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ORIGINAL ARTICLE

Frequency and MIC-based Antimicrobial Resistance Profile of MDR and XDR Gram Negative Isolates of Blood Culture Specimens in a Tertiary Care Setting

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ABSTRACT

The escalation of antimicrobial resistance (AMR) among Gram-negative bacteria, particularly multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens, posed a significant challenge in tertiary care settings. This paper comprehensively analyzed the antimicrobial resistance profile of MDR and XDR Gram-negative isolates from blood culture specimens at Jinnah Hospital, Lahore, a leading tertiary care facility. Over a period of one year, 9600 blood culture specimens were processed, revealing a high incidence of resistance to a wide array of antibiotics, with notable findings including 100% susceptibility to Colistin and over 90% resistance to several commonly used antibiotics such as Ciprofloxacin and Piperacillin/Tazobactam. Among the positive isolates, 33.8% were classified as MDR and 66.2% as XDR. The study emphasized the critical role of Minimum Inhibitory Concentrations (MICs) in guiding therapeutic decisions, highlighting the wide variability in resistance patterns and the necessity for personalized antimicrobial therapy. The prevalence of MDR and XDR isolates underscored the urgent need for advanced treatment modalities, comprehensive surveillance, and robust antimicrobial stewardship initiatives. Additionally, the study pointed to the emergence of colistin resistance as a critical challenge in managing infections caused by these pathogens. Through detailed analysis and discussion, this study illuminated the grave challenge posed by AMR in tertiary care settings. It emphasized the importance of innovative approaches to antimicrobial stewardship, developing new antimicrobial agents, and ongoing surveillance to effectively combat this public health crisis.

Keywords: Antimicrobial resistance, Antimicrobial stewardship, Colistin resistance, Extensively drug-resistant, Multidrug-resistant, Minimum Inhibitory Concentrations, Public health challenge.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4] Critical analysis and manuscript review; [5,6]Data analysis; Manuscript Editing

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Introduction

Antimicrobial resistance (AMR) among Gram-negative bacteria presents a significant challenge to public health, notably within tertiary care settings where multidrug-resistant (MDR) and extensively drug-resistant (XDR) pathogens increasingly compromise the efficacy of available treatments[1]. The rise of carbapenem-resistant *Enterobacterales* and *Pseudomonas aeruginosa* has been identified as

a critical threat to human health, attributed to the high mortality rates associated with infections caused by these pathogens and the limited antimicrobial therapy options available[2,3].

Bloodstream infections (BSIs) caused by these resistant organisms are particularly alarming due to their association with severe morbidity and mortality. The management of BSIs, a frequent complication in hospitalized patients, is complicated further by the emergence of MDR and XDR strains, underscoring the need for accurate diagnostic tools and effective antimicrobial stewardship[4]. The determination of minimum inhibitory concentrations (MICs) plays a pivotal role in guiding the selection of effective antimicrobial therapy, offering a quantifiable measure of the extent of an organism's resistance to an antibiotic [5,6]

However, the interpretation and application of MIC values in clinical practice involve considerable complexity, particularly in the context of rapidly emerging resistance mechanisms and the variability of resistance profiles among different pathogens. This complexity is compounded in tertiary care settings, where the patient population often presents with a higher burden of comorbidities, exposure to healthcare-associated pathogens, and previous antimicrobial use, all of which contribute to the selection pressure favoring the emergence and spread of resistant bacteria[7]. Despite guidelines aiming to optimize the management of BSIs, significant variations in clinical practice and adherence to these guidelines have been observed among infection specialists, indicating an ongoing challenge in standardizing care to effectively combat AMR[8]. Moreover, the reliance on polymyxins and other last-resort antibiotics, despite their known nephrotoxicity and questionable efficacy against certain resistant organisms, underscores the desperate need for novel therapeutic options and more effective antimicrobial stewardship strategies[9].

In light of these challenges, this study was planned to explore the antimicrobial resistance profile of MDR and XDR Gram-negative isolates from blood culture specimens in a tertiary care setting, focusing on the frequency of resistance and the role of MIC in guiding therapeutic decisions. Through this investigation, we obtained valuable insights into the ongoing efforts to combat AMR, emphasizing the critical need for innovative approaches to antimicrobial stewardship and the development of new antimicrobial agents.

Methodology

Ethical Considerations

Ethical considerations were paramount throughout the study, in all procedures including approval from the hospital's Institutional Review Board (IRB).

