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RETROSPECTIVE CROSS-SECTIONAL ANALYSIS OF BACTERIAL PATHOGENS AND THEIR ANTIMICROBIAL PROFILE IN CLINICAL SAMPLES AMONG THE ADULT ICU PATIENTS

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ABSTRACT

Background: Nosocomial infections caused by multidrug-resistant (MDR) bacteria are major contributors to morbidity and mortality in ICU patients. Rising antibiotic resistance poses a serious challenge in their management. This study aimed to assess the resistance patterns of pathogens infecting adult ICU patients in a tertiary care hospital in Assam, India.

Materials and Methods: A cross-sectional retrospective study was conducted from January 2024 to December 2025 on 471 adult ICU patients. Based on clinical symptoms, samples were collected for antibiotic sensitivity testing. Various clinical samples received from the patients were analysed by standard laboratory methods. Organism isolated were tested for different antimicrobials and sensitivity pattern were observed by phenotypic and Vitek 2 compact system (bioMerieux). In total, 471 ICU specimens were obtained.

Results: Out of a total of 471 clinical samples, 90 yielded pathogens of which 21 (23%) were *Escherichia coli*, 20 (22%) were *Klebsiella pneumoniae*, 14 (15%) were *Enterococcus spp*. Majority of the patients were male. The other organisms isolated were, *Staphylococcus aureus*, *Acinetobacter baumannii*, Candida spp. and *Pseudomonas aeruginosa* respectively. Among the 21 Escherichia coli isolates, 7 (36%) isolates showed resistance to various antimicrobial groups. *Klebsiella pneumoniae* also showed a high degree of resistance to various group of antimicrobials. 9 (45%) isolates of *Klebsiella pneumoniae* have shown multidrug resistance. All the MDR *Escherichia coli* isolates and *Klebsiella pneumoniae* isolates were positive for beta-lactamases.

Conclusion: There are serious treatment issues as a result of the rise in MDR bacterial infections in adult intensive care units. Antibiotic management in preventing the fatal effects of MDR bacteria in critically ill patients requires regular analysis of antimicrobial resistance patterns.

Keywords: Antibiotic resistance, intensive care units, multidrug-resistant microorganisms, nosocomial infections and public health.

INTRODUCTION

Up to 30% of hospital nosocomial infections occur in intensive care units (ICUs) because of the severely compromised population, which has weakened host defenses, numerous procedures, and the use of invasive devices like urinary catheterization, central venous catheterization, endotracheal intubation, and mechanical ventilation. Catheter-associated bloodstream infections, ventilator-associated pneumonia, and catheter-associated urinary tract infections are the most significant hospital-acquired infections in the intensive care unit.[1,2] Infections with drug-resistant gram positive and gram negative bacteria including *Acinetobacter baumannii*, *E. coli*

and *Klebsiella* produce Extended Spectrum Beta lactamases (ESBLs), and Metallo Beta lactamases (MBL) are common in Indian intensive care units.[3] *E. Coli*, *Klebsiella spp*, and *Proteus* are becoming multidrug resistant (MDR) in hospital settings as a result, and reports of gram-negative isolates' declining effectiveness against cephalosporins, carbapenems, and fluoroquinolones have come from all over the world. The overuse of wide spectrum antibiotics, which can stimulate the expansion of pre-existing resistant flora or transmit resistance to susceptible bacteria through mutation, is frequently attributed to the onset of MDR.[3,4] Multidrug-resistant organisms are carried by healthcare personnel, and inadequate infection control practices may contribute to the spread of these strains. Because of their inherent resistance to antimicrobial drugs, hospital-acquired strains of *Pseudomonas* and *Acinetobacter spp*. are resistant to a wide variety of antibiotics. Under selection pressure, *Pseudomonas* quickly acquires resistance by hyper production of enzymes including DNA gyrases and beta lactamases, active efflux pumps, and permeability alterations. Critically ill patients may get an opportunistic infection from *Acinetobacter spp*. These organisms are naturally resistant to aminoglycosides, cephalosporins, and penicillins .[5,6] Age, length of stay in intensive care unit, previous antibiotic use, exposure to indwelling prosthetic devices, and the severity of illness and debility are all positively associated with the likelihood of contracting an infection with the resistant organism.[1,7] If the first antibiotic selection does not provide coverage, the poor outcome may be linked to infection with organisms resistant to antibiotics. For improved patient outcomes and to stop the establishment of multidrug resistance, strict infection control procedures, routine monitoring of the local antimicrobial flora and resistance pattern, and optimal antimicrobial usage in intensive care units are crucial.[7] Our intensive care unit (ICU) at a teaching hospital with tertiary care services has not recently had any nosocomial infection surveys. The frequency and trends of antibiotic resistance in patients in intensive care units are poorly understood, despite the fact that it is a significant issue in low- and middle-income countries. Antibiotic resistance patterns differed greatly between countries, medical facilities, and even different intensive care units in the same hospital. Determining the prevalence of bacterial infections and the pattern of antibiotic resistance in adult intensive care units (ICUs) of a tertiary care hospital was the aim of the current investigation.

