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A study on the Prevalence of ESKAPE Pathogens Isolated from the Blood Culture Specimens of Various Intensive Care Units Patients Admitted in a Tertiary Care Hospital

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A B S T R A C T

Hospital Acquired Infections are the rising threat in the health care facilities across the globe. Multidrug resistance is a serious problem all across the world. The ESKAPE (*Enterococcus faecium, Staphylococcus aureus* including MRSA, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) pathogens in the hospital are the commonly acquired infections. As most ICU patients are frequently on broad spectrum antibiotics this induces selective antibiotic pressure which leads to development of antimicrobial resistance among the microorganisms of various ICU. Aim: To determine the prevalence of ESKAPE pathogens isolated from blood culture of patients admitted in various Intensive Care Units. This was a cross-sectional study conducted in the Department of Microbiology and Intensive care units (MICU, PICU and NICU) at UCMS & GTBH; Delhi was conducted from January 2021 to April 2022. The Inadequate quantity of sample and contaminated blood culture samples were not enrolled for this study. The antibiotic susceptibility pattern of the isolated organisms was performed by Kirby–Bauer disk diffusion method on mueller–Hinton agar plates, and the results were recorded as per the CLSI 2022 guidelines. Out of these 611 blood culture samples growth of microorganisms was observed in 115 (18.82%) samples. 496 (81.18%) blood culture samples showed no growth. One blood culture sample was taken from each patient. These 115 blood culture isolates were further tested for species identification by standard conventional phenotypic methods and their antimicrobial susceptibility testing (AST) was performed manually by Kirby Bauer disc diffusion method. Among these, 611 blood culture samples, 350 (57.28%) samples were from NICU, 211(34.53%) were from MICU, and 72 (8.18%) were from PICU isolated respectively. Conclusion: Our results from a relatively large dataset highlight the pathogenicity of ESKAPE bacteria and quantify their impact on patient length of stay, cost of care, and mortality. We also found that amongst all blood culture orders, employing simple word algorithms can predict ESKAPE infections better than random guessing.

Introduction

Hospital Acquired Infections (HAIs) are the rising threat in the health care facilities across the globe. (Mool chandani, 2017) Multidrug resistance (MDR) is a serious problem all across the world. (Mool chandani, 2017) It is estimated that 1.75 to 3 million(5 % to 10%) of the 35 million patients admitted annually to acute care hospitals in the United States of America acquire an infection that was neither present nor was in the prodromal (incubation) stage when they entered the hospital. (Loomba *et al.,* 2010) These infections are called Heath care -associated infections (HCAI). Earlier terms like nosocomial, hospital - acquired, hospital - onset infections were also used. (Mool chandani, 2017; Loomba *et al.,* 2010) Patients admitted in Intensive Care Units (ICU) are at a higher risk to catch infections due to longer stay in ICUs, central line insertion, mechanical ventilation, catheterization and underlying respiratory and neurological illnesses.[3] Out of all the routes of infection bloodstream infections (BSIs) are among the leading cause of infections in critically ill patients. (Loomba *et al.,* 2010; Ding *et al.,* 2009) The ESKAPE (*Enterococcus faecium, Staphylococcus aureus* including MRSA, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species) pathogens in the hospital are the commonly acquired infections.[4] Presence of HAIs in ICU patients causes a statistically significant increase in the length of hospital stay, mortality and financial burden to the society. (Loomba *et al.,* 2010; Ding *et al.,* 2009; Mackie and McCartney)

As most ICU patients are frequently on broad spectrum antibiotics this induces selective antibiotic pressure which leads to development of AMR (Antimicrobial resistance) in the microorganisms of ICU. (Loomba *et al.,* 2010) This is one of the greatest challenges in health care. This leads to extended hospital stay and increased cost of treatment for the patient. (Mackie and McCartney) According to the data of the 2 million patients developing antibiotic resistance around 23,000 patients die. Hence, the microbiology profile of the HAIs in the ICU often reveal multidrug resistant ESKAPE pathogens. (Ding *et al.,* 2009; Mackie and McCartney) Patients with resistant ESKAPE bacteremia more often received inappropriate empirical antibiotic therapy than the others (41% vs. 21.6%; P=0.01). (Oriol *et al.,* 2015) Overall case-fatality rate (30 days) was higher in patients with resistant ESKAPE bacteremia (35.2% vs. 14.4%; P=0.001). (Chakrabarti *et al.,* 2015; Gunasekaran and Mahadevaiah, 2020) There are few Indian studies regarding ESKAPE pathogens documented in their geographical location. However studies are lacking in prevalence of these pathogensin blood isolates from India. The present study was aimed to determine the prevalence of ESKAPE pathogens isolated from blood culture of patients admitted in various Intensive Care Units of a tertiary care hospital.

