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Bacteriological Profile of Sepsis and Their Antibiogram in Adult Patients in A Tertiary Care Center

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ABSTRACT

Bacteria causing sepsis show multi-drug resistance which increases the morbidity and mortality in sepsis patients. Antibiotic selection is an important determinant of multi-drug resistance. Material and Methods Patients with septicemia were included in a cross-sectional observational study for 2 years to analyze their bacteriological profile and antibiogram pattern.

Result: Total of 130 blood culture positive samples were included in the study. Out of 130 isolates, 73% were Gram-positive cocci and 26.9% were Gram negative bacilli, predominantly Gram-positive cocci were isolated in our study. Staphylococcus aureus 60% was most frequent isolate from blood culture, followed by 11.5% of coagulase negative Staphylococcus and only 1.5% isolates were Enterococcus spp. In Gram Negative isolates, most frequent isolate was klebsiella pneumoniae 10% followed by E. coli 8.4%, Pseudomonas aeruginosa 4% and Acinetobacter species 5%. Staphylococcus aureus showed maximum susceptibility to Linezolid 85% and Vancomycin 76%. Out of 78 Staphylococcus aureus, 67% were MRSA. Klebsiella pneumoniae showed maximum susceptibility to Amikacin 92.3% and Cefoperazone 84.6%. Phenotypic tests for gram positive and gram negative organisms were performed such as MRSA, ESBL and MBL Out of 35 Gram negative organisms, 63% showed ESBL production and 60% organisms showed MBL production.

Conclusion: In this study, we aim to highlight the fact that Gram-positive organisms, particularly Staphylococcus aureus, are the most common cause of hospital-acquired septicaemia and are sensitive to Linezolid and Vancomycin. This suggests that these drugs may be effective as a treatment.

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Keywords: *Sepsis, Antibiogram, Multi drug resistance, MRSA, ESBL, MBL*

INTRODUCTION

Sepsis is leading cause of death among critically ill patients admitted in intensive care unit (ICU) other than cardiac causes. Bloodstream infection can lead to sepsis. Multidrug resistant organisms can cause high risk of death. Sepsis differs from bacteremia, which includes life-threatening organ dysfunction caused by dysregulated host response to infection.[1] Septicemia is clinical syndrome characterized by fever, chills, malaise, hyperventilation and toxicity. Current guidelines recommended starting antibiotic therapy within one hour of identification of septic shock. Infection leading to sepsis are usually bacterial, but maybe fungal or viral.[2] Septicemia indicate systemic symptoms caused by bacteria or their toxins in blood. Blood culture is a gold standard for diagnosis. Life threatening septicemia remain one of the most important causes of morbidity and mortality worldwide.[3] They need urgent treatment with antimicrobial drugs. Bacteremia, viremia, fungemia all can lead to sepsis. Automated blood culture systems are also available. Still, conventional blood culture methods are the dominant approach to isolate bacteria in sepsis patient. The use of early and appropriate antibiotic therapy is essential to improve the survival rates in patient with the severe sepsis and septic shock.[1] Appropriate antibiotic selection is an important determinant of multidrug resistance. Severe sepsis is most common in Indian for higher mortality rate as compared to Western literature.[1] This study aims to determine the bacteria causing sepsis and their antibiotic susceptibility pattern in adult patients.[1]

MATERIAL AND METHODS

The present study was carried out in the Department of Microbiology, Krishna Institute of Medical Sciences, Krishna Hospital and Medical Research Centre. Ethical clearance was obtained from ethical committee, Krishna Institute of Medical Sciences Deemed to be University, Karad. All patients older than 18 years were included from different wards with symptoms of sepsis. In total 130 culture positive blood samples were collected. Blood was collected from patients admitted in the hospital who presented with signs and symptoms of sepsis.

