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Bacteriological Spectrum and Antimicrobial Resistance Burden of Bloodstream Infection Isolates in Intensive Care Unit Patients

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A B S T R A C T

Bloodstream infections (BSIs) are a significant cause of morbidity and mortality in intensive care unit (ICU) patients. In hospital settings, bloodstream infections are associated with mortality rates of approximately 16% in general wards and 40% in intensive care units. This study aimed to identify the bacterial isolates causing BSIs in the ICU and their antimicrobial susceptibility profiles. A cross-sectional observational study was conducted on 224 patients with clinically diagnosed BSI in the ICU between December 2017 and October 2019. Blood samples were collected and subjected to culture. Isolates were identified using conventional methods, and antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disc diffusion method. Of the 224 samples, 54 (24.10%) were culture-positive, with a higher prevalence in males (57.40%) and the age group of 51-60 years (29.63%). Gram-positive bacteria (55.56%) were more common than gram-negative bacilli (37.04%). The most common isolates were *coagulase-negative staphylococci* (CoNS) (37.03%), *Klebsiella pneumoniae* (14.82%), *Staphylococcus aureus* (12.97%), and Escherichia coli (11.12%). Gram-positive isolates showed high susceptibility to vancomycin and linezolid, while gram-negative isolates were highly susceptible to imipenem and piperacillin-tazobactam. Methicillin resistance was observed in 57% of S. aureus and 55% of CoNS isolates. Among the gram-negative isolates, 50% were extended-spectrum beta-lactamase (ESBL) producers and 20% were metallo-beta-lactamase (MBL) producers. This study highlights the importance of early recognition and prompt initiation of appropriate antimicrobial therapy to reduce morbidity and mortality associated with BSIs in ICU patients. Continuous surveillance and adherence to antimicrobial stewardship are crucial to combat the emerging threat of antibiotic resistance.

Introduction

Bloodstream infections (BSI) are a major cause of illness and mortality. (1) Accounting for approximately 15% of attributable deaths, these are a significant cause of mortality in both industrialized and developing nations.(2) In hospital settings, bloodstream infections are associated with mortality rates of approximately 16%

in general wards and 40% in intensive care units. (3) Bloodstream infections are often accompanied by sepsis and septic shock that necessitate admission to the intensive care unit (ICU). (4)BSI accounts for close to 20% of all admissions in ICU. (5) Bloodstream infections lead to higher death rates, extended stays in intensive care units and hospitals, and heightened financial burden on the healthcare system. (6)

A wide range of bacteria that cause bloodstream infections has been delineated, and this range is subjected to geographical alteration. (7) Studies from the West show that ICUs have more gram-positive bacteria causing infections as related to Asian ICUs, counting India, where gram-negative infections are predominant. (8, 9)

The cornerstone of treatment consists of timely and suitable antibiotic administration coupled with supportive care measures. (1) One of the main complications of managing BSI is the increasing resistance of bacteria to antibiotics. The rise of drug-resistant pathogens in the bloodstream limits treatment options and complicates patient care. Delay in diagnosis and ineffective early empiric antimicrobial therapy are important predictors of death in patients with bloodstream infections. (10)

The predominant type of pathogens, including multidrug-resistant organisms (MDROs) and antimicrobial susceptibility, is variable between institutions also across and inside countries. (11) Knowledge of the common isolates and their susceptibility patterns in the ICU is necessary to produce the most effective empiric antibiotic protocol.

Considering the above facts, this study was conducted to identify the various bacterial isolates causing bloodstream infections in the ICU and their antimicrobial susceptibility profiles.

