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BACTERIOLOGICAL PROFILE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BLOOD CULTURE ISOLATES FROM ADULT SEPSIS PATIENTS AT A TERTIARY CARE HOSPITAL

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Abstract

Background: Bacteria causing sepsis show multi drug resistance which increases morbidity and mortality in sepsis patient. The present study was conducted to identify the organisms causing sepsis in adult patients and their antibiogram. **Material and methods:** A total number of 390 blood samples of adult patients with sepsis were processed by using standard microbiological procedures. Identification of organisms was carried out by using standard biochemical test and antibiotic susceptibility testing of all isolates was performed by Kirby Bauer's disc diffusion method on Mueller Hinton agar. Antibiotic sensitivity results were interpreted as per CLSI guideline. **Results:** Total 390 blood samples were processed in which 87(22.30%) were culture positive isolates. Out of 87 culture positive isolates, 23(26.43%) were Gram positive isolates and 60(68.96%) were Gram negative isolates. *Escherichia coli* and *Staphylococcus aureus* were predominant bacterial isolates among gram negative and gram positive bacteria respectively. *Staphylococcus aureus* showed maximum susceptibility to Linezolid 90% and Vancomycin 89%. *E. coli* isolates were susceptible to Amikacin (89%) and Cefoperazone (62.96%).

Key words: Sepsis, Multi drug resistance, Antibiotic susceptibility testing

Introduction

The term "bacteraemia" refers to the presence of bacteria in blood that have not multiplied. When Bacteria circulate and actively proliferate in the circulation then the condition is called as septicaemia. [1] Sepsis differs from bacteraemia, which includes life-threatening organ dysfunction caused by dysregulated host response to infection. [2] Septicaemia is clinical

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syndrome characterized by fever, chills, malaise, hyperventilation and toxicity. Severe sepsis continues to rank among the leading causes of morbidity and death globally. [3]

The diagnosis of sepsis is based on presence of suspected infection and clinical or microbiological evidence of infection in the presence of at least two of the four systemic inflammatory response criteria (SIRS).[4]

Blood stream infections and sepsis can be initiated by any microorganism, like bacterial, fungal, viral, parasitic, or by microbial products/ toxins and then propagated by a complex network of inflammatory mediators and cellular dysfunction. Multi-drug resistant organism infections are more likely to require more expensive antibiotics for treatment, lengthen hospital stays, and increase mortality rates.

Blood culture is a gold standard for diagnosis. The detection of microorganisms in a patient's blood not only has great diagnostic and prognostic significance, but also provides essential information for the evaluation of a variety of diseases like endocarditis, pneumonia, pyrexia of unknown origin and others. [5] Isolation of the pathogens and determination of antimicrobial susceptibility pattern of the isolates from blood culture remain the main stay of definitive diagnosis and management of Septicaemia. [6] The use of early and appropriate antibiotic therapy is essential to improve the survival rates in patient with the severe sepsis and septic shock. [2] Appropriate antibiotic selection is an important determinant of multidrug resistance.

In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicemia, right choice of empiric therapy is of importance.

Therefore, the present study is used to analyze the various organisms causing adult sepsis and their antibiotic susceptibility patterns, as it will be a useful guide to formulate effective empirical treatment.

Materials and Methods

The study was carried out in the Department of Microbiology, B.K.L. Walawalkar Rural medical college and hospital, Sawarde. during the period of January 2018 -December 2020.

Total 390 blood culture samples from patients of Sepsis were collected and processed according to the standard Microbiological procedures. Blood was collected from patients admitted in the hospital who presented with signs and symptoms of sepsis.

All patients older than 18 years with signs and symptoms of sepsis were included in study

Blood samples collected from suspected neonatal sepsis cases were excluded in this study.

Specimen Collection- The specimen must be collected using sterile techniques to reduce the chances of contamination. The recommended specimen volume is 8-10ml. In order to facilitate accurate interpretation of a positive blood culture, a minimum of 2 blood cultures drawn from different sites should be collected whenever possible. Blood was collected as soon as possible before administration of antibiotics.

All the samples were inoculated into BACTEC bottles using sterile techniques and processed in BACTEC 9050 instrument.