About 5-10 ml of blood from adult patients were collected aseptically. Blood was collected as soon as possible before administration of antibiotics. Blood was collected into Brain Heart Infusion broth. Blood culture bottles were incubated at 37°c & subcultured after 48hrs onto Blood & MacConkey's agar Identification of isolate was done by Gram stain, catalase, coagulase, oxidase and biochemicals tests. If there was no growth then subcultured on 7th day. No growth then samples were reported as sterile.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was done for all isolates on Muller – Hinton agar. Susceptibility testing was carried out by standard Kirby Bauer disc diffusion method as per CLSI Guidelines.[24]

Phenotypic test for Staphylococcus aureus

Cephoxitin screening test for detection of MRSA

The test was used for detection of Methicillin-resistant Staphylococcus aureus. Methicillin-resistant was determined by using cephoxitin (30µg) disc on Muller Hinton agar followed by incubation at 37°c for 24 hrs. Zone diameter of 22 mm or more was taken as sensitive and 21 mm or less was considered as resistant. Staphylococcus aureus ATCC 25923 was used as a control strain for the test.[24]

Phenotypic tests for Gram negative organisms

Double disc synergy test for detection of extended spectrum of beta lactamase (ESBL)

This test was specifically designed to detect ESBL production in Enterobacteriaceae.A Ceftazidime disc and a ceftazidime + Clavulanic acid (30 µg + 10 µg) disc were placed, 20 mm apart, centre to centre and incubated aerobically at 37^0 c for 16-18 hrs.A positive test result was defined as a 5mm or greater increase in the size of the zone diameter for ceftazidime tested in combination with clavulanic acid versus the zone for either antibiotic tested alone. Zone of inhibition around the Ceftazidime disc increases towards the Clavulanic acid disc, in an ESBL producer.[24]

Double disc synergy test for detection of metallo beta lactamase (MBL)

This test was used to detect gram negative bacteria which produce metallo - Beta lactamase.The screening test for the MBL production was performed by using Imipenem disc (10µg). The screened isolates (Imipenem resistant) were further confirmed by combined disc method using Imipenem (10µg) alone and in combination with EDTA. After overnight incubation at 37°c, if the increase in inhibition zone with Imipenem EDTA disc was greater than or equal to 7 mm than the Imipenem (10µg) alone, it is interpreted as MBL producer.[24]

Observations

A total 130 culture positive blood samples were collected. Out of total 130 isolates, majority were from Medicine ward 33.1%, followed by Surgery ward 31.5%, Neurology ICU 15.3%, Oncology Ward 15.3% and Casualty ICU 4.6%, as shown in fig no .1

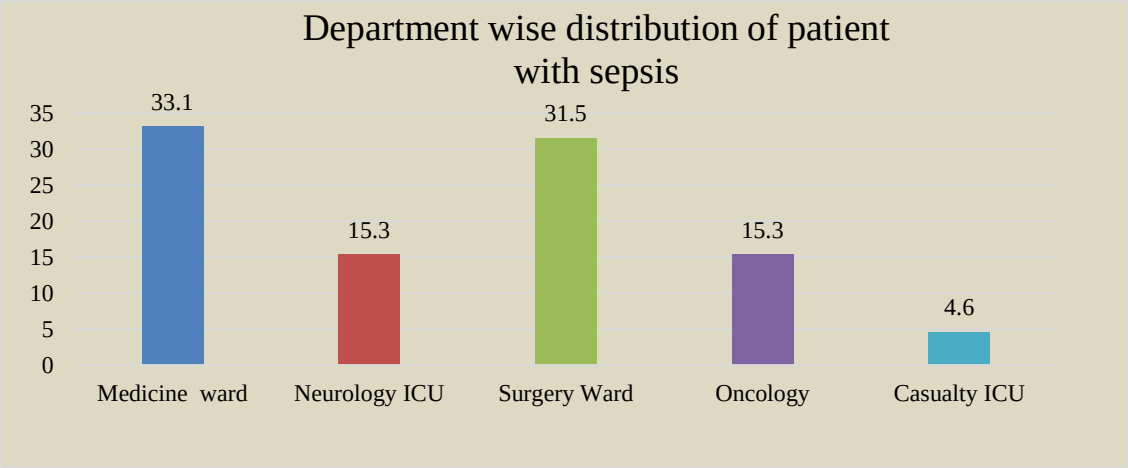

FIG 1: Department wise distribution of patient with sepsis

Males were 57.6% & 42.3% were females. Male were more as compare to females. Majority of patients i.e., 22.3% were from the age group 31-40 followed by 21.5% from the age group 21-30. Maximum Male patient's i. e., 25.3% were from age group 31-40 whereas 27.2% of females were above ≥60 years of age, as shown in Table. No. 1

