ORIGINAL ARTICLE

Bacteriological Profile of Sepsis and Their Antibiotic Susceptibility Pattern in Adult Patients in a Tertiary Care Hospital of Madhya Pradesh, India

Ruchi Agrawal[1], K P Ranjan[2]

Authors' affiliation: [1]PG student; [2]Asso. Prof., Dept. of Microbiology, Gajra Raja Medical College, Gwalior
Correspondence: Dr. K. P. Ranjan, Email: drkpranjan@gmail.com, Mobile No.: +91-9009021907

ABSTRACT

Introduction: Blood stream infections can lead to sepsis. Bacteria causing sepsis show multi-drug resistance which increases the morbidity and mortality in sepsis patients. The present study conducted to isolate the bacteria causing sepsis and their antibiotic susceptibility pattern in adult patients.

Material and methods: A total number of 296 blood samples of adult patients with sepsis were taken and processed as per standard protocol. Identification of bacteria was carried out according to the standard biochemical tests. Antimicrobial susceptibility test was carried out on Mueller Hinton agar plates by the Kirby-Bauer disk diffusion method, according to the Central Laboratory Standards Institute guidelines.

Results: Bacteria were isolated in 79 samples (26.69%). 54 isolates (68.35%) were gram negative bacilli and 25 isolates (31.65%) were gram positive cocci. Klebsiella spp. and Staphylococcus aureus were the predominant isolates among gram negative and gram positive bacteria respectively. Imipenem and linezolid were the most sensitive antibiotics for gram negative and gram positive bacteria respectively while ampicillin showed maximum resistance.

Conclusion: Gram negative bacilli were more common for causing sepsis in adults. Multi-drug resistance is shown by most of the causative bacteria and may be an important factor for high mortality in sepsis patients.

Key words: Sepsis, Bacteria, Antibiotic susceptibility testing

INTRODUCTION

Severe sepsis is one of the leading cause of death even in the developed nation among critically ill patients admitted in intensive care units (ICU) other than cardiac causes. Organism causing sepsis is an important determinant of the outcome.[1] Blood stream infections can lead to sepsis. Organisms causing such infections shows multi-drug resistance and are associated with high risk of death in these patients. [2]

Sepsis differs from bacteremia, which includes life-threatening organ dysfunction caused by dysregulated host response to infection. Organ dysfunction can be represented by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 points or more. Septic shock should be defined as a subset of sepsis in which profound circulatory, cellular, and metabolic abnormalities are present and are associated with higher hospital mortality rates.[3]

There is high variation in incidence of sepsis in ICU patients with in most countries about one out of every 10 ICU patients has severe sepsis. Variation in incidence is due to different availability of ICU resources and facilities. Incidence of sepsis in the US is about 240 cases per 100,000 populations with mortality rate ranging from 17.9% for sepsis to 28.6% for severe sepsis. In a study on patients admitted to European ICUs occurrence rate of severe sepsis was found to be 14.7% with about 50% mortality rate. Hospital mortality rate was much higher in infected patients (53.6%) than in non-infected patients (16.9%). In the first epidemiological study based on the ACCP/SCCM Consensus Conference criteria among the patients with systemic inflammatory response syndrome, 26% developed sepsis, 18% developed severe sepsis, and 4% developed septic shock with mortality rate associated with severe sepsis was 20%.[4] In a multicentre study conducted in four intensive therapy units in India the incidence of severe sepsis was found to be 16.45% of all admissions, with ICU mortality was 12.08% of all admissions and that of severe sepsis was 59.26%. Severe sepsis was more common in Indian ICUs with higher mortality rate as compared to western literature. [5]

Common sources of infection are the respiratory tract, genitourinary tract, intravascular line, intra abdominal and the skin but in some cases the focus of infection remained obscure. [6,7] Intra abdominal and respiratory sources of infection, increase the risk of

clinical progression to more severe stages of sepsis independently of SOFA and APACHE II scores. [8]

