

Original Research Article

<https://doi.org/10.20546/ijcmas.2019.809.198>

Bacteriological Profile and Antibiogram of isolates from Bloodstream Infections in Patients Admitted in ICU from a Tertiary care hospital, Nerul, Navi Mumbai, India

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ABSTRACT

Bloodstream infections are frequent and life – threatening, can lead to increase in morbidity, mortality and health care cost of patients admitted in intensive care unit (ICU). In addition to this, infections due to emerging multidrug resistant (MDR) microorganisms, the treatment becomes challenging. With the rising problem of drug resistance, the present study was undertaken to evaluate the most prevalent bacterial pathogen causing Bloodstream infections in adult patients admitted to an Intensive Care Unit (ICU) with their antimicrobial sensitivity pattern. A retrospective analysis of data was done on the blood cultures received from 817 patients with clinically suspected bloodstream infections, admitted in Medical ICU of tertiary care hospital, Navi Mumbai, between October 2016 and October 2018. All the samples were received and processed in the Department of Microbiology, using standard microbiological techniques and antimicrobial sensitivity was done according to CLSI guidelines. From 817 patients, the positive growth for pathogen was observed in 165 (20.19%) patients. 167 isolates were identified, maximum isolates were Gram – negative 120 (71.86%), Gram – positive were 31 (18.56%) and *Candida* spp. were 16 (9.58%). Among bacterial isolates, there was a predominance of *Klebsiella pneumoniae* 37 (22.15%) followed by *Acinetobacter* spp. 31 (18.56%), *Escherichia coli* 29 (17.36%), *Pseudomonas aeruginosa* 16 (9.58%) & *Enterococcus* spp. 14 (8.38%). Gram – negative bacterial pathogens showed decreasing sensitivity to Imipenem, Piperacillin – tazobactam, Aminoglycosides, Third – generation Cephalosporins & Cephalosporin. Whereas all gram – positive bacterial isolates were sensitive to Vancomycin and Linezolid while resistant to Penicillin. This study showed the high prevalence of multi drug resistant gram – negative pathogens causing bloodstream infections in our ICU setting. Thus a continues surveillance of prevalent etiological pathogens of BSI along with their antibiotic susceptibility pattern will be helpful to the clinicians in choosing the proper antimicrobials. And clinical management of BSI will minimize the emergence of multi drug resistance.

Keywords

Bloodstream infections (BSI), Intensive care unit (ICU), Multi drug resistant (MDR), Blood cultures, and Antimicrobial sensitivity.

Article Info

Accepted:

18 August 2019

Available Online:

10 September 2019

Introduction

Bloodstream infections, frequent and life – threatening, lead to increase in mortality and morbidity among critically ill patients admitted in ICU⁽¹⁾. Critically ill patients are

particularly predisposed to the acquisition of BSIs, which occur in approximately 7% of all patients within the first month of hospitalization in Intensive care units (ICUs). The acquisition of a Bloodstream infection also results in increased length of ICU stay

and Healthcare related cost^(2,3). Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20 – 50%^(4, 5).

The intensive care unit (ICU) often is called the epicentre of infections, due to its extremely vulnerable population (reduced host defences deregulating the immune responses) and increased risk of becoming infected through multiple procedures and use of invasive devices (intubation, mechanical ventilation, vascular access, etc.). In addition, several drugs may be administered, which also predispose for infections, such as pneumonia, e.g., by reducing the cough and swallow reflexes (sedatives, muscle relaxants) or by distorting the normal non-pathogenic bacterial flora (e.g., stress ulcer prophylaxis^(6, 7)). Consequently, the ICU population has one of the highest occurrence rates of (nosocomial) infections (20-30% of all ICU-admissions)^(8, 9), leading to an enormous impact on morbidity, hospital costs, and often, survival⁽¹⁰⁻¹²⁾.

Pattern of organisms causing infections and their antibiotic resistance pattern vary widely from one country to another, as well as one hospital to other and even among ICUs within one hospital⁽¹³⁾.

Among gram negative bacteria, *Acinetobacter spp.*, *Pseudomonas aeruginosa*, *E.coli*, *Klebsiella*, *H. influenza*, *Neisseria meningitides* are responsible for BSI along with CONS, *S.aureus*, *Enterococci* and alpha haemolytic *Streptococci* among gram positive bacteria^(14, 15). In the last few years, clinicians have witnessed a growing incidence of BSIs by bacteria with resistance against commonly used antimicrobials.

