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RESEARCH ARTICLE
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MULTIDRUG RESISTANT ACINETOBACTER SPECIES: A RISING CONCERN: RESTORATION OF OLD ANTIBIOTICS.

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Acinetobacter stands out as a significant and emerging nosocomial threat. Its remarkable resilience across diverse temperatures, pH levels, and surfaces—both dry and moist—coupled with its adeptness in forming biofilms and colonizing medical apparatus like catheter lines and ventilators, contributes to its rapid transmission within hospital ICUs. Moreover, the organism's intrinsic and multi-drug resistance presents a formidable worldwide health issue. The waning efficacy of once-reliable carbapenems in combating these infections has notably heightened mortality rates. Recognizing the myriad factors influencing mortality, there's a pressing need to optimize existing antibiotics for more effective treatment.

ABSTRACT

Background

Acinetobacter species, resilient in the face of multiple drugs, have become a prevalent presence in hospital settings and among patients. The formidable array of resistance mechanisms they accumulate not only restricts treatment options but also amplifies the challenge of combating infections, significantly elevating the risk of mortality.

Materials and Methods

This prospective study took place in the Department of Microbiology at GEMS and Hospital from November 2022 to June 2023,

Results: 150 multidrug-resistant Acinetobacter isolates were collected from the ICU and various Wards. Maximum MDR isolates were found in the ICU. Pus samples showed the highest multidrug-resistant followed by ET aspirate and Blood. Among 150 MDR isolates, our isolates showed high resistance to ceftriaxone85 (100%), cefoperazone sulbactam 85(100%), amikacin 85(100%), gentamicin 83 (97.6%), cotrimoxazole 75 (88.2%) and ciprofloxacin78 (91.7%) followed by imipenem 78 (91.7%), Piperacillin – tazobactam 81 (95.2%), tigecycline 68 (80%), colistin 61 (71.7%). Susceptibility rates of MDR Acinetobacter strains to minocycline showed better susceptibility by E-test method 78 (91.7%) compared to disk diffusion method 71 (83.5%) and highest susceptibility of minocycline in pus samples followed by ET aspiration and blood and least susceptibility in urinary tract infection where minocycline does not respond well.

Conclusion: This research underscores the enduring effectiveness of minocycline as a therapeutic choice against MDR-AB. Notably, the E-test demonstrates superior sensitivity when contrasted with the disk diffusion test, adding a layer of precision to our understanding of treatment dynamics.

Keywords: Minocycline; Multi-Drug Resistant Acinetobacter.

INTRODUCTION –

Globally, the increase of extensively drug-resistant Acinetobacter baumannii poses a formidable challenge. These resilient bacteria not only defy standard antimicrobial agents but also exhibit resistance to colistin as indicated by Taneja N et al[1,] tigecylcine, and sulbactam. Carbapenem-resistant Acinetobacter species are particularly dangerous in critical care settings and are increasingly recognized as important causes of nosocomial infections. Their repertoire includes biofilm-associated device infections, urinary tract infections, pneumonia, and bloodstream infections. In the United States, Acinetobacter species account for 8.4% of ventilator-associated pneumonia 2.2% of bloodstream infections linked to central lines, and 8.4% of ventilator-associated pneumonia. In the USA and Europe, 65% of A. baumannii pneumonia is attributed to carbapenem resistance. Recent findings from Asian countries reveal that Carbapenem resistance is seen in 60% of A. baumannii cases of hospital-acquired pneumonia as suggested by Farrell DJ et al[2]. The worldwide rise of A. baumannii which is resistant to drugs (MDR-AB) has forced a reassessment of treatment approaches, turning attention to old yet reliable options like minocycline as recommended by Bishburg E et al[3]. The proposition of intravenous minocycline for treating drug-resistant A. baumannii gains significance, given its approximately 80% global susceptibility rate as showed by Castanheira M et al [4]. Functioning by inhibiting protein synthesis in bacteria, the 30S subunit of the bacterial ribosome interacts with minocycline, often resulting in a bacteriostatic effect. Noteworthy is its improved lipophilicity compared to previous tetracyclines, facilitating better tissue penetration. With a long half-life unaffected by renal or liver impairment as showed by Agwuh KN, Welling PG et al [5,6], minocycline intravenous is now FDA-approved for tackling minocycline-susceptible Acinetobacter species infections, offering a beacon of hope in the face of multidrug resistance as indicated by Jingyi Shi et al [7].