Sample Collection

Blood specimens were collected from patients admitted to Jinnah Hospital, Lahore, a leading tertiary care facility grappling with a high incidence of infectious diseases. Patient selection adhered to clearly defined criteria, including individuals diagnosed with or suspected of having infectious diseases, while excluding those who had received antimicrobial therapy within 48 hours. The collection was conducted using standardized blood culture bottles (Bactec), specifically designed for the optimized recovery of pathogens from blood samples while minimizing contamination from the skin flora. The collection was conducted in accordance with stringent aseptic techniques, ensuring that skin antisepsis with chlorhexidine gluconate was performed prior to venipuncture to reduce the potential for contamination. To ensure the integrity of the samples, healthcare professionals were trained in the latest phlebotomy protocols, emphasizing skin antisepsis and proper collection methods. The volume of blood drawn was carefully measured (standardized at 4 mL for adults and 3 mL for paediatric patients) to meet the recommended quantity for the Bactec bottles. Upon acquisition, these bottles were immediately transported under controlled conditions,

maintaining a temperature range of 2-8°C to preserve sample integrity until analysis[10].

Inoculation and Monitoring

Upon arrival at the laboratory, every specimen containing bottle was scanned with barcode and loaded into the Bact/Alert automated blood culture system, which fosters optimal growth conditions for any present organisms. This system, equipped with advanced sensors, continually monitored the culture bottles for signs of microbial growth, such as color changes or turbidity resulting from CO_2 production and other metabolic activities. Once a specimen was flagged positive by the system, indicating the potential presence of pathogens, it was immediately subjected to subculturing to isolate and identify the microorganisms[11].

Subculturing for Isolation and Identification

The subculturing involved spreading the contents of each positive blood culture bottle onto two distinct types of agar plates. Blood agar, a non-selective, nutrient-rich medium, was utilized to support the growth of a broad spectrum of bacteria, providing an inclusive environment for organism recovery. Conversely, MacConkey agar, a selective and differential medium, was explicitly employed to isolate Gram-negative enteric bacilli. This medium facilitated the differentiation of organisms based on lactose fermentation. A standardized inoculum of 0.1 mL from each positive blood culture was spread onto the agar surfaces for subculturing. This volume was chosen based on preliminary studies indicating its effectiveness in yielding discernible, countable colonies[12]. The inoculated agar plates were then incubated in an aerobic environment at 37°C for 18-24 hours. Post-incubation, colonies indicative of Gram-negative organisms were meticulously selected for further analysis. This was based on their growth characteristics, colour, and morphology on the selective media. Morphologically distinct colonies were selected for further identification to ensure that a diverse representation of the microbial flora in the blood cultures was analyzed[13].

Antimicrobial Susceptibility Testing

Following their initial isolation, bacterial specimens underwent confirmation and identification using the Vitek automation system. This platform was selected for its proven accuracy and efficiency in bacterial identification, using metabolic reactions to a variety of substrates to match isolates to a comprehensive database. Concurrently, the system assessed the antimicrobial susceptibility of each isolate, precisely determining Minimum Inhibitory Concentrations (MIC) by exposing bacteria to a spectrum of antibiotics across various concentrations. Interpretation of the MIC data adhered strictly to guidelines set forth by authoritative bodies like the Clinical and Laboratory Standards Institute (CLSI), ensuring accuracy and relevance[14]. Based on these metrics, isolates were classified according to their resistance profiles: multi-drug resistant (MDR) isolates demonstrated non-susceptibility to at least one agent in three or more antimicrobial categories, whereas Extensively Drug-Resistant (XDR) isolates were non-susceptible to one or more agents in all but two or fewer categories[15].

Data Analysis

The frequency of MDR and XDR isolates and their corresponding MIC values were cataloged and analyzed. Descriptive statistics were used to summarize the data, providing a comprehensive overview of the antimicrobial resistance landscape within the sampled population. The distribution of MICs was plotted to visualize the spectrum of drug susceptibilities, offering valuable insights into the prevailing resistance patterns. Statistical analyses were performed using SPSS Version 25 (IBM Corp., Armonk, NY, USA).