Bacteremia, viremia, fungemia all can lead to sepsis.[9] Various studies found different proportion of gram positive and gram negative bacteria as etiological agent for sepsis. Staphylococcus aureus , Coagulase negative Staphylococcus (CONS) and Streptococcus pneumoniae are the predominant gram positive bacteria while Escherichia coli, Enterobacter cloacae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter spp., Citrobacter spp. and Salmonella Typhi are the predominant gram negative bacteria isolated in blood cultures of sepsis patients.[7,10,11,12,13]

Blood culture currently represents the "gold standard" for diagnosis of septicemia. Various commercial sources are available for conventional manual systems and media. Both aerobic and anaerobic blood culture bottles are inoculated with blood and usually incubated for 7 days. Instrumented blood culture systems are also commercially available. [14] Various newer blood culture techniques for the diagnosis of blood stream infections and drug susceptibility testing includes API, BBL systems, BACTEC systems, BacT/Alert, BacT/Alert 3D, VITEK systems, and Versa TREK system. [15] Molecular methods like nucleic acid amplification assays, DNA microarrays, DNA sequencing approaches and Probe hybridization are newer tools for identification of microorganism and to select antibiotics. Still conventional blood culture methods are the dominant approach to isolate bacteria in sepsis patients.[16] The use of early and appropriate antibiotic therapy is essential to improve the survival rates in patients with severe sepsis and septic shock. Early antimicrobial therapy and supportive resuscitation measures are necessary to avoid the further cellular and organ dysfunction. [17,18]

Antibiotic susceptibility pattern of bacteria causing sepsis show multi-drug resistance which increases the morbidity and mortality in sepsis patients. [13,19,20] Inappropriate antibiotic selection is an important determinant of multi-drug resistance. [21] Colistin, imipenem, showed the highest sensitivity toward gram-negative isolates. [19] Maximum resistance was shown against ampicillin.[22] Most gram positive cocci show susceptibility for vancomycin and linezolid.[23] This study determines the bacterial isolates causing sepsis in adult patients and their antibiotic susceptibility pattern.

MATERIAL AND METHODS

The prospective study was conducted in Department of Microbiology, Gajra Raja Medical College and J.A. Group of Hospitals, Gwalior from 1st September 2017 to 30th June 2018. A total number of 296 blood samples of suspected sepsis adult patients were collected from different wards and critical care unit under strict aseptic conditions and transferred to blood culture bottles. Bottles were properly labelled and transported to bacteriology section of department of Microbiology, Gajra Raja Medical College with minimal delay and processed in the laboratory as per standard protocol by conventional method. Blood culture bottles were incubated overnight at 37°C then sub-cultured onto Blood agar and MacConkey agar to look for growth. Identification of isolated bacteria was done by gram staining and standard biochemical tests. Blood culture bottle which shows no sign of growth was further incubated at 37°C and subculture was done on 2, 4 and 7 day. After 7 days samples were reported as no growth. Antimicrobial susceptibility test was done by Kirby- Bauer disc diffusion method as per CLSI guidelines. [24]

RESULTS

Bacteria were isolated in 79 blood samples (26.69%). 54 isolates (68.35%) were gram negative bacilli and 25 isolates (31.65%) were gram positive cocci. Klebsiella spp. and Staphylococcus aureus were the predominant isolates among gram negative and gram positive bacteria respectively.

Table: 1. Distribution according to culture positivity in sepsis patients

Culture status	Patients (%)
Positive	79 (26.69)
Negative	217 (73.31)
Total	296 (100)

Table: 2. Gram positive cocci and Gram negative bacilli in total isolates of sepsis patients

Culture status	Positive blood culture (%)
Gram negative bacilli	54 (68.35)
Gram positive cocci	25 (31.65)
Total	79 (100)