During the past decades, a shift in the MDR dilemma has been noted from gram-positive to gram-negative bacteria, especially due to the

scarceness of new antimicrobial agents active against resistant gram-negative microorganisms⁽¹⁶⁾.

Among gram-positive organisms, the most important resistant microorganisms in the ICU are currently methicillin-(oxacillin - resistant *Staphylococcus aureus*, and vancomycin-resistant enterococci^(6, 16, and 17). In gram-negative bacteria, the resistance is mainly due to the rapid increase of extended-spectrum Beta-lactamases (ESBLs) in *Klebsiellapneumoniae*, *Escherichia coli*, and *Proteus mirabilis*; high level third-generation cephalosporin Beta-lactamase resistance among *Enterobacter spp.* and *Citrobacter spp.*, and MDR in *Pseudomonas aeruginosa*, *Acinetobacter spp.*, and *Stenotrophomonasmaltophilia*^(6,17).

This rising problem of emerging drug resistance among bloodstream pathogens limits the therapeutic options and complicate patient's management.

With this background, the present study was undertaken to identify the most prevalent bacteria isolated from patients suspected with Blood stream infections along with antibiotic sensitivity pattern of isolates thus providing useful guidance to clinicians to modify antibiotic therapy thus minimizing morbidity, mortality and emergence of resistant organisms.

Materials and Methods

The study was carried out in the Department of Microbiology of Dr. D.Y. Patil Medical College and Hospital, Nerul, Navi Mumbai wherein the retrospective analysis of blood cultures received during two years period from October 2016 to October 2018, was done.

A total of 817 blood samples for culture were received from clinically suspected adult

patients with bloodstream infections who were admitted in MICU.

Inclusion criteria

Patients who had a blood cultures that grew aerobic bacterial isolate from two sets of blood cultures taken at different intervals of time with their antibiogram during their stay in Medical ICU were eligible for the study.

Exclusion criteria

Negative blood cultures, fungal isolates and contaminant growths were excluded from the study.

Sample Collection

Blood specimens were obtained according to the standard sample collection protocol followed in hospital by a trained phlebotomist.

Sample processing

Blood for culture samples collected from clinically suspected bacteraemia cases under strict aseptic precautions. The venepuncture site was disinfected with 70% alcohol and 2%

Tincture of iodine, before drawing blood. A volume of 10 ml of blood from adult patient was collected and inoculated into Adult BACTEC blood culture bottles and incubated in an automated BACTEC 9050 blood culture instrument (Becton – Dickenson, USA) at 37⁰ C.

All Bactec positive samples were subjected to inoculation on 5% Sheep Blood Agar, Chocolate Agar and MacConkey's Agar, followed by Gram staining and the plates were incubated at 37⁰ C for 24 hours and plates were observed for growth. The growth was identified by colonial characteristics (phenotypic identification),

Gram's stain and standard biochemical tests (18, 19).

Antibiotic susceptibility testing was done for the pathogenic isolates on Mueller – Hinton agar by Kirby-Bauer disc diffusion method and interpreted according to CLSI guidelines (20).

Control strains of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* 27853 and *Staphylococcus aureus* ATCC 25923 were used.

Statistical Analysis

Data was entered in MS-Excel worksheet for calculation purposes. Further data was analysed using Statistical software *IBM SPSS Statistics version 21.0* and results were presented using frequency and percentages. The results were summarised using graphical and tabular presentation. The chi-square test was used to assess the association between variables. Also z-test for two proportions was used to compare the proportions. A p-value of less than 0.05 was considered as statistical significant.

Results and Discussion

During the study period from October 2016 to October 2018, a total of 817 blood samples from patients suspected of blood stream infections were received and analysed. Positive growth of pathogen was observed in 163 (19.95%) blood samples.

Negative growth was seen in 640 (78.34%) blood samples whereas from 14 (1.71%) blood samples, the contaminants were recovered.

Most of the culture positive samples were of monomicrobial aetiology (97.55%) and from four samples (2.45%) more than one organism were isolated.