OBJECTIVES

1. To identify the multidrug-resistant Acinetobacter species isolate.
2. To identify the best method of determining the susceptibility of minocycline against MDR-Acinetobacter isolates
3. To evaluate the activity of minocycline against multidrug-resistant Acinetobacter clinical isolates.

MATERIALS AND METHODS

This prospective study was carried out at GEMS and Hospital. for one and half years from November 2022 to 2023 june.

Drug-resistant bacteria isolates – Selection

This study delved into the vibrant world of bacterial culture-positive isolates, sourced from the respiratory tract (sputum), soft tissue, sputum, blood, and skin of samples in-patients samples of in-patients. The Microbiology laboratory became a hub of exploration, with samples reaching from diverse hospital wards—Burns unit, ICU, surgery, and medicine. Notably, our subjects encompassed a wide spectrum, embracing both female and male patients throughout all age ranges adding a dynamic dimension to our research.

Bacterial identification

Blood culture samples, pus swabs, sputum, urine, Endotracheal aspirate, and other specimens underwent inoculation on blood, and MacConkey agar plates, with a 24-hour aerobic incubation period at 37°C. Conventional microbiological techniques, such as evaluating colony shape, Gram stain, and biochemical testing, were utilized to identify the species of Acinetobacter. A macroscopic analysis of the plates, concentrating on particular features of the bacteria, such as color, size, and form, directed our investigation. Biochemical tests, including Indole, Urease, Citrate, and Triple Sugar Iron (TSI) played a key role. The Acinetobacter species displayed traits such as being non-lactose fermenters (NLF), Gram-negative coccobacillus, oxidase-negative, catalase-positive, and having an alkaline/alkaline TSI profile without gas or H2S. They added distinguishing characteristics to their identification by being citrate-positive but indole- and urease-negative.

A total of 1520 samples were processed, out of which 673 were culture positives, of which 150 isolates of Acinetobacter were identified using biochemical reactions and culture techniques

Antimicrobial susceptibility testing

The Kirby-Bauer disc diffusion method was used to assess the antibiotic susceptibility of the 150 confirmed isolates of Acinetobacter species. Using a turbidimeter to measure the 0.5 McFarland turbidity, we prepared a suspension of pure colonies of the organism and 2 milliliters of regular saline. To achieve confluent growth, this suspension was equally dispersed using the lawn culture technique on Mueller Hinton agar plates. Antibiotics were positioned on the plates by the Clinical and Laboratory Standards Institute (CLSI) 2023 standards. After 24 hours of aerobic incubation at 37°C, the Mueller Hinton agar plates were checked for zones of inhibition, which were measured in millimeters and classified as sensitive, intermediate, or resistant. The antibiotics included ceftriaxone (30 µg), cefoperazone sulbactam, Amikacin AMK (30 µg), Gentamicin GEN (10 µg), Co-trimoxazole- SXT (1.25/23.75 µg), Ciprofloxacin CIP (5 µg), Imipenem IMP (10 µg), Piperacillin–tazobactam (110 µg), tigecycline, and colistin.

Quality control (QC)

Escherichia coli (E. coli) ATCC25922 were used in quality control (QC) with all of the employed previously mentioned antibiotics

Determination of carbapenems resistance in *Acinetobacter species*

Following the criteria of the Clinical and Laboratory Standards Institute (CLSI), broth microdilution procedures were used to determine the bacterial minimum inhibitory concentrations (MICs). The isolates were examined for their resistance to ampicillin, amoxicillin-clavulanic acid, ceftazidime, ciprofloxacin, amikacin, cotrimoxazole, imipenem, colistin, piperacillin-tazobactam, and polymyxin B. Multi-drug resistant isolates were those that show resistance to at least three drug classes, including aminoglycosides, fluroquinolones, and penicillins and cephalosporins.

Susceptibility testing – Freshly prepared media and antimicrobial solutions were utilized for conducting minocycline susceptibility tests. The testing methodologies encompassed both disk diffusion and the E-test method. Minocycline (30 µg) was placed onto the discs in disk diffusion experiments. Meanwhile, the E test assays covered concentration ranges from 0.016 to 256 µg/ml. The strains of Escherichia coli ATCC 25922 were used as quality control.

Interpretive criteria - Currently used interpretation standards based on CLSI recommendations were applied, indicating susceptibility to minocycline with MICs of ≤4 µg/ml (≥16 mm), intermediate susceptibility at 8 µg/ml (13 to 15 mm), and resistance at ≥16 µg/ml (≤12 mm). In the case of E-test MICs, values below 4 µg/ml were deemed sensitive, 8 µg/ml indicated intermediate susceptibility and MICs exceeding 16 µg/ml were considered resistant.

