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RESEARCH ARTICLE
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TO STUDY THE IN VITRO EVALUATION OF MINOCYCLINE EFFECTIVENESS AGAINST MULTIDRUG-RESISTANT GRAM-NEGATIVE BACILLI ISOLATES IN TERTIARY CARE CENTER OF LUCKNOW, INDIA

Parinita Raj[1]*, Dr. Shweta Kumari[2], Sandeepika Dubey[3]

[1]*Msc. Student, Department of Microbiology, Integral Institute of Medical Science and Research, Lucknow, Uttar Pradesh, India.
[2]Assistant professor, Department of Microbiology, Integral Institute of Medical Science and Research, Lucknow, Uttar Pradesh, India.
[3]Tutor, Department of Microbiology, Integral Institute of Medical Science and Research, Lucknow, Uttar Pradesh, India.

***Corresponding Author:** Ms Parinita Raj
*Email ID: parinitaraj@gmail.com

ABSTRACT

Background: Multidrug-resistant gram-negative bacilli (MDR-GNB) represent a critical global health challenge due to their association with hospital-acquired infections and limited therapeutic options. Minocycline, a second-generation tetracycline, has re-emerged as a potential treatment option.

Aim: To evaluate the in vitro effectiveness of minocycline against MDR-GNB isolates and compare its activity with meropenem.

Methods: A prospective observational study was conducted over six months in the Department of Microbiology, IIMSR, Lucknow. Sixty-six MDR-GNB isolates from various clinical samples were identified using standard microbiological methods. Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method following CLSI guidelines.

Results: Of the 66 isolates, the majority were obtained from urine (43.9%) and pus (27.3%). The predominant organisms included Escherichia coli (30.3%), Acinetobacter spp. (22.7%), and Citrobacter spp. (19.7%). Minocycline demonstrated sensitivity in 34.8% of isolates, intermediate susceptibility in 10.6%, and resistance in 54.6%. Comparatively, meropenem showed 16.7% susceptibility and 83.3% resistance. Notably, 45.5% of isolates were susceptible to minocycline, including many strains resistant to meropenem.

Conclusion: Minocycline demonstrated promising in vitro activity against MDR-GNB, particularly against meropenem-resistant strains. Its role as a potential alternative therapeutic option warrants further clinical validation.

Keywords: Minocycline, multidrug-resistant, gram-negative bacilli, antimicrobial resistance, meropenem.

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most critical global health challenges of the 21st century. The World Health Organization (WHO) has declared AMR as a top public health

threat, with multidrug-resistant (MDR) pathogens causing significant morbidity, mortality, and financial burden worldwide [1]. Hospital-acquired infections (HAIs), often caused by MDR gram-negative bacilli (MDR-GNB), are particularly concerning because of their association with longer hospital stays, increased treatment costs, and high mortality rates [2]. These infections are especially problematic in intensive care units (ICUs), where invasive procedures, immunocompromised patients, and widespread antibiotic use contribute to the rapid spread of resistance [3].

Gram-negative bacteria such as Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Citrobacter spp., and Pseudomonas aeruginosa are recognized as major MDR pathogens [4,5]. Their structural features, including the outer membrane barrier, efflux pumps, and enzymatic mechanisms like extended-spectrum beta-lactamases (ESBLs) and carbapenemases, confer resistance to multiple drug classes [6,7]. The increasing prevalence of carbapenem-resistant Enterobacterales (CRE) and carbapenem-resistant A. baumannii has created an urgent need for effective therapeutic alternatives [8].

The development of new antibiotics has not kept pace with the spread of resistance. In fact, the antibiotic pipeline has remained limited, with few novel classes introduced in recent decades [9]. Consequently, repurposing older agents with favorable pharmacokinetic and safety profiles is gaining renewed attention. Minocycline, a semisynthetic second-generation tetracycline, first introduced in 1967, has resurfaced as a potential option against MDR-GNB [10]. Its lipophilic structure enhances cell penetration and confers activity against a wide spectrum of pathogens, including multidrug-resistant A. baumannii, Stenotrophomonas maltophilia, and Enterobacterales [11,12].