Table: 3. Distribution of bacteria in total 79 isolates of sepsis patients

Bacterial Isolates	Isolates (%)
Gram negative bacteria	
Escherichia coli	13 (16.45)
Klebsiella pneumoniae	12 (15.19)
Klebsiella oxytoca	6 (7.6)
Citrobacter koseri	4 (5.06)
Citrobacter freundii	1 (1.27)
Enterobacter aerogenes	3 (3.8)
Pseudomonas aeruginosa	6 (7.59)
Acinetobacter baumannii	8 (10.13)
Acinetobacter lwoffii	1 (1.27)
Gram positive bacteria	
Staphylococcus aureus	19 (24.05)
Coagulase negative staphylococcus	6 (7.59)

Table: 4. Antibiotic susceptibility pattern of gram negative isolates of sepsis patients

Antibiotics	Percentage of sensitive strains								
	Escherichia coli (n=13)	Klebsiella pneumonia (n=12)	Klebsiella oxytoca (n=06)	Citrobacter koseri (n=04)	Citrobacter freundii (n=01)	Enterobacter aero-genes (n=03)	Pseudomonas aeru-ginosa (n=06)	Acinetobacter bau-manii (n=08)	Acinetobacter lwoffii (n=01)
Amikacin	69.23	41.67	66.67	75	100	33.33	50	50	100
Ampicillin	15.38	08.33	00	25	00	00	16.67	12.50	00
Cefepime	53.85	33.33	33.33	25	00	33.33	50	37.50	00
Ciprofloxacin	23.08	50	50	50	100	66.67	33.33	12.50	00
Ceftriaxone	30.77	16.67	16.67	50	00	00	16.67	25	00
Cefoperazone + Sulbactam	38.46	33.33	50	50	100	66.67	33.33	50	100
Ceftazidime	38.46	41.67	50	50	00	33.33	16.67	25	00
Doxycycline	76.92	66.67	66.67	75	100	66.67	33.33	50	100
Gentamicin	46.15	33.33	50	50	00	33.33	33.33	25	00
Levofloxacin	46.15	41.67	50	50	100	66.67	50	25	00
Piperacillin + Tazobactam	46.15	33.33	50	75	100	66.67	50	37.50	100
Imipenem	92.31	75	100	75	100	100	83.33	100	100

Table: 5. Antibiotic susceptibility pattern of gram positive isolates of sepsis patients

Antibiotics	Percentage of sensitive strains	
	Staphylococcus aureus (n=19)	Coagulase negative staphylococcus(n=06)
Ampicillin	10.53	16.67
Amoxycillin + clavulanic acd	42.11	50
Azithromycin	36.84	33.33
Ceftriaxone	31.58	33.33
Cefotaxime	42.11	50
Ciprofloxacin	57.89	50
Doxycycline	68.42	83.33
Erythromycin	36.84	33.33
Gentamicin	63.16	83.33
Levofloxacin	63.16	66.67
Linezolid	94.74	100
Vancomycin	89.47	100

Imipenem and linezolid were the most sensitive antibiotics for gram negative and gram positive bacteria respectively while ampicillin showed maximum resistance.

DISCUSSION

In our study, culture positivity was found to be 26.69%. The rate of culture positivity in septicemia cases nearly similar to our study were reported in the study of Wasihun et al [12] (28%) and Sahoo et al [22] (26%) while not in concordance with the study by Khara et al [25] (49.03%), Kante et al [11] (17%) and Gupta et al [19] (16.5%).

In our study, gram-negative bacteria were found to be 68.35% of total isolates, whereas the gram- positive bacteria were 31.65% which is similar to studies of Kante et al [11] (gram-negative bacteria 67.64% and gram- positive bacteria 32.36%) and Sahoo et al [22] (gram-negative bacteria 69.2% and gram- positive bacteria 30.8%) while in contrast to study of Dagnew et al [26] (gram-negative bacteria 31% and gram-positive bacteria 69%).

In present study, among gram-negative bacteria Klebseilla spp. 22.79% was the most frequent gram-negative isolate followed by E. coli 16.45% to studies of Khara et al[25] (Klebseilla spp. 22.38%, E. coli 11.19%). Sweta et al[23], Jadhav et al26, Sonawane J et al[28] also found Klebseilla spp. as the most frequent gram-negative isolate. Sahoo et al[22] and Gupta et al[19] found E. coli while Kante et al[11] found Pseudomonas aeruginosa as the most frequent gram-negative isolates from blood culture samples.