Statistical analysis

Data was entered and managed by Spss software version 22., which was also used for the analysis.

Ethical Approval

The study was conducted after ethical clearance was obtained from the GEMS ethical committee with 14/IEC/GEMS&H.

RESULTS

Table -1

Age	No.of non-MDR isolates (n=65)	No.of MDR isolates (n=85)
18-30 yrs	18	8 (9.4%)
31-45 yrs	14	11 (12.9%)
46-60 yrs	13	15 (17.6%)
61-75 yrs	9	28 (32.9%)
>75 yrs	11	23 (27%)

Table 1 shows the highest number of MDR cases in the age group 61 -75 years 28 (32.9%) followed by > 75 years of age 23 (27%)as shown by Jingyi Shi, et al[7], S. Jayashree et al[8], and Zeina A. Kanafani et al [9].

Table 2- Gender-wise distribution

sex	No.of non- MDR isolates (n=65)	No.of MDR isolates (n=85)
Male	27	57 (67%)
Female	38	28 (32.9%)

Table 2 shows that the number of MDR isolates was high in males compared to females as demonstrated by S. Jayashree et al [7] and A Jingyi Shi, et al[8]

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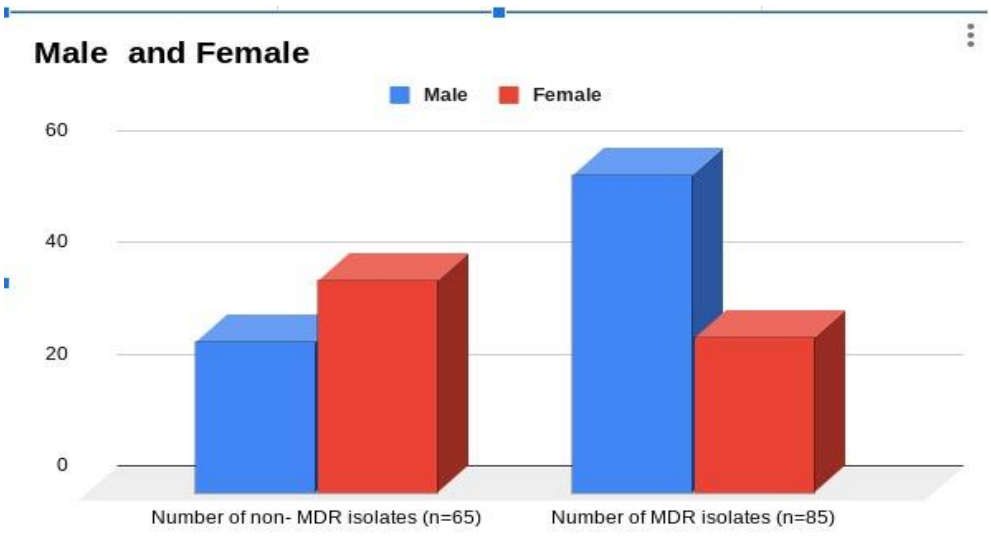