Principle- If micro- organisms are present in the test sample inoculated in to the BD BACTEC vial, CO2 Will be produced when the organism metabolize the substrates present in the vial. Increases in the fluorescence of the vial sensor caused by the higher amount of Co2

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are monitored by the BD BACTEC fluorescent series instrument. Analysis of the rate and amount of Co2 increase enable the BD BACTEC fluorescent series instrument to determine if the vial is positive. i.e. that the test sample contains viable organisms.

When BACTEC 9050 detected fluorescence, that specimens were cultured on to the MacConkey agar, blood agar plates(Himedia) and Saboraud‟s Dextrose Agar (SDA) .Culture plates incubated aerobically for 24hrs. overnight at 37°C.

The culture plates were examined for bacterial growth and identified using standard microbiological techniques by using Motility, catalase test, oxidase test and standard biochemical tests. Antibiotic susceptibility testing for isolates were performed by Kirby Bauer's disc diffusion method on Mueller Hinton agar. Antibiotic sensitivity results were interpreted as per CLSI guideline. [7]

Statistical analysis: Data was tabulated and analysed by using Microsoft excel

Observations and Results

Table 1: Culture Positivity from blood culture specimens

Culture	Frequency	Percentage(%)
Growth	87	22.30
No Growth	303	77.69
Total	390	99.99

Table 2: Distribution of aerobic bacterial isolates from adult sepsis patients

Sr.No	Organism	Frequency	Percentage (%)
1	Escherichia coli	27	31.03
2	Staphylococcus aureus	17	19.54
3	Klebsiella species	13	14.94
4	Acinetobacter species	11	12.64
5	CONS (Coagulase negative Staphylococcus)	6	6.89
6	Enterobacter species	3	3.44
7	Pseudomonas aeruginosa	2	2.29
8	Salmonella typhi	2	2.29
9	Salmonella species	2	2.29
10	Candida species	4	4.59
Total		87	100

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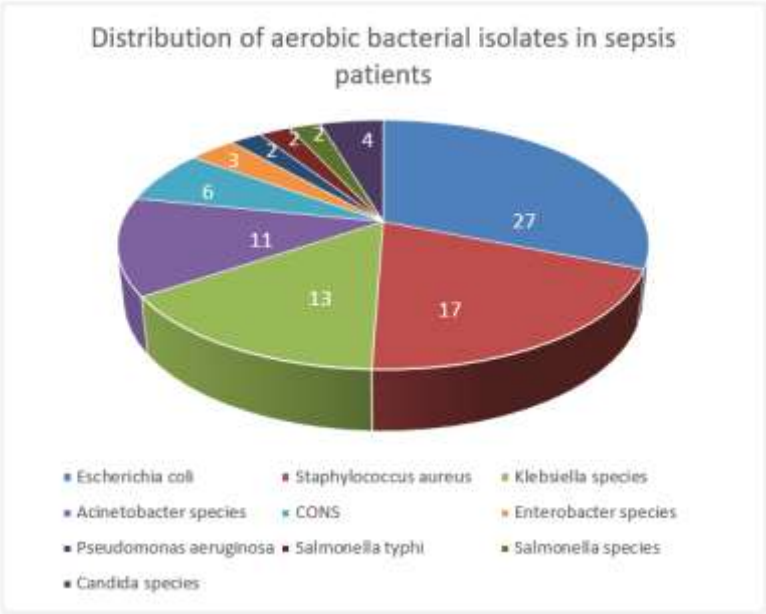
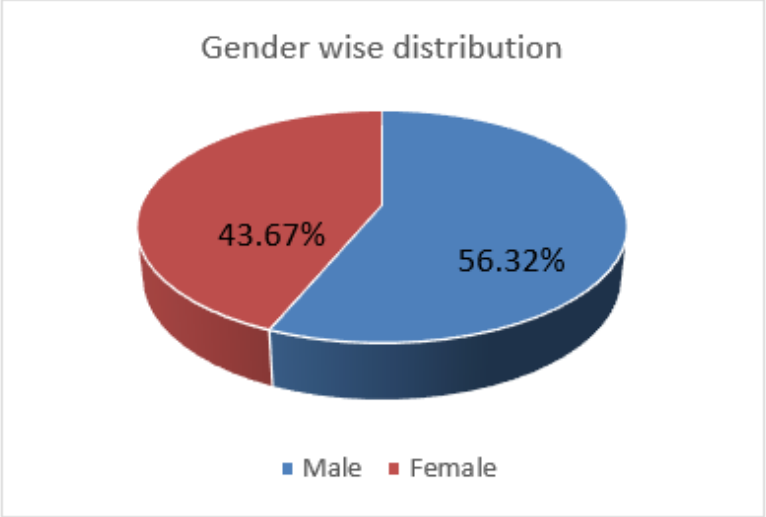


