Antibiogram of Bacterial Isolates from Critical Care Patients in Sharda Hospital - A Tertiary Care Hospital

Abstract

Background:

Antimicrobial resistance (AMR) poses a major threat to global public health, particularly in developing nations with high infection burdens and widespread antibiotic misuse. Intensive Care Units (ICUs) are critical sites for the emergence and transmission of multidrug-resistant (MDR) organisms, with critically ill patients at increased risk due to invasive procedures and compromised immunity. ESKAPE pathogens and other Gram-negative bacteria are frequently

implicated in ICU infections, complicating empirical treatment and contributing to adverse outcomes. Despite this, there is limited surveillance data on resistance trends in ICUs, especially in low- and middle-income countries like India.

Materials and Methods:

A hospital-based cross-sectional observational study was conducted over six months (October 2023- March 2024) in the Department of Microbiology, Sharda Hospital, Greater Noida. Clinical samples from ICU patients including blood, urine, respiratory secretions, and pus—were processed using standard microbiological techniques. Bacterial identification involved Gram staining, colony morphology, and biochemical testing. Antimicrobial susceptibility testing (AST) was carried out using the Kirby-Bauer disc diffusion method, interpreted as per CLSI 2023 guidelines.

Results:

A total of 2,125 ICU samples were analyzed, including blood (46%), urine (30%), respiratory samples (20%), and pus (4%). From these, 285 clinical isolates were recovered: respiratory samples yielded the highest proportion (46%), followed by blood (23%), urine (20%), and pus (11%). The most common isolates included Klebsiella pneumoniae**,** Acinetobacter baumannii**,** Pseudomonas aeruginosa**,** Escherichia coli**,** Staphylococcus aureus**, and** Enterococcus spp.**,** consistent with the global ESKAPE pathogen profile.

Conclusion:

The study highlights a predominance of Gram-negative organisms in ICU infections, with significant antimicrobial resistance, emphasizing the need for regular surveillance and updated antibiograms. Tailored empirical therapy based on local resistance patterns is crucial to improve patient outcomes and support antimicrobial stewardship efforts in resource-limited settings.

Keywords- Antimicrobial, multidrug resistant, enterococcus

Introduction

Antimicrobial resistance (AMR) has emerged as a critical global public health challenge, especially in developing countries where the burden of infections is high and the unregulated use of antibiotics is widespread. Hospitals, particularly intensive care units (ICUs), serve as hotspots for the emergence and spread of multidrug-resistant (MDR) pathogens.[1] In these settings, critically ill patients are more susceptible to infections due to invasive procedures, prolonged hospitalization, and weakened immune defenses.

A significant proportion of ICU infections are caused by a group of highly virulent and resistant organisms known collectively as **ESKAPE pathogens**, which include *Enterococcus*

faecium, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species.[2] Numerous epidemiological studies have reported a predominance of Gram-negative bacteria in ICU infections, with *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa* being the most frequently isolated. Among Gram-positive organisms, *Staphylococcus aureus* and *Enterococcus* spp. are often encountered.[3]

Although no specific bacterial species have been conclusively linked to higher ICU mortality rates, adverse outcomes are commonly associated with factors such as advanced age, comorbidities, and delayed initiation of appropriate antibiotic therapy. The increasing prevalence of antimicrobial resistance, fueled by the overuse and misuse of antibiotics, has significantly complicated the management of infections in ICUs worldwide.

An important strategy to improve clinical outcomes in ICUs involves timely administration of empirical antibiotics, guided by regularly updated local antibiograms. These antibiograms are essential components of antimicrobial stewardship programs and play a crucial role in ensuring appropriate and effective empirical therapy, ultimately reducing morbidity and mortality rates in critically ill patients.

Despite the high burden of AMR in low- and middle-income countries, data regarding resistance patterns among ICU patients remain limited. Resistance trends often vary not only between countries but also between hospitals and even among different ICUs within the same hospital. At our tertiary care teaching hospital, no recent surveillance study had been conducted to document the antibiotic susceptibility patterns of ICU pathogens. Hence, the present study was undertaken to determine the spectrum of bacterial isolates from ICU patients and to analyze their antibiotic resistance profiles.

Materials and Methods

This hospital-based, cross-sectional observational study was conducted in the Department of Microbiology, Sharda Hospital, Greater Noida, over a six-month period from October 2023 to March 2024. The study included all clinical samples received from ICU patients, including blood, central line tips, bronchoalveolar lavage (BAL), pleural fluid, cerebrospinal fluid (CSF), ascitic fluid, endotracheal aspirates, pus or tissue, sputum, throat swabs, and urine.