Minocycline acts by binding to the 30S ribosomal subunit, thereby inhibiting protein synthesis [13]. Compared to older tetracyclines, it has higher lipid solubility, better tissue distribution, and lower rates of efflux-mediated resistance [14]. It is clinically used for respiratory tract infections, urinary tract infections, skin infections, and as a second-line therapy for patients with penicillin allergy [15]. More importantly, it retains activity against strains resistant to multiple antibiotic classes, including carbapenems, positioning it as a valuable salvage therapy [16].

Recent studies have highlighted its clinical utility in treating ventilator-associated pneumonia, bloodstream infections, and urinary tract infections caused by MDR-GNB [17,18]. A systematic review demonstrated that minocycline achieved favorable outcomes in infections due to extensively drug-resistant A. baumannii, with acceptable safety and tolerability [19]. Furthermore, its availability in both intravenous and oral formulations increases its practical applicability in both hospital and outpatient settings [20].

The global rise of antimicrobial resistance poses one of the most significant threats to public health. Multidrug-resistant gram-negative bacilli (MDR-GNB) are of particular concern due to their ability to cause life-threatening hospital-acquired infections (HAIs), including urinary tract infections, pneumonia, bloodstream infections, and wound sepsis. These pathogens, such as Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, exhibit resistance to multiple antibiotic classes, limiting therapeutic options and increasing morbidity, mortality, and healthcare costs.

The World Health Organization (WHO) has identified carbapenem-resistant Enterobacteriaceae, Acinetobacter spp., and Pseudomonas aeruginosa among the “critical priority pathogens” requiring urgent development of new therapies. With the stagnation in novel antibiotic discovery, repurposing older antibiotics such as minocycline offers a potential strategy to address this crisis.

Minocycline, introduced in 1967 as a second-generation tetracycline, demonstrates broad-spectrum activity against both Gram-positive and Gram-negative bacteria. Its mechanism involves binding to the 30S ribosomal subunit, thereby inhibiting protein synthesis. Minocycline is characterized by high lipid solubility, excellent tissue penetration, and favorable pharmacokinetics. Importantly, it retains activity against several MDR-GNB, including Acinetobacter spp. and Stenotrophomonas maltophilia.

In low- and middle-income countries (LMICs) like India, where diagnostic capacities are limited and newer antibiotics are often unavailable, evaluating the efficacy of older drugs such as minocycline becomes crucial [21]. Surveillance studies have shown variable susceptibility patterns of MDR isolates to minocycline, underlining the importance of local data to guide empiric and targeted therapy [22]. This study was undertaken to evaluate the in vitro effectiveness of minocycline against MDR-GNB isolates in a tertiary care hospital in Lucknow, India, thereby contributing to antimicrobial stewardship and informing therapeutic strategies.
Despite its long-standing use in clinical practice, the role of minocycline against MDR-GNB remains underexplored in many regions, including India. This study evaluates the in vitro effectiveness of minocycline against MDR-GNB clinical isolates in a tertiary care hospital in Lucknow, and compares its activity with meropenem, a widely used last-line antibiotic.

MATERIAL AND METHODS

Study Design and Setting:

A prospective observational study was conducted in the Department of Microbiology, Integral Institute of Medical Sciences and Research (IIMSR), Lucknow, over six months following ethical approval.

Inclusion Criteria:

All properly labeled and consented clinical samples yielding gram-negative bacilli resistant to at least one antibiotic in three or more antimicrobial categories.

Exclusion Criteria:

Improperly labeled or leaking samples
Gram-positive bacterial isolates
Samples from patients/guardians unwilling to provide consent

Sample Processing:

Clinical specimens including urine, pus, sputum, blood, and tissue were collected aseptically and processed per standard protocols. Gram staining was performed, followed by inoculation on Blood agar and MacConkey agar. Identification of isolates was carried out using conventional biochemical tests.