Graph 1: Distribution of aerobic bacterial isolates from sepsis patients

Table 3: Distribution of Gram positive and Gram negative isolates from sepsis patients.

Sr.No.	Isolates	Frequency	Percentage(%)
1.	Gram positive isolates	23	26.43
2.	Gram negative isolates	60	68.96
3.	Candida species	04	4.59



Graph 2: Gender wise distribution of adult patients with sepsis

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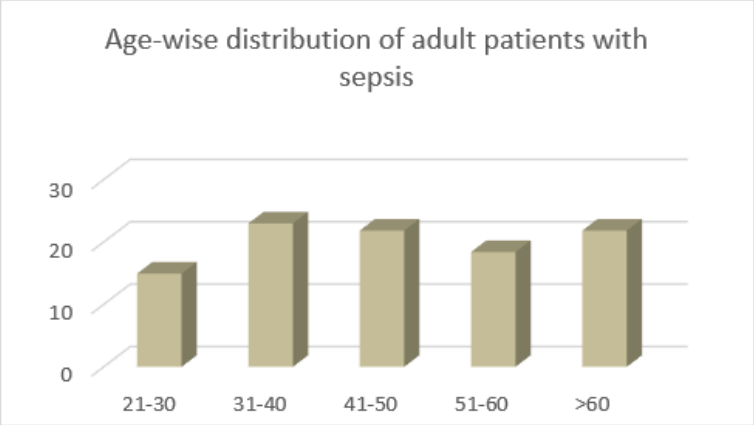
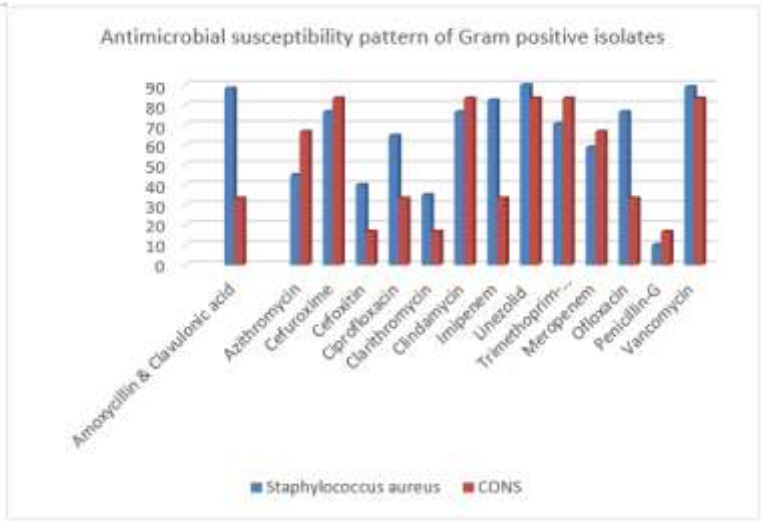
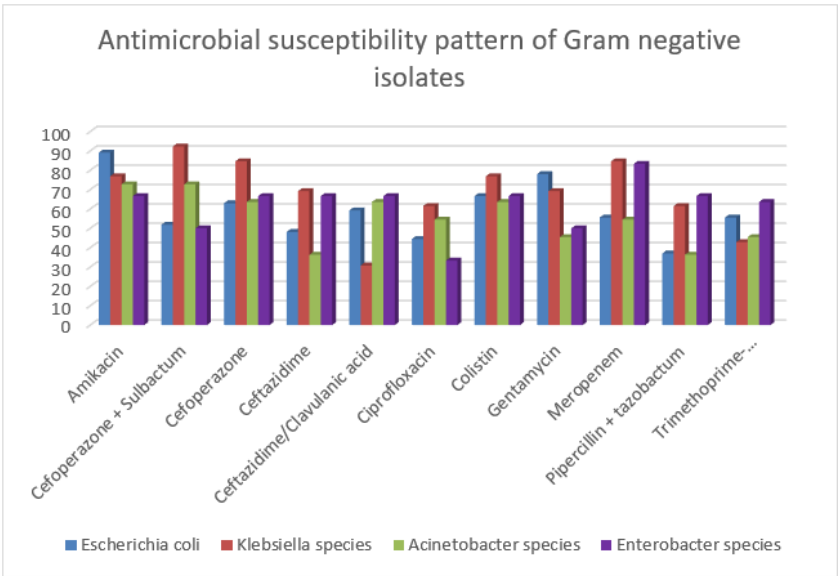