Table 3

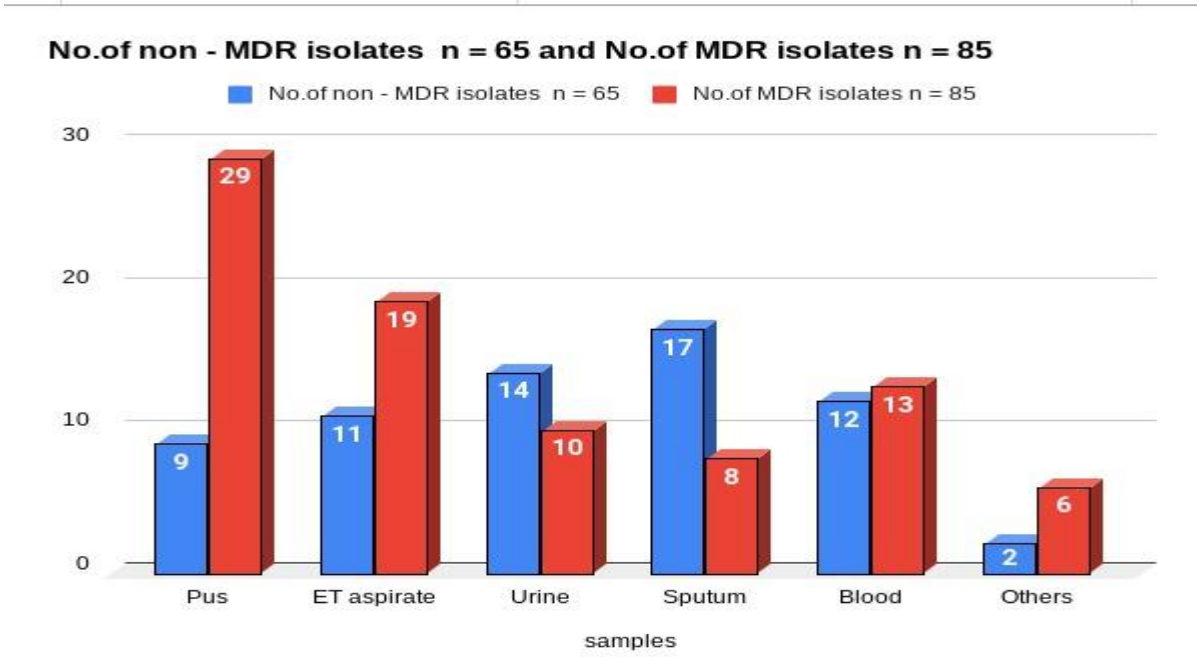
Acinetobacter isolates distribution among various wards.Ward	**No.of non- MDR isolates (n=65)**	**No.of MDR isolates (n=85)**
ICU	15	30 (35.2%)
General Surgery	18	23 (27%)
Orthopaedics	9	17 (20%)
Obstetrics and Gynaecology	7	9 (10.5%)
General Medicine	11	6 (7%)
Pediatrics	3	0
Urology	2	0

Table 3 shows the number of MDR isolates in ICU 30 (35.2%) is high followed by General Surgery 23 (27%) and Orthopaedics 17 (20%) as shown by other studies Zeina A. Kanafani et al [9]and Taghreed A. Hafiz et al[10]

Table 4 Distribution of isolated Acinetobacter from several samples

Samples	**No.of non- MDR isolates (n=65)**	**No.of MDR isolates (n=85)**
Pus	9	29 (34.11%)
ET aspirate	11	19 (22.3%)
Urine	14	10 (11.7%)
Sputum	17	8 (9.4%)
Blood	12	13(15.2%)
Others	2	6 (7%)

Table 4 shows the number of MDR isolates in Pus 29 (34.11%) followed by ET aspirate 19(22.3%) and Blood 13(15.2%) as shown by Zeleke Ayenew et al[11]