Antimicrobial Susceptibility Testing (AST):

The Kirby-Bauer disc diffusion method was employed on Mueller-Hinton agar as per CLSI guidelines. Discs included minocycline and meropenem. The zone diameters were interpreted as Sensitive (S), Intermediate (I), or Resistant (R).

Sample Size Estimation:

Based on prevalence data (p = 1.4%) and a 95% confidence level, the minimum calculated sample size was 66 isolates.

RESULTS

A total of 66 MDR-GNB isolates were analyzed.

Sample-wise distribution: Urine (43.9%), pus (27.3%), others (19.7%), sputum (6.1%), and blood (3%).

Gender distribution: Male patients 60.6% (n=40), females 39.4% (n=26).

Age distribution: Majority were elderly (>60 years).

Ward/Department distribution: ICU (21.2%), urology (13.6%), surgery (12.1%), medicine (12.1%), emergency (10.6%), NICU (9.1%), others.

Organism distribution: E. coli (30.3%), Acinetobacter spp. (22.7%), Citrobacter spp. (19.7%), K. pneumoniae (13.6%), P. aeruginosa (4.5%), Enterobacter spp. (4.5%), Proteus spp. (4.5%).

Minocycline susceptibility: Sensitive 34.8%, Intermediate 10.6%, Resistant 54.6%.

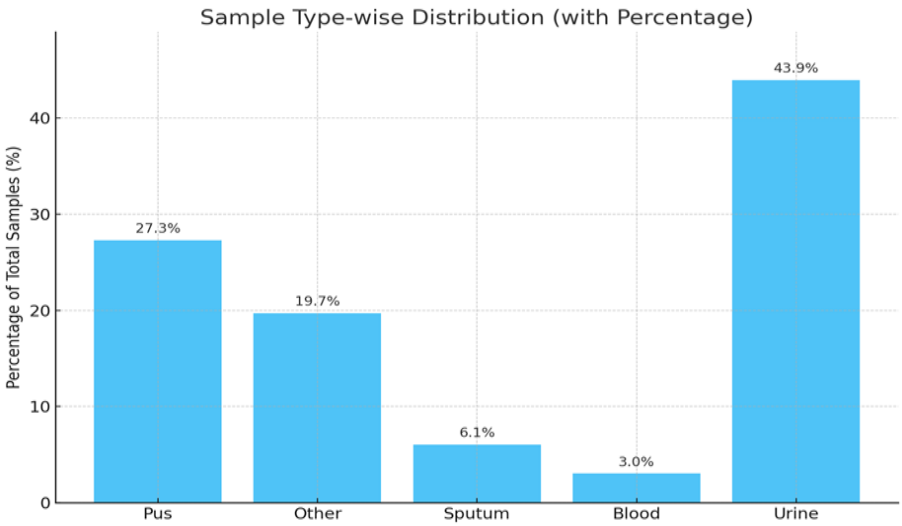
Comparison with meropenem: Meropenem sensitivity 16.7% vs. minocycline 45.5%. Importantly, 25 of 55 (45.5%) meropenem-resistant strains were susceptible to minocycline.

Sample type wise distribution-

During the study period, 66 MDR, gram-negative bacilli(MDRGNB),isolated specimens were analyzed. The majority of the MDR GNB were isolated from urine (29(43.9%)) samples, followed by pus (18(27.3%)), sputum (4(6.1%)) samples, blood (2(3.0%)) samples,and others (13(19.7%)) samples.