Among 163 patients, 109 (66.87%) were males and 54 (33.13%) were females.

The maximum bloodstream infections were observed in above 60 years of age group. The chi-square analysis indicates bloodstream infection was maximum in higher age groups (p <.01).

From 163 patients, 167 isolates were recovered. Out of 167 isolates, 120 (71.86%) were Gram-negative, 31 (18.56 %) were Gram-positive and 16 (9.58%) were *Candida* spp

Among Gram-negative isolates, predominant pathogen was *Klebsiella pneumoneae* 37 (22.15%) followed by *Acinetobacter spp.* 31 (18.56%), *E.coli* 29 (17.36%) and *Pseudomonas aerugenosa* 16 (9.58%) (p <.01). Whereas among Gram-positive isolates, maximum isolation was of *Enterococcus spp.* 14(8.38%) and *Staphylococcus aureus* 11(6.59%) (p <.01).

Antimicrobial sensitivity patterns for Gram – positive isolates and Gram – negative isolates were interpreted according to CLSI guidelines and are represented in TABLE 3 and TABLE 4 respectively. All gram-positive isolates showed 100% sensitivity towards Vancomycin and Linezolid (p<.001). More than 90% enterococcal isolates were resistant to Gentamicin, Ciprofloxacin, Penicillin and

Erythromycin (p<.01). Among *S.aureus*, Methicillin resistance (MRSA) was observed in 54.55% of the isolates and 100% were resistant to Penicillin, Erythromycin whereas low level resistance was shown to Ciprofloxacin & Gentamicin (p<.01).

75% strains of Coagulase – negative Staphylococcal spp. (CONS) were Methicillin resistant and 100% resistance to Penicillin, Erythromycin, Ciprofloxacin and Gentamicin (p<.01).

2 Streptococcal spp. showed 100% sensitivity to all antibiotics.

Among Gram-negative isolates, maximum isolation was of *Klesiella pneumoniae* and *Acinetobacter* spp (p<.01)

Among Gram – negative isolates, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter* spp. showed decreasing sensitivity to Imipenem, Piperacillin + Tazobactum, Aminoglycosides, Ciprofloxacin, third – generation cephalosporins. (p<.01).

35/37 (94.59%) of *Klebsiella* spp. were resistant to Extended spectrum β - lactamases while 28/37 (75.68%) and 21/37(56.76%) resistant to Piperacillin – Tazobactum and Imipenem respectively. (p<.01).

Table.1 Demographic characteristics of the patients

(n = 163).

AGE (years)	MALES	FEMALES	TOTAL
13 - 20	01	01	02
21 - 40	25	11	36
41 - 60	39	19	58
Above 60	44	23	67
TOTAL	109	54	163

The number of males were significantly higher than females (p <.01). Male to Female ratio was approximately 2: 1.

Table.2 Distribution of bacterial isolates from positive blood cultures

(n = 167).

Causative pathogens	NUMBER (%)
<i>Klebsiella pneumoniae</i>	37 (22.15%)
<i>Acinetobacter spp.</i>	31 (18.56%)
<i>E.coli</i>	29 (17.36%)
<i>Pseudomonas aeruginosa</i>	16 (9.58%)
<i>Enterobacter spp.</i>	02 (1.20%)
<i>Proteus spp.</i>	02 (1.20%)
<i>Salmonella typhi</i>	02 (1.20%)
<i>Citrobacter spp.</i>	01 (0.60%)
<i>Enterococcus spp.</i>	14 (8.38%)
<i>S.aureus</i>	11 (6.59%)
CONS	04 (2.40%)
<i>Streptococcus spp.</i>	02 (1.20%)
<i>Candida spp.</i>	16 (9.58%)
TOTAL	167 (100%)

Table. 3 Antimicrobial Susceptibility pattern in Gram-positive isolates

(n = 31)