Results

Characteristics of Isolates

During the study period from February 2023 to January 2024, a total of 9,600 blood culture specimens were processed at our tertiary care

setting. The most common pathogens were *Klebsiella*, *Enterobacter cloacae* complex, *Serratia marcescens* and *Acinetobacter baumannii* complex. Among these, 2,688 specimens tested positive for Gram-negative isolates; a notable finding was the identification of 154 antimicrobial resistance isolates. Among these, a significant portion demonstrated resistance to multiple antibiotic classes, underscoring the challenges faced in clinical management and selecting effective therapeutic options. MDR Salmonella enterica is defined as resistant to first line antibiotics; Ampicillin, Chloramphenicol and Co-trimoxazole. XDR Salmonella is defined as MDR and resistant to Quinolones and third generation cephalosporins, (following the Centers for Disease Control and Prevention guidelines). All the isolates were 100% susceptible to Colistin, while 97.4% of isolates were non-susceptible to Ciprofloxacin (Cip). Other important findings included high resistance rates to commonly used antibiotics such as 90.6% were resistant to Ticarcillin (Tic), 82.2% resistant to Ticarcillin/Clavulanic Acid (Tic/CA), 89.6% resistant to Piperacillin (PIP), 81.3% resistant to Piperacillin/Tazobactam (TZP), 87.1% resistant to Ceftazidime (CAZ), 89.3% resistant to Cefepime (CEP), 88.3% resistant to Aztreonam (AZT), 76.3% resistant to Imipenem (IMI), 74.6% resistant to Meropenem (MEM), 85.3% resistant to Amikacin (AMK), 88.3% resistant to Gentamicin (GEN), 90.6% resistant to Tobramycin (TOB), 96.2% resistant to Minocycline (MIN) and 94.8% resistant to Trimethoprim/Sulfamethoxazole (SXT). The overall resistance rate among the tested isolates was approximately 5.73%, while the mean resistance rate across all antibiotics mentioned in the study was approximately 88.6%.

Frequency of MDR and XDR Gram-negative Isolates

In our investigation of Gram-negative bacterial isolates, we observed a concerning prevalence of antimicrobial resistance. Multidrug-resistant (MDR) isolates, defined as non-susceptibility to at least one agent in three or more antimicrobial categorized

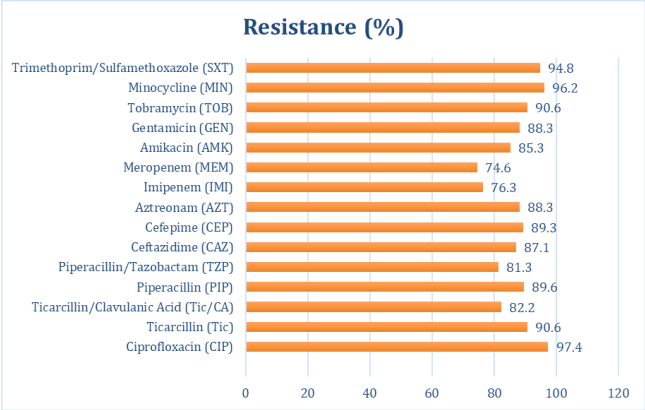

Figure 1: Bar chart illustrating the percentage of bacterial resistance to various antibiotics.

Notably, 102 isolates were classified as Extensively Drug-Resistant (XDR), indicating non-susceptibility to at least one agent in all but two or fewer antimicrobial categories, based on our stringent criteria adapted from World Health Organization recommendations.

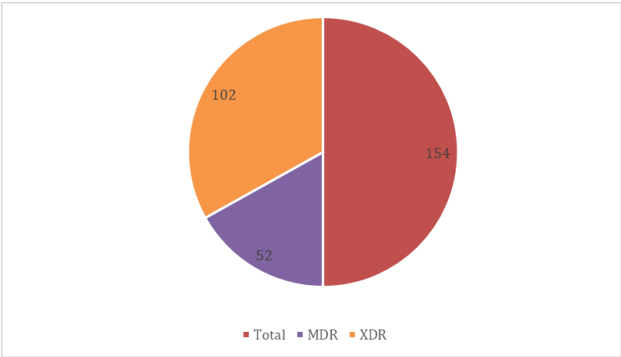

Figure 2: Proportion of Multidrug-Resistant (MDR) vs Extensively Drug-Resistant (XDR)

(Following the Centers for Disease Control and Prevention guidelines), were identified in 52 out of 154 isolates. The proportion of MDR isolates among the total isolates tested was about 33.8%, while the proportion of XDR isolates among the total isolates tested was 66.2%. These findings underscored the urgent challenge posed by these pathogens, emphasizing the necessity for advanced treatment modalities, comprehensive surveillance, and robust antimicrobial stewardship initiatives.