Sample Type	Number of Isolates	Percentage (%)
Urine	29	43.9%
Pus	18	27.3%
Other	13	19.7%
Sputum	4	6.1%
Blood	2	3.0%
Total	**66**	**100%**

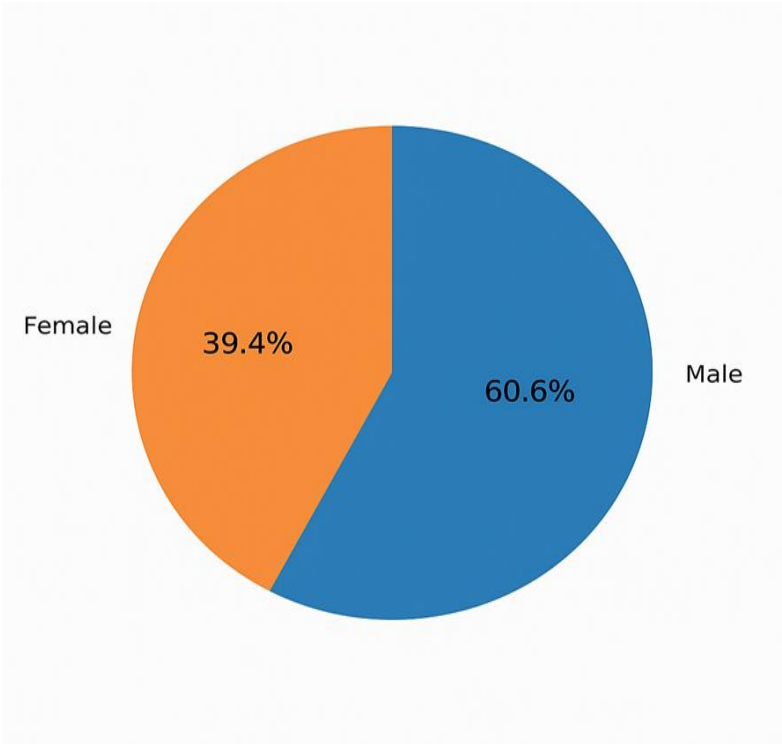
TABLE: Displaying sample wise distribution



GRAPH: Column displaying displaying distribution of sample

Gender wise distribution-

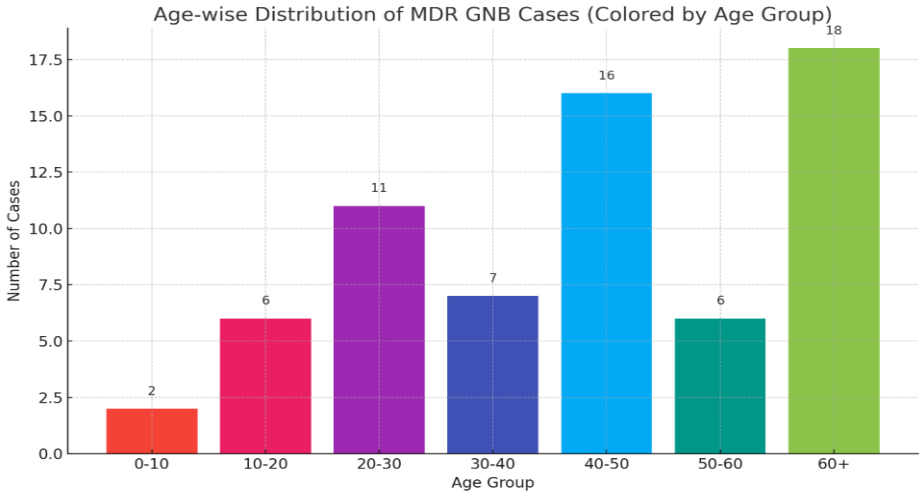
In those, 66 (MDR GNB) were isolated from 40(60.6%) male patients are 40 and 26 (39.4%) female patients.



PIE CHART-Gender wise distribution of MDR

Age wise distribution-

Age group-wise distribution is done and the majority of the patients were belonging to old age specifically above 60 years.



GRAPH: Column displaying age wise distribution

Location wise distribution-

The source of sample receiving was analyzed and found that 21.2% ofspecimens were received from intensive care unit (ICU) patients, followed by urology(URO) 13.6%.