Materials and Methods

This was a cross-sectional study conducted in the Department of Microbiology and Intensive care units (Multidisciplinary Adult Intensive Care Unit:MICU, Pediatrics Intensive Care Unit:PICU and Neonatal Intensive Care Unit:NICU) at University College of Medical Sciences and Guru Teg Bahadur Hospital, Delhi. The present study was conducted from January 2021 to April 2022. Considering the prevalence of ESKAPE pathogens 15% in specimen to estimate the Relative marginal error of 20% on either side at α= 5%, a sample of 550 was required. According to the recent data of last one year we enrolled 611 blood culture samples isolated from MICU, PICU & NICU respectively. The institutional ethical committee (IEC) had approved this study. The Inadequate quantity of sample and contaminated blood culture samples were not enrolled for this study.

Procedure of Blood culture specimen

8–10 ml of venous blood was drawn from adults and 1-3 ml from children following the necessary aseptic precautions. Each 8 to 10 ml of blood was manually

inoculated into a blood culture bottle containing 50 ml of brain heart infusion broth for adults, and 1 to 3 ml for pediatric patients in a 30 ml blood culture bottle. The blood culture samples were immediately transported to the bacteriology laboratory and incubated at 37°C for overnight incubation in an ambient environment. The samples were manually sub cultured onto 5% sheep blood agar and Mac-Conkey agar at 24 hours, 48 hours, and on the 5th day of incubation. The growth obtained was identified by colony morphology, gram stain of the isolated colonies, and biochemical identification tests were put as per the standard protocol followed in our laboratory. (Patricia, 2014)

Antimicrobial susceptibility testing of blood culture isolates

The antibiotic susceptibility testing of the isolated organisms were performed by Kirby–Bauer disk diffusion method on Mueller–Hinton agar plates, and the results were recorded as per the CLSI 2022 guidelines. (CLSI, 2022) The antibiotic disks that were used to identify the susceptibility pattern of the gram-negative pathogens were Ciprofloxacin (15ug), Ampicillin (10ug), Gentamicin (10ug), Piperacillin-Tazobactam (100/10ug), Imipenem (10ug), Meropenem (10ug), Ceftriaxone (30ug), Cefotaxime (30ug), Ceftazidime (30ug), Aztreonam (30ug) and Trimethoprim-sulfamethoxazole (1.25/23.75ug) (Himedia Laboratories, Mumbai, India). The antibiotics susceptibility results were interpreted as per the latest CLSI guidelines.

The antibiotics discs (Himedia laboratories) used were commercially procured. The discs were stored at 2 degree to 8 degree C for a short time (maximum one week) in a tightly - sealed containers and stock were stored at -20 degree C in order to ensure that the potency of the drugs is maintained. All antibiotics were not tested for all the microorganisms. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) control strains were used for the Kirby Bauer disc diffusion method.

Results and Discussion

A total of 611 blood samples from patients admitted in the various Intensive Care Units (Neonatal Intensive Care Unit, Pediatric Intensive Care Unit& Multidisciplinary Adult Intensive Care Unit) were included in the study to observe the prevalence of ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter species*). Out of these 611 blood culture samples growth of microorganisms was observed in 115 (18.82%) samples. 496 (81.18%) blood culture samples showed no growth. One blood culture sample was taken from each patient. These 115 blood culture isolates were further tested for species identification by standard conventional phenotypic methods and their antimicrobial susceptibility testing (AST) was performed manually by Kirby Bauer disc diffusion method.

Among these, 611 blood culture samples, 350 (57.28%) samples were from NICU, 211(34.53%) were from MICU, and 72 (8.18%) were from PICU isolated respectively. Which were statistically significant. (chi-square test, $p < 0.5$)(Figure 1)

Out of the 350 blood cultures specimens of the patients admitted to NICU, microbial growth was seen in67 (19.1%) patients. Out of 72 specimens of PICU, microbial growth was seen in 15(20.83%) blood specimens. Out of 189 blood culture specimens of MICU, 33 specimens (17.46%) showed growth. Therefore the blood culture positivity in MICU, PICU and NICU were 17.46%, 20.83%, 19.1% respectively.

