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Research Article

# Bacteriological Profile and Antibiotic Susceptibility of Blood Culture Isolates in Intensive Care Units of a Tertiary Care Hospital.

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**Abstract:** Intensive Care Units infections have been found to be highest amidst the nosocomial infections. Sepsis results in increased morbidity and mortality rates. In order to decrease mortality and morbidity rates associated with sepsis, intensivists should have a keen knowledge of the existing bacteriological flora and their antibiotic susceptibility pattern. In this study, we aim to determine the prevalence of current bacteriological profiles in blood cultures, along with their antibiogram from Intensive Care Unit patients. This project was a retrospective cross-sectional study carried out for the duration of one year from December 2018 to 2019 in the Central lab, Microbiology department at tertiary care hospital, from patients suspicious of some blood stream infections like sepsis or other risk factors for it. From ICU, the number of blood samples received was 1440 in our Central Microbiology laboratory. All the samples received in the microbiology central lab were processed, and laboratory data, including bacteriological profile and antimicrobial susceptibilities were analysed. From ICU, the number of blood samples totally received were 1440 in our microbiology laboratory. Identification of isolates was done by colony morphology, Gram stain and standard biochemical reactions. Antibiotic susceptibility pattern was conferred by Clinical and Laboratory Standards Institute guidelines. Total number of positive cultures present in the study was 156(10.8%). Among these culture growth organisms, 82 were Gram positive (55.4%) isolates and 74 were Gram negative (50%). The most common isolate was Coagulase negative *Staphylococcus* trailed by *Escherichia coli* and *Staphylococcus aureus, Acinetobacter species* and *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus* species. Majority of the Gram positive isolates were susceptible to Linezolid, vancomycin and clindamycin. All the Gram negative isolates were susceptible to Carbapenems ,with susceptibility rate of 97% for *E. coli*, 93% for Acinetobacter, 93% for *Pseudomonas aeruginosa* and almost all these isolates showed 100% susceptibility to Colistin and Polymyxin-B.

**Keywords:** Blood stream infections, Intensive care unit, Antimicrobial resistance, Coagulase negative *Staphylococcus*, *E. coli*.

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# 1. INTRODUCTION

WHO estimated the burden of occurrence of nosocomial infection globally around 7-12%, the statistics from India are distressing, with a frequency of occurrence varying between 11% to 83% for diverse kinds of infections that are acquired in the hospital[1]. There is an escalating trend of bacterial infections in ICU, because of serious morbidities that are associated with impaired immunity, increased usage of intrusive diagnostic procedures, delay in sterilization and disinfection, and random usage of antibiotics. Beta lactam antibiotics are most commonly used antibiotics globally in ICU, because of their extent of action, wide spectrum activity and low toxicity [2]. In 1928, Alexander Fleming discovered the first beta lactam antibiotic Penicillin, which remained antibiotic of choice for many years. But later bacteria learnt new incentive mechanisms by which they can resist the antibiotics acting on them. One such mechanism is by production of enzymes called beta lactamases. In 1960, the first plasmid beta lactamase discovered was TEM-1. Over the last 20 years, several novel antibiotics belonging to the beta-lactam group were developed which were resistant to hydrolysis action by beta lactamases. But in due course of time these beta lactam antibiotics developed resistance by mutation of enzymes continuously, due to pressure that is imposed selectively by the antibiotic usage [3]. Sepsis is a life threatening preventable morbidity, which is encountered in Intensive care units. These succumb patients to stay in the ICU of hospital for longer duration, with increased mortality and also burdening patients with lots of financial constraints.[4.] Surveys from India suggest mortality rate is on the higher side ranging from 35.2% to 44.9% which is usually high when compared to other countries like United States[5.] It is also shocking that 30% of poor outcomes of patients is due to inappropriate empirical therapy with antibiotics [6,7]. These blood stream infections are due to, either of the organisms which can be either Gram positive or Gram negative. With the emergence of antimicrobial drug resistance in ICUs, it is very challenging for physicians to treat such patients and has become a threat to public health [8-10.] The aim of the study was to know the current spectrum of aerobic organisms isolated from blood samples in Intensive Care Units, their antibiotic susceptibility patterns and their trends which is more commonly prevailing among these isolates in our institute. Blood cultures is one of the most valuable diagnostic tests carried out to rule out blood stream infections. Earlier identification of blood stream infections and right choice of antimicrobial treatment can reduce the liability of sepsis in intensive care units. The objective of the study was, to know the prevalence of aerobic blood culture isolates commencing from intensive care units, and thereby be vigilant in using the right antibiotics for the right bug so that the right drug-bug combination is helpful in saving patients from mortality resulting due to sepsis.

