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BACTERIOLOGICAL AND ANTIBIOTIC RESISTANCE PROFILE OF ISOLATES AMONG INTENSIVE CARE UNIT INA TERTIARY CARE TEACHING HOSPITAL: A CROSS-SECTIONAL STUDY.

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Abstract:

Background: Antibiotics are the cornerstone of modern medicine, playing a crucial role in the treatment of bacterial infections. Research identify specific resistance patterns among bacterial isolates, crucial for guiding treatment decisions, enhancing infection control strategies, and addressing the broader public health challenge of antibiotic resistance.

Objective: To analyse the antibiotic sensitivity and resistance profile in an Intensive Care Unit (ICU) of a tertiary care hospital.

Materials and Methods: This cross-sectional, prospective study was conducted over a three months period in January 2023 to January 2024 and involved 150 participants admitted to the ICU

of a tertiary care hospital. The culture and sensitivity patterns of clinical isolates from blood, urine, sputum, endotracheal tube (ET) aspirates, catheter sites, and wound swabs were analyzed. Positive cultures were isolated, and their antibiotic sensitivity testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI).

Results: Cultures were obtained from 150 participants. Among these, 121 patients had positive cultures, while 29 had negative cultures. The isolated bacteria were predominantly gram-negative bacilli, with Escherichia coli being the most common (18.06%), followed by Acinetobacter (13.25%), Klebsiella (10.23%), Pseudomonas (8.9%), and Proteus (2.31%). Among gram-positive organisms, coagulase-negative staphylococcus (CoNS) was the most frequently isolated (17.62%), followed by Streptococcus (3.25%). Fungal growth was observed in 29 samples (18.32%). The distribution of samples with positive cultures included blood (n = 44), sputum (n = 15), urine (n = 36), ET aspirate (n = 32), pus (n = 14), catheter sites (n = 3), ear swabs (n = 4), and stool (n = 2).

Conclusion: The prevalence of gram-negative bacterial infections is rising in ICUs, complicating the selection of appropriate antibiotics. Therefore, studying the antibiotic sensitivity and resistance patterns in a hospital setting is crucial for guiding clinicians in initiating empirical antibiotic treatment in critical cases.

Introduction

Antibiotics have long been the cornerstone of modern medicine, playing a crucial role in the treatment of bacterial infections. However, the emergence of antibiotic resistance represents a significant public health crisis globally, posing a severe threat to human health. In India, which bears one of the highest burdens of infectious diseases worldwide, the inappropriate and irrational use of antimicrobial agents has exacerbated the development of antimicrobial resistance (AMR). Several factors, including poor financial conditions, inadequate healthcare infrastructure, a high disease burden, and the unregulated sale of inexpensive antibiotics, have intensified the AMR crisis in the country. AMR crisis in the country.

Nosocomial infections, particularly in critical care settings, are a common cause of hospitalization. The rate of such infections ranges from 5% to 30% among patients in intensive care units (ICUs). The increased risk of infection in these settings is associated with the severity of patient illness, prolonged exposure to invasive devices and procedures, frequent patient contact with healthcare personnel, and extended hospital stays. Over the past two decades, infection control practices and the development of new antimicrobials have primarily focused on controlling and treating infections caused by gram-positive organisms. However, there has been a recent rise in infections caused by gram-negative bacteria in ICUs, with some multi-drug-resistant (MDR) strains presenting a significant challenge due to the limited availability of effective treatment options. Infections caused by MDR gram-negative organisms are associated with high morbidity and mortality rates. (10)

The present study addresses a critical concern in modern healthcare: the escalating problem of antibiotic resistance among bacteria, particularly in high-risk environments such as intensive care units (ICUs). With the widespread use of antibiotics, bacteria have developed mechanisms to resist these drugs, rendering once-effective treatments ineffective. This phenomenon not only complicates patient care but also poses a significant public health threat by limiting treatment options and increasing healthcare costs.

Despite the well-documented global challenge of antibiotic resistance, there remains a crucial gap in understanding the specific resistance profiles of bacterial isolates within ICUs of tertiary care hospitals. ICUs are unique environments where patients with severe illnesses are often treated with multiple antibiotics, creating a selective pressure that promotes the emergence and spread of resistant bacteria. Understanding the prevalence and patterns of antibiotic resistance in this setting is

essential for guiding empirical therapy decisions, implementing effective infection control measures, and ultimately improving patient outcomes.