In the present study, the Male: female ratio was 1:1 from blood culture isolates from various ICUs. Out of the 611samples received for blood culture, 57% (350) samples were from neonates, 12% (72) samples were from the pediatric age group (1 month to 14 years) and31% (189) samples were from patients belonging to the age 15to>60years of age. This was not statistically significant. (Table: 1)

In our study, out of these 115blood culture-positive isolates, 54 (46.95%) isolates were gram-negative bacilli (GNB), 49 (42.60%) isolates were gram-positive cocci (GPC) and 12 (10.43%) isolates were *Candida species.* (Table 2). This finding was not statistical significant (p>0.089, paired t test). Further processing of *Candida* species were not processed in this study.

Among gram-negative blood culture isolates, *Acinetobacter baumannii* and *Klebsiella pneumoniae* isolated were 14 % (16 isolates) each respectively. Out of 42 % gram-positive blood culture isolates, 34 isolates (30 %) were *Staphylococcus aureus* followed by CONS 13 (11%). 12 blood culture isolates were *Candida species*. (Figure 2)

Total ESKAPE Pathogens isolated were 75 and *Candida* isolates were 12 in number. Out of the total 115 isolates from ICUs, ESKAPE pathogens accounted for 65.2% (75) and 10.4% (12) were *Candida* species. Among the total blood infections caused by ESKAPE pathogens, majority of the infection was caused by *Staphylococcus aureus* 45.3%, followed by *Acinetobacter baumannii* and *Klebsiella pneumoniae* both contributing 21.3% each, *Pseudomonas aeruginosa* caused8% infection, followed by *Enterococcus faecium* 2.7%, and infection from *Enterobacter species* was 1.3% only (Figure.3).

Among 34 *Staphylococcus aureus isolates,* 14 (41.17%, n=34), were Methicillin Resistant *Staphylococcus aureus* (MRSA) and 20 (58.83%, n=34), were (Methicillin Sensitive *Staphylococcus aureus* (MSSA). Majority of MRSA was isolated in NICU (34%) patients, followed by MICU (17%) & PICU (5.8%). (Figure 4)

Overall the ESKAPE pathogens showed 100% susceptibility to High Gentamicin in *Enterococcus faecium*. Among Gram- positive cocci Vancomycin showed 100% susceptibility while, Aztreonam, Erythromycin, Meropenem and Ciprofloxacin were (60%-80%) susceptible for all the ESKAPE pathogen isolates. (Figure: 5)

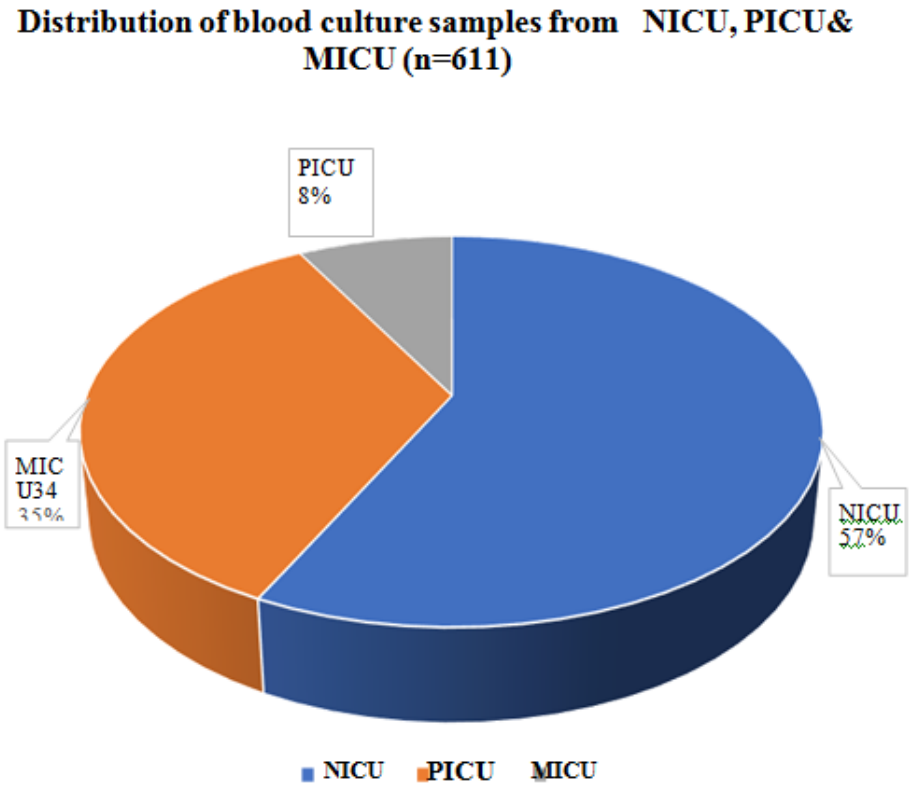
Healthcare-associated infections (HAI) are a major problem in the current scenario in hospitals. Infection with multidrug-resistant organisms is another serious concern. The patients admitted to Intensive Care Units (ICU) have frequently on broad spectrum antimicrobials even in absence of evidence of infection. This creates a selective antibiotic pressure leading to further development of antimicrobial resistance. Presence of HAIs in ICU patients causes a statistically significant increase in the length of hospital stay, morbidity, mortality, and financial burden. Taking this into consideration the study was designed to determine the prevalence of ESKAPE pathogens in blood specimens of ICU patients admitted to our hospital. In our study, the overall percentage growth positivity rate from all the ICUs was 18.82%, which is similar to a study done in San Giovanni Hospital in Bellinzona, Switzerland by Previsdomini *et al.,* (2012). They performed a descriptive retrospective study over two years (2007-2008) in the medico-surgical Intensive Care Unit (ICU), where the percentage culture positivity rate of 19.5% was observed (Previsdomini *et al.*, 2012). In the present study out of 115 isolates, 54 were of gram-negative bacilli (46.95%), 49 (42.6%) isolates were gram-positive cocci and 12(10.43%) isolates were of *Candida* species. Chand Wattal *et al.,* (2014) had publisheda study in New Delhi, conducted from years 2008 to 2011, observed that the distribution of bloodstream infections in ICU. The infections by Gram-negative bacilli (GNB), Gram-positive cocci (GPC), and fungi were 49%, 33%, and 18% respectively. The distribution of GNB in their ICU is comparable to ours finding though the distribution of GPC, and fungi differ, which may be due to the difference in the duration of the study, setting of study.