TABLE 1: Age & Sex wise distribution of patient with sepsis

Age	Male	Female	%
21-30	24	8.1	21.5
31-40	25.3	18.1	22.3
41-50	20	22	21
51-60	14.6	14.5	15
≥60	16	27.2	21
Total	57.6	42.3	100

Out of 130 isolates, 73% were Gram-positive cocci and 26.9% were Gram negative bacilli, predominantly Gram-positive cocci were isolated in our study. Staphylococcus aureus 60% was most frequent isolate from blood culture, followed by 11.5% of coagulase negative Staphylococcus and only 1.5% isolates were Enterococcus spp. In Gram Negative isolates, most frequent isolate was klebsiella pneumoniae 10% followed by E. coli 8.4%, Pseudomonas aeruginosa 4% and Acinetobacter species 5%, as shown in table No. 2

TABLE 2: Bacterial isolates with percentage

Organisms	No of isolates	Percentage (%)
Gram-positive organisms		
Staphylococcus aureus	78	60
Coagulase Negative staphylococcus	15	11.5
Enterococcus species	02	1.5
Gram-Negative organisms		
Klebsiella pneumoniae	13	10
E. coli	11	8.4
Pseudomonas Aeruginosa	5	4
Acinetobacter species	6	4.7

Among gram-positive organisms Staphylococcus aureus showed maximum resistance to Penicillin 93.3% and 83.3% to Erythromycin. Maximum susceptibility was seen to Linezolid 85% and Vancomycin 76%. Followed by Coagulase Negative Staphylococcus showed maximum susceptibility to Linezolid 86.6% and Vancomycin 80% and maximum resistance was seen to Penicillin 93.3%. And Enterococcus spp. showed 100% resistance to Erythromycin, Tetracycline, Ampicillin and Cefoperazone and 100% susceptibility to Linezolid and Vancomycin, as shown in table No. 3

TABLE 3: Antimicrobial susceptibility pattern of Gram-Positive organisms

Antibiotics	**Staphylococcus aureus (78)**		**Coagulase Negative Staphylococcus (15)**		**Enterococcus Faecium (02)**	
	S	**R**	**S**	**R**	**S**	**R**
Penicillin G	05(6.4)	73(93.5)	01 (6.6)	14(93.3)	01(50)	01(50)
Linezolid	66(85)	12(15.3)	13(86.6)	02(13.3)	02(100)	00(00)
Ciprofloxacin	18(23.07)	60(76.9)	6(40)	9(60)	00(00)	02(100)
Erythromycin	13(17)	65(83.3)	4(26.6)	11(73.3)	00(00)	02(100)
Tetracycline	39(50)	39(50)	7(46.6)	8(53.3)	00(00)	02(100)
Ampicillin	20(26)	58(74.3)	3(20)	12(80)	00(00)	02(100)
Cefoperazone	04(27)	45(57.6)	4(26.6)	11(73.3)	00(00)	02(100)
Ceftriaxone	20(26)	58(74.3)	3(20)	12(80)	00(00)	02(100)
Ofloxacin	31(40)	47(60.2)	7(46.6)	08(53.3)	01(50)	01(50)
Vancomycin	59(76)	19(24.3)	12(80)	03(20)	02(100)	00(00)
Amikacin	25(32.1)	53(67.9)	08(53.3)	7(46.6)	01(50)	01(50)