This study aims to fill this knowledge gap by systematically analyzing the antibiotic sensitivity and resistance profiles of bacterial isolates obtained from patients admitted to the ICU of a tertiary care hospital. By characterizing the resistance mechanisms and identifying trends in antibiotic resistance, the study seeks to provide clinicians with critical data to optimize antibiotic prescribing practices and combat the growing threat of resistance. Furthermore, the findings from this study may contribute to the development of targeted interventions and policies aimed at preserving the efficacy of existing antibiotics and ensuring better patient care in ICU settings.

Materials and Methods:

This prospective observational study was conducted at a teaching tertiary care hospital in April 2024. A total of 150 adult patients admitted to the ICU during this period were included. Data were collected from the participants and included participant identity, diagnosis, comorbidities, source of infection, results of microbial culture, antibiotic sensitivity and resistance patterns, antibiotic use, duration of hospital stay, and clinical outcomes.

Various diagnostic tests were employed to analyze different specimens collected from participants, including blood, sputum, urine, endotracheal (ET) aspirate, pus, central venous catheter tips, ear swabs, and stool. Blood cultures were used to detect bacteria or fungi in the bloodstream. Sputum cultures identified respiratory pathogens. Urine cultures diagnosed urinary tract infections by identifying bacterial colonies and determining their antibiotic sensitivity. ET aspirate cultures, collected from mechanically ventilated patients, helped diagnose ventilator-associated pneumonia. Pus cultures identified organisms in abscesses or wounds, guiding effective antibiotic selection. Central venous catheter tip cultures detected colonization or infection by identifying bacteria or fungi that might cause bloodstream infections. Ear swab cultures identified pathogens causing ear infections. Stool cultures detected enteric pathogens like Salmonella, Shigella, and certain strains of Escherichia coli. Each of these tests played a crucial role in identifying causative organisms, understanding their antibiotic sensitivity and resistance patterns, and guiding effective clinical management of infections.

All collected data were statistically analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 23.0. The analysis utilized appropriate statistical methods to interpret the data. Frequencies and percentages were calculated for the demographic profiles of the participants, while means and standard deviations were used to describe quantitative data that followed a normal distribution.

For comparisons between two independent continuous groups, a parametric independent Student's t-test was employed. Discrete (categorical) groups were compared using the chi-square (χ^2) test. Statistical significance was defined as a p-value of ≤ 0.05 , and a p-value of ≤ 0.01 was considered highly significant.

Results:

Out of the 150 ICU admissions, 81 samples were gram-negative and 48 were gram-positive organisms and 21 were positive for fungal growth as depicted in Figure 1. The distribution of specimens that yielded microbial growth included blood (n = 34), sputum (n = 23), urine (n = 28), endotracheal (ET) aspirate (n = 32), pus (n = 17), central venous catheter tip (n = 7), ear swab (n = 4), and stool (n = 3) and vaginal swab (n = 2).

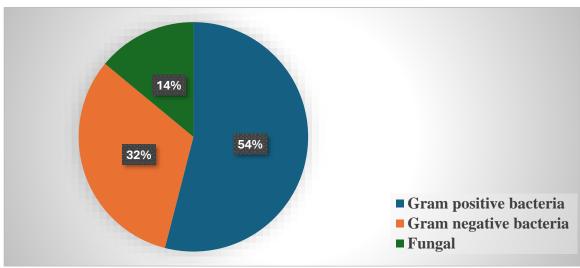


Fig 1: Gram's staining and organism isolated.