Table.1 Age-wise distribution of blood culture samples from patients admitted to various ICUs patients.

ICU's	Age	Number of patients n=611 (%)
NICU	<1Month	350(57%)
PICU	1Month-1year	50(8%)
	1-14year	22(4%)
MICU	15-30year	35(6%)
	30-45year	27(4%)
	45-60year	67(11%)
	>60year	60(10%)

*Neonatal Intensive care unit (NICU) *Pediatric Intensive care unit (PICU) *Multidisciplinary Adult Intensive care (MICU)

Fig.1 Distribution of blood culture specimens from various ICUs patients. (n=611)



*Neonatal Intensive care unit (NICU) *Pediatric Intensive care unit(PICU)*Multidisciplinary Adult intensive Care (MICU)

Table.2 Distribution of blood culture positive microorganisms in various ICUs patients.(n=115)

Blood cultures isolated Microorganisms	Number of isolates(n=115)			Total n=115(%)	
	NICU n=67(%)	PICU n=15 (%)	MICU n=33 (%)		
Acinetobacter baumannii	11(16.4)	3(20)	2(6.06)	16(14)	**54 (46.9%)**
Citrobacter species	5(7.4)	0(0)	2(6.06)	7 (6)	
Enterobacter species	0(0)	0(0)	1(3.03)	1(0.8)	
Escherichia coli	5(7.4)	1(7)	2(6.06)	8 (7)	
Klebsiella pneumoniae	8(11.9)	1(7)	7(21.2)	16(14)	
Pseudomonas aeruginosa	2(2.9)	1(7)	3(9.09)	6 (5)	
Staphylococcus aureus	22(32.8)	4(26)	8(24)	34(30)	**49 (42%)**
Enterococcus faecium	2(2.9)	0(0)	0(0)	2(1.7)	
***Coagulase Negative Staphylococcus aureus* (CONS)**	7(10.4)	3(20)	3(9.09)	13(11)	
***Candida* species**	**5(7.4)**	**2(13)**	**5(15.1)**	**12(10)**	**12 (10.4%)**

Fig.2 Percentage wise distribution of blood culture pathogens in patients admitted in various ICUs in the study group.(n=115)