Among gram-negative organisms, Klebsiella pneumoniae showed 100% resistance to Ampicillin and 92.3% to Co-trimoxazole. Maximum susceptibility was seen to Amikacin 92.3% and Cefoperazone 84.6%. Followed by E. coli showed maximum resistance to Gentamycin 90.9% and Ceftazidime 82%. Maximum susceptibility was seen to Cefoperazone 90.9% and Ampicillin 82%, Acinetobacter spp. showed maximum resistance to Gentamicin 100% and Co-trimoxazole 83.3%. Maximum susceptibility was seen to Ampicillin 100% and Amikacin 83.3%. And Pseudomonas aeruginosa showed maximum resistance to Gentamicin 100% and Cotrimoxazole 100%. Maximum susceptibility was seen to Amikacin 100%, as shown in Table No- 4

TABLE 4: Antimicrobial susceptibility pattern of Gram-negative organisms

Antibiotics	**Klebsiella pneumoniae (13) N (%)**		**E. coli (11) N (%)**		**Acinetobacter Species (6) N (%)**		**Pseudomonas aeruginosa (5) N (%)**	
	S	R	S	R	S	R	S	R
Gtamicin	03(23.0)	10(76.9)	01(9)	10(90.9)	00(00)	06(100)	00(00)	05(100)
Cefepime	04(31)	09(69.2)	03(27.2)	08(73)	02(33.3)	04(66.6)	00(00)	05(100)
Cotrimoxazole	01(8)	12(92.3)	04(36.3)	07(64)	01(16.6)	05(83.3)	00(00)	05(100)
Cefuroxime	05(38.4)	08(61.5)	03(27.2)	08(73)	04(66.6)	02(33.3)	00(00)	05(100)
Ceftazidime	03(23.07)	10(77)	02(18.1)	09(82)	04(66.6)	02(33.3)	01(20)	04(80)
Ampicillin	00(00)	13(100)	09(82)	02(18.1)	06(100)	00(00)	00(00)	05(100)
Ceftriaxone	04(31)	09(69.2)	04(36.3)	07(64)	02(33.3)	04(66.6)	01(20)	04(80)
Ofloxacin	05(38.4)	08(61.5)	06(54.5)	05(45.5)	04(66.6)	02(33.3)	00(00)	05(100)
Cefoperazone	11(84.6)	02(15.3)	10 (90.9)	01(9)	02(33.3)	04(66.6)	01(20)	04(80)
Amikacin	12(92.3)	01(7.6)	05(45.5)	06(54.5)	05(83.3)	01(16.6)	05(100)	00(00)

Out of 78 Staphylococcus aureus, 67% were MRSA and 33% were MSSA, as shown in Table No – 5

TABLE 5: Distribution of Methicillin Resistance in S. aureus

Staphylococcus Aureus	No. of isolates	Percentage
MRSA	**52**	**67**
MSSA	**26**	**33**
Total	**78**	**100**

Out of 35 Gram negative organisms, 63% showed ESBL production as a major form of resistant mechanism. Total 41% was contributed by E. coli, followed by 40% Klebsiella pneumoniae and 27% Acinetobacter spp. and smaller percentage of 15% by Pseudomonas aeruginosa, as shown in Table No – 6 While assessing organism with ESBL producers and non-producers, it was found that there was significant association with chi square value is 7.861 and P value < 0.05.

TABLE 6: Detection of ESBL producers among Gram -negative organisms

Organisms	ESBL Producer	Non ESBL producers
Klebsiella pneumoniae (13)	**8(36.3)**	**5(38.4)**
E. coli (11)	**9(41)**	**2(15.3)**
Pseudomonas Aeruginosa (5)	**4(18.1)**	**1(7.6)**
Acinetobacter species (6)	**1(4.5)**	**5(38.4)**
Total	**22**	**13**

χ^2 **: 7**.816 P value < 0.05

χ^2

Among 35 Gram negative bacilli, 60% were MBL producers. Out of these, major MBL producers were Klebsiella pneumoniae 52.3%, followed by E. coli 28.5%. Only 14.2% Pseudomonas aeruginosa were MBL producers and least were Acinetobacter species 5%, as shown in Table No – 7. MBL producers and non-producers, it was found that there was significant association with chi square value is 8.113 and P value < 0.05