# 2. SURVEY METHODS

## 2.1. *Study Design and Data Collection*

This project was a retrospective cross-sectional study, carried out for duration of one year from December 2018 to 2019 in the Central lab, Microbiology department at tertiary care hospital, from patients suspicious of some blood stream infections like sepsis or other risk factors for it. From the ICU, "the total number of blood samples received was 1440" in our Central Microbiology laboratory. Before drawing blood, the skin is disinfected with 10% Povidone-iodine solution for 2 min, followed by 0.5% Chlorhexidine solution for 1 minute. One to three milliliters of blood are taken aseptically from a peripheral vein and injected into the BACTEC culture vials. About 5 to 10 ml of blood was collected among adults and then inoculated into the 50 ml brain heart infusion broth. Blood culture bottles were incubated at 37-degree C aerobically for 24 hrs, followed by subcultures onto nutrient, blood and Mac-conkey agar plates. Blood culture bottles that did not show any signs of growth (hemolysis, turbidity) were subcultured again, on 2$^{nd}$, 3$^{rd}$, and 7$^{th}$ day, and were reported negative on 7$^{th}$ day after final subculture. Isolate identification was done by colony morphology, gram stain and standard biochemical reactions [11.] The antibiotic susceptibility testing was done on Muller Hinton Agar by Kirby-Bauer disc diffusion method, with commercially available antibiotic discs namely Amoxicillin-

clavulanate(20/10mcg), Ceftriaxone(30mcg), Cefuroxime(30mcg), Gentamicin(10mcg), Ciprofloxacin(5mcg), Ceftazidime(30mcg), Amikacin(30mcg), Cefotaxime(30mcg), Tobramycin(10mcg), Piperacillintazobactam(100/10mcg), Cefazolin(30mcg), Ampicillin(10mcg), Ciprofloxacin(5mcg), Cotrimoxazole(1.25/23.75mcg), Aztreonam(30mcg), Cefepime(30mcg), Chloramphenicol(30mcg), Tetracycline(30mcg), Penicillin(10units), Cefoxitin(30mcg), Erythromycin(15mcg), Linezolid(30mcg), Clindamycin(2mcg), Vancomycin(30mcg), Highlevelgentamycin(120mcg), Aztreonam(30mcg), Meropenem(10mcg,) Imipenem(10mcg), Cotrimoxazole(1.25/23.75mcg).

Interpretation of results of antibiotic susceptibility pattern were according to CLSI guidelines[12] Bacterial isolate identification confirmation was also done by automated identification systems, and also the antibiotic susceptibility pattern with Minimum Inhibitory Concentration values were known with our automated i.e. Vitek-2 compact machine.

## 2.2. *Inclusion criteria*

All the pathogens isolated from blood culture specimens of immunocompromised patients were included in the study.

## 2.4 *Exclusion criteria*

Contaminated specimens, mixed growth specimens, patients who were on prior antibiotics within the last 2 weeks of visiting the hospital, specimens from immuncompetent patients were excluded from the study.

## 2.4 *Ethical approval*

The study was conducted in accordance with the Declaration of Helsinki, the protocol was approved by the local ethics committee with reference number 002/SBMC/IHEC/2017/1018.