Department	Number of Cases	Percentage (%)
ICU	14	21.2%
URO	9	13.6%
Surgery	8	12.1%
Medicine	8	12.1%
Emergency	7	10.6%
NICU	6	9.1%
M/M2	5	7.6%
FMED	4	6.1%
Ortho	3	4.5%
Pediatrics	1	1.5%
Neuro	1	1.5%
Total	**66**	**100%**

TABLE:Displaying area wise sample distribution

Organism type distribution-

Of 66 isolates, 7 different types of GNB were isolated. Escherichia coli(30.3%) was the highest, followed by Aceinetobacter species(22.7%),Citrobacter species (19.7%), klebsiella pneumoniae (13.6%),pseudomonas aeruginosa, Enterobacter species and proteus (4.5%) each.

Organism	Number of Isolates	Percentage (%)
Escherichia coli	20	30.3%
Acinetobacter species	15	22.7%
Citrobacter species	13	19.7%
Klebsiella pneumoniae	9	13.6%
Pseudomonas aeruginosa	3	4.5%
Enterobacter species	3	4.5%
Proteus	3	4.5%
Total	**66**	**100%**

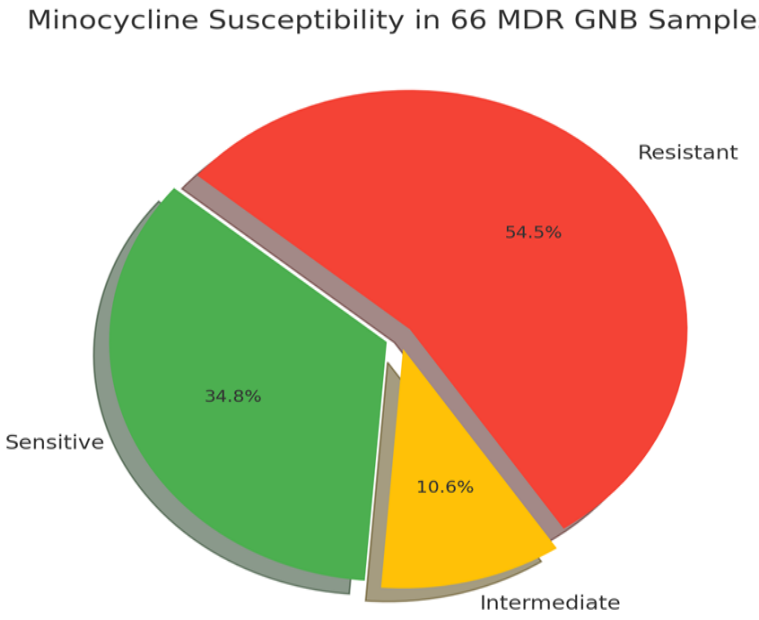
TABLE: Displaying organism wise distribution

Minocycline sensitive, intermediate, resistance distribution-

During the study period ,66 MDR, gram- negative bacilli (MDR GNB), isolated specimens were analyzed. Out of 66 isolates, 23(34.8%) isolates are sensitive to minocycline, 36 (54.6%) isolates were resistant, and the remaining 7(10.6%) showed intermediate susceptibility.

Minocycline Susceptibility	Number of Samples	Percentage
Sensitive	23	34.8%
Intermediate	7	10.6%
Resistant	36	54.6%
Total	**66**	**100%**

TABLE: Displaying the percentage distribution of minocycline sensitive, resistance inetermediate.