Materials and Methods

The present cross-sectional observation study was conducted at the Department of Microbiology, Tertiary Care Center. A total of 224 patients clinically diagnosed with bloodstream infections in the ICU between December 2017 and October 2019 were included in the study. From all the patients, blood samples were collected (12) and subjected to culture. Culture-positive isolates are identified using conventional methods such as Gram staining, motility testing, and various biochemical tests. The isolates underwent antimicrobial susceptibility testing using a modified version of the Kirby-Bauer disk diffusion technique on Mueller-Hinton agar. The interpretation of zone diameters followed the guidelines set by the Clinical and Laboratory Standards Institute (CLSI). (13). Gram-negative bacilli that showed the resistance to Cefotaxime (30 µg)/ Ceftriaxone (30 µg)/ Ceftazidime(30µg)to the drug were likely to produce ESBL and this isolate were subjected to Phenotypic Confirmation combination disc test method by ceftazidime (CAZ 30µg) and ceftazidime/clavulanic acid (CAZ/CA 30µg/10 mg), interpreted as per CLSI guidelines(13). Imipenem-resistant isolates were evaluated for metallo-β-lactamase (MBL) production using the imipenem-EDTA combination disc test. For Enterobacteriaceae and *Pseudomonas*, a positive MBL result was indicated when the inhibition zone of the imipenem-EDTA disc exceeded that of the imipenem disc alone by 7mm or more. (14, 15) In *Acinetobacter* isolates, MBL production was confirmed when the zone of inhibition expanded by more than 17 mm for an imipenem-EDTA disc with imipenem alone. (16) Frequency and percentage were used to present the qualitative data. Microsoft Excel was used for statistical analyses of the data. Ethical approval for the study was obtained from the Institutional Ethics Committee of Swami Ramanand Teerth Rural Government Medical College, Ambajogai.

Results and Discussion

The study showed that the maximum number of patients were in the age group of 41–50 years (30.35%), followed by the age group of 31–40 years (21.48%), age group of 51–60 years (15.62%), age group of 21–30 years (12.9%), above 60 years (11.16%), and age group of 13–20 years (8.49%). Details of Culture-Positive Cases by Age and Sex given in table 1.

Of the 54 culture-positive samples, 23 (42.60%) were from females and 31(57.40%) were from males, whereas more culture positivity (29.63%) was observed in the age range of 51 to 60 years. Details of Culture-Positive and Culture-negative shown in graph 1.

Among the 50 bacterial isolates, 30(55.56%) were gram-positive and 20 (37.04%) were gram-negative bacilli. Among the 30(55.56%) gram-positive isolates, *CoNS* was the most frequent (37.03%), followed by *Staphylococcus aureus* (12.97%) and *Enterococcus faecalis* (5.55%). Among the 20(37.04%) gram-negative

isolates, the most frequently found pathogens were *Klebsiella pneumoniae* (14.82%), followed by *E. coli* (11.12%), *Acinetobacter baumannii* (7.40%), and *Pseudomonas aeruginosa* (3.70%). All four fungal isolates belonged to *Candida* species. Antimicrobial Susceptibility Profile of gram-positive bacteria, Enterobacteriaceae and non-fermenters are shown in graph 1,2 and 3 respectively.

In our study, *Staphylococcus aureus* and *coagulase-negative Staphylococcus* were found to be resistant to methicillin in 57% and 55% of patients, respectively. As confirmed by a combined disk test, among the gram-negative isolates, *Klebsiella pneumoniae* 6(75%) was the highest ESBL producer, followed by *Pseudomonas aeruginosa* 1(50%), *Acinetobacter baumannii* 2(50%), and *E. coli* 1 (16.6%). Among the 20 gram-negative bacteria, 10(50%) were ESBL-producers. As confirmed by a combined test, among the gram-negative isolates, *Pseudomonas aeruginosa* 1(50%) and *Acinetobacter baumannii* 2(50%) were the highest MBL producers, followed by *Klebsiella pneumoniae* 1(12.5%). This study revealed that among the total gram-negative bacterial isolates, 4 (20%) were MBL producers.

Bloodstream infections are a significant cause of mortality in both industrialized and developing countries. It can be life-threatening for patients in the intensive care unit (ICUs). Multiple factors contribute to the development of BSIs in ICU settings, with outcomes influenced by the specific pathogens involved, existing risk factors, prompt intervention, and the timely administration of antibiotics.