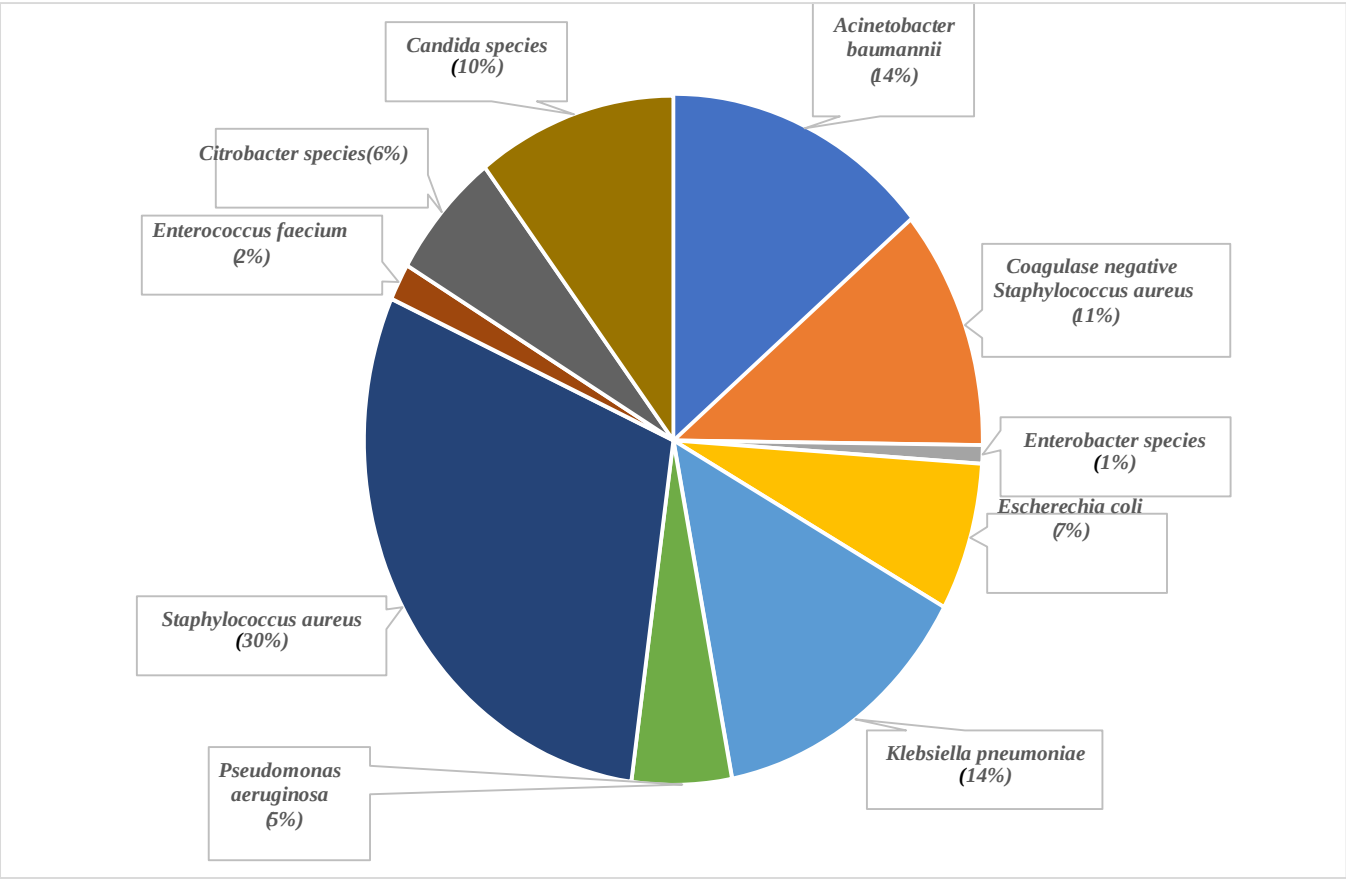


Fig.3 Distribution of ESKAPE pathogens in blood culture from patient admitted in various ICUs.(n=75)