Samples were processed using standard bacteriological techniques. Isolates were identified based on colony morphology, Gram staining, and a series of conventional biochemical tests

such as catalase, oxidase, coagulase, urease, citrate, indole, methyl red (MR), Voges-Proskauer (VP), oxidative-fermentative (OF) test, triple sugar iron (TSI) test, nitrate reduction, and amino acid decarboxylation reactions.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method on Mueller-Hinton agar, following Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines. Commercially available antibiotic discs (HiMedia Laboratories, Mumbai, India) were used, and results were interpreted based on CLSI breakpoints. Ethical clearance was obtained from the institutional review board prior to the study.

Results

Study population. During the study period, a total of 2,125 samples were received from various ICUs and analysed in the Bacteriology Lab at Sharda Hospital, Greater Noida. The distribution of these samples were as follows- 988 blood samples (46%), 627 urine samples (30%), 420 respiratory samples (20%), and 90 pus samples (4%). (Table 1)

ICU Sample and Pathogen Distribution Overview

Total ICU Samples Collected (n=2125):

- Blood: 988 (46%)
- Urine: 627 (30%)
- Respiratory: 420 (20%)
- Pus: 90 (4%)

Total Clinical Isolates (n=285):

- Respiratory: 131 (46%)
- Blood: 64 (23%)
- Urine: 58 (20%)
- Pus: 32 (11%)

Table No. 1 Organism Distribution by Site

	Blood (n=64)	**Urine (n=58)**	**Respiratory (n=131)**	**Pus (n=32)**	**Total (n=285)**

E. coli	**8 (12%)**	**23 (40%)**	**27 (21%)**	**11 (35%)**	**69 (24%)**
Acinetobacter spp.	**6 (9%)**	**1 (2%)**	**54 (41%)**	**4 (12%)**	**65 (23%)**
Klebsiella spp.	**10 (16%)**	**6 (9%)**	**17 (13%)**	**5 (16%)**	**38 (13%)**
Pseudomonas spp.	**2 (3%)**	**3 (5%)**	**21 (16%)**	**2 (6%)**	**28 (10%)**
Staphylococcus aureus (MRSA/MSSA)	**17 (27%)**	**1 (2%)**	**8 (6%)**	**2 (6%)**	**28 (10%)**
Enterococcus spp.	**4 (6%)**	**23 (40%)**	**0**	**1 (3%)**	**28 (10%)**
CONS	**17 (27%)**	—	—	—	**17 (6%)**
Citrobacter spp.	—	**1 (2%)**	**4 (3%)**	**5 (16%)**	**10 (3%)**
Proteus spp.	—	—	—	**2 (6%)**	**2 (1%)**

Table No.2 Antibiotic susceptibility profile of Enterobacteriaceae (n=119)

Antibiotics	Sensitivity (%)	Resistance (%)
Ampicillin	8%	92%
Gentamicin	42%	58%
Tobramycin	31%	69%
Amoxyclav	22%	78%
Ceftriaxone	26%	74%
Cefotaxime	26%	74%
Cefuroxime	12%	88%
Cefepime	22%	78%
Ciprofloxacin	26%	74%
Levofloxacin	26%	74%
Amikacin	41%	59%
Imipenem	43%	57%
Meropenem	43%	57%
Piperacillin/tazobactum	27%	73%

Cotrimoxazole	35%	65%
Ceftazidime	13%	87%
Aztreonem	19%	81%
Tetracycline	26%	74%
Minocycline	39%	61%
Tigecycline	43%	57%
Nitrofurantoin	70%	30%
Fosfomycin	66%	34%
Norfloxacin	10%	90%