Studies have shown that the incidence of BSI in the ICU ranges from 0.47% to 20.9%. (17) Our study revealed that 54 (24.10%) of 224 cases of bloodstream infections yielded positive cultures. This was comparable to other studies from India: Patel *et al.,* and Gill *et al.,* (18,19) showed 24.8%, Neeta *et al.,*(17)showed 22%, and Bhabhor *et al.,* showed 18.35% (20) This variation in the positive rates may be due to factors like patient type, geographical locations, risk factor distribution like invasive procedures, underlying co-morbid conditions such as e.g., diabetes mellitus, timing and a number of blood cultures or differences in blood culture system like usage of conventional or automated blood culture methods (21–23), variations in the population under study, study duration and hospital setups for intensive care management(17). In our study, there was a male predominance, accounting for 57.40% positivity and females accounting for 42.60% positivity. This is comparable with other studies that showed a male predominance of 54.33% by Bhabhor *et al.,* (20) and 55.77% by Bhatia *et al.,* (24) and 57.6% by Sarvepalli *et al.,* (25). Most studies have shown that the predominance of men appears without explanation (26), but this could be due to unidentified anatomical differences between females and males or due to variations in skin colonization (27).

Our study revealed that the age group between 51 and 60 years (29.63%) was mostly culture-positive, followed by > 61 years (24.08%). This observation was comparable with other studies that show the mean (±SD) age of commonly affected patients were 55.6 (±19.9) and 56.16(±15) years by Panahi Yunes *et al.,* (28) and Sarvepalli AK *et al.,* (25)respectively. More positivity in elderly patients is explained by reports from different countries, showing that the range of ICU-treated elderly patients was 26-52% (29), which may be due to the declining immunity pasting middle age and exposure of elderly individuals to a high risk of contracting infectious diseases, including bacteremia. (24)

In the present study, among 54 positive cultures, 55.56% were gram-positive isolates, 37.04% were gram-negative, and 7.40% were fungi. Overall, Gram-positive organisms were more common, and this finding was consistent with those reported by Patel *et al.,* Gill *et al.,* (18,19) (53%), and Stéphane *et al.,* (30)(69.6%), and Bhabhor *et al.,* (20)(51.25%). But in contrast to our findings, other studies showed gram-negative as predominant isolates.(17,25) While earlier US surveillance studies indicated a higher prevalence of gram-positive bacteria in bloodstream infections (BSIs) compared to gram-negative bacteria, recent observations have noted an increase in BSIs caused by gram-negative bacteria.

In the present study, *coagulase-negative Staphylococcus* (37.04%) was the most common Gram-positive isolate. It was comparable with studies by Patel K *et al.,* Gill M *et al.,* (18,19), and Orsini *et al.,* (31) who showed 35% and 38.8% of isolation rates respectively. Valles *et al.,* also revealed that CoNS are common among isolates (20-30%), causing bloodstream infections (BSIs) in ICU patients. (32) This was clarified by the fact that CoNS are skin commensal and appear as common contaminants in blood culture and can be prevented by careful skin disinfection (33). The clinical correlation of these isolates can help distinguish between possible and true pathogens. (34,35) Studies show that frequent use of

indwelling devices is a major source of an increasing incidence of CoNS-causing BSIs.(36) In our study, the isolation rate of *S.aureus* was 12.97%. It is comparable to studies by Patel k *et al.,* Gill m *et al.,* with (14.3%), (18, 19), and Orsini *et al.,* with (20.8%) (31). In our study, *Enterococcus faecalis* was isolated in 3(5.5%) cases. Other studies by Orsini *et al.,* reported 16.6% (31), while stéphane *et al.,* reported 6% (30) of *Enterococcus faecalis*.