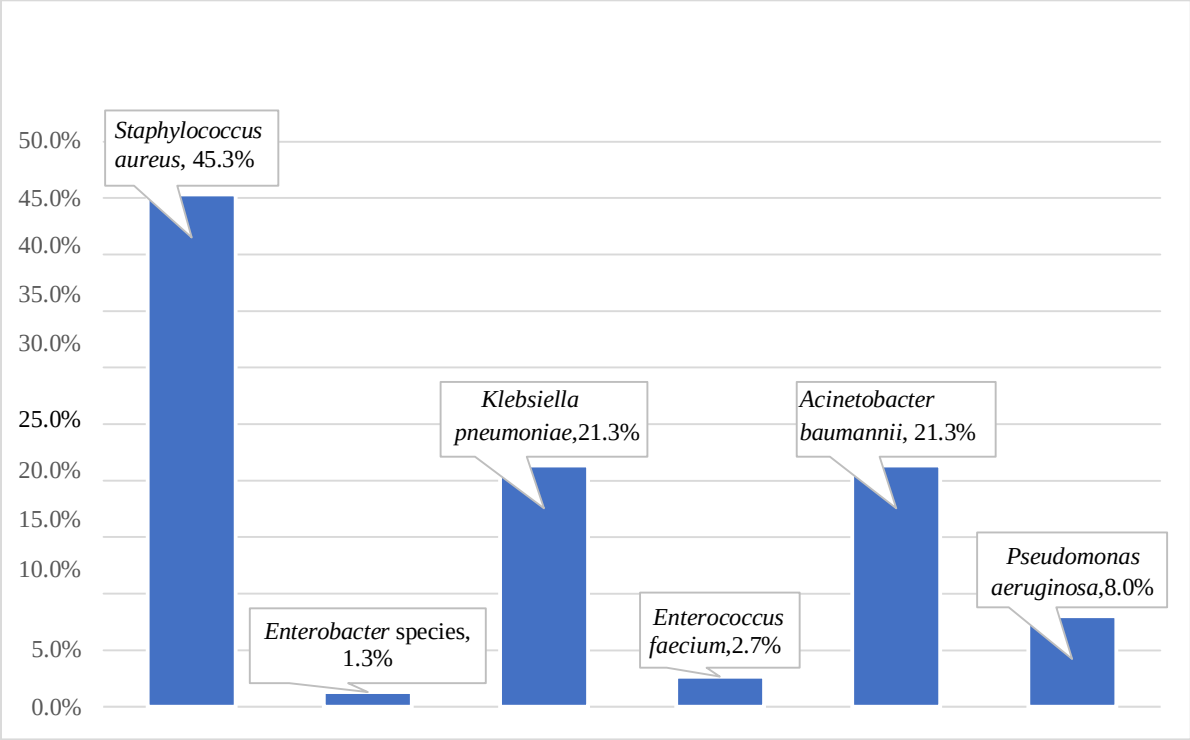


Fig.4 Distribution of Methicillin resistant *Staphylococcus aureus* from blood culture isolates in NICU, PICU & MICU patients (n=34)