# 3. RESULTS

A total of 1440 blood samples from suspected patients of bacteremia, admitted in critical units of our tertiary care institute were regularly processed for blood culture in the Microbiology laboratory from December 2018 to 2019.

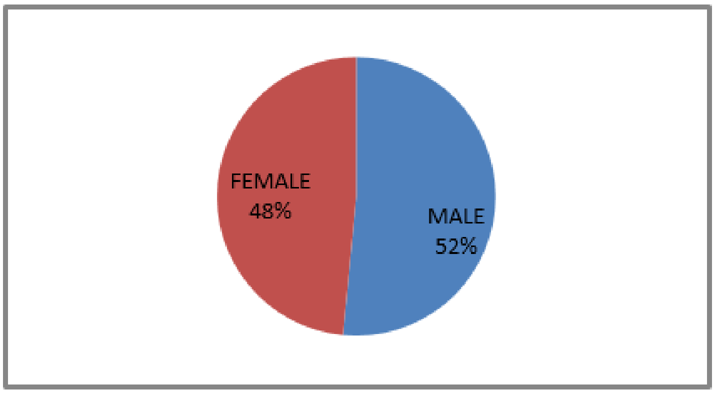



**Fig 1: Gender Distribution**

Out of these samples male prepositions were 742(51.5%)] higher compared to female with female preponderance around 698(48.5%).

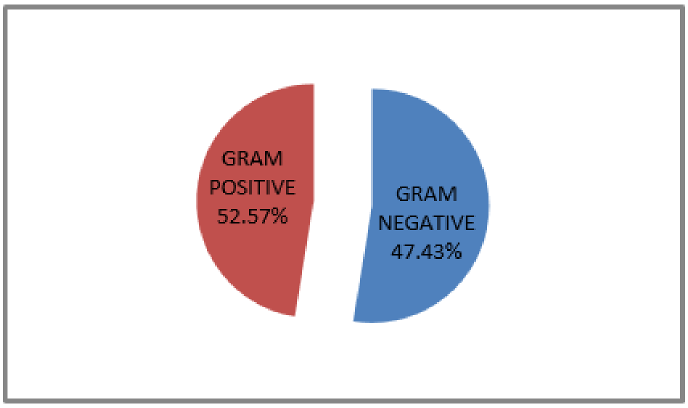



**Fig 2: Culture Positive Distribution**

Total number of positive cultures present in the study is 156 (10.8%). Among these total number of culture positive isolates, 82 were gram positive(52.56%) isolates with higher preponderance[13] and 74 were gram negative isolates (47.4%).

| Table 1: General characteristic of Patients with positive Blood cultures | | |
|---|---|---|
| **Age(In years)** | **No. of positive culture patients(n=156)** | |
| | **MALE** | **FEMALE** |
| | **N %** | **N %** |
| **18-40 years** | **27** | **21** |
| **40-60 years** | **37** | **29** |
| **>60 years** | **24** | **18** |
| **TOTAL** | **88** | **68** |

In Table 1, among 156 positive culture isolates, the majority of males and females belonged to the age group of 40-60 years and the next age group common were 18-40 years.

| Table 2: Clinical profile of patients with positive blood cultures | | |
|---|---|---|
| **Clinical suspicion** | **N** | **Percentage %** |
| **Suspected sepsis** | **59** | **37.82%** |
| **Pyrexia of unknown origin** | **27** | **17.30%** |
| **Unexplained leukocytosis/ or leukopenia** | **29** | **18.58%** |
| **Suspicion of infective endocarditis** | **18** | **11.53%** |
| **Systemic/localized infections including suspected meningitis/osteomyelitis/septic arthritis/acute untreated bacterial pneumonia or other possible bacterial infection.** | **23** | **14.74%** |

Table 2 shows a higher percentage of suspected sepsis, followed by unexplained leukocytosis/leukopenia/pyrexia of unknown origin/systemic or localized infections and finally suspicion of infective endocarditis.