In our study, among the gram-negative isolates, the most common were *Klebsiella pneumoniae* (14.82%) and *E. coli* (11.12%). It is comparable with studies by Orsini *et al.,* which shows (26.3%), (21%) (31) and Neeta *et al.,* showed (15.15%) and (9.0%)(17) of *Klebsiella pneumoniae* and *E. coli*, respectively. Among non-fermenter isolates, *Acinetobacter baumannii* was isolated from 4(7.40%) samples, which was comparable to that reported by Bhatia *et al.,* (7.69%) (24) and Patel *et al.,* (5.6%) (18). In our study, *Pseudomonas aeruginosa* was isolated in two (3.70%) samples, which is comparable with the results of another study by Gill *et al.,* (3.6%). (19) In our study, Four (7.40%) isolates of fungi. This finding was comparable with that of a study by Orsini *et al.,* who isolated 12 patients (9.8%). (31)

In the present study, all gram-positive isolates were susceptible to vancomycin and linezolid. This is comparable to the results of Sangwan *et al.,* (37), Khan *et al.,* (38) which shows 100% susceptibility to linezolid and vancomycin. In our study, gram-positive isolates were less susceptible to penicillin; findings were similar to the study by Chand Wattal *et al.,* which shows (0%). (35) In the present study, CoNS was highly susceptible to clindamycin (100%), ciprofloxacin (95%), gentamicin (95%), and erythromycin (90%), findings were similar to those Bhabhor *et al.,* (20).In our study, *S. aureus* isolates showed 71.42% sensitivity to gentamicin and ciprofloxacin, and 57.1% to erythromycin and clindamycin. Neeta *et al.,* (17) showed 100% susceptibility to gentamicin, Amit *et al.,* (39) showed 76.5% susceptibility to erythromycin, and Khan *et al.,* showed 100% susceptibility to erythromycin (38). Khan *et al.,* showed 60% susceptibility, (38) and Sangwan *et al.,* showed 40.4% susceptibility to clindamycin. (37). Ullas Bhabhor *et al.,* showed 69.44% sensitivity to ciprofloxacin. (20) In our study, *Enterococcus faecalis* showed moderate susceptibility to penicillin, high-level gentamicin, and ciprofloxacin (66.6%). These findings were correlated to studies by Sarvepalli *et al.,* (25) and Sangwan *et al.,* (37)

Among *coagulase-negative Staphylococcus* isolates, 55% were methicillin-resistant, which is consistent with the results of other studies by Zadeh *et al.,* (66.6%). (40) Methicillin-resistant *Staphylococcus aureus* in our study was 57.14%which similar with other studies by Jyoti Sangwan *et al.,* (48%) (37) Orsini *et al.,* (40%). (31) A study conducted in Delhi revealed that the isolation rate of MRSA strains from blood samples collected in the ICU was 43%. (35) Compared to MSSA isolates, MRSA isolates have been observed to be more resistant to different antibiotics, which were shown in other studies. (41) In our study, MRSA isolates showed a significant difference in resistance to erythromycin, ciprofloxacin, and gentamicin. For the treatment purpose of MSSA bacteremia, β-lactams are considered superior compared to vancomycin. (42) So once if culture and sensitivity reports are available, Staphylococcus aureus, which is methicillin-sensitive, needs to be de-escalated to beta-lactam antibiotics from vancomycin. Therefore, antibiotics like vancomycin can be used for MRSA infection.

Isolates of the Enterobacteriaceae family were highly susceptible to imipenem(87.5% *for Klebsiella pneumoniae* and 100% for *E.coli*), it correlates with Jyoti Sangwan *et al.,* study (37), Patel *et al.,* (43) study. *Klebsiella pneumoniae, Escherichia coli* was showing a good rate of sensitivity to piperacillin-tazobactam(75%and 83.3%), amikacin(62.5% and 66.6%), amoxicillin-clavulanate(50%each) respectively; these findings were nearly matching with studies by Patel *et al.,* (43).In contrast to our study, Sangwan *et al.,* showed high susceptibility to amikacin (37). In our study, *Escherichia coli* and *Klebsiella pneumoniae* isolates were moderately sensitive to gentamicin (33.3% and 50%, respectively), ciprofloxacin (50% and 25%, respectively), and cotrimoxazole(33.3% and 12.5%, respectively). These findings are similar to those of a study by Zadeh *et al.,* (40) *E.coli* showed less susceptibility to ampicillin(16.6%), and these findings were similar to those of a study by Chand Wattal *et al.,* (4%). (35) Our study revealed that ceftriaxone was effective against 25% of *Klebsiella pneumoniae* isolates and 66.6% of *Escherichia coli* isolates. This finding showed a resemblance to Cortes *et al.,* study. (44) But a high level of resistance was reported by study of Neeta *et al.,* (17) Chand Wattal *et al.,* (35)Among Enterobacteriaceae compared to *Escherichia coli, Klebsiella pneumoniae* has shown more resistance and emerged as a significant problematic organism.