MATERIALS AND METHODS

Sample collection: Between January 2024 and December 2025, a retrospective, cross-sectional study was carried out on 471 patients who were hospitalized to the intensive care units of Lakhimpur Medical College and Hospital, a tertiary care facility in Assam, India. In these patients complete medical histories and related risk factors were obtained. A total of 471 clinical specimens from the adult intensive care unit were received. All clinical samples were taken at the patient's bedside in accordance with the standard procedure,[8] and sent as soon as possible to the Microbiology Laboratory of the Department of Microbiology, Lakhimpur Medical College and Hospital, North Lakhimpur, Assam, India. Blood, urine, sputum, tracheal aspirate, throat swab and pus swabs were received from various patients. All the samples were processed according to standard laboratory method. Blood agar, MacConkey agar plate were used to process the samples and plates were incubated aerobically at 35°C ± 1°C overnight. A healthcare-associated infection is defined as a localized or systemic condition that arises from an adverse reaction to the presence of an infectious agent or its toxin(s) that was not present at the time of admission to the acute care facility for the purposes of Centers for Disease Control and Prevention (CDC)/National Healthcare Safety Network (NHSN) surveillance in the acute care setting. Hospital and community-acquired illnesses are often distinguished using a 48-hour time cut-off following admission.[9] Blood culture: - For aerobic culture, blood cultures were injected in a BacT/ALERT bottle. After being inoculated on blood agar and MacConkey agar, positive flagged blood culture bottles and other samples were first incubated aerobically at 35°C ± 1°C overnight. Regular biochemical assays like indole, triple sugar iron agar, urease, and citrate utilization test, as well as common fast tests like catalase and oxidase, motility, and colony features on MacConkey and blood agar were used to identify the bacterial species.

Antibiotic susceptibility test: To evaluate the susceptibility of isolates to various antibiotics, the Kirby Bauer's disk diffusion method was used [Figure 1].

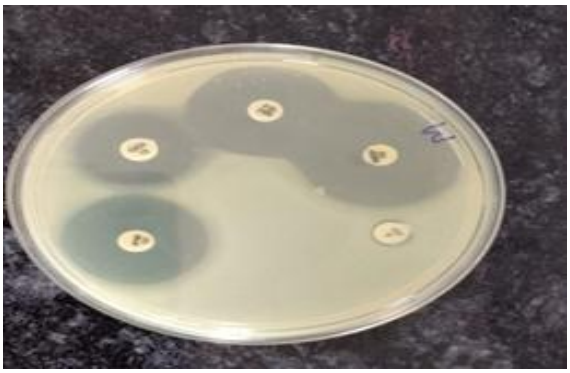

Figure 1: Antibiotic sensitivity of different antimicrobials by Kirby-Buer disc diffusion method

Using a panel including ceftazidime (30µg), ceftazidime/clavulanate (30/10µg), cefoxitin (30µg), cefepime (30µg), levofloxacin (5µg), aztreonam (30µg), gentamicin (10µg), meropenem (10µg), piperacillin/tazobactam (100/10µg), tigecycline (15µg), fosfomycin (200 µg), nitrofurantoin (300