Graph 3: Age (in yrs)- wise distribution of adult patients with sepsis



Graph 4: Antimicrobial susceptibility pattern of Gram positive isolates from sepsis patients



Graph 5: Antimicrobial susceptibility pattern of Gram negative isolates from sepsis patients

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Discussion

Total 390 blood samples from adult sepsis patients were collected and processed according to the standard Microbiological procedures.

In this study out of 390 blood samples, 87(22.30%) were culture positive isolates and 303(77.69%) were culture negative isolates. (Table 1)

Out of 390 blood samples examined ,22.30% blood culture samples showed pure growth of organisms indicating culture proven cases of Sepsis.

Among 87 culture positive samples, *Escherichia coli* was predominant bacterial isolates 27 (31.03 %) followed by *Staphylococcus aureus*17(19.54%), *Klebsiella species* 13(14.94%), Acinetobacter species 11(12.64%), CONS 6(6.89%), *Enterobacter species* 3(3.44%). *Pseudomonas aeruginosa* 02(2.29%), and *Salmonella typhi* 02(2.29%), *Salmonella species* 02(2.29%), *and Candida species* 4(4.59%). (Table 2)

Out of 87 culture positive isolates, 23(26.43%) were Gram positive isolates and 60(68.96%) were Gram negative isolates. (Table 3)

Our results are matched with study done at Sahoo D *et al.* [8] in which they noted Culture positivity of Gram negative isolates was 69.23% while Gram positive isolates was 30.76%

However, our results are in agreement with Ruchi Agrawal e al. [9] who reported culture positive isolates of Gram negative bacilli 68.35% and Gram positive cocci was 31.65%.

Our findings are not matched with Dagnew M et.al. [10] in which they found culture positivity of Gram-positive organisms were (69%) and Gram-negative organisms were (31%).

Our results are not in agreement with Meghna Palewar et.al in which they reported incidence of positivity for gram-negative *Enterobacteriaceae*, Gram-negative non-fermenters and Gram-positive cocci were 38%, 31%, and 31%, respectively [11].

In our study, male predominance was seen 56.32% as compared to females 43.67%. (Graph2)

Similar to present study, there was a preponderance of male patients in the studies conducted by Praneetha Jain *et al.* [12]., Aroop Mohanty *et al.*, [13] Age group 31–40 was found to be 22.98% which was comparable with Nikita Vasudeva *et al.* 20.7%. [14] (Graph 3)

Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates showed sensitivity towards Amoxicillin and clavulanic acid (88.23%) followed by Cefuroxime (76.47%), Ciprofloxacin (64.70%), Clindamycin (76.47%), Imipenem (82.35%), Linezolid (90%), Trimethoprim-sulphamethaxazole (70.58%) ,Ofloxacin(76.47%) and Vancomycin (89%). (Graph 4)

The resistance pattern amongst antimicrobial agents used against *Staphylococcus aureus* isolates was Penicillin (90%) Cefoxitin (60%), Clarithromycin (65%) and Azithromycin (55%) in decreasing order of resistance

Staphylococcus aureus showed maximum susceptibility to Linezolid 90% and Vancomycin 89%. These findings are similar to study done by Rohit Tiwari *et al.* [15] in which they detected maximum susceptibility to Linezolid 85% and Vancomycin 92.8%.