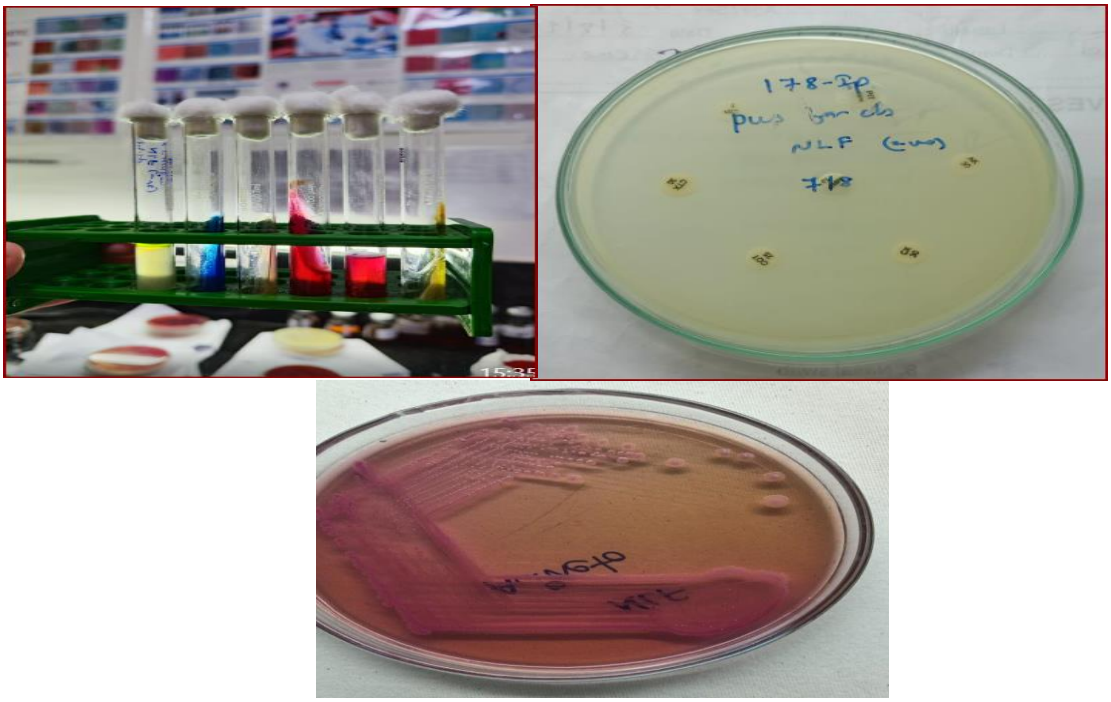


Figure 1 – cultural characteristics and biochemical reactions of Acinetobacter spp.

Table 5 Susceptibility of carbapenem-resistant Acinetobacter strains to minocycline based on testing methods (n = 85)

samples	No. of MDR isolates (n=85)	Minocycline					
		Disk Diffusion			E-Test		
		sensitive	intermediate	resistant	sensitive	intermediate	resistant
pus	33	28 (84.8%)	5(15.1%)	0 (0%)	33 (100%)	0 (0%)	0(0%)
ET aspiration	26	25 (96%)	1 (3.8%)	0(0%)	26 (100%)	0(0%)	0(0%)
urine	7	2 (28.5%)	2 (28.5%)	3 (42.8%)	2 (28.5%)	2 (28.5%)	3(42.8%)
sputum	6	5(83.3%)	1(16.6%)	0 (0%)	6 (100%)	0(0%)	0(0%)
blood	9	8 (88.8%)	0 (0%)	1(11.1%)	7 (78%)	2 (22.2%)	0(0%)
others	4	3(75%)	0 (0%)	1(25%)	4 (100%)	0(0%)	0(0%)

Table -5 showed Susceptibility rates of carbapenems-resistant Acinetobacter strains to Minocycline based on testing methods i.e., E-test method and disk diffusion method, were 71 (83.5%) and 78 (91.7%) respectively, and the highest susceptibility of minocycline by E-test method was seen in pus samples 33 (100%) and ET aspiration 26(100%) and blood 7 (77.7%), and least susceptibility was seen in urine 2(28.5%) as different from a study done by Peng Wang et al[15].

Figure 2- strain showing resistant to minocycline in both methods (Disk diffusion and E-test

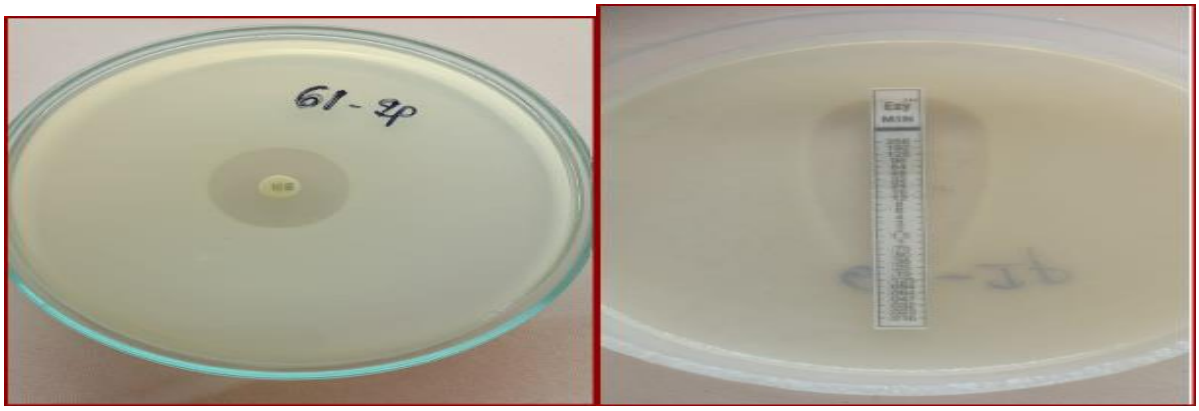


Figure 3 – strain showing suspectible to minocycline by E-test method

Discussion

This cross-sectional prospective study was conducted on patients with laboratory-confirmed multi-drug resistant Acinetobacter isolates. Patients in whom samples were found to have colonizing bacteria without clinically significant infection or contaminants were excluded.