Table I. Percentage frequency,Multi-Drug Resistant (MDR)& Extensively Drug-Resistant (XDR) percentages of individual organisms			
Organism	**Frequency %**	**MDR%**	**XDR%**
Serratia Marcescens	22		100
Klebsiella pneumoniae	20.7	6.2	93.7
Salmonella enterica	16.8	100	
Acinetobacter baumannii complex	12.9	30	70
Enterobacter cloacae complex	12.9	10	90
Escherichia coli	2.6	100	
Burkholderia cepacia	2.59	50	50
Pseudomonas species	1.29		100

Pathogen Identification and Resistance Pattern

Our study identified a broad spectrum of Gram-negative resistant species, with the most commonly tested being *Klebsiella pneumoniae sp. pneumoniae* (n=32), *Enterobacter cloacae* complex (n=20), *Serratia marcescens* (n=34) and *Acinetobacter baumannii* complex (n=20). The *Enterobacter spp.*, *Klebsiella pneumoniae*, and *Serratia marcescens* were among the most prevalent XDR organisms. These pathogens contributed significantly to the overall burden of XDR infections, complicating the management of bloodstream infections and increasing the risk of adverse outcomes. The analysis indicated a high level of resistance across various organisms. The resistance was particularly notable for antibiotics such as Ciprofloxacin, Trimethoprim/Sulfamethoxazole, and Ticarcillin/Clavulanic Acid, suggesting that these organisms had developed strong resistance mechanisms. The high resistance rates to a broad spectrum of antibiotics suggested a significant presence of multi-drug resistant (MDR) organisms within the samples. The effectiveness of Colistin against the pathogens for which data was available indicated that it could be a valuable treatment option against MDR organisms. However, its use is typically reserved for highly resistant infections due to its toxicity profile.

Table II. Key findings of antimicrobial resistance patterns among Gram-negative isolates		
Metric/Category	**Result (%)**	**Details**
Mean Antibiotic Resistance Rate	88.6	It was calculated across all tested antibiotics, indicating widespread resistance.
Proportion of Multidrug-Resistant (MDR) Isolates	33.8	Isolates resisted at least one agent in three or more antimicrobial categories.
The proportion of Extensively Drug-Resistant (XDR) Isolates	66.2	Isolates resisted at least one agent in all but two or fewer antimicrobial categories.
Percentage of Isolates Resistant to Colistin (MIC < 4 mg/L)	Nil	Indicated all isolates sensitive to Colistin, a last-resort antibiotic.
Organism-specific Highlights		
Klebsiella pneumoniae and related species	Prevalence of XDR	Demonstrated high levels of resistance, particularly to carbapenems and third generation cephalosporins.
Acinetobacter baumannii complex	Significant resistance	It resisted nearly all tested antibiotics, highlighting its role as a critical concern in healthcare settings.

Complete Susceptibility to Colistin in Antibiotic-Resistant Isolates

This research evaluated the resistance profiles of 154 bacterial isolates, which contained reference strains alongside representatives of MDR and XDR strains, against the antibiotic Colistin. Known as a

last-line defence in the treatment against infections by highly resistant bacteria, the significance of Colistin cannot be ignored. Our investigation was firmly anchored on determining the Minimum Inhibitory Concentrations (MIC) for Colistin, adhering to the criteria set by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI). Our findings reveal a critical insight: The resistance to carbapenem antibiotics underscored the challenge in treating infections caused by these pathogens. For Imipenem, MIC values ranged from 8 to 16 µg/mL while for Meropenem, MIC values were consistently reported at 16 µg/mL for the isolates indicating a high level of resistance in these isolates. All of the 154 isolates analyzed demonstrated complete susceptibility to Colistin, with no instances of resistance (MIC values < 4 mg/L). This outcome underscored the unparalleled efficacy of Colistin in combating pathogens that exhibit resistance to multiple drugs. The absolute susceptibility rate of 100% highlighted the essential role that Colistin continues to play in the management of infections caused by MDR and XDR pathogens.