The predominant gram positive isolate in our study was S. aureus 24.05%, similar to studies of Gupta et al[19], Jadhav et al[27] , Rajeevan et al[29] , Khara et al[25] while studies of Mohanty et al[30] and Sweta et al[23] found CONS as the most common gram positive isolate from blood culture specimens of sepsis patients.

As multi drug resistance is emerging problem and an important determinant of high mortality in sepsis patients, it is essential to find out susceptibility pattern of bacteria for particular antibiotic so that sepsis can be managed effectively by eliminating the causative bacteria to reduce morbidity and prevent mortality.

In our study when isolates were tested for antimicrobial susceptibility pattern, it was seen that, Klebsiella pneumoniae was highly sensitive for imipenem (75%), followed by doxycycline (66.67%). 41.67% for levofloxacin and least sensitivity for ampicillin

(08.33%). Klebsiella oxytoca showed highest sensitivity for imipenem (100%), 50% sensitivity for levofloxacin, while 100 % resistance for ampicillin.

Study by Radha Rani et al[31] showed that sensitivity to imipenem and meropenem in Klebsiella spp. was 53.84% and 56.41% respectively. Study of Sweta et al[23] showed that Klebsiella pneumonia was least resistant for levofloxacin (10%) and imipenem (37 %) while 100% resistant for amoxicillin, amoxicillin + clavulanic acid. So, present study showed higher sensitivity for imipenem in Klebsiella spp. than both the studies while lower sensitivity for levofloxacin than study of Sweta et al.

In the present study Escherichia coli showed 92.31% sensitivity to imipenem, amikacin 69.23%, cefepime 53.85% and levofloxacin 46.15%. Only 15.38% of isolates were sensitive to ampicillin. In the study by Radha Rani et al[31] E. coli isolates showed 83.60%, 67.21% and 65.57% sensitivity for amikacin, meropenem and imipenem respectively. Only 18.03% of isolates were sensitive to cefepime and 13.11% were sensitive to levofloxacin. So present study showed comparable sensitivity for amikacin while higher sensitivity for imipenem, cefepime and levofloxacin in Escherichia coli than study of Radha Rani et al.

In the present study it was observed that Pseudomonas aeruginosa showed maximum sensitivity for imipenem (83.33%) followed by 50% sensitivity for amikacin, cefepime and piperacillin + tazobactam combination. Low level of sensitivity of 33.33% was observed for ciprofloxacin. P. aeruginosa showed least sensitivity against ampicillin, ceftriaxone and ceftazidime (16.67%). Study of Kante et al[11] showed Pseudomonas spp. was highly sensitive to ciprofloxacin (100%), piperacillin (80%), ceftazidime (80%) while 50% sensitivity for imipenem, 30% for amikacin and 20% for cefepime. So present study showed higher sensitivity for imipenem, amikacin and cefepime in Pseudomonas aeruginosa while lower sensitivity for other drugs than study of Kante et al.

In the present study Acinetobacter baumannii showed highest sensitivity to imipenem (100%), 37.50% sensitivity with cefepime, 25% sensitivity to ceftazidime while least sensitivity of 12.50% with ampicillin and ciprofloxacin. Acinetobacter lwoffii showed 100% sensitivity to imipenem, while 100% resistance for ampicillin, cefepime, ciprofloxacin and ceftazidime. Study of Kante et al [11] showed Acinetobacter spp. was highly sensitive to ciprofloxacin (100%) followed by ceftazidime (60%), cefepime (38.3%), imipenem (10%). So in Acinetobacter spp. sensitivity for cefepime was comparable while contrasting for ciprofloxacin, ceftazidime and imipenem with the present study. But as only one isolate of A. lwoffi was found in present study actual sensitivity pattern is difficult to determine.