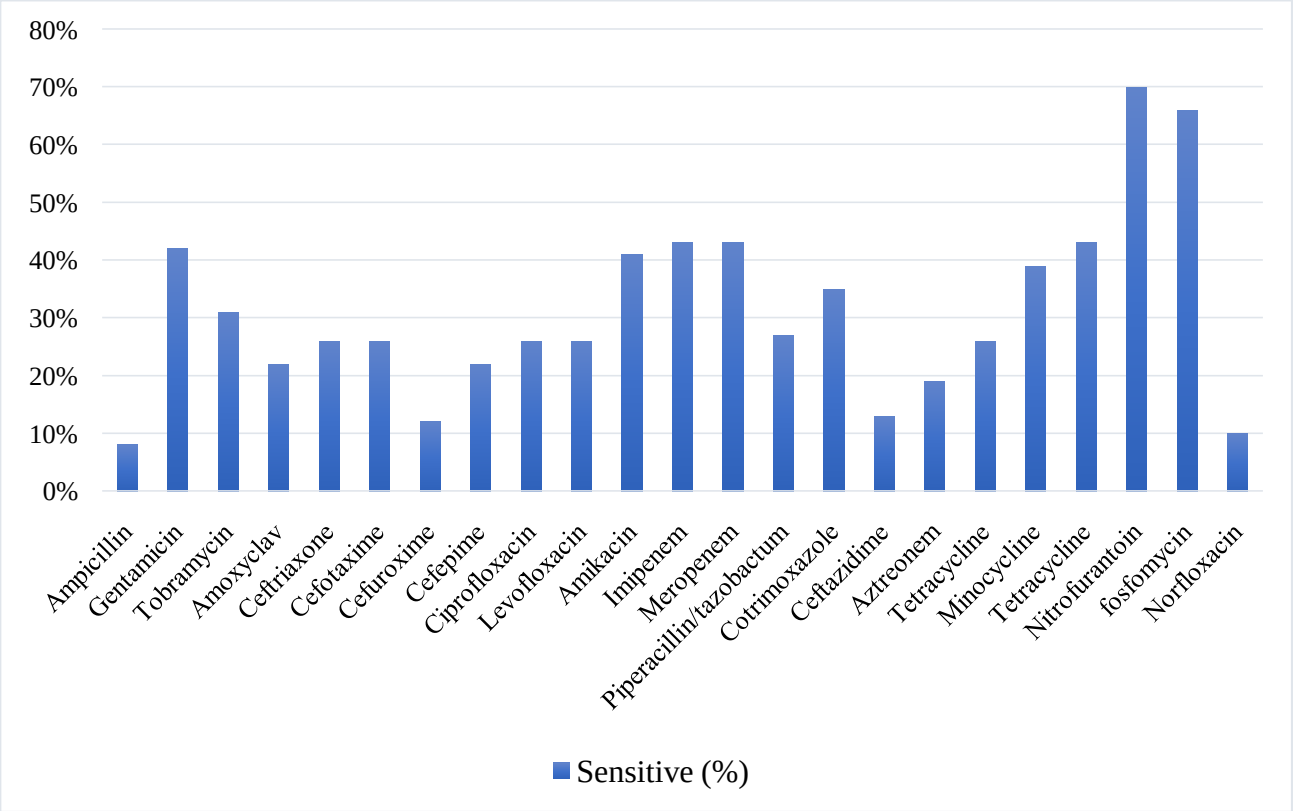

Fig 1-Antibiotic sensitive profile of *Enterobacteriaceae* (n=119)

Non- Enterobacteriaceae- Among the 285 isolates, 65 were as *Acinetobacter spp.*, and 28 as *Pseudomonas spp.*

1-*Acinetobacter spp.*

- ✓ A total of 65 *Acinetobacter species* were isolated from 285 isolates. *Acinetobacter spp.* were found to be highly effective for Minocycline (68%) followed by Tigecycline (45%), Cotrimoxazole (15%), Ceftriaxone and Cefotaxime (14%)

whereas, Meropenem, Imipenem, Cefepime and Cefuroxime were least effective (1%) among all the antibiotic agents tested.

- ✓ Nitrofurantoin and Norfloxacin showed resistant to all the isolates of *Acinetobacter spp*. in urinary tract infection. (Table 6)

Table No 3: Antibiotic susceptibility pattern of *Acinetobacter spp.* (n=65)

Antibiotics	Sensitivity (%)	Resistance (%)
Ceftazidime	4%	96%
Ciprofloxacin	4%	96%
Levofloxacin	4%	96%
Gentamicin	4%	96%
Tobramycin	5%	95%
Imipenem	1%	99%
Meropenem	1%	99%
Ceftriaxone	14%	86%
Cefotaxime	14%	86%
Cefepime	1%	99%
Amikacin	7%	93%
Piperacillin/tazobactam	7%	93%
Minocycline	68%	32%
Cotrimoxazole	15%	85%
Tigecycline	45%	55%
Cefuroxime	1%	99%
Tetracycline	6%	94%
Nitrofurantoin	0%	100%