CoNS is the most frequent isolate from blood culture, E. coli and fungal growth from urine culture, and Klebsiella and Acinetobacter from ET secretions. E. coli (28%) was the most common organism isolated, followed by Acinetobacter (11.33%), Klebsiella (9.33%), Pseudomonas (7.33%), Enterococcus (1.33%), Staphylococcus(2%) and Proteus (2%). Among the gram-positive organisms, CoNS (20.66%) was the most common organism followed by Streptococcus (2.66%) and Nonfermenting gram-negative Bacillus (1.33%). In all, 22 samples, i.e., (14.66%) were positive for fungal growth (Table 1). E. coli was most sensitive to colistin (97.52%), followed by tigecycline (81.23%), nitrofurantoin (74.62%), aztreonam (69.36%), and meropenem (62.36%) (Table 2 and Fig. 2). Acinetobacter showed highest sensitivity to colistin (66%) followed by tigecycline (66%) (Fig. 3). Klebsiella demonstrated highest sensitivity to colistin(74%) (Fig 4). CoNS documented more sensitivity to togecycline(74.12%) and teicoplanin (74.23%)(Fig 5). Enterococcus was showed greater sensitivity to linezolid (85.56%), tigecycline (76.23%) and vancomycin (75.32) (Fig. 6). Streptococcus was produced more sensitivity to cefepime, ceftazidime, clindamycin, vancomycin and linezolid (78% and Fig. 9). Staphylococcus showed 100% sensitivity to tigecycline and nitrofurantoin (Tabe 2 and Fig. 10). Similarly, Table 2 and Figs.5 and 6 depicted the sensitivity pattern of other isolated organisms. E. coli, Acinetobacter, Pseudomonas, Proteus, and Enterobacter showed resistance to cephalosporins and piperacillin-tazobactam. Resistance to colistin was observed more in Proteus, and CoNS Staphylococcus showed 100% resistance to vancomycin and clindamycin, as depicted in Table 3.

Table 1: Frequency of Organisms isolated.

No.	Organisms	Frequency
1	Escherichia coli	32 (21.33%)
2	Acinetobacter	15 (10%)
3	Klebsiella	16 (10.66%)
4	Pseudomonas	13 (8.66%)
5	Coagulase negative Staphylococcus	22(14.66%)
6	Enterococcus	4(2.66%)
7	Proteus	5 (3.33%)
8	Staphylococcus	4 (2.66%)
9	Nonfermenting gram-negative Bacillus	3 (2%)
10	Streptococcus	19(12.66%)
11	Fungal	21 (14%)
	Total	150 (100%)

Table 2.	Antibiotio	-Resistance	Dottown of	Flaclaton
I anie 3:	Antibiotic	- Kesistance	Pattern of	i isolates

Antibiotic	E. coli	Acineto	Kleb	Pseud	CoNS	Entero	Prot	Strepto	Staph
Ak	16.23	0	50	72.56	78.56	56.32	56.23	45.23	45
Gm	22.25	0	61	52.36	45	78.56	65.36	26	45
Amx	38	0	85	92	78.26	60.23	63.23	0	0
Amp	38.56	0	0	0	0	0	0	0	0
Cfm	63.23	92	62	72	56	0	85	35	40
Ctx	52	85	56	58	80	95	35	56	48
Ctzm	63.25	90	60	72	71.25	80	100	28	44
Cfpz	42.35	90.	60.23	74	72	78	100	38	45
Cxm	48.25	85	55	70	63.23	100	35	45	51
Cfu	45	90	50	69	59	85	95	38	52
Срх	18.25	90	75	48	71.26	70	66.25	70.25	43
Lfx	18.28	0	0	85.26	65.23	0	0	0	0
Ofx	19.23	0	0	0	0	0	0	0	0
Ctmx	45.26	95	68.25	71.25	55.23	75.28	66.36	40	53
Cl	19	23.62	26.26	0	0	51.18	0	0	0
Col	4.25	39	25	55.26	85.23	70.25	92	45	28
Ip	25.36	18.14	41	62.25	75.29	58.29	59.63	49.62	45
Mp	25.36	69.54	75.28	52.36	42.58	71.28	69.36	60.28	45
Nf	10.57	0	0	95	48.36	79.36	62.54	98	0
Ptz	48.56	78.58	0	0	88.25	75.28	60.35	100	48
Tig	0	38	45	74.12	39.28	28.54	95	78.36	0
Tpn	19.57	0	69	95.25	28.54	36.25	98	97	48
Mcn	0	50	0	75.05	38.25	80.25	100	29.63	45
Cli	18.25	0	0	95.45	26.25	59.63	95	29.23	90.25
Vmn	16.25	0	80	90.23	38.54	26.52	95	29.56	95
Lzd	18.25	0	95.23	85	45.23	18.25	68.56	39.25	49
Doxy	18.25	0	95.	98	45	82.3	0	100	48
Rif	12.35	0	85.63	95	25.36	74.25	0	98	45
Aznm	18.56	0	0	0	0	0	0	0	0