ANTIBIOTICS	<i>S.aureus</i> (n=11)		CONS (n=4)		<i>Enterococcus spp.</i> (n= 14)		<i>Streptococcus</i> <i>spp.</i> (n= 2)	
	S(%)	R(%)	S (%)	R (%)	S(%)	R (%)	S (%)	R (%)
Penicillin (10 units)	00	100 %	00	100%	1(7.69%)	13(92.86%)	100	00
Erythromycin (15mcg)	00	100%	00	100%	00	14(100%)	100	00
Cefoxitin (30mcg)	5(45.45%)	6(54.55%)	1(25%)	3(75%)	-	-	-	-
Gentamicin (10mcg)	3(27.27%)	8(72.73%)	1(25%)	3(75%)	1(7.69%)	13(92.86%)	100	00
Vancomycin (30mcg)	11(100%)	00(00%)	4(100%)	00	14(100%)	00	100	00
Linezolid (30mcg)	11(100%)	00(00%)	4(100%)	00	14(100%)	00	100	00
Cotrimoxazole (1.25/23.75 mcg)	2(18.18%)	9(81.82 %)	00	100%	-	-	100	00
Ciprofloxacin (30mcg)	3(27.27%)	8(72.73 %)	00	100%	1(7.69%)	13(92.86%)	100	00

Table.4 Antimicrobial susceptibility of Gram-negative isolates

(n = 120)

Antibiotics	<i>Kleb.spp</i> (n=37)	<i>E.coli</i> (n=29)	<i>Enterobac</i> <i>ter spp.</i> (n=2)	<i>Citrobacter</i> <i>spp.</i> (n=1)	<i>Proteus</i> <i>spp.</i> (n=2)	<i>Acinetobac</i> <i>ter spp.</i> (n=31)	<i>Pseudomonas</i> <i>aeruginosa</i> (n=16)	<i>S.typhi</i> (n=2)
Amikacin	15(40.54%)	19 (65.52%)	00	00	1 (50%)	8 (25.81%)	7 (43.75%)	1(50%)
Gentamicin	15(40.54%)	14 (48.26%)	00	00	1 (50%)	7 (22.58%)	00	1(50%)
Ciprofloxacin	11(29.73%)	4 (13.79%)	00	1 (100%)	00	9 (29.03%)	10 (62.5%)	2(100%)
Co-tromoxazole	9(24.32%)	6 (20.69%)	00	1 (100%)	00	9(29.03%)	00	1(50%)
Ampicillin	-	-	-	-	-	-	-	2(100%)
Piperacillin+ Tazobactam	10 (27.03%)	15 (51.72%)	00	00	2 (100%)	9(29.03%)	10(62.5%)	1 (50%)
Imipenem	16 (43.24%)	16 (55.17%)	1 (50%)	00	2 (100%)	15 (48.39%)	9 (56.25%)	2 (100%)
Ceftazidime- clavulanic acid	2 (5.41%)	3 (10.34%)	00	00	1 (50%)	2 (6.45%)	-	2(100%)
Ceftazidime	2(5.41%)	3 (10.34%)	00	00	1 (50%)	2 (6.45%)	4 (25%)	2(100%)
Ceftriaxone	2(5.41%)	3 (10.34%)	00	00	1 (50%)	2 (6.45%)	-	2 (100%)
Cefotaxime	2(5.41%)	3 (10.34%)	00	00	1 (50%)	2 (6.45%)	-	2 (100%)
Tobramycin	-	-	-	-	-	-	7(43.75%)	-
Aztreonam	-	-	-	-	-	-	1(6.25%)	-
Cefepime	-	-	-	-	-	-	5 (31.25%)	-

Fig.1 Demographic Characteristics of the Patients (n= 163).