Norfloxacin	0%	100%

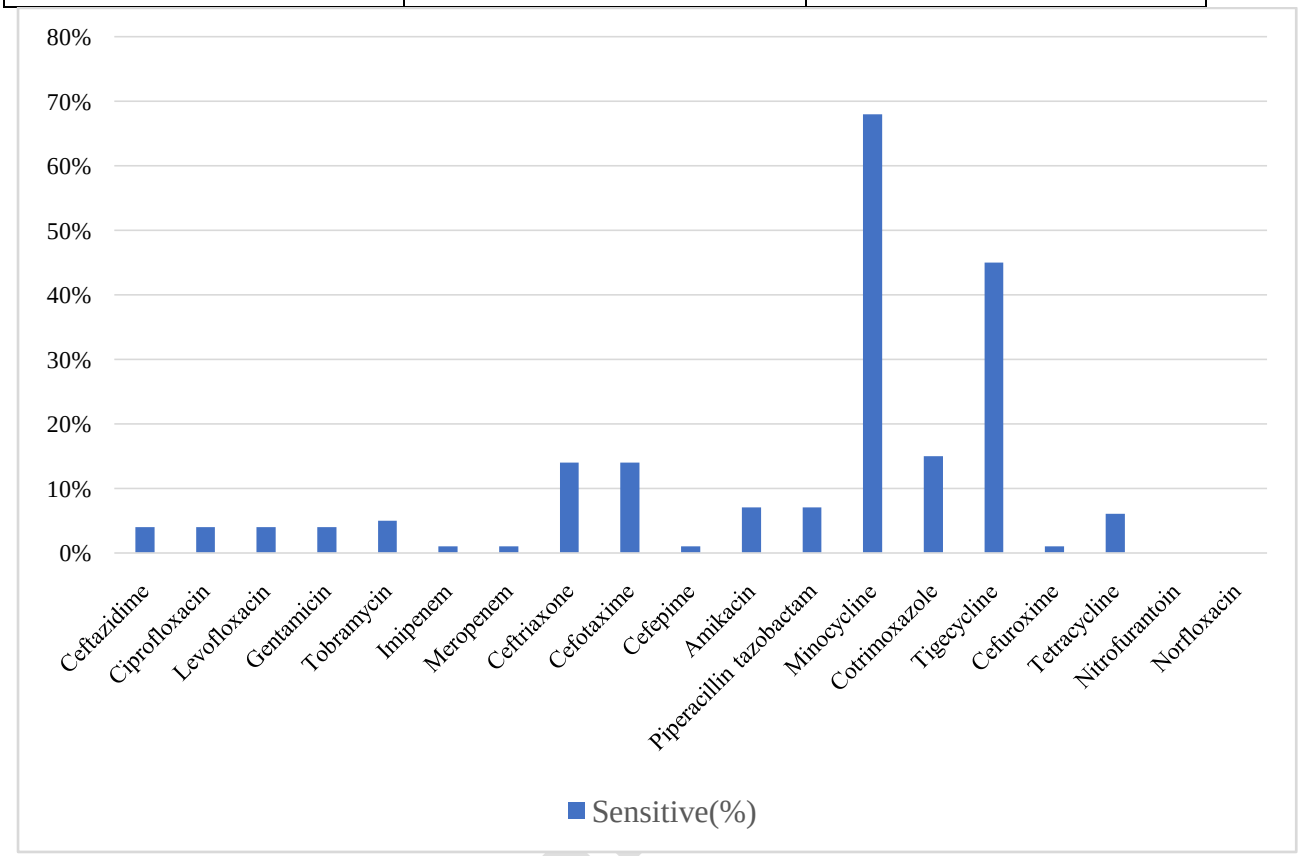

Fig 2- Antibiotic sensitive pattern of *Acinetobacter spp.* (n=65)

2-*Pseudomonas aeruginosa*

- ✓ Aminoglycosides (Amikacin, Gentamicin, Tobramycin) were found to be highly effective (79%, 75%, 75%) followed by Carbapenems (meropenem, imipenem) (75%) Cefepime (75%) and flouroquinolones (Ciprofloxacin, Levofloxacin) (60%) respectively. whereas, Piperacillin-tazobactum was least effective (53%).
- ✓ Nitrofurantoin and Norfloxacin showed resistant to all the isolates of *Pseudomonas aeruginosa* in urinary tract infection. (Table 7)

Table No. 5 Antibiotic susceptibility pattern of *Pseudomonas aeruginosa* (n=28)

Antibiotics	Sensitivity (%)	Resistance (%)
Ceftazidime	58%	42%
Gentamicin	75%	25%
Tobramycin	75%	25%
Piperacillin-tazobactum	53%	47%
Cefepime	75%	25%
Ciprofloxacin	60%	40%
Levofloxacin	60%	40%
Amikacin	79%	21%
Imipenem	75%	25%
Meropenem	75%	25%
Aztreonem	67%	33%
Nitrofurantoin	0%	100%
Norfloxacin	0%	100%

