Definitive culture results take at least 48–72 h or even more, resulting in treatment delay. However, the use of improved bacteriological techniques such as the BACT/ALERT blood culture system, which was used in this study, shows bacterial growth within 12–24 h. A sustainable antibiotic susceptibility surveillance program with infection control practices and rational antibiotic use will reduce infection rates and prolong the efficacy of available antimicrobials. Rapid identification results and antimicrobial susceptibility tests are essential for guiding clinicians in the selection of the most appropriate treatment for patients with bloodstream infections.

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Funding: Nil; Conflicts of Interest: None Stated.

How to cite this article: Nirmal K, Gupta P, Ahmad N, *et al*. Bacteriological profile and their antimicrobial susceptibility pattern among clinical suspected adult septicemia admitted patients: A study from tertiary care and teaching hospital. Eastern J Med Sci. 2024;9(1):4-9.