In our study, 50% isolates of *Pseudomonas aeruginosa* were susceptible to cefepime, ceftazidime, gentamicin, amikacin, imipenem, and ciprofloxacin. These findings were similar to those reported by Wattal *et al.,* (35). In other studies, Pseudomonas aeruginosa showed high resistance to most antibiotics. (17) In our study, 50% of *Acinetobacter baumannii* isolates were susceptible to amikacin, trimethoprim-sulfamethoxazole, ceftriaxone, ceftazidime, and imipenem. These findings are correlated with studies by Zadeh *et al.,* (40) and Sangwan *et al.,* (37). A total of 25% of *Acinetobacter baumannii* isolates were sensitive to cefepime, gentamicin, piperacillin-tazobactam, and ciprofloxacin, and these findings were correlated with studies by Chand Wattal *et al.,* (35) and Patel *et al.,* (43). Management of opportunistic infections with Acinetobacter species may be the biggest challenge faced by clinicians today, particularly in intensive care units, because resistance among clinical isolates has developed rapidly.

The production of extended-spectrum beta-lactamases (ESBL) stands as the primary mechanism of resistance against third-generation cephalosporins. Studies in India have shown that ESBL production in Enterobacteriaceae organisms associated with bloodstream infections (BSI) can reach levels as high as 89%.(45) In our study, 50% of gram-negative isolates were confirmed as ESBL producers.

These findings were consistent with those of Sangwan *et al.,* (18.6%). (37). ESBL-producing Enterobacteriaceae play an important role in the ineffectiveness of antimicrobial therapies. Consequently, the main cause for increasing the mortality rate and prolonging the hospital stay. Therapeutic picks for ESBL-producing organisms are very limited. ESBL-producing strains are resistant to most β-lactam antibiotics except carbapenems and cephamycins. Plasmid-encoded ESBLs frequently encode resistance to trimethoprim, sulphonamides, tetracyclines, and aminoglycosides.(46)

In the present study, the rate of MBL (Metallo beta-lactamase) producers was 20% in gram-negative bacteria and common among *Acinetobacter baumannii* (50%) and *Pseudomonas aeruginosa* (50%). Neeta *et al.,* reported that 83.33% and 50% of P. aeruginosa and Acinetobacter baumannii isolates were MBL producers, respectively. (17)Widespread use of carbapenem in ICUs is considered the cause of the emergence of carbapenem resistance. Plasmids encoding metallo-β-lactamase (MBL) genes are easily acquired, and dissemination of these strains is a worrisome problem. These strains are not susceptible to all β-lactams, including carbapenems and serine β-lactamase inhibitors (such as sulfones and clavulanate). (47) Early recognition and rapid initiation of infection control are essential to prevent the dissemination of this MBL genetic locus to other gram-negative bacteria. Studies indicate that antibiotic use in intensive care units (ICUs) is approximately ten times higher than in regular hospital wards. (48)This results in the development of pathogens resistant to multiple drugs, creating a significant challenge for physicians and critical care specialists, as viable treatment options are often unavailable in such cases.

Available data from the present study were helpful in developing a protocol for empirical treatment of patients with BSI in the ICU. Prompt initiation of suitable antibiotics plays a crucial role in decreasing the morbidity and mortality due to bloodstream infections. The formulated protocol of empirical treatment for clinically suspected BSIs in the ICU is based on the information available about the likely pathogen causes of BSIs in the ICU and its antibiotic susceptibility pattern.