Gupta et al[19] study showed among Enterobacteriaceae high degree of resistance observed to amoxicillin + clavulanic acid combination, second and third generation cephalosporins. Among cephalosporins, cefoperazone + sulbactam combination was found to be highly sensitive for Enterobacteriaceae (81.14%). So resistance pattern for cephalosporins was comparable with present study.

In the present study Staphylococcus aureus showed maximum sensitivity of 94.74% for linezolid and vancomycin is 89.47% sensitive while showed minimal sensitivity for ampicillin (10.53%). Similar results were seen in study of Sweta et al[23] showed Staphylococcus aureus showed high resistance towards ampicillin. All gram positive cocci isolates were susceptible to vancomycin, linezolid and cefoperazone + sulbactam.

Present study showed that coagulase negative staphylococcus was 100% sensitive to linezolid and vancomycin while showed 33.33% sensitivity for erythromycin. Ampicillin was found to be the least sensitive antibiotic. Mohanty et al[30] showed CoNS exhibited high levels of resistance to penicillin and erythromycin while all gram positive bacteria were susceptible to vancomycin which is similar to our study.

Resistance for multiple antibiotics was observed in all the bacteria isolated from blood culture of sepsis patients in the present study. While on comparison of antibiotic susceptibility pattern with various studies it is found that susceptibility pattern though show similarity for some antibiotics, also show wide difference for others. It also varied with the bacteria isolated.

CONCLUSION

Gram negative bacilli were more common for causing sepsis in adults. As the culture positivity is quite less by conventional method, other methods for isolation of bacteria should be considered for assessing the etiology. Multi-drug resistance is shown by most of the causative bacteria and may be an important factor for high mortality in sepsis patients. Selection of antibiotics for the treatment of sepsis patients should be individualized to improve outcome.

REFERENCES

1. Mayr FB, Yende S et al. Epidemiology of severe sepsis, Virulence 2014; 5:1, 4–11
2. Gohel K, Jojera A. et al. Bacteriological Profile and Drug Resistance Patterns of Blood Culture Isolates in a Tertiary Care Nephrourology :Teaching Institute, BioMed Research International, 2014; 5.
3. Singer M, Deutschman CS et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) JAMA. 2016; 315(8):801-810