Ak, amikacin; Amx, amoxicillin; Amp, ampicillin; Gm, gentamicin; Cfm, cefepime; Ctx, ceftriaxone; Czm, ceftazidime; Cpz, cefaperazone; Cfx, cefexime; Cfu, cefuroxime; Cpx, ciprofloxacin; Lfx, levofloxacin; Ofx, oflaxacin; Ctmz, cotrimoxazole; Cl, clarithromycin; Col, colistin; Ip, imepenem; Mp, meropenem; Nf, nitrofurantoin; Ptz, piperacillin—tazobactam; Tig, tigecycline; Tpn, tiecoplanin; Mcn, minocycline; Cli, clindamycin; Vmn, vancomycin; Lzd, linezolid; Doxy, doxycycline; Rif, rifampicin; Aznm, aztreonam; NT, not tested; E. coli, Escherichia coli; Acineto, Acinetobacter; Kleb, Klebsiella; Pseud, Pseudomonas; Entero, Enterococcus; Prot, Proteus; Strepto, Streptococcus; Staph, Staphylococcus.

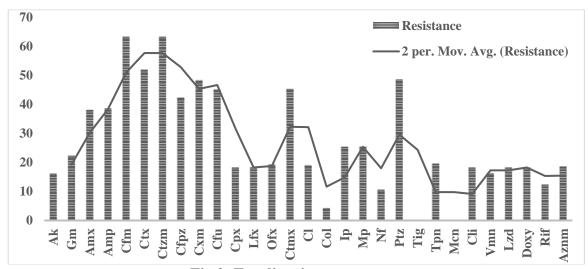


Fig 2: E. coli resistance pattern

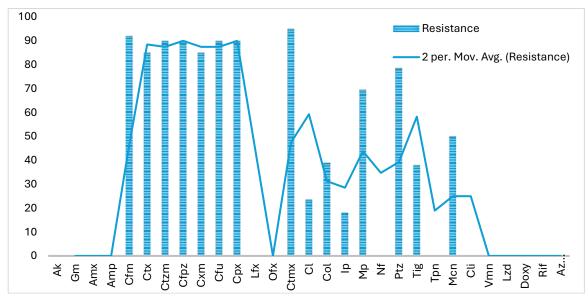


Fig 3: Acinetobacter resistance pattern

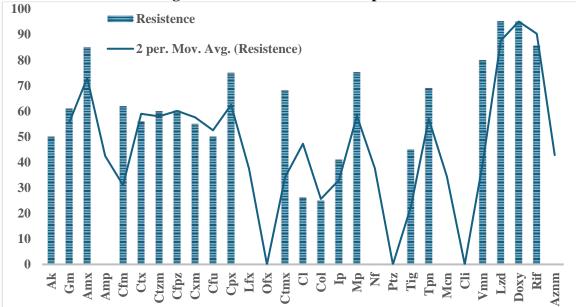


Fig 4: Klebsiella resistance pattern



Fig 5: Pseudomonas resistance pattern

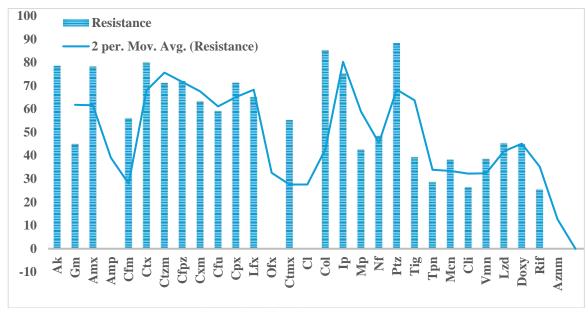


Fig 6: CoNS resistance pattern

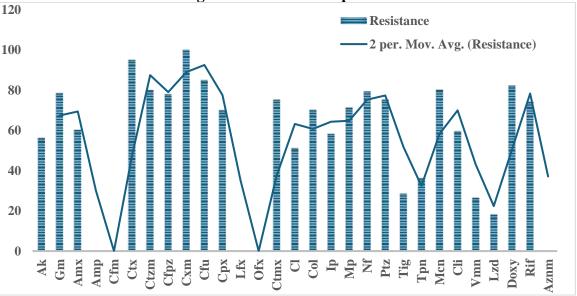


Fig 7: Enterococcus resistance pattern



Fig 8: Proteus resistance pattern

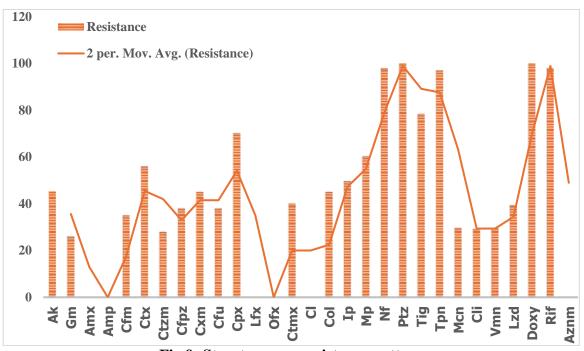


Fig 10: Staphylococcus resistance pattern.