TABLE 7: Detection of MBL Producer among Gram- Negative organisms

Organisms	MBL Producer	Non – MBL producer
Klebsiella pneumoniae (13)	**11(52.3)**	**2(14.2)**
E. coli (11)	**06(28.5)**	**5(35.7)**
Pseudomonas Aeruginosa (5)	**03(14.2)**	**2(14.2)**
Acinetobacter species (6)	**1(5)**	**5(35.6)**
Total	**21**	**14**

χ^2 **:** 8.113, P value < 0.05

DISCUSSION

Septicemia, also called bacteremia or blood poisoning, is a life-threatening condition with multiple complications of the blood and is extremely dangerous.[4] It usually occurs when untreated or severe bacterial infection that exists elsewhere in the body, like in the lungs or skin, which enters the bloodstream.[5] Through the bloodstream, the causative organism spreads throughout the body, resulting in multiple organ failure, primarily the heart and brain.[6]

There's no way to tell what infection caused septicemia by its etiology, but multiple infections can also lead to septicemia if left untreated, including urinary tract infections, abdominal infections, lung infections, or many others. A medical emergency like septicemia can be effectively treated with antibiotics.[6] Septicemia, also called bacteremia or blood poisoning, is a life-threatening condition with multiple complications of the blood and is extremely dangerous.[4] It usually occurs when untreated or severe bacterial infection that exists elsewhere in the body, like in the lungs or skin, which enters the bloodstream.[5]

Through the bloodstream, the causative organism spreads throughout the body, resulting in multiple organ failure, primarily the heart and brain.[7] There's no way to tell what infection caused septicemia by its etiology, but multiple infections can also lead to septicemia if left untreated, including urinary tract infections, abdominal infections, lung infections, or many others. A medical emergency like septicemia can be effectively treated with antibiotics.[6]

In our study, out of 130 isolates from IPD, majority were from Medicine ward 33.1%. Our study findings can be compared with study done by Meghna Palewar et al.[8] who reported maximum isolates from medicine ward 27.5%. This could be due to serious underlying disease or due to exposure to life saving invasive procedures. Sepsis associated infection in the age group 31–40 was found to be 22.3% which was comparable with Nikita Vasudeva et al.[9] 20.7% and Divyashanti CM et al.[10] 19%. This could be due to admission to ICU or longer hospital stay. In our study, male predominance was seen 57.6% as compared to females 42.3%. Similar to present study, there was a preponderance of male patients in the studies conducted by Aroop Mohanty et al.,[11] Praneetha Jain et al.[12] and Aida Salehi Nobandegani et al.[13]

Majority of isolates were Gram positive organisms as compared to Gram negative organisms. This finding is in agreement with the study by Kabi et al.[14] who has reported majority of Gram-positive organisms than Gram negative organisms. 73% were Gram positive organisms. These findings can be co-related with study conducted by Muleta et al.[15] in which they have reported 69% of Gram-positive organisms. Among 26.9% Gram negative isolates, maximum isolates were Klebsiella pneumoniae 10.11%. Similar to present study, Sharon Nyesiga et al.[16] has isolated 12.7 % Klebsiella pneumoniae. 8.4% isolates were of E. coli which is similar to the study done by Mulat Dagnew et al.[15] has isolated 7.0 % and 4.7% isolates were of Acinetobacter species. Similar findings were noted by Debananda Sahoo et al.[17] which showed 7.7% of Acinetobacter species and 4% isolates were of Pseudomonas aeruginosa. According to Dassalegn Muleta et al.[18], in their study, 3.3% Pseudomonas aeruginosa were isolated.