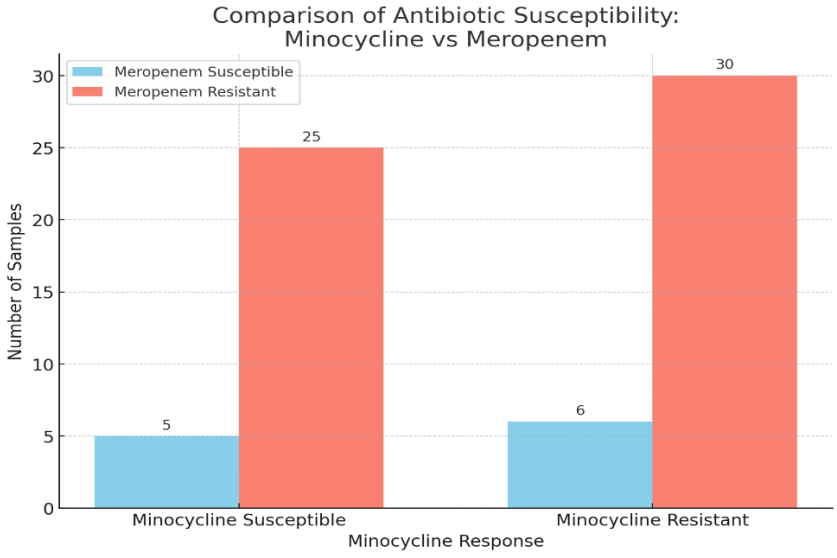
PIE CHART: Distribution of minocycline effectivness

COMPARISON OF MINOCYCLINE AND MEROPENEM: The comparison of susceptibility of minocycline and meropenem is shown in table. Among the 11 meropenem susceptible strains, 5(7.6%) showed susceptible to minocycline. A total of 25(37.9%) out of 55 the meropenem resistant strains susceptible to minocycline.

Drug	Susceptible (n)	% Susceptible	Resistant (n)	% Resistant	Total
Meropenem	11	16.7%	55	83.3%	66
Minocycline	30	45.5%	36	54.5%	66

	Minocycline Susceptible	Minocycline Resistant	Total
Meropenem Susceptible	5 (7.6%)	6 (9.1%)	11 (16.7%)
Meropenem Resistant	25 (37.9%)	30 (45.5%)	55 (83.3%)
Total	30 (45.5%)	36 (54.5%)	66 (100%)

Table:-The comparison of susceptibility of minocycline and meropenem for MDR gram negative bacilli



DISCUSSION

This study highlights the alarming burden of MDR-GNB in a tertiary care setting, with urine and pus being the predominant sources of isolates, reflecting the high incidence of urinary tract and wound infections. Male predominance and higher rates among the elderly are consistent with global trends, as older age and comorbidities increase susceptibility to resistant infections.

ICUs were the most affected units, which aligns with literature reporting that critically ill patients undergoing invasive procedures and broad-spectrum antibiotic exposure are at heightened risk of acquiring MDR organisms. The predominance of E. coli and Acinetobacter spp. is in line with global surveillance reports, as these organisms frequently harbor extended-spectrum beta-lactamases (ESBLs), carbapenemases, and efflux-mediated resistance mechanisms.

The present study evaluated the in vitro effectiveness of minocycline against 66 multidrug-resistant gram-negative bacilli (MDR-GNB) isolated from diverse clinical samples. The majority of isolates were recovered from urine (43.9%) and pus (27.3%), followed by sputum and blood. These findings are consistent with previous studies where urinary and wound infections were reported as major sources of MDR pathogens due to prolonged hospitalization, catheterization, and invasive procedures [1,2]. Similar to earlier reports, males and elderly patients constituted the predominant group affected, reflecting the higher prevalence of comorbidities, immunosenescence, and greater healthcare exposure in these populations [3].

In terms of hospital location, the highest burden of MDR isolates was observed in the intensive care unit (ICU), which aligns with findings from global surveillance data where ICUs were recognized as hotspots for resistant infections [4]. Frequent antibiotic usage, mechanical ventilation, and invasive interventions contribute significantly to the emergence and spread of MDR pathogens in these units [5]. Among the organisms isolated, Escherichia coli (30.3%) was the most common, followed by Acinetobacter species (22.7%) and Citrobacter species (19.7%). This distribution corresponds with several Indian and international studies where Enterobacterales and Acinetobacter baumannii were highlighted as the predominant MDR pathogens [6,7].