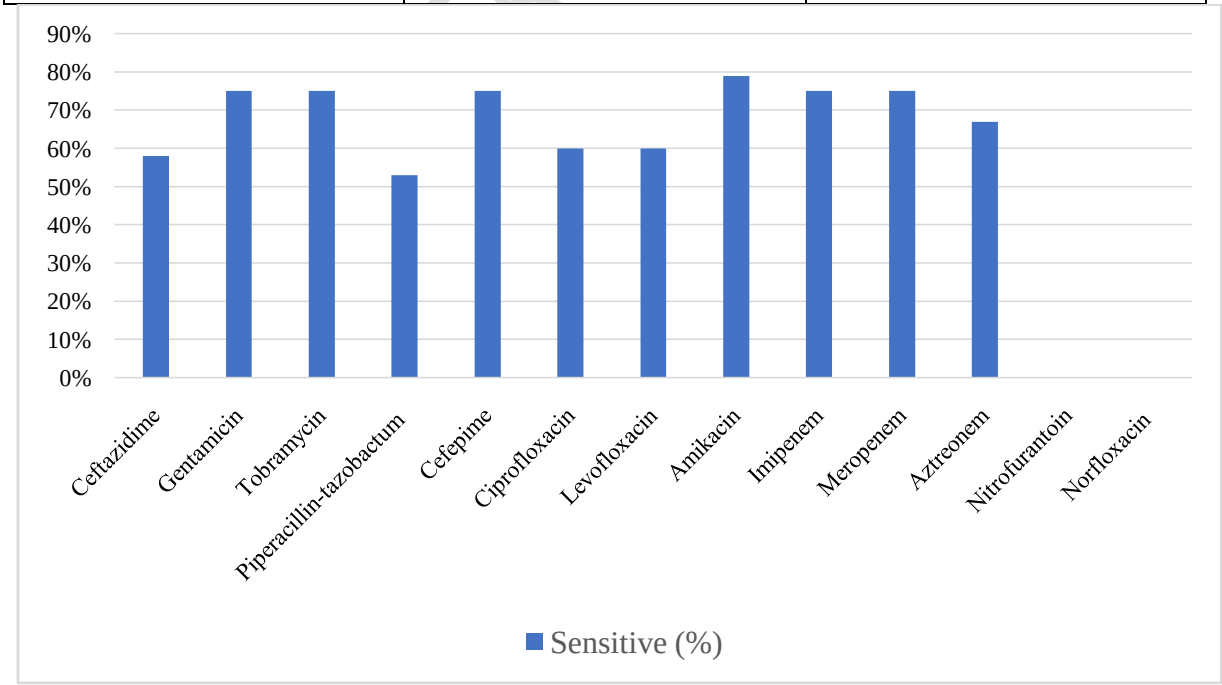

Fig 3- Antibiotic sensitive pattern of *Pseudomonas aeruginosa* (n=28)

Among the 285 isolates, 28 were as *Staphylococcus aureus*, 28 as *Enterococcus spp.* and 17 as *Coagulase negative Staphylococci (CoNS).*

1-*Staphylococcus aureus and CoNS*

- ✓ Linezolid was found to be highly effective (95%) followed by Vancomycin (86%), Tetracycline (81%), Cotrimaxazole (68%), Gentamicin (63%) and Teicoplanin (46%) whereas, Penicillin was least effective (15%) respectively.
- ✓ Nitrofurantoin and Norfloxacin was found to be highly effective (100%) in urinary tract infections.
- ✓ Fosfomycin was found to be highly effective (66%) in urinary tract infections and respiratory tract infections. (Table 8)

Table 4

Antibiotics	Sensitivity (%)	Resistance (%)
Penicillin	15%	85%
Cefoxitin	37%	63%
Vancomycin	50%	50%
Teicoplanin	46%	54%
Linezolid	95%	5%
Gentamicin	63%	37%
Azithromycin	34%	66%
Erythromycin	28%	72%
Tetracycline	81%	19%
Ciprofloxacin	32%	68%
Levofloxacin	32%	68%
Nitrofurantoin	100%	0%
Clindamycin	28%	72%
Cotrimaxazole	68%	32%
Norflox	100%	0%
Fosfomycin	66%	34%

Antibiotic susceptibility pattern of *Staphylococcus aureus* and *CoNS* (n=45)