Bacterial isolates were tested against antimicrobial agents and their susceptibility pattern was observed. Staphylococcus aureus showed maximum susceptibility to Linezolid 85% and Vancomycin 76%. These findings are similar to study conducted by Rohit Tiwari et al.[21] which showed maximum susceptibility to Linezolid 85% and Vancomycin 92.8%. Maximum resistance was seen to Penicillin 93.5% and erythromycin 83.3% which is similar

to Sharon Nyesiga et al.[16] where Penicillin 100 % and erythromycin 71 % resistance was seen. Coagulase Negative Staphylococcus showed maximum susceptibility to Linezolid 86.6% and Vancomycin 80% which is similar to Meghna Palewar et al.[8] Linezolid 100% and Vancomycin 97.5%. Coagulase Negative Staphylococcus showed resistance to Penicillin 93.3% which is similar to Tariq Mahmud Tariq et al.[19] who reported 93.5% resistance to Penicillin.

Klebsiella pneumoniae showed maximum susceptibility to Amikacin 92.3% and Cefoperazone 84.6%. These findings can be corelated to the study by Amit Banik et al.[22] which showed Amikacin 95.23% and Cefoperazone 80.95% susceptibility. Klebsiella pneumoniae showed maximum resistance to Ampicillin 100% and Co-trimoxazole 92.3% which is similar to Sharon Nyesiga et al.[16] Ampicillin 100% and Co-trimoxazole 100%. E. coli showed maximum susceptibility to Cefoperazone 90.9% and Ampicillin 82% which is Similar to Amit Banik et al.[22] Cefoperazone 100%. E.coli showed maximum resistance to Gentamycin 90.9% and Ceftazidime 82% which is similar to Sharon Nyesiga et al.[16] Gentamycin 100%, Kalpesh Gohel et al.[23] Ceftazidime 92%. Acinetobacter spp. showed maximum susceptibility to Ampicillin 100% and Amikacin 83.3%. Amit Banik et al.[22] reported 100% susceptibility to Ampicillin and 76.7% to Amikacin. These findings are similar to the present study. Acinetobacter spp. showed maximum resistance to Gentamicin 100% and Cotrimoxazole 83.3% which is similar to Rohit Tiwari et al.[21] Gentamicin 100% and Cotrimoxazole 75%. Pseudomonas aeruginosa showed maximum susceptibility to Amikacin 100%. These findings can be corelated to the studies done by Amit Banik et al.[22] and Aida Salehi Nobandegani et al.[13] where 100% susceptibility was reported to Amikacin. Pseudomonas aeruginosa showed maximum resistance to Gentamicin 100% and Cotrimoxazole 100%. According to Aroop Mohanty et al.[11], 100% resistance was seen to Gentamicin and Cotrimoxazole which are comparable with the present study.

In present study, 67% of MRSA and 33% of MSSA has been documented among 60% of total Staphylococcus aureus isolates. Above data co-relates with results of Meghna palewar et al.[8] who has documented 70% MRSA. ESBL producers was found to be 62.8%. Similarly, Tariq Mahmud Tariq et al.[19] reported 51.9% and Shweta oza et al.[20] 51.3% of ESBL producers respectively. MBL producers was found to be 60% in present study.

CONCLUSION

The present study emphasized the burden of sepsis in patients at our hospital. The determination of antibiotic susceptibility patterns and screening for MRSA, ESBL and MBL among isolates are important for controlling the sepsis. Having examined the findings of this study, we can conclude that it is our duty as microbiologists to not only identify the organism, but also to tell clinicians about how to prevent infection. It is very important to treat septicaemia caused by multi drug resistant organisms as soon as possible in order to achieve a favourable outcome. In this study, we aim to highlight the fact that Gram-positive organisms, particularly Staphylococcus aureus, are the most common cause of hospital-acquired septicaemia in our institution, and are sensitive to Linezolid and Vancomycin. This suggests that these drugs may be effective as a treatment. The antibiotic therapy must be chosen with great caution. It is important to continually assess the sensitive-resistance patterns of the isolates. According to the changing bacterial resistance in the hospital and population of western Maharashtra, the study might be helpful in preparing antibiotic policy in our institute. Indiscriminate use of antibiotic can be minimized. The judicious use of antibiotics, rotational use of antibiotic therapy and educational programs will be useful in preventing sepsis.

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