The susceptibility testing revealed that 34.8% of isolates were sensitive to minocycline, 10.6% were intermediate, and 54.6% were resistant. These results highlight variable activity of minocycline against MDR-GNB, suggesting its potential role as a salvage option rather than a first-line therapy. A study by Lashinsky et al. [8] demonstrated favorable in vitro susceptibility of MDR A. baumannii to minocycline, reporting resistance rates lower than carbapenems. Similarly, Asadi et al. [9] emphasized that minocycline retains activity against non-fermenters, particularly Stenotrophomonas maltophilia and carbapenem-resistant A. baumannii. However, in our study, nearly half of the isolates were resistant, indicating regional differences in resistance patterns and the necessity of local surveillance data before therapeutic use.

Interestingly, comparison with meropenem showed that a substantial proportion of carbapenem-resistant isolates remained susceptible to minocycline. This finding is supported by Fragkou et al. [10], who reported that minocycline can serve as an alternative in the management of carbapenem-resistant A. baumannii infections, particularly in critically ill patients where therapeutic options are limited. The activity of minocycline against some carbapenem-resistant strains in our study suggests its potential role in combination regimens to improve treatment outcomes and delay the emergence of further resistance.

Several mechanisms may explain the partial resistance observed in this study. Gram-negative bacilli possess multiple resistance strategies, including efflux pumps, enzymatic degradation, and alteration of ribosomal binding sites, all of which reduce the effectiveness of tetracyclines [11]. Roberts [12] described the role of tet genes in conferring tetracycline resistance, which may be a contributing

factor in the high resistance rates seen in our isolates. Furthermore, regional misuse of tetracyclines in both human and veterinary medicine could also contribute to selective pressure and resistance development [13].

The clinical implications of our findings are significant. Although minocycline demonstrated only moderate susceptibility rates, its effectiveness against a subset of carbapenem-resistant strains highlights its relevance as a therapeutic alternative, particularly in resource-limited settings where access to newer agents such as tigecycline or ceftazidime-avibactam may be restricted. Our study underscores the importance of continuous local antimicrobial surveillance to guide empirical therapy and strengthen antibiotic stewardship programs.

Overall, this study contributes to the growing body of evidence supporting the reconsideration of older antibiotics like minocycline for MDR infections. However, the high resistance rates observed also emphasize the need for judicious use, preferably guided by susceptibility testing, to prevent further escalation of resistance. Future research should focus on clinical trials, pharmacodynamic evaluations, and combination therapy strategies to define the optimal role of minocycline in managing MDR-GNB infections.

The study found that while more than half of isolates were resistant to minocycline, a significant proportion (34.8% sensitive, 10.6% intermediate) remained susceptible. Importantly, minocycline retained activity against nearly half of meropenem-resistant isolates, underscoring its therapeutic potential as a salvage option when carbapenems fail. These findings corroborate previous reports highlighting minocycline's effectiveness against MDR Acinetobacter baumannii and Stenotrophomonas maltophilia.

Given the paucity of novel antibiotics, minocycline represents a valuable re-purposed agent. However, its moderate resistance rates emphasize the need for judicious use within antimicrobial stewardship programs. Further large-scale studies and clinical trials are required to determine optimal dosing strategies, combination therapies, and clinical outcomes in MDR infections.

CONCLUSION

MDR-GNB pose a major threat in healthcare settings, particularly in ICUs and among elderly patients. Minocycline demonstrated notable in vitro activity against MDR isolates, including meropenem-resistant strains, suggesting its role as an alternative therapeutic option. Rational antibiotic use, infection control practices, and continued surveillance are essential to curb the spread of resistance and optimize the role of minocycline in clinical practice.

Declarations:

Conflicts of interest: There is no any conflict of interest associated with this study

Consent to participate: We have consent to participate.

Consent for publication: We have consent for the publication of this paper.

Authors' contributions: All the authors equally contributed the work

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