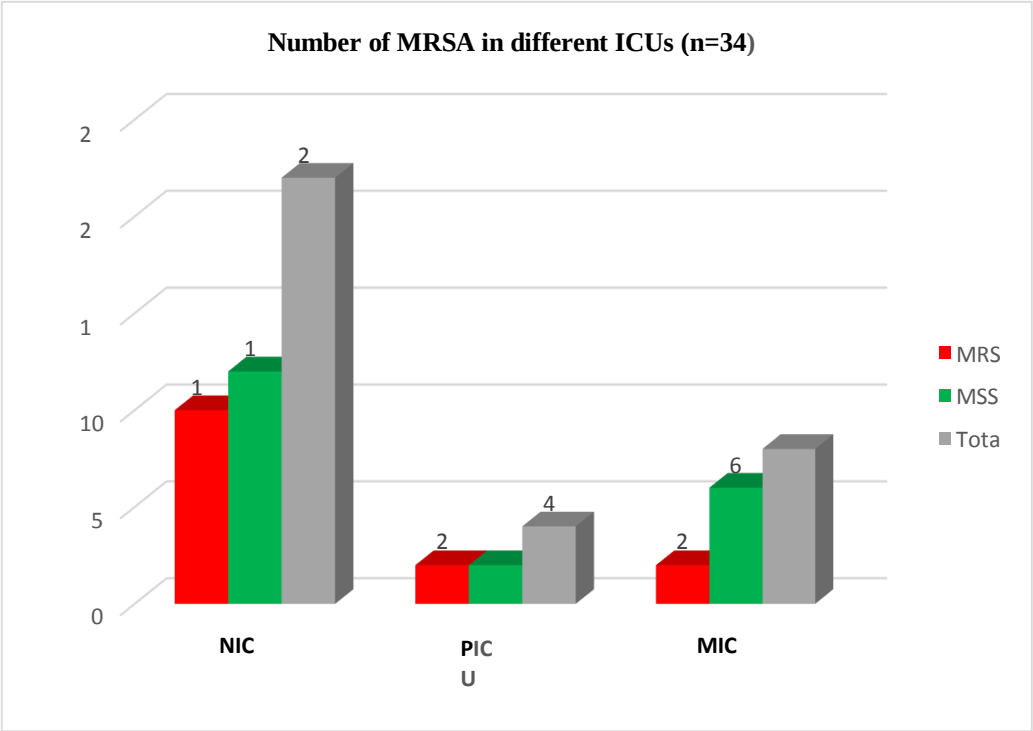
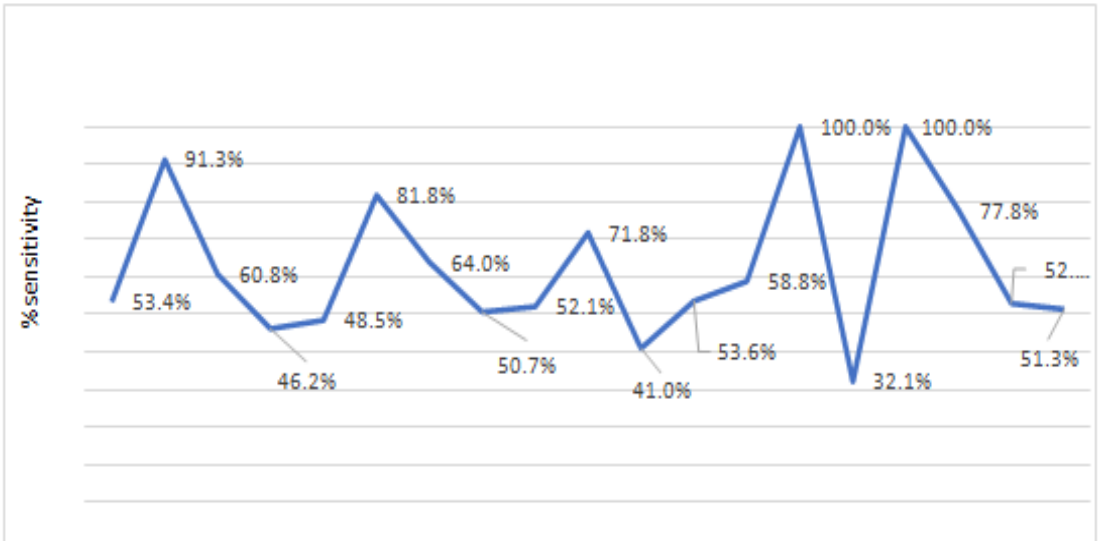


Fig.5 Antibiotic susceptibility pattern of ESKAPE blood culture isolates invarious admitted ICUs patients in the study group.



In our study the Male: Female ratio was 1:1, which is similar to a study done in Brazil in the year 2017 to study Prevalence and antimicrobial susceptibility profile of ESKAPE pathogens from the Federal District, Brazil, by Daniely M. Silva *et al.,* (2017) where 52% of the patients were male and 48 % patients were female on which the study was done.

Though the samples they studied included urine, rectal swab, nasal swab, blood and others and we only studied blood samples (Daniely M. Silva *et al.,* 2017). In a study done by Saed Al-Musawi *et al.,* (2020) to screen the prevalence of ESKAPE pathogens group among pediatric patients in Iraq in the year 2020, the ratio between male and female was 1:1 which is similar to our study, though the age group on which they studied belonged to the age 1 day to 14 years, while we studied samples from age 1 day to>60 years. (Saed Al-Musawi *et al.,* 2020) In the study done by Daniely M. Silva *et al.,* (2017) the nmber of patients belonging to >=60 years constituted 48.18% of the total positive samples, while in our study only10% of the samples were from patients belonging to age >60 years (Daniely M. Silva *et al.,* 2017).

In the present study, ESKAPE pathogens accounted for 75/115 (65.2%) of the total isolates in which *Enterococcus faecium*- 2/115(1.7%), *Staphylococcus aureus* 34/115(29.5%), *Klebsiella pneumonia* 16/115 (13.9%), *Acinetobacter baumannii* 16/115 (13.9%), *Pseudomonas aeruginosa*6/115 (5.2%) and *Enterobacter species* 1/115(0.87%) were present, which is more than half of the infections in the ICU. In our study, the most frequent organism among ESKAPE pathogens was *Staphylococcus aureus* 34/75 (46%), followed by *Acinetobacter baumannii* 16/75 (21%), *Klebsiella pneumoniae* 16/75 (21%) and *Pseudomonas aeruginosa* 6/75(8%). Our finding coincides with various published studies.