µg), norfloxacin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), ampicillin (10 µg), linezolid (30 µg), vancomycin (30 µg) and co-trimoxazole (25 µg). The test organism was suspended in normal saline, adjusted to 0.5 McFarland standards, and streaked with a sterile swab stick on Mueller Hinton Agar (MHA). Antibiotic-impregnated disks were applied and incubated at 35°C ± 1°C overnight. Zone of inhibition diameters were recorded and interpreted following CLSI guidelines 2024 and 2025

RESULTS

Out of a total of 471 clinical samples, 90 yielded pathogens of which 21 (23%) were *Escherichia coli*, 20 (22%) were *Klebsiella pneumoniae*, 14 (15%) were *Enterococcus spp.* Majority of the patients were male. The other organisms isolated were, *Staphylococcus aureus*, *Acinetobacter baumanii*, *Candida spp*. and *Pseudomonas aeruginosa* respectively [Table 1&2]

Table 1: Organism isolated from various clinical samples

Organism isolated	No of isolates (n= 90)	Percentages (%)
Escherichia coli	21	23%
Klebsiella pneumoniae	20	22%
Enterococcus spp.	14	15%
Methicillin-sensitive Staphylococcus	8	9%
Methicillin resistant Staphylococcus (MRSA)	4	4%
Acinetobacter baumanii	9	10%
Candida spp.	7	7%
Pseudomonas spp.	7	7%

Table 2: Number of different microorganisms isolated from different clinical samples

Clinical samples	Number of clinicalsamples	Organism isolated							
		Klebsiella pneumoniae	*Acinetobacter baumani*	*Escherichia coli*	*Staphylococcus aureus*	*Enterococcus spp.*	*Staphylococcus (MRSA*	*Pseudomonas aeruginosa*	*Candida species*
Urine	367	4	3	15	2	14	2	0	7
Tracheal aspirate	16	3	2	2	0	0	0	4	0
Sputum	37	12	2	0	2	0	2	2	0
Pus	9	0	0	4	4	0	0	1	0
Throat swab	13	1	0	0	0	0	0	0	0
Ascitic fluid	7	0	0	0	0	0	0	0	0
Cerebrospinal fluid	20	0	0	0	0	0	0	0	0
Pleural fluid	2	0	0	0	0	0	0	0	0
Total	471	20	9	21	8	14	4	7	7

Among the 21 *Escherichia coli* isolates, 7 (36%) isolates showed resistance to various antimicrobials group. *Klebsiella pneumoniae* also showed a high degree of resistance to various group of antimicrobials. Nine (45%) isolates of *Klebsiella pneumoniae* have shown multidrug resistance [Table 3].

Table 3: Susceptibility profile of common gram negative pathogens

Antimicrobials	Bacterial pathogens N (%)			
	Escherichia coli	*Klebsiella pneumoniae*	*Pseudomonas aeruginosa*	*Acinetobacter baumanii*
Gentamicin	11(55)	14 (72)	7 (100)	1 (12)
Ceftazidime	12 (60)	6 (33)	3 (50)	2 (25)
Cefoxitin	16 (80)	8 (42)	1(25)	2 (25)
Levofloxacin	17(85)	12 (63)	7 (100)	9 (100)
Piperacillin-Tazobactam	14 (70)	10 (54)	7 (100)	4 (50)
Meropenem	17 (88)	13 (66)	7 (100)	2 (25)
Aztreonam	10 (52)	11 (55)	7 (100)	2 (25)
Cefepime	8 (42)	8 (40)	3 (50)	0(0)
Nitrofurantoin	15 (76)	17 (85)	7 (100)	3 (37)
Fosfomycin	18 (92)	17 (87)	NA	NA
Norfloxacin	13 (65)	12 (62)	3 (50)	6 (75)
Cotrimoxazole	18 (90)	16 (82)	7 (100)	3 (37)
Tobramycin	NA	NA	7 (100)	7 (87)