### 3.1 Bacteriological profile

The majority of bacterial isolates were Gram-negative. Among the total isolates, *Escherichia coli* was most common isolated followed by *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter species.* The commonest isolate among gram positive was Coagulase negative *Staphylococcus*, followed by *Staphylococcus aureus* and *Enterococcus species*.

**Table 3 Distribution of bacterial isolates with their relative frequency Bacterial isolate**

| Gram negatives | Number | Percentage |
|---|---|---|
| *E . coli* | **38** | **51.35%** |
| *Klebsiella pneumoniae* | **8** | **10.81%** |
| *Pseudomonas aeruginosa* | **14** | **18.91%** |
| *Acinetobacter Species* | **14** | **18.91%** |
| **Gram positives** | **Number** | **Percentage** |
| CONS | **53** | **64.63%** |
| *S. aureus* | **24** | **29.26%** |
| *Enterococcus species* | **5** | **6.09%** |

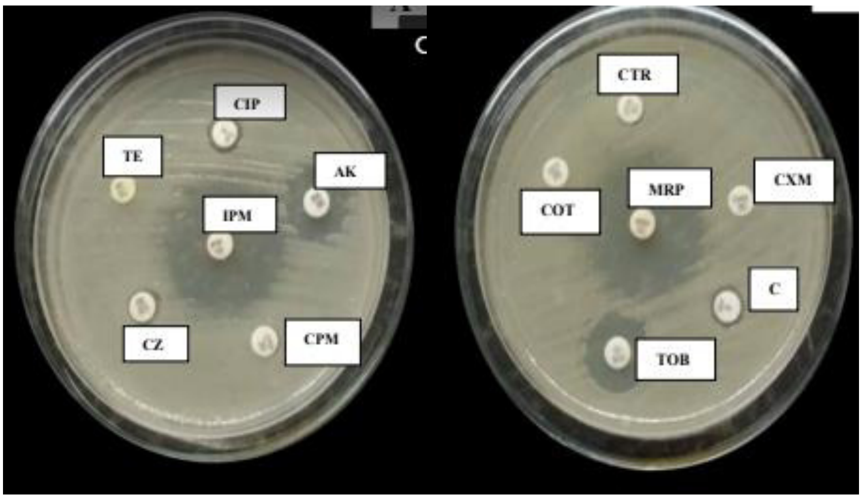
Isolate which was most commonly identified among the entire list as illustrated in Table 3 was Coagulase negative *Staphylococcus* species followed by *Escherichia coli* and *Staphylococcus aureus, Acinetobacter species* and *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Enterococcus species*. All bloodstream infections were due to the presence of a single organism only. Among Gram positive bacterial isolates 100% of Coagulase negative Staphylococcus species, 29% of *Staphylococcus aureus*, 80% of *Enterococcus species* were resistant to the penicillin group of antibiotics. However, the majority of the Gram positive isolates were sensitive to Linezolid, Vancomycin and Clindamycin. Among Gram negative isolates, *E. coli* (57.5%), *Acinetobacter species* (18.9%), Pseudomonas species (18.9%) were dominant species. All these Gram negative isolates showed weak activity against third generation cephalosporins, but good activity against all carbapenems with susceptibility of 97% for *E. coli*, 93% for *Acinetobacter*, 93% for *Pseudomonas aeruginosa*. Moreover, all these isolates showed 100% susceptibility to Colistin and Polymyxin-B.