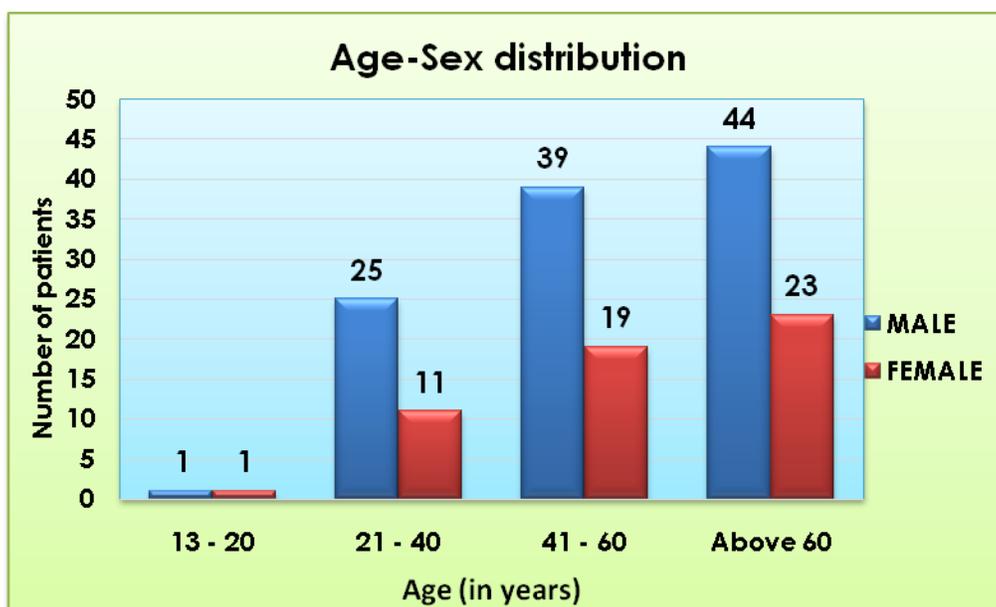


Fig.2 Percentage of isolates (n = 167)

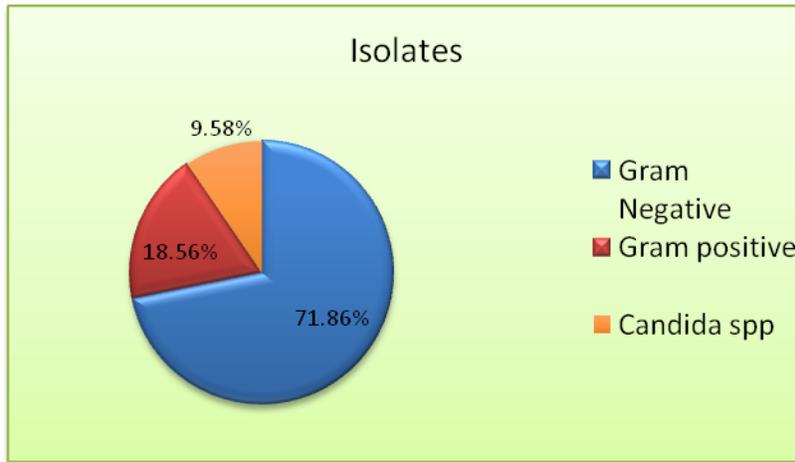


Fig.3 Antimicrobial Susceptibility Pattern of Gram – positive isolates

(n = 31)