In 2012 Monterrey, Mexico, (Llaca *et al.,* 2012), One-year surveillance in an intensive care unit was done to observe the prevalence of ESKAPE pathogen. The prevalence of ESKAPE pathogens observed in their study was 64.5%, which is similar to our study. Although the most frequent organisms among ESKAPE pathogens in their study were *Acinetobacter baumannii* (15.8%) and *Pseudomonas aeruginosa* (14.3%) whereas in present study the most common organisms was *Staphylococcus aureus* (46%) followed by *Acinetobacter baumannii* (21%) and *Klebsiella pneumoniae* (21%) (Llaca *et al.,* 2012). The study conducted by Wattal *et al.,*

(2014) showed that the common gram-negative organisms isolated organism were *Klebsiella pneumoniae*, followed by *Acinetobacter baumannii*, which was similar to our study (Wattal *et al.*, 2014).

However Coagulase-negative *staphylococcus* was the most common Gram-positive organism isolated in their study whereas *Staphylococcus aureus* was the most common GPC. Similarly, another study published by Ashopa *et al.,* (2020) conducted at Umaid hospital, Jodhpur, Rajasthan in 2019 observed the prevalence of 86.87% for ESKAPE pathogens isolated from various samples including blood culture, CSF culture, urine culture, vaginal culture, pus, throat swab, Endotracheal culture, and other culture etc. over the period of 6 months. They have collected samples from various wards and ICUs. The most prevalent isolate observed in blood isolates in their study was *Staphylococcus aureus* (41.42%) which is comparable to our study. The above study was on various specimens, including blood specimens from various wards and ICUs of hospitals, however our study is more specific to ICUs (Ashopa *et al.,* 2020).

In our study, among the S*taphylococcus aureus* the MRSA accounts for 41.17% (14/34) of infections in ICU which is comparable to a study documented by Seas *et al.,* (2018) conducted at Hospital Cayetano Heredia, Lima, Peru. They observed 44.7% of MRSA causing significant bacteremia in adults over the duration of 4 years (2011 to 2014) (Garcia *et al.*, 2018).

The percentage of *Enterococcus faecium* among ESKAPE isolates in our study was (2/75) 2.67%, which is almost similar to another study conducted in Massachusetts, USA (Marturano *et al.,* 2019) in 2019 for 4 years. They observed 1.7% of *Enterococcus faecium* among ESKAPE isolates from bloodstream infections (Marturano *et al.*, 2019). The most susceptible antimicrobials for all the Gram positive ESKAPE pathogen in our study was Vancomycin(100%). While for Gram negative isolates Aztreonam (91.3%), followed by Cefixime (81.8%) were the most sensitive antimicrobials. The most resistant antimicrobials were Ampicillin (67.9% resistant) followed by, Piperacillin-Tazobactam (59% resistant), and Ceftazidime (53.8%). In a study done by Shrestha *et al.,* in 2016 to analyze the drug resistance bacteria in respiratory ICU, Antibiotic resistance of Gram-positive microorganisms isolated from infections in the ICU showed that Vancomycin had the most sensitivity which is similar to our finding in our study and Penicillin was the most resistant antibiotic. Antibiotic resistance Pattern of Gram-negative microorganisms isolated from infections in the ICU revealed the least resistant drug being Amikacin and the highest resistance was observed in the Piperacillin –Tazobactum. While in our study the 2nd most resistant drug was Piperacillin – Tazobactum. The slight difference in the antimicrobial susceptibility pattern can be because the samples taken for the study were blood, CSF, BAL, Sputum, pleural, and urine, while in our study we took only blood culture specimens.

The design of their study was a retrospective cohort study, while ours was a cross-sectional study. Also, their study was conducted in China, so the difference in the geographical area also may lead to the difference in the susceptibility pattern of antimicrobials. Mool chandani *et al.,* (2017) in their study had maximum resistance to Ceftriaxone and Ceftazidime and was most sensitive to Meropenem and Amikacin. The prevalence of ESKAPE pathogens in ICU was 65.2%. Among the ESKAPE pathogens, Our results from a relatively large dataset highlight the pathogenicity of ESKAPE bacteria and quantify their impact on patient length of stay, cost of care, and mortality.

The present study also found that amongst all blood culture orders, employing simple word algorithms can predict ESKAPE infections better than random guessing. Given the steady rise of antibiotic resistance, and the high cost to treat sepsis, rapidly identifying patients infected with ESKAPE pathogens will continue to be a major healthcare priority. Present study understanding the local epidemiology of bacterial pathogens may provide benefits in establishing local empirical treatment protocols.

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