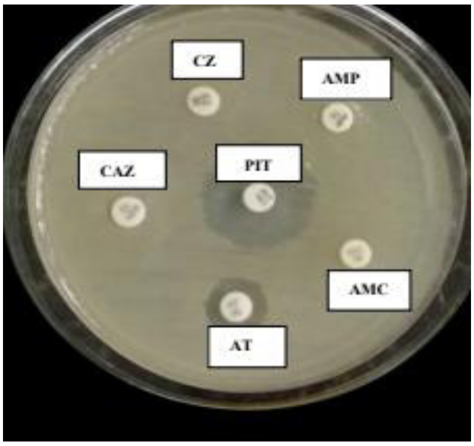
**Table 4: Antibiotic Susceptibility Pattern Of Gram Positive Isolates**

| Isolates (n=82) | Penicillin | Cefoxitin | Erythromycin | Clindamycin | Cotrimoxazole | Linezolid | Vancomycin |
|---|---|---|---|---|---|---|---|
| CONS (n=53) | 0 | 0 | 5(9.4%) | 38(71.6%) | 17(32.0%) | 53(100.0%) | NA |
| *S. aureus* (n=24) | 17(70.8%) | 19(79.1%) | 6(25.0%) | 13(54.1%) | 13(54.1%) | 24(100.0%) | NA |
| *Enterococcus species* (n=5) | 1(20%) | 2(40%) | 3(60%) | 4(80%) | 3(60%) | 5(100%) | 5(100%) |

***Not applicable (NA). Routine susceptibility testing with Vancomycin disc is no more preferred for Staphylococcus species since 2017 CLSI guidelines.***

Table 4 shows that CONS were 100% susceptible to linezolid followed by clindamycin, cotrimoxazole and erythromycin. *Staphylococcus aureus* were 100% susceptible to linezolid followed by cefoxitin, penicillin, clindamycin, cotrimoxazole and erythromycin. *Enterococcus* species were 100% susceptible to linezolid, vancomycin, clindamycin, erythromycin, cotrimoxazole, cefoxitin followed by penicillin.






*TE-Tetracycline, CIP-Ciprofloxacin, AK-Amikacin, IPM-Imipenem, CPM-cefepime, CZ-Cefazolin, CTR-ceftriaxone, MRP-meropenem, CXM-cefuroxime, COT-Trimethoprim-sulfamethoxazole, C-chloramphenicol, TOB-Tobramycin, CZ-cefazolin, AMP-Ampicillin, PIT-Piperacillin-tazobactam, CAZ-ceftazidime, AMC-amoxicillin –clavulanic acid, AT-Aztreonam.*

**Fig 3 Antibiotic Susceptibility Pattern of Gram Negative Isolates**

**Table 5: Antibiotic Susceptibility Pattern of Gram Negative Isolates**

| Isolates (n=74) | MRP | IMP | PIT | CIP | COT | DO | AK | AMC | GEN |
|---|---|---|---|---|---|---|---|---|---|
| *E. coli* (n=38) | 37 (97.3%) | 36 (94.7%) | 35 (92.1%) | 21 (55.2%) | 28 (73.6%) | 15 (39.4%) | 21 (55.2%) | 11 (28.9%) | 17 (44.7%) |
| *Klebsiella pneumoniae* (n=8) | 6 (75%) | 6 (75%) | 3 (37.5%) | 5 (62.5%) | 6 (75%) | 3 (37.5%) | 4 (50%) | 2 (25%) | 3 (37.5%) |
| *Pseudomonas aeruginosa (n=14)* | *13 (92.8%)* | 11 78.5%) | 6 (42.8%) | 7 (50%) | 11 (78%) | 11 (78.5%) | 6 (42.8%) | 3 (21.4%) | 7 (50%) |
| *Acinetobacter* Species (n=14) | 11 (78.5%) | 13 (92.8) | 2 (14.2%) | 4 (28.5%) | 9 (64.2%) | 7 (50%) | 4 (28.5%) | 2 (14.2%) | 1 (7.1%) |

Fig 3 and Table 5 represents *Escherichia coli* and *Klebsiella pneumoniae* to have higher susceptibility to Carbapenems, followed by Piperacillin –tazobactam (Beta Lactam-Beta Lactamase Inhibitors), Cotrimoxazole, Ciprofloxacin, and Amikacin. *Pseudomonas aeruginosa* and *Acinetobacter* have higher susceptibility to Meropenem, imipenem, Cotrimoxazole, and Doxycycline.