Discussion

The findings of this study illuminated the grave challenge antimicrobial resistance (AMR) posed in tertiary care settings, particularly emphasizing the threat from multidrug-resistant (MDR) and extensively drug-resistant (XDR) Gram-negative isolates. The study's comprehensive analysis, spanning over a year and involving 9,600 blood culture specimens, underscored a significant public health concern: the alarming frequency of resistance and the critical role of minimum inhibitory concentrations (MICs) in guiding therapeutic decisions amidst this resistance crisis. The high prevalence of MDR and XDR isolates, which constituted approximately 33.8% and 66.2% of the resistance isolates, respectively, represented a clarion call for the medical and scientific communities. This alarming distribution indicated the evolving nature of bacterial pathogens in response to antibiotic pressure. The pronounced resistance to a broad spectrum of antibiotics, including beta-lactams and carbapenems, further complicated clinical management, narrowing the therapeutic window and limiting effective treatment options. Identifying these resistant strains in a tertiary care setting, where the patient population often presented with multiple comorbidities and higher exposure to healthcare-associated pathogens, posed additional complexity to treatment[7,8].

The variability in resistance patterns, as evidenced by the wide range of MIC values, underscored the complexity of AMR and the necessity for personalized antimicrobial therapy. The elevated MIC values for beta-lactam and carbapenem antibiotics, in particular, indicated the severe limitation in treatment options available for infections caused by these resistant organisms. This supported the argument for the crucial role of MIC determination in guiding therapeutic decisions, a point also emphasized by Diallo *et al.* and Havenga *et al*[5,6]. However, due to the absence of viable alternatives, the reliance on polymyxins, with known nephrotoxicity, highlighted the desperate need for novel antimicrobial agents[9].

The identification of crucial pathogens, such as Klebsiella pneumoniae and Acinetobacter baumannii complex, as prevalent XDR organisms was consistent with global trends, indicating these species as significant contributors to the burden of XDR infection[2,3]. The high resistance rates to a broad spectrum of antibiotics underlined the substantial presence of MDR organisms within the sampled population, corroborating the findings of Cavalieri *et al.*[12] regarding the challenges in the clinical management of these infections. The one-size-fits-all approach is inadequate in the face of such diversity in resistance mechanisms. This underscored the importance of innovative

treatment strategies, including the development of new antimicrobial agents and the judicious use of existing antibiotics, such as Colistin, despite its known adverse effects.

The sensitivity to Colistin, a last-resort antibiotic, in 100% of the isolates highlighted the colistin as a critical antibiotic in managing infections caused by MDR and XDR pathogens. It echoed the necessity for ongoing surveillance of colistin susceptibility and the development of alternative treatments to address this emerging threat[4]. The escalated challenge of AMR necessitates a multifaceted approach, integrating research, clinical practice, and public health policy. The findings of this study illuminated the critical challenges posed by the prevalence of MDR and XDR Gram-negative bacteria in a tertiary care setting. They emphasized the importance of antimicrobial stewardship, infection control measures, and the urgent need for innovative therapeutic options. Additionally, the study highlighted the critical role of ongoing surveillance and research in understanding and tracking the evolving landscape of AMR. Future research should focus on elucidating the genetic mechanisms underlying resistance, exploring alternative therapeutic options (such as bacteriophage therapy, antimicrobial peptides, and the use of adjuvants to potentiate the efficacy of existing antibiotics), and developing predictive models to inform clinical decision-making and stewardship practices.

Conclusion

In conclusion, it is alarming to observe that MIC values for all antibiotics are increasing among all isolates. This trend suggests a potential surge in antimicrobial resistance rates, highlighting the urgent need for effective antimicrobial stewardship measures. Furthermore, the emergence of common pathogens as MDR and even XDR strains is concerning. This upward trend underscores the necessity for robust interventions to control these evolving patterns of resistance. Moreover, the identification of rare pathogens becoming more prevalent in our healthcare setting is noteworthy. These pathogens, already challenging to treat due to their complex nature and intrinsic drug resistance capabilities, pose significant challenges. These findings emphasize the importance of implementing rigorous infection prevention strategies to curb their spread and mitigate the impact on patient outcomes. The findings from this study served as a stark reminder of the complexities and challenges in managing infections caused by MDR and XDR Gram-negative bacteria. The escalated challenge of AMR necessitates a multifaceted approach, integrating research, clinical practice, and public health policy. While the study provided valuable insights into the resistance profiles of these pathogens, it also highlighted the urgent need for concerted efforts in antimicrobial stewardship, research, and policy-making to address this growing public health crisis. Collaboration across disciplines will be paramount in devising effective strategies to mitigate the spread of AMR and ensure the availability of effective therapeutic options for future generations. Only through concerted efforts could there be a hope to mitigate the impact of AMR and safeguard the efficacy of existing and future antimicrobial agents.

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