Discussion:

Antibiotic resistance is a growing challenge in the management of critically ill patients, significantly impacting their prognosis and survival, as well as contributing to prolonged hospital stays and increased treatment costs (11-13). In the present study, 76% of the 150 clinical samples collected yielded positive cultures, a higher rate compared to the 46.4% positivity reported by Chakravarthi et al. (14). Among the positive cultures, Gram-negative organisms constituted 32% of the isolates, Gram-positive organisms accounted for 54%, and fungal growth was observed in 14% of the samples (Fig. 1). The most prevalent pathogens identified in this study were *Escherichia coli* (21.33%), *Klebsiella* species (1.66%), *Acinetobacter* species (10%), and *Pseudomonas* species (8.66%). These findings are consistent with other studies where Gram-negative bacteria were predominantly isolated (10). Among Gram-positive isolates, coagulase-negative staphylococci

(CoNS) emerged as the most common pathogen (14.66%). Fungal growth was observed in 14% of the samples (Table 1).

In alignment with similar research conducted in Asian countries, including India, the majority of ICU isolates were Gram-negative bacteria such as *E. coli*, *Klebsiella*, and *Acinetobacter*, followed by Gram-positive organisms like *Staphylococcus* ^(15–17). Specifically, CoNS was the most frequently isolated pathogen in blood cultures (48.96%), with *E. coli* and *Pseudomonas* following, which corroborates findings from studies by Vanitha Rani et al. ⁽¹⁸⁾, Javeed et al. ⁽¹⁹⁾, Jain et al. ⁽²⁰⁾, Rajeevan et al. ⁽²¹⁾, and Shrestha et al. ⁽²²⁾.

E. coli (46%) was commonly isolated from urine, followed by fungal growth and Acinetobacter. In other studies, such as Bajaj et al. (23) and Sheth et al., (24) Klebsiella was commonly isolated from urine culture. Fungal urinary tract infection has become a significant nosocomial problem over the past decade; (21) however, laboratory yield of yeast in urine and its significance may be difficult to differentiate from colonization and infection. (24–27) Klebsiella was commonly isolated from ET aspirate culture (31%) followed by Acinetobacter and Pseudomonas. In most other studies done in respiratory ICU, Acinetobacter was commonly isolated followed by Klebsiella and Pseudomonas. (28–30) E. coli showed highest resistance to ceftazidime (63.25%), and cefepime (63.23%). This was identical to the study by Hsu et al., (31) Mangaiarkkarasi et al., (32) and Oteo et al. (33) (Fig. 2). Acinetobacter showed high resistance to cephalosporins (96%) followed by piperacillin–tazobactam (84%) as also reported by Chakraverti et al. (14) (Fig. 3).

Klebsiella showed high resistance to cephalosporins (75%),linezolid (95.23%), doxycycline (95%), rifampicin (85.63), amoxycillin (85%), vancomycin (80%) and meropenem (75.28%), teicoplanin (69%%), and cotrimoxazole (68.25%) (Fig. 4). The resistance of Klebsiella to cephalosporins was also observed in other studies by Sheth et al., ⁽²⁴⁾ Javeed et al. (Fig. 13). ⁽¹⁹⁾ Pseudomonas showed the highest resistance to antipseudomonal drugs suchasdoxycycline (98%),clindamycin (95.45%), teicoplanin (95.25%), rifampin (95%), nitrofurantoin (95%) vancomycin (90.23%), and levofloxacin (85.26%)(Fig. 5). This pattern of resistance was observed by Mohana Sundaram et al. ⁽³⁴⁾. Enterococcus showed highest resistancetoce fexime (100%), cotrimoxazole (95%), cefuroxime (85%), minocycline (80.25%), ceftazidime(80%) and nitrofurantoin (79.36%). (Fig. 7) Streptococcus showed 100% resistance to piperacillin-tazobactam and doxycycline. (Fig. 9).