Ciprofloxacin	10 (52)	8 (42)	3 (50)	5 (62)
Tigecycline	NA	NA	0(0)	NA

Table 4: Susceptibility profile of common gram positive pathogens

Antimicrobials	Bacterial pathogens N (%)		
	Enterococcus spp.	*Staphylococcus aureus (MSSA)*	*Staphylococcus aureus (MRSA)*
Gentamicin	11 (80)	6 (80)	20(80)
Cefoxitin	NA	4 (50)	4 (100)
Levofloxacin	9 (67)	5 (67)	2 (72)
Nitrofurantoin	11 (85)	6 (78)	3 (92)
Fosfomycin	11 (85)	NA	NA
Norfloxacin	10 (72)	5 (72)	2 (67)
Cotrimoxazole	11 (82)	6 (78)	3 (82)
Erythromycin	7 (52)	4 (58)	2 (60)
Clindamycin	10 (72)	5 (66)	2 (72)
Ciprofloxacin	9 (67)	4 (62)	2 (62)
Linezolid	14 (100)	8 (100)	4 (100)
Vancomycin	10 (71)	NA	NA
Tigecycline	11 (80)	NA	NA
Ampicillin	8 (63)	4 (60)	2 (57)
High level Gentamicin	9 (66)	NA	NA

Figure 2: Phenotypic confirmatory test for ESBL using Kirby-Bauer disk diffusion method. Positive test if the zone of inhibition around cefotaxime+ clavulanate and ceftazidime+clavulanate is increased by ≥ 5mm as compared to that of cefotaxime or ceftazidime on MHA plate.

All the 7 MDR *Escherichia coli* isolates and 9 *Klebsiella pneumoniae* isolates were positive for ESBL beta-lactamases [Figure 2].

Four (4) isolates of *Klebsiella pneuoniae* were screening positive for AmpC production by phenotypic methods.

Methicillin resistant *Staphylococcus aureus* (MRSA) was isolated in 4 (4%) number of patients. Among the 14 *Enterococcus* isolates, Vancomycin resistant were seen in 4 isolates by disc diffusion method [Table 4]. Further confirmation cannot be done by microbroth dilution method.

DISCUSSION

Multidrug-resistant organisms that cause nosocomial infections constitute a global issue because they put critically sick patients at risk for death and increase healthcare costs. In our analysis of the data on antibiotic resistance patterns, we discovered that *Enterococcus* species isolated from intensive care units (ICUs) exhibited 35% resistance to fluoroquinolone compounds and 40% resistance to ampicillin, respectively. MRSA strains exhibited 28% cotrimoxazole resistance and 35% resistance to fluoroquinolones, respectively [Table 4]. The urgent need is to prevent this ICU infection. We must be aware of the local bacteriological profile, antibiotic resistance trend, and infection rates in order to accomplish this. The current study focus on antibiotic resistance in the intensive care units (ICUs) of a tertiary care facility in Assam, India. We have examined a variety of infections and antibiotic treatments in this study, enabling a reasonably thorough documentation of antibiotic resistance in intensive care unit patients. A total of 471 samples were collected from various patients; 20% of these samples exhibited bacterial growth, and tested positive for bacteria. Among all the isolates *Enterococci spp*. was the 3rd most common organism which belongs to the ESKAPE group of pathogens. The majority of patients had a gram-negative bacterial infection. Our results are consistent with previous research that found Gram-negative bacteria were responsible for over 50% of nosocomial infections in hospital intensive care units.[10,11] Frequent ICU infections worldwide may be primarily caused by the high prevalence of Gram-negative bacteria in hospital environments.[11] Biofilm production is triggered by a wet environment and waste materials disposed of in hospital sinks or drains. These biofilms contain MDR bacteria, which can lead to nosocomial infections.[12] Hospital settings and surveillance techniques used to detect nosocomial infections influence the incidence of nosocomial infections.[12] 18.9% of patients had at least one ICU-acquired infection, according to a large multicentric study, with a frequency that varied from 2.3% to 49.2% across the centers.[13] Based mostly on the patient group under study, other research,[14,15] have previously found prevalence rates ranging from 9% to 37%. Nosocomial infections were discovered in 51% of patients in an international investigation by Vincent et al,[12] that included 1265 intensive care