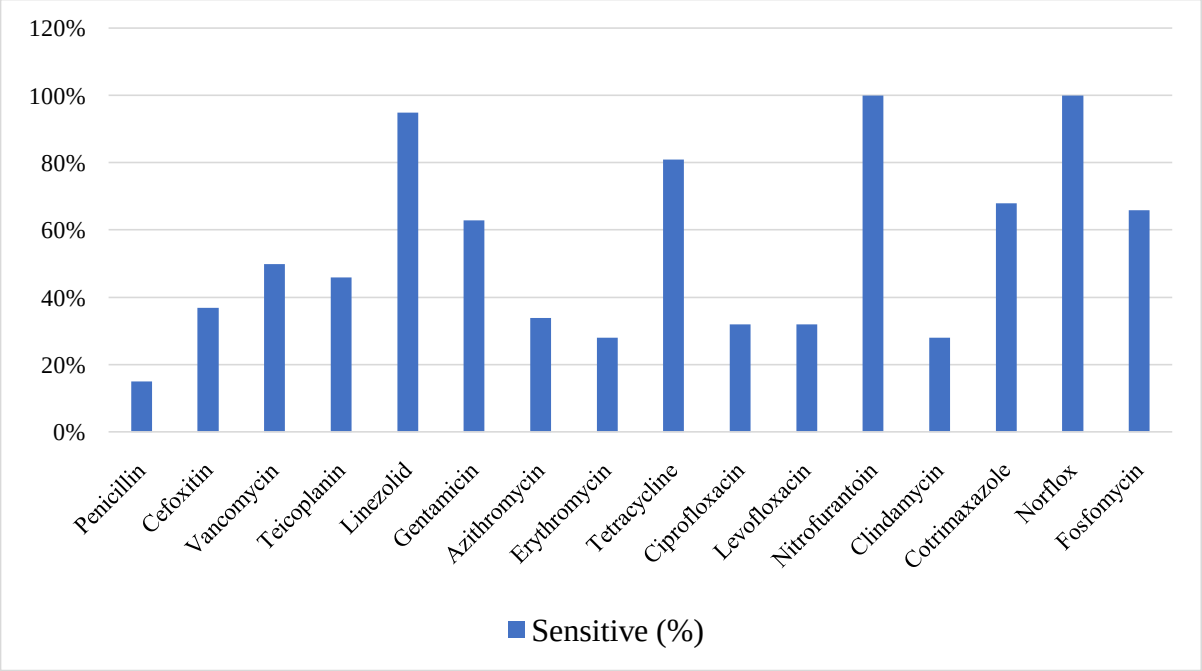

Fig 4- Antibiotic sensitive pattern of *Staphylococcus aureus and CoNS* (n=45)

2-*Enterococcus spp.*

- ✓ Linezolid was found to be highly effective (100%) followed by Teicoplanin (90%), Vancomycin (78%), Ampicillin (33%), High level gentamycin (15%) and High level streptomycin (15%) respectively. Erythromycin showed resistant to all the isolates of *Enterococcus spp.*
- ✓ Nitrofurantoin was found to be highly effective (60%) whereas, Fosfomycin was least effective (18%) respectively and Norfloxacin showed resistant to all the isolates of *Enterococcus spp.* in urinary tract infections. (Table 9)

Antibiotics	Sensitivity (%)	Resistance (%)
Ampicillin	33%	67%
Penicillin	15%	85%
Linezolid	100%	0%
Vancomycin	52%	48%
High level gentamycin	15%	85%
High level streptomycin	15%	85%
Ciprofloxacin	8%	92%
Levofloxacin	8%	92%
Fosfomycin	18%	82%
Nitrofurantoin	60%	40%
Tetracycline	9%	91%
Teicoplanin	90%	10%
Erythromycin	0%	100%
Norflox	0%	100%

Table No.5: Antibiotic susceptibility pattern of Enterococcus *spp.* (n=28)

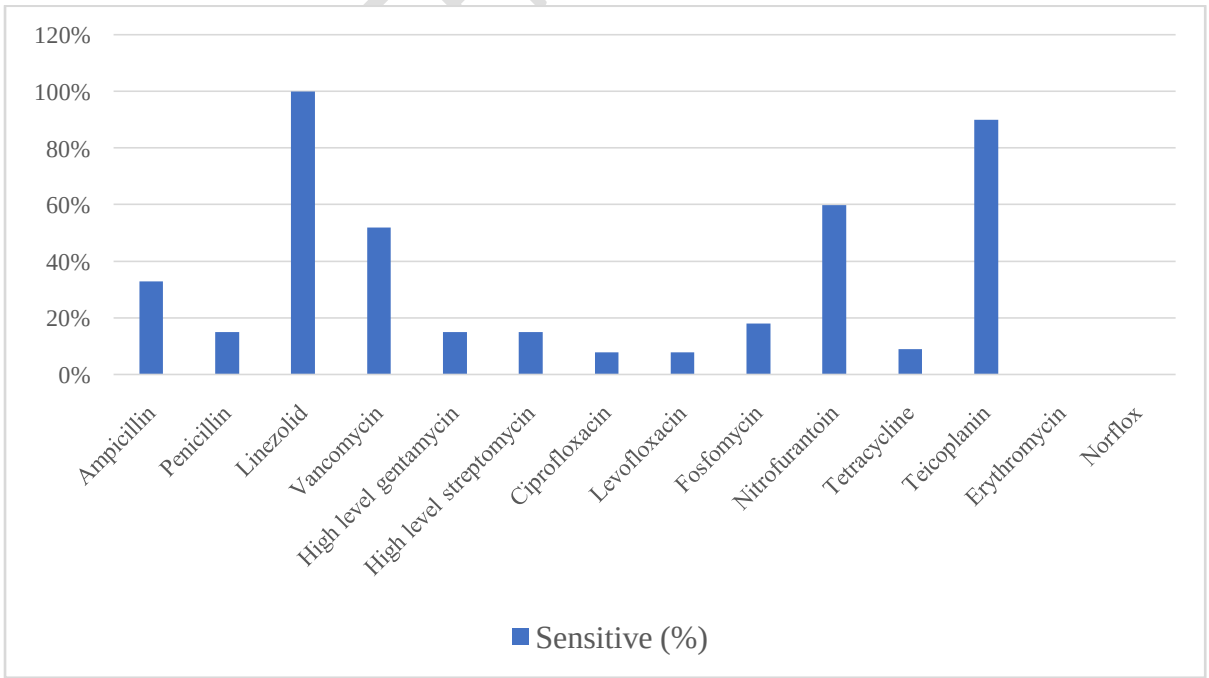

Fig 5- Antibiotic sensitive pattern of *Enterococcus spp.* (n=28)