Piperacillin-tazobactam has traditionally been a cornerstone of empirical antibiotic therapy for severely ill ICU patients, with carbapenems often used as subsequent treatment options. The Indian Council of Medical Research (ICMR) guidelines advocate the use of β -lactam agents combined with β -lactamase inhibitors, such as piperacillin-tazobactam, as a recommended empirical therapy for critically ill patients ⁽¹⁾. However, our study revealed alarmingly high resistance rates to piperacillin-tazobactam, with resistance observed in 60% to 86% of both Gram-negative and Grampositive pathogens, as determined by culture and sensitivity testing.

Over the past decade, there has been a marked increase in the prevalence of carbapenem-resistant Enterobacteriaceae, including *Klebsiella*, *E. coli*, and *Acinetobacter* species ⁽²⁻³⁾. This trend is evident in our findings, which indicate that *E. coli* exhibited a high sensitivity of approximately 97.52% to colistin, whereas *Acinetobacter*, *Klebsiella*, and *Pseudomonas* demonstrated lower sensitivities of 66%, 74%, and 49.23%, respectively (Table 1). The rising resistance to piperacillintazobactam underscores the critical need for continuous surveillance and the adaptation of treatment protocols to address the challenges posed by multidrug-resistant pathogens.

The prevalence of multidrug-resistant organisms in our ICU can be attributed to factors such as prior antibiotic usage, previous severe Gram-negative infections, inappropriate antibiotic courses, and the high acuity of patients presenting with severe sepsis and septic shock, characteristic of a tertiary care hospital. The resurgence of older antibiotics, such as colistin, is a response to the increasing resistance of these organisms.

The present study revealed a notable resistant to all tested antibiotics including carbapenems, colistin, and minocycline, highlights a significant and emerging threat in the management of severe

infections. The presence of such PDR organisms underscores the urgent need for reassessment of current treatment strategies and the development of novel therapeutic approaches.

In light of these findings, it is imperative to establish and regularly update local antibiograms in every ICU setting, ideally on a quarterly basis, to inform empirical antibiotic therapy decisions. A robust antibiotic stewardship program is essential for curbing the rise of multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens. Effective stewardship should include strategic use of broad-spectrum empirical antimicrobials followed by timely de-escalation based on susceptibility testing to minimize collateral damage to the microbial flora.

Additionally, stringent adherence to infection control practices is crucial for preventing nosocomial infections. This includes the meticulous application of sterile techniques during device insertion, rigorous hand hygiene protocols, and the appropriate use of personal protective equipment such as gowns and gloves. Implementing these measures will not only help in preventing the emergence of resistant organisms but also improve patient outcomes and optimize clinical responses in the ICU environment.

Conclusion:

Antibiotic resistance has become a significant challenge in modern clinical practice, increasing the complexity of treatment for healthcare providers and imposing serious financial burdens on patients and their families. The rising prevalence of Gram-negative infections resistant to antibiotics in intensive care units (ICUs) is linked to worsened patient outcomes, including higher morbidity and mortality rates. To address these issues, it is crucial to implement regular antibiogram monitoring and develop effective antibiotic stewardship programs. These programs play a key role in accurately identifying pathogens and their resistance profiles, which supports the appropriate initiation of empirical antibiotic therapies in emergency situations. Equally important is the practice of antibiotic de-escalation, which aims to reduce unnecessary antibiotic use and prevent further resistance development. Effective stewardship and optimal antimicrobial use are essential for maintaining the efficacy of antibiotics for future generations.

The study has several notable limitations that must be considered when interpreting the findings. Firstly, the relatively small sample size of 150 clinical cases and the short duration of the study may not fully represent the broader resistance patterns across different healthcare settings or capture long-term trends in antibiotic resistance. Additionally, the single-center design limits the generalizability of the results to other institutions or regions. The focus on only blood culture samples restricts the scope of the study, as resistance patterns can vary across different infection sites and sample types. Furthermore, the reliance on standard microbiological methods for susceptibility testing introduces potential variability in results, and the study did not explore the specific genetic mechanisms underlying antibiotic resistance. The absence of detailed patient outcome data and a lack of longitudinal analysis further constrain the study's ability to assess the long-term impacts of resistance and the effectiveness of treatment regimens. Lastly, the limited range of antibiotics tested and the potential for selection bias due to the study's focus on cases with ordered blood cultures may not fully capture the complexity of antibiotic resistance in the ICU setting. Addressing these limitations in future research through multi-center studies, broader sampling methods, and comprehensive outcome evaluations would provide a more robust understanding of resistance dynamics and improve clinical strategies for managing resistant infections.

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