units from 76 countries. Nonetheless, there were notable differences in the prevalence of these illnesses among the various nations.[16] Depending on the severity of their sickness, patients from a single hospital may be at multiple risk of contracting infections.[17] According to a recent study by Negm et al,[18] the most common condition among the ICU patients at Zagazig University Hospitals in Egypt was bacteremia (32%) though in our study we didn't isolate any pathogenic organism in blood. Majority of the organisms we isolated from respiratory samples and urinary samples (in both ventilated and nonventilated patients).[19] Gram-negative bacteria are the pathogens that cause respiratory tract infections in our study. In the past, pneumonia was the most common infection found in intensive care units (62.07%), according to Dasgupta et al. In the First Affiliated Hospital in Zhejiang Province, China, respiratory tract infections made up 64.75% of all nosocomial infections, according to Shao et al. We discovered that *Escherichia coli* and the *Enterococcus spp* were the most often isolated pathogens among the patients in urine samples though the history of UTI couldn't be traced out in those patients. Majority of the patients were on urinary catheters. *Klebsiella pneumoniae* were the most prevalent isolates in respiratory samples. The frequency of various nosocomial illnesses can change depending on the study and hospital context, according to the US National Nosocomial illnesses Surveillance System. The degree of antibiotic resistance among significant pathogens was one of the study's key findings. Remarkably, the majority of the isolated bacteria exhibited high resistance to various antibiotic classes [Tables 3 and 4]. Given that these antibiotic classes are the most crucial for treating nosocomial infections, this poses a major public health risk. Human health and the healthcare system are at risk from antibiotic-resistant ESKAPE (*Enterococcus fecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and E*nterobacter* species) pathogens. ESKAPE pathogens acquisition of antibiotic resistance genes has progressively decreased the available treatments for severe nosocomial infections, raised the prevalence of infectious disorders, and raised hospital treatment failure mortality rates. Patterns of antibiotic resistance in intensive care unit patients admitted to a tertiary hospital in Vietnam were recently reported by Tran GM et al. Ventilator-associated pneumonia (VAP) was the main topic of their investigation. *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were shown to be resistant to ceftazidime, ceftriaxone, piperacillin, imipenem, meropenem, ertapenem, ciprofloxacin, and levofloxacin; additionally, large percentages (>70%) of *Klebsiella spp.* resistant to ceftriaxone and ceftazidime were also noted. Yadav et al also recently reported the appearance of a non-fermentative, multidrug-resistant Gram-negative bacterial infection in hospitalized patients in a Nepalese tertiary care center. Contaminated hands or hospital equipment, as well as contaminated environments, can readily spread the MDR bacterium to various patient groups admitted to intensive care units. During an extended hospital stay, nosocomial infections may increase as a result of cross-infections brought on by bacterial transmission. The rise in MDR bacterial strains in hospital intensive care units may be caused by improper use of antibiotics, poor hygiene, improper hospital environment cleaning, and most importantly newer antibiotic-resistant mechanisms that opportunistic pathogenic bacteria have acquired. Therefore, the cornerstones of preventing ICU infections by multidrug-resistant organisms are the formation of the local antibiogram, frequent monitoring of the nosocomial infection rates, and thorough cleaning of the ICU environment. Based on the hospital antibiogram's sensitivity pattern, we recommended using a combination therapy for empirical treatment, which consists of one bactericidal and one bacteriostatic medication. To ascertain the synergy between the two antibiotics, more research on fractional inhibitory concentration is possible.[20-22]

CONCLUSION

Our study illustrated the prevalence of antibiotic resistance in a tertiary care hospital in adult intensive care units. Compared to Gram-positive cocci, non-fermenters and Gram-negative *Enterobacterales* were found to be the most frequently isolated pathogens in adult intensive care units. ICU-derived bacterial infections shown strong resistance to several clinically significant drug classes. The management of critically ill patients is discouraged by the high percentage of antibiotic resistance seen. Our research has important clinical ramifications for how ICU patients are managed and treated. The issue of antibiotic resistance in hospital settings could be lessened by minimizing the use of broad-spectrum antibiotics and using antibiotics appropriately when treating patients with nosocomial infections. Based on the hospital antibiogram, we recommend empirical treatment with a combination of one bactericidal and one bacteriostatic medication. The occurrence of antibiotic resistance in hospital intensive care units can be reduced by implementing an effective infection control program and an active surveillance system.

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