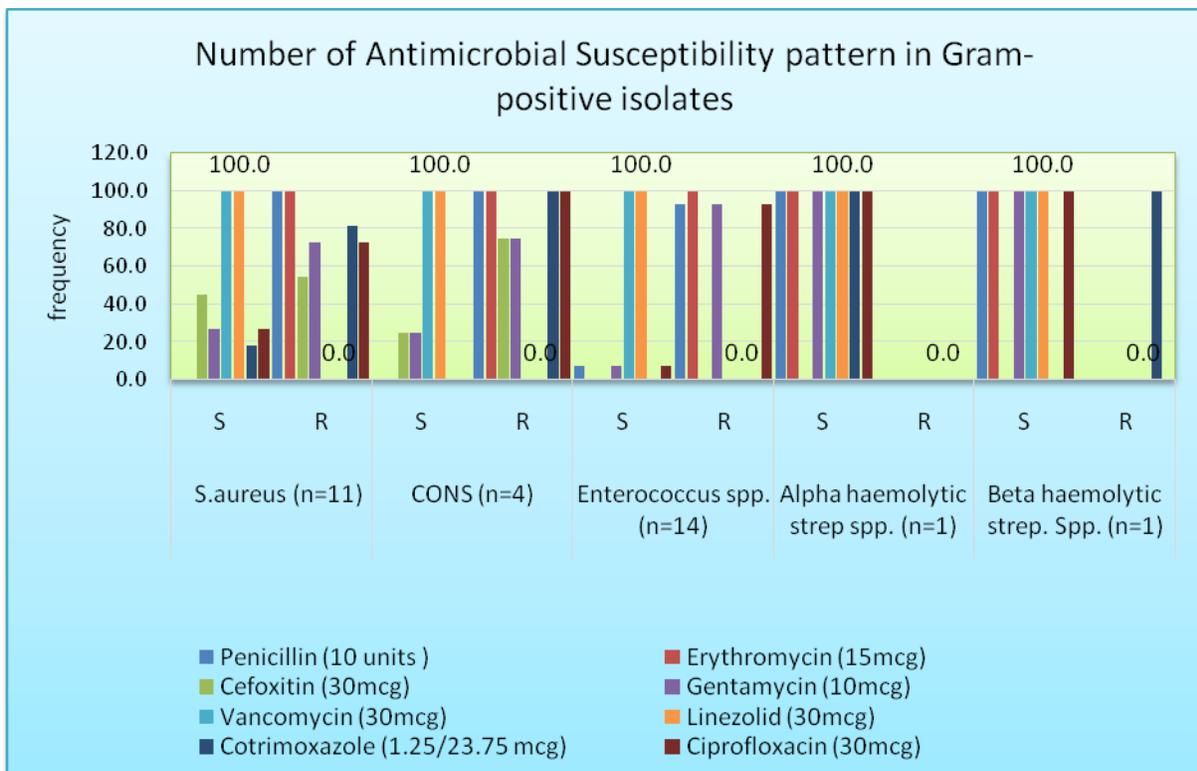


Fig.4 % of antimicrobial susceptibility of Gram – negative isolates

(n=120)

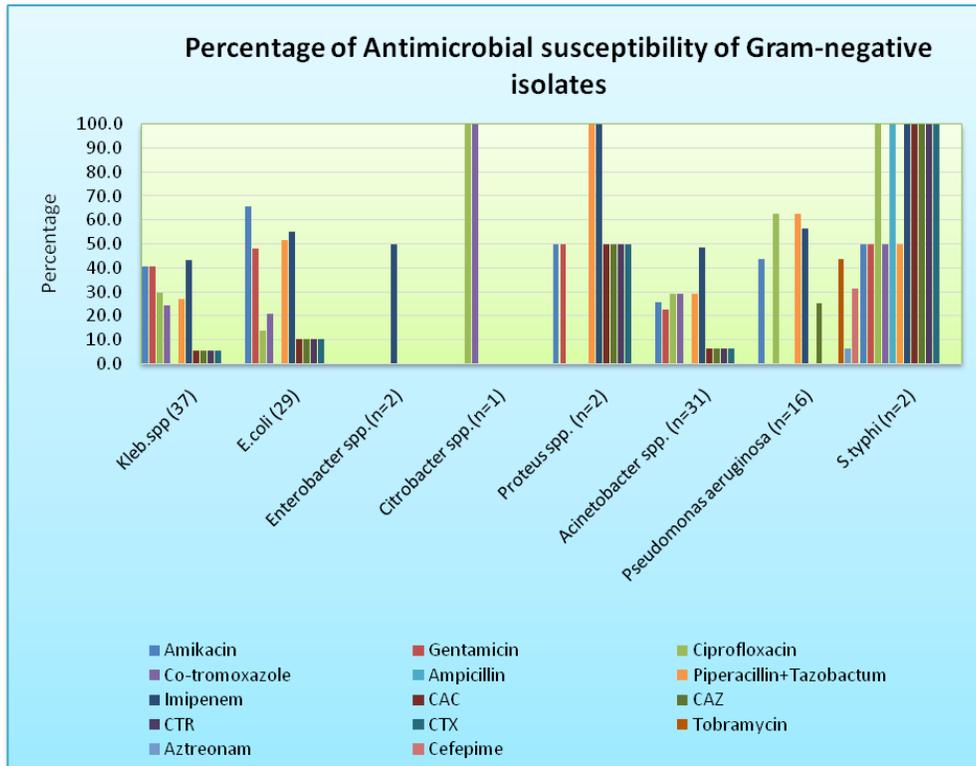
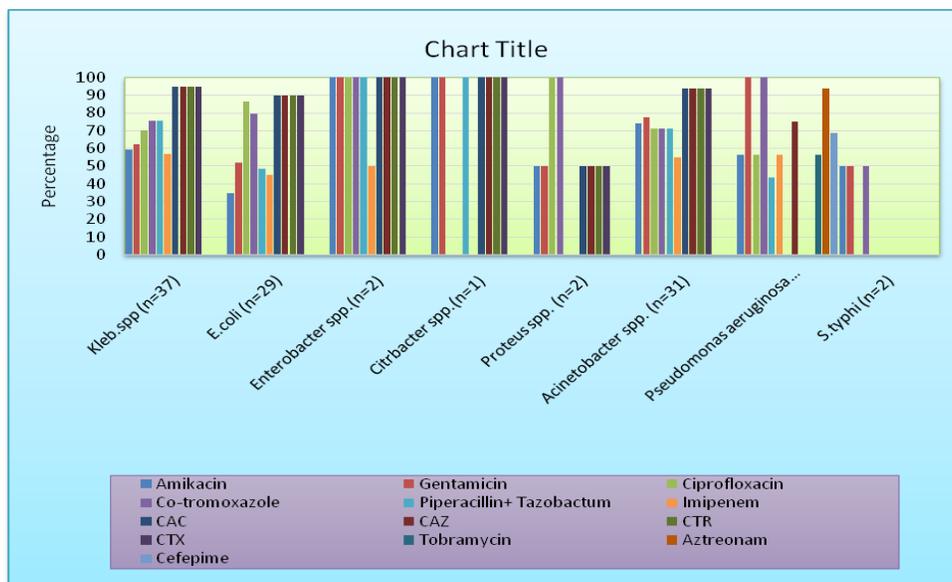