Coagulase negative Staphylococcus showed sensitivity towards Cefuroxime and Clindamycin was 83.33%followed by Linezolid (66.66%), Meropenem (66.66%) and Amoxicillin and Clavulanic acid (33.33%)

Coagulase negative Staphylococcus showed maximum susceptibility to Vancomycin (83.33%) and Linezolid (83.33%)

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In our study, *E. coli* isolates were susceptible to Amikacin (89%), Cefoperazone (62.96), Colistin (66.66%), Gentamycin (78.03%) and Meropenem (55.55%). (Graph 5)
Resistance pattern of *E. coli* showed resistance towards Piperacillin& tazobactum (62.97%), Ciprofloxacin (55.56%), Ceftazidime (51.86%), Cefoperazone and Sulbactum (48.15%), to Ceftazidime & clavulanic acid (40.75%), Trimethoprim – Sulphamethaxazole (44.45%)
Klebsiella species were 92.30% sensitive to Cefoperazone and Sulbactum followed by Cefoperazone (84.61%), Meropenem (84.61%), Amikacin (76.92 %), Colistin (76.92%), Ceftazidime (69.23%) and Ciprofloxacin (61.53%) (Table 5)
Similar finding was seen in study done by Asmita Patil *et al.* [16] in which 66.66% susceptibility to Ceftazidime was observed.
Klebsiella species showed resistance towards Ceftazidime & clavulanic acid was (69.24%), Trimethoprim – Sulphamethaxazole (57.15%)
Acinetobacter species were 72.72% sensitive to amikacin and Cefoperazone & Sulbactum, Followed by Colistin and Cefoperazone (63.63%), Ceftazidime & clavulanic acid (63.63%)
Antimicrobial resistance pattern of Acinetobacter species showed resistance towards Ceftazidime and Piperacillin& tazobactum (63.64%) followed by Trimethoprim – Sulphamethaxazole and Gentamycin (54.55%) and Meropenem (45.46%) (Graph5)
Pseudomonas aeruginosa (2) were 100% sensitive to Cefoperazone, Cefoperazone & Sulbactum, Trimethoprim – Sulphamethaxazole and Gentamycin ,50% sensitive to Ceftazidime &clavulanic acid, Meropenem, Piperacillin& tazobactum and polymyxin B. Salmonella typhi (2) and Salmonella species (2) showed sensitivity towards Ciprofloxacin (100%), Meropenem (100%), Amikacin (50%), Gentamycin (50%) and Trimethoprim – Sulphamethaxazole (50%)
In this study, Candida species were 4.59% which is not matched with study done by Radhika Khara *et al.* in which 10.02% candida species isolated. [17]
In present study, we found that most of the gram negative isolates showed resistance against Piperacillin -tazobactum, Ciprofloxacin, Ceftazidime, Ceftazidime and clavulanic acid.
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.

Conclusion

The study detected 87(22.30%) culture positive isolates among 390 blood samples from in adult patients with sepsis. Out of 87 culture positive isolates, 23(26.43%) were Gram positive isolates and 60(68.96%) were Gram negative isolates. *Staphylococcus aureus* showed maximum susceptibility to Linezolid 90% and Vancomycin 89%. E*scherichia coli* was more common bacterial isolate among gram negative organisms.
Most of the gram negative isolates showed resistance against Piperacillin-tazobactum, Ciprofloxacin, Ceftazidime, Ceftazidime and clavulanic acid. Therefore, we suggest continuous monitoring & surveillance is needed due to transient changes in organisms and their antimicrobial susceptibility pattern in sepsis cases. The increase in the prevalence of multi drug resistance bacteria emphasize the urgent need for rational use of antibiotics, formulation of antibiotic policy, and implementation of infection control practices for the

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effective management. This antibiogram of isolate will improve the therapeutic outcome and prevent antibiotic resistance.

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