4. Angus DC, Pereira CAP et al. Epidemiology of Severe Sepsis Around the World, Endocrine, Metabolic & Immune Disorders - Drug Targets, 2006, 6, 7-16
5. Todi S, Chatterjee S et al. Epidemiology of severe sepsis in India: an update,Crit Care. 2010; 14(1): P382.
6. Seifert H, The Clinical Importance of Microbiological Findings in the Diagnosis and Management of Bloodstream Infections, Clinical Infectious Diseases 2009; 48: S238–45.
7. Crowe M, Ispahani P. et al. Bacteraemia in the adult intensive care unit of a teaching hospital in Nottingham, UK, European Journal of Clinical Microbiology and Infectious Diseases, 1998; 17 (6): 377–384.
8. Luz León A, Andrea Hoyos N. et al. Clinical course of sepsis, severe sepsis, and septic shock in a cohort of infected patients from ten Colombian hospitals, BMC Infectious Diseases , 2013; 13: 345-9.
9. Pal N, Sujatha R. Microbiological Profile and Antimicrobial Resistant Pattern of Blood Culture Isolates, Among Septicaemia Suspected Patients, National Journal of Laboratory Medicine, 2016; Vol 5(1): 17-21.
10. Tak V, Mathur P. et al. Staphylococcal blood stream infections: Epidemiology, resistance pattern and outcome at a level 1 Indian trauma care center. J Lab Physicians. 2013; 5: 46–50.
11. Kante M, Uma P. et al. Bacterial profile of blood stream infections and antibiotic susceptibility pattern of isolates, Int. J. Curr. Microbiol. App. Sci 2014; 3(12): 222-233.
12. Wasihun AG, Wlekidan LN. et al. Bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates among febrile patients in Mekelle Hospital, Northern Ethiopia, Springer Plus,2015; 4:314.
13. Kumalo A, Kassa T. et al. Bacterial Profile of Adult Sepsis and their Antimicrobial Susceptibility Pattern at Jimma University Specialized Hospital, South West Ethiopia, Health Science Journal, 2016; 10 (2) 3: 1-8.
14. Weinstein MP. Current Blood Culture Methods and Systems: Clinical Concepts, Technology, and Interpretation of Results, Clinical Infectious Diseases, 1996; 23: 40-6.
15. Dilnessa T, Demeke G. et al. Emerging Blood Culture Technologies for Isolation of Blood Pathogens at Clinical Microbiology Laboratories. J Med Microb Diagn, 2016; 5 (2): 1-7.
16. Kirn TJ, Weinstein MP. Update on blood cultures: how to obtain, process, report, and interpret, Clinical Microbiology and Infection, 2013; 19 (6): 513-520.
17. Martin-Loeches I, Levy MM. et al. Management of severe sepsis: advances, challenges, and current status, Drug Design, Development and Therapy, 2015: 9: 2079–2088.
18. Vincent JL, José Pereira A. et al. Early management of sepsis, Clin Exp Emerg Med 2014; 1(1): 3-7.
19. 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; 19:94-9.
20. Inglis TJJ, Urosevic N. Where sepsis and Antimicrobial resistance countermeasures converge, Frontiers in Public Health, 2017; 5 (6) : 1-6.
21. Zilberberg MD, Shorr AF. et al. Multi-drug resistance, inappropriate initial antibiotic therapy and mortality in Gram-negative severe sepsis and septic shock: a retrospective cohort study, Critical Care, 2014;18:596-13.
22. Sahoo D, Mohanty L. et al. Bacteriological analysis of blood culture isolates in patients with sepsis in a tertiary care hospital of eastern India, International Journal of Contemporary Medical Research, 2016; 3(12): 3448-3450.
23. Sweta S.Oza, Mehta SJ. et al. Bacteriological profile and antibiogram of blood culture isolates from patients of rural tertiary care hospital, International Journal of Microbiology and Mycology, 2016; 4 (3): 1-7.
24. CLSI. Performance standards for antimicrobial disc susceptibility tests: approved standard, 13th ed., CLSI standard M02. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
25. Khara R, Lakhani SJ. Bacteriological Profile of Blood Culture from Adult Sepsis Patients from a Rural Based Tertiary Care and Teaching Hospital, Piparia, Vadodara, India, Int. J. Curr. Microbiol. App. Sci 2018; 7(5): 3173-3182.
26. Dagnew M, Yismaw G. et al. Bacterial profile and antimicrobial susceptibility pattern in septicemia suspected patients attending Gondar University Hospital, Northwest Ethiopia, BMC Research Notes 2013, 6:283.
27. Jadhav S, Gandham N. et al. Bacteriological Profile of Septicaemia and Antimicrobial Susceptibility of Isolates from Tertiary Care Hospital in India, Research Journal of Pharmaceutical, Biological and Chemical Sciences, 2012; 3 (4): 1100-1108.
28. Sonawane J, Kamnath N. et al. Bacteriological Profile and Antimicrobial Susceptibility Testing of Blood Culture Isolates from a Tertiary Care Hospital, Navi Mumbai. JMSCR; 2016; 4 (10): 13116-13124.
29. Rajeevan S, Mustaq Ahmad S. et al.Study of prevalence and antimicrobial susceptibility pattern in blood isolates from a tertiary care hospital in North Kerala, India, Int.J.Curr.Microbiol.App.Sci, 2014; 3(4): 655-662.
30. Mohanty A, Singh TS, Bacteriological Profile and Antibiotic Sensitivity Pattern of Hospital-Acquired Septicemia in a Tertiary Care Hospital In North East India, Asian J Pharm Clin Res, 2017; 10 (11) : 186-189.
31. Radha Rani D, Sridevi Chaitanya B. et al. Retrospective Analysis of Blood Stream Infections and Antibiotic Susceptibility Pattern of Gram Negative Bacteria in a Tertiary Care Cancer Hospital, International Journal of Medical Research &Health Sciences, 2017, 6(12): 19-26.