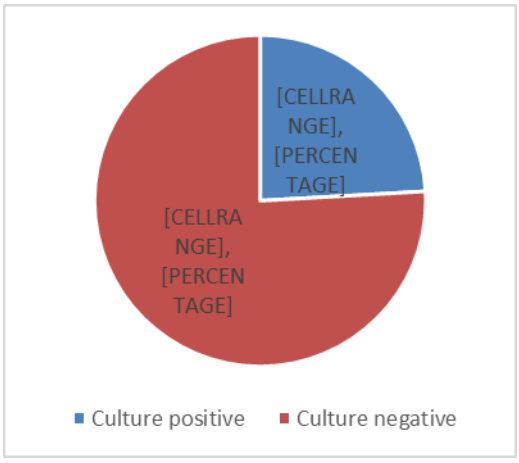
Susceptibility analysis of the bacterial isolates indicated that linezolid and vancomycin were highly active against gram-positive bacteria, whereas piperacillin-tazobactam and imipenem were highly active against gram-negative organisms, causing bloodstream infections in our hospital ICU environment. However, the findings are limited by the single-center study, small sample size, and lack of molecular methods to characterize the resistance mechanisms. Further multicenter studies are needed to confirm these observations and enhance their generalizability.

In conclusion, Bloodstream infections may progress to sepsis, which is a life-threatening condition. Prompt initiation of treatment reduces the mortality rate. Information about common isolates causing BSIs in the ICU setup and their susceptibility pattern provides knowledge to the clinician to start empirical therapy sooner to reduce the consequences of BSIs. Currently, antibiotic resistance is a serious threat. Prudent use of antibiotics, strict implementation of antimicrobial stewardship programs, and ongoing monitoring can contribute to limiting further proliferation of these superbugs.

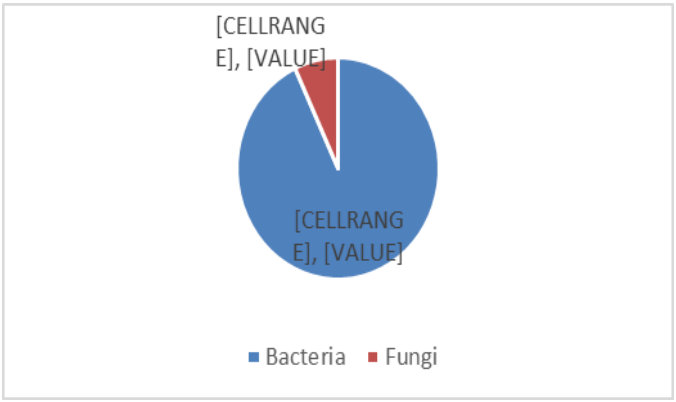
Table.1 Distribution of Culture-Positive Cases by Age and Sex

Age	No. Of cases		Total	Percentage (%)
	Male	Female		
13-20years	2	3	5	**9.25%**
21-30 years	2	4	6	**11.12%**
31-40years	3	2	5	**9.25%**
41-50 years	6	3	9	**16.67%**
51-60 years	11	5	16	**29.63%**
>61 years	7	6	13	**24.08%**
Total	**31 (57.40%)**	**23 (42.60%)**	**54**	**100%**