**Table 6: Overall status of antibiotic resistance among the Gram positive and Gram-negative isolates**

| ANTIBIOTICS TESTED | GRAM POSITIVE | | GRAM NEGATIVE | |
|---|---|---|---|---|
| | R | R % | R | R % |
| Meropenem | Nil | Nil | 7 | 9.45% |
| Imipenem | Nil | Nil | 8 | 10.81% |
| Piperacillin tazobactam | Nil | Nil | 28 | 37.83% |
| Ciprofloxacin | Nil | Nil | 37 | 50% |
| Cotrimoxazole | Nil | Nil | 20 | 27.02% |
| Doxycycline | Nil | Nil | 38 | 51.35% |
| Amikacin | Nil | Nil | 39 | 52.70% |
| Amoxicillin clavulanic acid | Nil | Nil | 56 | 75.67% |
| Gentamicin | Nil | Nil | 46 | 62.16% |
| Penicillin | 64 | 78.04% | Nil | Nil |
| Cefoxitin | 61 | 74.39% | Nil | Nil |
| Erythromycin | 68 | 82.92% | Nil | Nil |
| Clindamycin | 55 | 67.07% | Nil | Nil |
| Cotrimoxazole | 49 | 59.75% | Nil | Nil |
| Vancomycin | 0 | 0 | Nil | Nil |
| Linezolid | 0 | 0 | Nil | Nil |

Table 6 shows Overall higher percentage of antibiotic resistance with respect to Gram negative isolates were seen with Amoxicillin clavulanic acid, Gentamicin, Amikacin, Doxycycline, Ciprofloxacin least percentage of antibiotic resistance seen among Meropenem. Higher percentage of Antibiotic resistance with respect to gram Positive isolates were seen with erythromycin, penicillin, cefoxitin, clindamycin and cotrimoxazole.