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194 **Images**

195 Growth of most isolated Bacteria are shown in the figures given below

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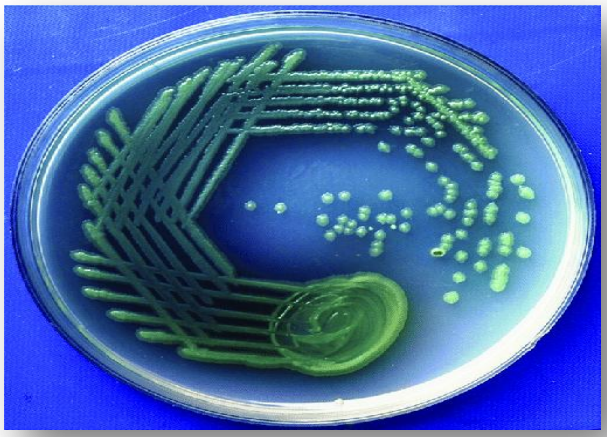

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Klebsiella spp. on MacConkey's Agar

E. coli on MacConkey's Agar

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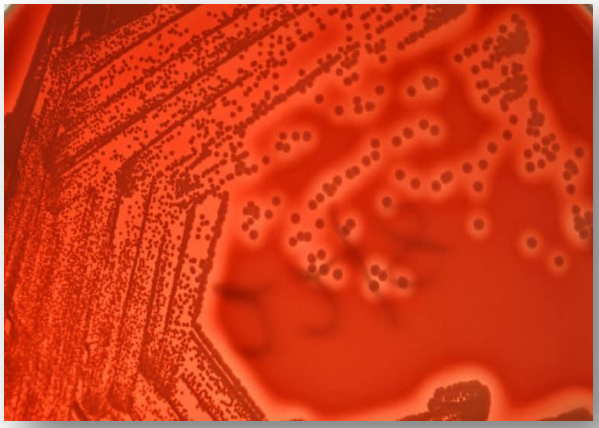

Staphylococcus aureus on Blood Agar

Enterococcus spp. on Blood Agar

Kirby-Baur AST on Muller Hinton Agar

Discussion:

In this study, out of 2,125 samples, bacterial growth was observed in 285 (13.4%). Among these 285 isolates, the highest frequency was noted in respiratory samples 131 (46%), followed by blood 64 (23%), urine 58 (20%), and pus 32 (11%). A study done by **Negm et al (2021)**[4] bacterial growth was observed in 45,221. Among these 45221 isolates, the highest frequency was noted in Blood 14,637 (32.37%), followed by Sputum 13,106 (28.98%), Urine 7379(16.32%), Wound swab 1240 (2.74%), Pus 5349 (11.83%), Pleural fluid 155 (0.34%), Peritoneal fluid 412 (0.91%), CSF 190 (0.42%), BAL 504 (1.11%), CVC tip 2166 (4.79%), Vaginal swab 33 (0.07%), Stool 50 (0.11%). [(11)] In another study by **Savanur SS and Gururaj H (2019)**[5] found bacterial growth in 127 (77.0%) out of 165 samples. In their findings, the highest isolation rate was recorded in blood (48), followed by ET aspirates (40), urine (39), sputum (17), pus (11), catheter tips (4), stool (1), ear swabs (2), and vaginal swabs (1).

Among the 64 blood samples analyzed in this study, the most frequently isolated organisms were *Staphylococcus aureus* (including both *MRSA* and *MSSA*) and Coagulase-negative staphylococci (CONS), each with 17 isolates (27%). This was followed by *Klebsiella* spp. with 10 isolates (16%), *E. coli* with 8 isolates (12%), *Acinetobacter spp.* with 6 isolates (9%), and *Enterococcus spp.* with 4 isolates (6%). The least isolated organism was *Pseudomonas spp.*, with only 2 isolates (3%) in bloodstream infections. A study done by **Jain Set al. (2022)**[6] involving 89 isolates reported that the most frequently isolated organism was Coagulase-negative staphylococci with 31 isolates (34.83%), followed by *Staphylococcus aureus* with 21 isolates (23.59%), *Pseudomonas* spp. with 12 isolates (13.48%), *Klebsiella* spp. with 7 isolates (7.87%), and *E. coli* with 6 isolates (6.74%). The least isolated organisms (2.25%) in their study included *Streptococcus spp*., *Enterococcus spp*., *Citrobacter spp.*, and *Burkholderia cepacian.* [(13)] In another study by **Katyal A et.al (2018)**[7] A total of 2,028 blood cultures were received from various ICUs. Total positive cultures were obtained in 504 (24.86%) cases. Among the Gram-positive (GP) isolates 288 (57.14%), *coagulase-negative Staphylococci (CoNS)* 55.5% was the most common followed by *Staphylococcus aureus* 34% and *Enterococcus spp.* 10.4%. *Acinetobacter baumannii* 52.3% was the most common Gram-negative (GN) isolate, 216 (42.85%), followed by *E.coli* 27.7%, *Klebsiella pneumoniae* 14.35%, and *Pseudomonas aeruginosa* 5.5%.