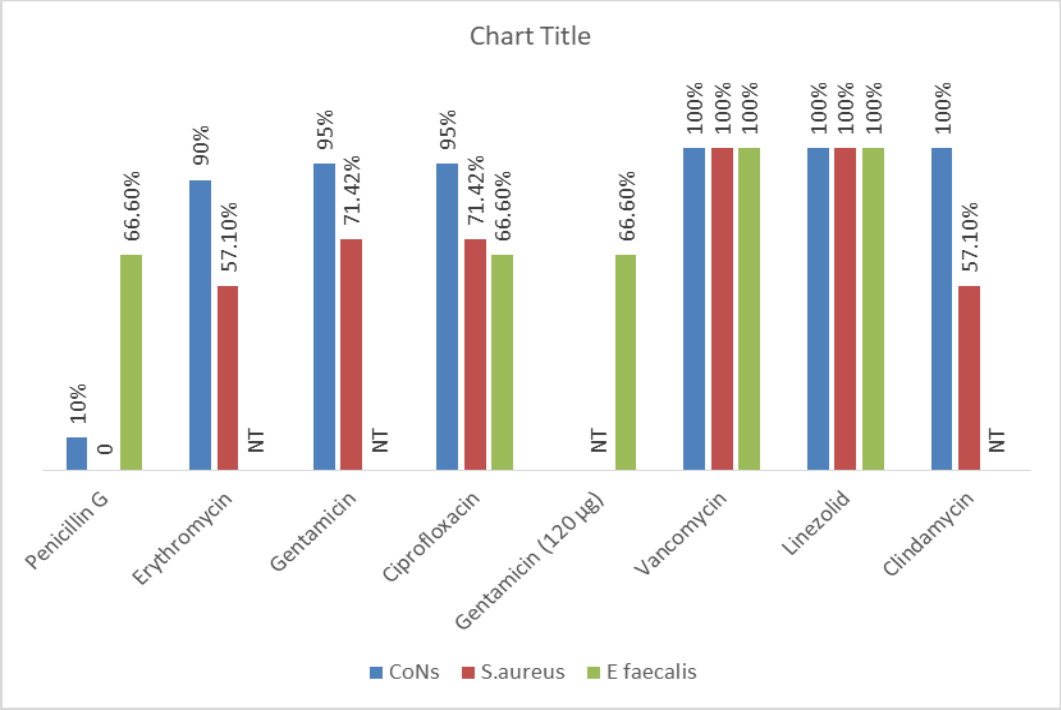
Graph.1 Overall Culture Positive and Culture Negative Rate (N=224)



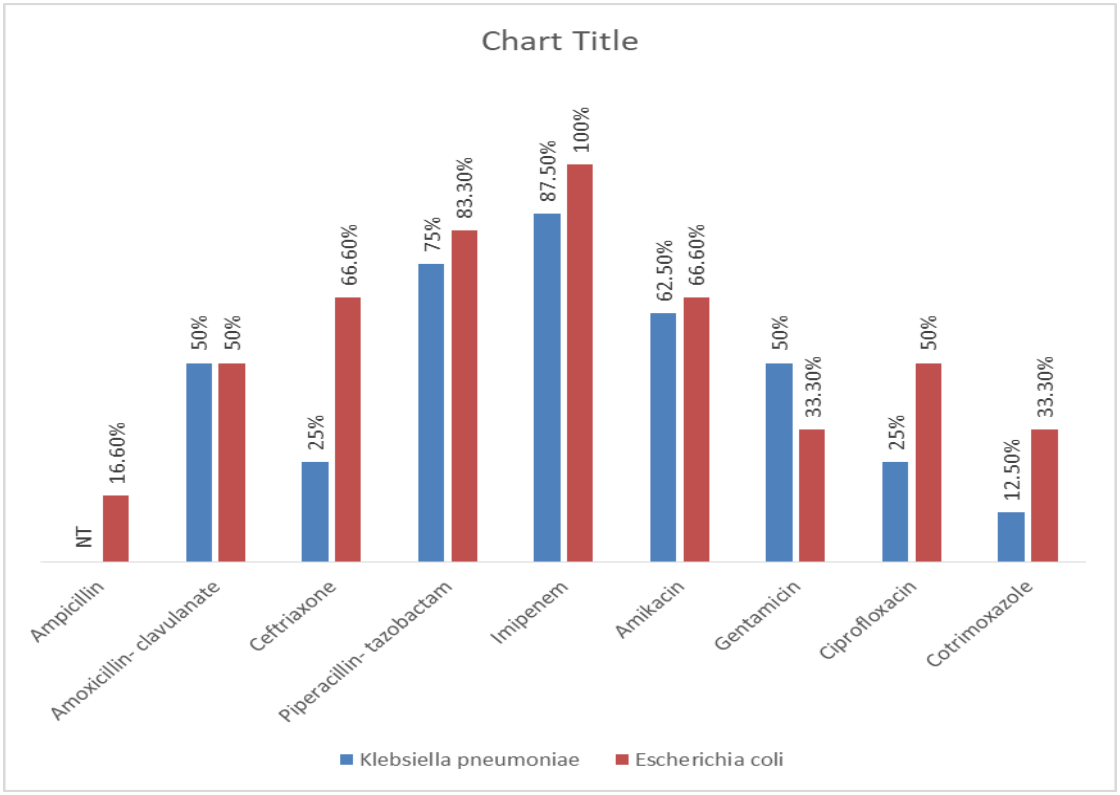
Graph.2 Percentage of Culture-Positive Isolates