In our study, the highest number of cases was found in the age group 61 -75 years similar to Jingyi Shi, et al[7], S. Jayashree et al.[8], and Zeina A. Kanafani et al.[9] revealed a higher prevalence of MDR-Ab in patients who belonged to above 65 years age group, the reason might be due to prolonged hospital stay, or frequent use of empirical antibiotic for illness.

Our study's high male-to-female ratio is consistent with the findings of multiple other research that have produced comparable findings. The reason might be due to pre-existing diseases such as heart

disease, chronic lung disease, and cancer, this study is comparable to those carried out by S. Jayashree et al. and A Jingyi Shi7, et al.[8]

In our investigation, the greatest concentration of multidrug-resistant Acinetobacter was identified in areas experiencing heightened antibiotic usage, particularly in ICUs, followed closely by general surgery. This trend aligns with findings from studies by Zeina A. Kanafani et al [9] and Taghreed A. Hafiz et al[10]. The probable reasons behind this pattern include inadequate hand hygiene among healthcare personnel and the critical health condition of patients with multiple comorbidities. Contributing factors to the reported prevalence are also the usage of invasive devices and the bacterium's capacity to produce biofilm and colonize medical devices like ventilators and catheter lines.

Our study showed the highest number of isolates from the pus sample followed by Endotracheal aspirate followed by blood similar to the article by Zeleke Ayenew [11]et al whereas the study conducted by Mostof S[12] et al showed the highest number of samples were from blood.

Among 85 MDR isolates, Our isolates showed high resistance to ceftriaxone85 (100%), cefoperazone sulbactam 85(100%), amikacin 85(100%), gentamicin 83 (97.6%), cotrimoxazole 75 (88.2%) and ciprofloxacin78 (91.7%) followed by imipenem 78 (91.7%), Piperacillin – tazobactam 81 (95.2%), tigecycline 68 (80%), colistin 61 (71.7%)respectively comparable to the research carried out by Vijayan Sivaranjani et al [12, 13,] but the study by Shakibaie MR et al[14] that found high resistance to third-generation cephalosporins and enhanced resistance to piperacillin-tazobactam.

In our study among Susceptibility rates of Acinetobacter strains resistant to carbapenem to minocycline based on testing methods i.e., disk diffusion method and E-test method, were 71 (83.5%) and 78 (91.7%) respectively in contrast to the study conducted by Peng Wang et al[15] which showed higher susceptibility rate by disk diffusion method.

In our investigation, the E-test method revealed the highest susceptibility of minocycline in pus samples (100%), ET aspiration (100%), and blood (77.7%). Conversely, the lowest susceptibility was observed in urine samples (28.5%). This aligns with a study by H. Adibhesami et al[16], suggesting that, unlike isolates from different body sites, urine samples exhibited low susceptibility to minocycline. This implies that minocycline might not be the preferred antibiotic for urinary tract infections.

Conclusion

In the context of Acinetobacter gaining prominence as "superbugs" within modern hospitals, causing a significant portion of infections, particularly among critically ill ICU patients, and considering the limited availability of new antibiotics, there's an increasing imperative to optimize the utilization of existing antibiotics for infection treatment. This study emphasizes the continued efficacy of minocycline as a valuable therapeutic option against MDR-AB. Regular susceptibility assessments of minocycline, following CLSI criteria, should be a routine practice in laboratories. Continuous monitoring and utilization of older antibiotics should be regarded with the same significance as the evaluation of new drugs. Clinical trial results and additional laboratory research should be used to inform minocycline use, either alone or in combination. Notably, the lower susceptibility of minocycline in urinary tract infections suggests that it might not be the preferred antibiotic for such cases. Beyond antibiotic considerations, implementing precautions like maintaining robust hand hygiene among healthcare workers and restricting broad-spectrum antibiotic use contribute significantly to infection reduction. Active surveillance and thorough environmental cleaning play vital roles in sustaining a decrease in the rates of MDR and XDR Acinetobacter, ultimately leading to reduced hospitalization costs and mortality rates.

Limitations-

The absence of clinical trials directly comparing the clinical, safety, and cost-effectiveness of old antibiotics is a notable gap. The prescription of these older antibiotics requires regulation through antibiotic stewardship programs and should be informed by prevailing resistance rates. Additionally, it's crucial to acknowledge that our study lacks the inclusion of medication resistance gene detection, limiting a comprehensive understanding of antibiotic resistance mechanisms.

Conflict of interest

The research was conducted without any commercial or financial relationships that could be seen as a conflict of interest, as declared by the authors**.**

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