## 4. DISCUSSION

Patients admitted in intensive care units are likely to progress to nosocomial bloodstream infections,which can lead to increased morbidity rates and mortality rates[14]. Our study provides information regarding dissemination of aerobic blood culture organisms, along with their antibiotic susceptibility pattern among patients admitted in intensive care units, which plays a major role in the management of these patients. Among the total 1440 samples collected, 156 samples were positive for blood culture, which is nearly 10.8%. Male preponderance was higher in the age group of 40-60 years compared to females. The blood culture positivity rate in our study was 10.8%[15,16] similar to a study done by Jyoti [15] *et al* showed positive blood cultures were obtained in 10.29 % (308/2994) and Khana et al[16] where 3,324 blood samples collected for culture and sensitivity and only 345 (10.3%) showed bacterial growth. A diverse group of organisms are responsible for bloodstream infections. In our study, among the culture positive isolates 82 were Gram positive[17] (55.4%) and 74 were Gram negative (50%). Among the Gram positive isolates, Coagulase staphylococcus species[18] was the most common followed by *Staphylococcus aureus*[19] and *Enterococcus species*. Among the Gram negative isolates *Escherichia coli*[15] most common followed by *Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Acinetobacter species*. Our study findings with gram positive bacteria predominance accounted for 64.63% of cases with Coagulase Negative Staphylococcus were similar to Gohel *et al* study,[20] which had positive blood cultures in 9.2% of cases of which gram-positive bacteria accounted for 58.3% of cases with *Staphylococcus aureus* predominance; gram negative bacteria accounted for 40.2% with Enterobacteriaceae predominance; and 1.5% were fungal isolates. The most sensitive drugs for gram-positive isolates were vancomycin, teicoplanin, daptomycin, linezolid, and tigecycline and for Gram-negative isolates were carbapenems, colistin, aminoglycosides and tigecycline. Similarly, CoNS were considered as contaminants in the past but nowadays they have become one of the leading cause of bloodstream infections due to increasing use of medical devices such as prosthetic heart valves, vascular grafts and indwelling catheters. This might be related with our study findings where CoNS contributed to nearly 64.63% of cases of bacteremia which was one of the important organisms related to blood stream infection[21]. All of the bacteremia episodes were caused by a single organism which is almost similar to a study done by Kumar[22] *et al* where most (93.2%) bacteremia episodes were caused by a single organism, while polymicrobial aetiology was observed in 52 (6.8%) cases. *E. coli* (57.5%) contributes to the major group among the gram negative isolate followed by *Acinetobacter* species (18.9%), *Pseudomonas* species (18.9%), and *Klebsiella* species (10.5%). The inappropriate use of antibiotics has led to a higher antimicrobial resistance. All the gram positive organisms were susceptible to Linezolid,[22] Vancomycin[24,25] and Clindamycin which was consistent with the study done by Wasihun *et al*, all gram-positive isolates in this current study were sensitive to vancomycin consistent a to study done by Fayyaz *et al*[26] and shreshtha *et al*[27] which showed gram positive cocci isolates showed 95% sensitivity to teicoplanin and 100% sensitivity to vancomycin and linezolid. Gram negative organisms like *Escherichia coli* and *Klebsiella pneumoniae* has higher susceptibility to Carbapenems[27], followed by Piperacillin – tazobactam (BetaLactam-BetaLactamase Inhibitors), Cotrimoxazole, Ciprofloxacin and Amikacin. *Pseudomonas aeruginosa* and *Acinetobacter* have higher susceptibility to Meropenem, imipenem, Cotrimoxazole, and Doxycycline. Over all higher percentage of Antibiotic resistance with respect to gram negative isolates were seen with Amoxicillin clavulanic acid, Gentamicin, Amikacin, Doxycycline, Ciprofloxacin least percentage of antibiotic resistance seen among Meropenem. Higher percentage of antibiotic resistance with respect to Gram Positive isolates were seen with erythromycin, penicillin, cefoxitin, clindamycin and cotrimoxazole. All the organisms identified by Gram stain as Gram negative revealed weaker susceptible activity to beta-lactam antibiotics, as it was the most commonly prescribed drugs for patients which contributed to such high levels of antimicrobial resistance. Among the isolates which are Gram negative, the family Enterobacteriales showed higher susceptibility to Carbapenems, Cotrimoxazole, Ciprofloxacin similarly non-fermenters in the study showed high susceptibility to Carbapenems, Co-trimoxazole and Doxycycline poor susceptibility to Amikacin, Amoxicillin-clavulanic acid, and Piperacillin Tazobactam. We observed 7 (9.2%) of gram negative isolates were not susceptible to Carbapenems, with 4(57.1%) and 3(42.8%) isolates among non-fermenters and members of Enterobacteriaceae respectively. The greatest menace with these infective microorganisms is the limited antibiotics which are available for treatment. With higher antimicrobial drug resistance and limited choices of drugs available for treatment, the health care practitioners are left with last resort of drugs like Polymyxin-B and Colistin which could eventually lead to pan drug resistance[29].

## 5. CONCLUSION

Our study findings revealed that the prevalence of aerobic blood culture isolates commencing from intensive care units is around 10.8% and also it is identified that both Gram positive and Gram negative organisms are responsible for causing bloodstream infections. This implies that blood culture is warranted in every other suspicious cases of septicemia and bacteremia, also when the antibiotic susceptibility profile of an organism is revealed de-escalation of higher end antimicrobial becomes mandatory to bring down the selective pressure of antimicrobials. Nevertheless, a good hospital infection prevention and control team with effective antibiotic stewardship policy can combat drug resistance.

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## 7. AUTHORS CONTRIBUTIONS STATEMENT

Dr.Nishanthy.M, Dr.Chitralekha saikumar made extensive contributions in the work design. First author has drafted the work, analysed, interpreted the data and second author has approved the version to be published.

## 8. CONFLICT OF INTEREST

Conflict of interest declared none.

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