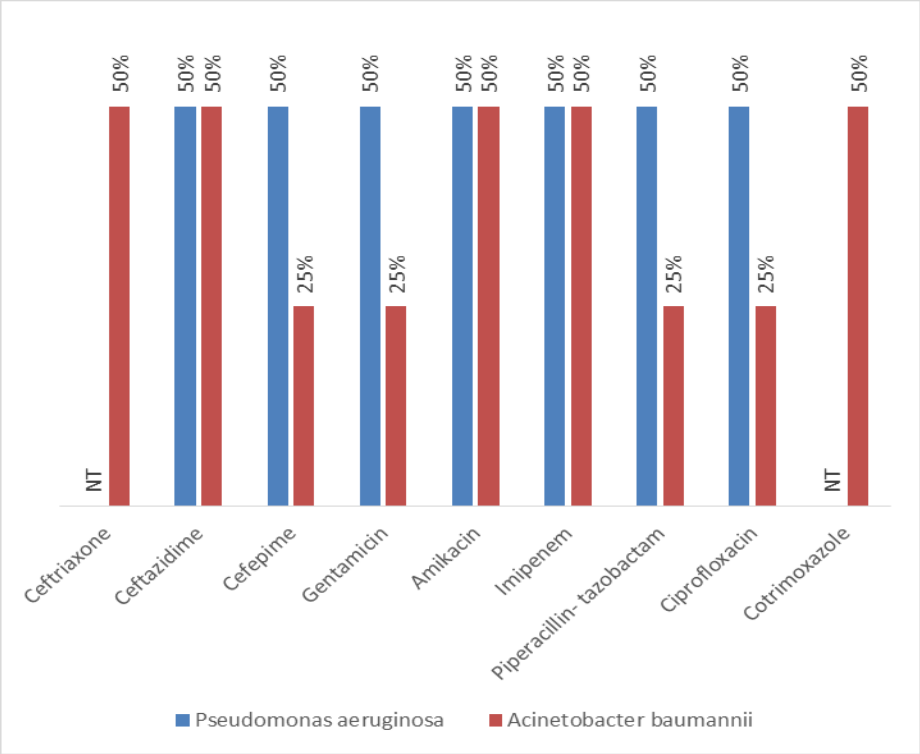
Graph.3 Antimicrobial Susceptibility Profile of Gram-Positive Bacteria



Graph.4 Antimicrobial Susceptibility Profile of Enterobacteriaceae



Graph.5 Antimicrobial Susceptibility Profile of Non-Fermenters



Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could potentially influence the work reported in this study.

Author Contributions Statement

Vidhya T.: MD Microbiology Senior Resident Dept. of Microbiology, SRTRGMC, Ambajogai Conceptualization, Study Design, Data Collection, Laboratory Investigation, Formal Analysis, Data Curation, Writing – Original Draft Preparation, Writing – Review & Editing. Deepali M Kulkarni, MD Microbiology Assistant Professor Dept. of Microbiology, SRTRGMC, Ambajogai Supervision, Methodology, Validation, Resources, Writing – Review & Editing, Project Administration, Final Approval of Manuscript

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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