Fig.5 Percentage of Antimicrobial resistance in Gram-negative isolates (p<.01).



Of 31 *Acinetobacter* spp., 29 (93.55%) were resistant to Extended spectrum β - lactamases while 22 (70.97%) and 17 (54.84%) were resistant to Piperacillin+Tazobactam and Imipenem respectively. (p<.01).

Among *E.coli*, 26/29 (10.34%) were resistant to Extended spectrum β - lactamases while 45 to 48% (13 to 14/29) *E.coli* were resistant to Piperacillin + Tazobactam and Imipenem.

S.typhi was isolated from 2 patients which showed almost sensitivity to all antimicrobials (p<.01).

Pseudomonas aeruginosa showed (50-100%) resistance towards Gentamicin, Cotrimoxazole, Aztreonam, Ceftazidime, Cefepime, Amikacin, Ciprofloxacin, Imipenem, Tobramycin, Piperacillin - Tazobactam (p <.01).

With underlying diseases and the risk factors like age, decreasing immunity, instrumentation, Patients admitted in the intensive care units or critical care units, are always at a higher risk of developing healthcare- associated infections, which result in high morbidity and mortality, ICU stay, cost among these patients. With over & indiscriminate use of antibiotics in ICU settings, pathogens isolated are emerging as multi-drug resistant under continues antibiotic pressure.

In view of this, the present study was done to know the most prevalent pathogen isolated along with their antimicrobial susceptibility patterns from adult patients with blood stream infections admitted in medical intensive care units.

The patients in this study were in the age group of 20 to above 60 years with Male to Female ratio was approximately 2: 1.

Similar ratio was also observed in the earlier study²¹. With maximum bloodstream infections were observed in above 60 years of age group. This may be because of sepsis which is common in aging population with underlying disease, declining immunity make them prone to new infections.^(22,23)

The epidemiology of microbial pathogens causing BSI's dramatically changed over years, with a concomitant increase in antimicrobial resistance.

A nationwide surveillance study conducted in 49 hospitals in USA showed a large prevalence of Gram-positive bacteria causing BSI's compared with Gram-negative organisms. However, a trend towards an increasing incidence of Gram-negative organisms causing BSI's has been observed more recently⁽²⁴⁾.

The present study showed that there was more Gram – negative isolates (71.86%) with predominance of *Klebsiella pneumoniae* (22.15%) followed by *Acinetobacter* spp. (18.56%), *E.coli* (17.36%) and *Pseudomonas aeruginosa* (9.58%) than the Gram – positive isolates (18.56 %) and *Candida* spp. (9.58%). Similar observations were also stated by earlier studies.^(22, 25, 26) The emergence of MDR often is dedicated to excessive use of broad-spectrum antimicrobial agents, since more than 60% of all ICU patients receive antimicrobials during their stay in critical care unit⁽²⁵⁾.