In urinary tract infections (UTIs) in this study, *Enterococcus spp*. and *E. coli* were the most frequently isolated organisms, each accounting for 23 isolates (40%). This was followed by *Klebsiella spp.* with 6 isolates (10%), *Pseudomonas spp.* with 3 isolates (5%), *Acinetobacter spp.* with 1 isolate (2%), and *Citrobacter spp.* with 1 isolate (2%). *Staphylococcus spp.* (*MSSA*) was the least isolated organism, with only 1 isolate (2%). A study done by **Deb J. and Debnath S. (2023)[8]** identified 45 microbial pathogens from 150 suspected UTI cases. Among the bacterial isolates, *Enterococcus spp*. was the most common, accounting for 33.3%, followed by *E. coli* (29%), *Staphylococcus aureus* (11.1%), *Klebsiella pneumoniae* (8.89%), *Acinetobacter spp.* (2.22%), *Citrobacter freundii* (2.22%), and *Enterococcus faecalis* (2.22%). **(15)**

In the analysis of respiratory samples in this study, *Acinetobacter* spp. was the most frequently isolated organism, accounting for 54 isolates (41%). This was followed by *E. coli* with 27 isolates (21%), *Pseudomonas spp.* with 21 isolates (16%), *Klebsiella spp.* with 17 isolates (13%), and *Staphylococcus aureus* (including both *MRSA* and *MSSA*) with 8 isolates (6%). *Citrobacter spp.* was the least identified organism, with only 4 isolates (3%). A study done by **Padmaja N. and Rao V. (2021)[9]** analyzed 135 respiratory samples, of which 52 (58%) showed positive growth. Their findings revealed a predominance of Gram-negative bacteria, with *Klebsiella pneumoniae* being the most common organism with 30 isolates (61%), followed by *Pseudomonas aeruginosa* with 15 isolates (30%) and *E. coli* with 4 isolates (8%). Additionally, the study identified three fungal isolates, all of *Aspergillus niger* (1%).

In skin and soft tissue infections in this study, *E. coli* was the most frequently isolated organism, accounting for 11 isolates (35%), followed by *Klebsiella spp.* and *Citrobacter spp.*, each with 5 isolates (16%). Other organisms included *Acinetobacter spp.* with 4 isolates, (12%), *Pseudomonas spp., Staphylococcus aureus (MSSA), and Proteus spp.*, each with 2 isolates (6%), while *Enterococcus spp.* was the least isolated organism with only 1 isolate (3%). A study done by **Kursheed F. and Tabassum A. (2023)[10]** analyzing 2,507 samples reported positive cultures in 1,242 cases (49.5%). Among these, 364 were Gram-positive cocci (GPCs) and 878 were Gram-negative rods (GNB). The most common isolate was *Methicillin-resistant Staphylococcus aureus (MRSA)* (23%), followed by *Klebsiella pneumoniae* (22.6%), *Pseudomonas aeruginosa* (16.9%), *Enterobacter spp.* (15.5%), and *E. coli* (14.2%). **(17)**

In this study, *E. coli* was the most frequently isolated organism, with 69 isolates (24%), followed by *Acinetobacter spp.* with 65 isolates (23%), *Klebsiella spp.* with 38 isolates (13%), *Pseudomonas spp.* with 28 isolates (10%), *Staphylococcus aureus (MRSA and MSSA)* with 28 isolates (10%), *Enterococcus spp.* with 28 isolates (10%), *CONS* with 17 isolates (6%), and *Citrobacter spp.* with 10 isolates (3%). *Proteus spp.* was the least isolated organism, with only 2 isolates (1%) among various ICU infections.

Summary & Conclusion:

A total of 2,125 ICU samples were processed, yielding 285 microbial isolates. The most common pathogens varied by infection site: Staphylococcus aureus, CONS, Klebsiella spp., and E. coli in bloodstream infections; Enterococcus spp. and E. coli in UTIs; Acinetobacter spp. in respiratory infections; and E. coli in skin/soft tissue infections. Overall, E. coli was the most frequent isolate (24%), followed by Acinetobacter spp. (23%).

Antimicrobial susceptibility patterns revealed carbapenems and tigecycline as the most effective against Enterobacteriaceae, with notable resistance trends in Acinetobacter spp. and Pseudomonas aeruginosa. Linezolid and vancomycin remained highly effective against Staphylococcus aureus, CONS, and Enterococcus spp.

The study highlights the urgent need for regular culture and sensitivity testing due to evolving resistance patterns, recommending hospital-specific antibiograms to guide empirical therapy in ICU settings.

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