In the present study, ESBL production was observed in 94 (78.33%) Gram negative isolates. The most common ESBL – producers were *Klebsiella pneumoniae* (35/37; 94.59%) followed by *Acinetobacter* spp. (29/31; 93.54%) and *E.coli* (26/29; 89.65%). Similar observation of maximum ESBL production in *Klebsiella pneumonia* and *Acinetobacter* spp. were also shown by previous studies^(22, 25).

ESBL-producing organisms have been described in USA since the 1980's and have been associated strongly with nosocomial infections.

Carbapenams antimicrobials are considered the first-line therapy for ESBL infections, but resistance to this antimicrobial class is becoming widespread. Since the first case of CRE occurred in North Carolina in 1996⁽²⁷⁾.

In this study, Carbapenem – resistant phenotype was found in 61/120 (50.83%) of Gram – negative isolates. It was most commonly found in *Klebsiella pneumoniae* and *Acinetobacter baumannii* isolates. 21/37 (56.76%) were *Klebsiella pneumoniae* and 17/31 (54.84%) were *acinetobacter spp.* similar observations were also found in the earlier studies^(22, 25, 28).

Whereas, Gram – negative isolates showed a variable susceptibility to Aminoglycosides, Piperacillin – tazobactam and Ciprofloxacin antibiotics. *S.typhi* was isolated from 2 patients which showed almost sensitivity to all antimicrobials. *Pseudomonas aeruginosa* showed (50-100%) resistance towards Gentamicin, Co-trimoxazole, Aztreonam, Ceftazidime, Cefepime, Amikacin, Ciprofloxacin, Imipenem, Tobramycin.

Of 31 (18.56%) Gram – positive isolates, maximum isolation was of *Enterococcus spp.* 14(8.38%) and *Staphylococcus aureus* 11(6.59%) and CONS 4 (2.40%). Whereas, the studies by Valles *et al.*,⁽²⁹⁾ reported maximum isolation of CONS (20-30%) causing BSI in ICU patients and Manmeet *et al.*,⁽²⁸⁾ reported 39.5% of CONS isolation.

Although, the CONS is also a very preventable cause of infection and these isolates are often skin colonizers and appear in blood cultures as common contaminants at the time of sample collection⁽²²⁾ but is now a well

described pathogen associated with the use of central venous lines, prematurity in neonates⁽²⁸⁾. In this study almost all strains of *Enterococcus spp.*, *CONS* & *S.aureus* showed 100% resistance to Penicillin. Methicillin resistance among *S.aures* isolates was (54.55%) which was comparable to the earlier studies by Amit Bhatia *et al.*,⁽²²⁾ who reported 67% *MRSA* & a rate of 52.9% described in the National Nosocomial Infections Surveillance (NNIS) data summary for the period 1992 - 2004⁽³⁰⁾. Whereas, 75% *Coagulase negative Staphylococalspp.* were Methicillin resistant. All gram-positive isolates showed 100% sensitivity towards Vancomycin and Linezolid.

The present study brings to light that the prior knowledge of the most prevalent multi – drug resistant pathogens causing blood stream infections in ICU and their antibiotic sensitivity patterns can be of help to the clinicians in choosing appropriate antimicrobial therapy thus reducing morbidity and mortality among admitted patients in ICU. With rise in the problem of emergence of multidrug – resistance in isolates, there should be continuous surveillance of data of clinical isolates with their sensitivity pattern along with the implementation of strict antimicrobial usage policies in health care setting. Thus In the absence of new antimicrobials, prevention of infections with optimal adherence to infection control measures, and a good antibiotic policy for the hospital through promotion of antimicrobial stewardship programmes is the need of the hour to stop or reduce drug resistance.

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How to cite this article:

Jyoti P. Sonawane, Keertana S. Shetty, N. Kamath, Nitin Bharos and Abhay S. Chowdhary 2019. Bacteriological Profile and AntibioGram of isolates From Bloodstream Infections in Patients Admitted in ICU from a Tertiary care hospital, Nerul, Navi Mumbai, India. *Int.J.Curr.Microbiol.App.Sci*. 8(09): 1752-1723. doi: <https://doi.org/10.20546/ijcmas.2019.809.198>