

# An Insight into *In vitro* Minocycline Susceptibility among Carbapenem Resistant *Acinetobacter baumannii*

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## ABSTRACT

**Introduction:** *Acinetobacter baumannii* especially Carbapenem Resistant *Acinetobacter baumannii* (CRAB) is one of the most virulent organisms causing a variety of nosocomial and community acquired infections. Minocycline, which is a second generation tetracycline is proven to have antibacterial activity against these strains and is available as oral and intravenous formulations. **Objective:** To evaluate the invitro Minocycline susceptibility among Carbapenem Resistant *Acinetobacter baumannii* by Kirby Bauer disc diffusion method and E-test. **Results:** The present study was conducted to evaluate the invitro Minocycline susceptibility against CRAB isolates from various samples where a total of 50 isolates were collected and tested according to CLSI guidelines where 40 isolates (80%) were sensitive to Minocycline by Disc diffusion method, and 43 isolates (86%) were susceptible by E-test. **Conclusion:** This study gives insight into invitro Minocycline susceptibility against CRAB isolates which is effective against these strains that were included in the study, but some isolates show resistance to Tigecycline and Minocycline which is of great concern especially in hospital settings.

**Keywords:** *A. baumannii*; CRAB; Minocycline susceptibility; Disc diffusion method; E- test; MDR

## Introduction

*Acinetobacter baumannii* which is an aerobic, catalase - positive, oxidase - negative, gram negative coccobacilli, although first described in 1911, taxonomy was published in 1986 after several studies according to which there are 54 species of the genus *Acinetobacter* in which *Acinetobacter baumannii* is one of the most virulent species in the genus causing a bulk of human infections including nosocomial infections as this bacterium can survive in environment for months and has simple nutritional requirements/not fastidious and also has the ability to grow in diverse pH and temperature<sup>[1,2]</sup>. They are also resistant to commonly used disinfectants and antiseptics and also have the ability to form biofilm in biotic and abi-

otic surfaces. *Acinetobacter baumannii* also exhibit increased antimicrobial resistance due to their ability to incorporate resistance determinants along with virulence determinants. Apart from *Acinetobacter baumannii* other species like *Acinetobacter pittii*, *Acinetobacter nosocomialis*, *Acinetobacter lwoffii* and *Acinetobacter radioresistens* are also identified as significant nosocomial pathogens<sup>[3]</sup>.

Health care associated infections are most common infections encountered by *Acinetobacter* species due to hospitalization of patients leading to colonisation, improper disinfection procedures and unnecessary antibiotic usage like third generation cephalosporins, fluoroquinolones and carbapenems, lack of hand hygiene, spray of contaminated fluids due to pulsatile lavage of wounds or bronchoscopy etc., which lead to heavy environmental contamination leading to transmission<sup>[4,5]</sup>.

In the healthcare setting *Acinetobacter* species is mainly associated with Ventilator associated pneumonia (VAP) accounting for 3-7% of cases who require mechanical ventilation for more than five

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days and also implicated in 26% of respiratory infections. They are also responsible for other nosocomial infections like blood stream infections associated with intravascular catheters, Surgical site infections (SSIs), Urinary tract infections (UTIs), wound infections, meningitis after neurosurgery procedure and soft tissue infections in burns patients<sup>[6]</sup>.

*Acinetobacter* species are also responsible for community-associated infections like pneumonia in tropical regions during summer season and bacteremia which is often associated with respiratory infection. Meningitis is rare but they are associated with invasive soft tissue infections especially during warfare and natural disasters<sup>[7]</sup>.

*Acinetobacter* is well known for its antimicrobial resistance mechanisms which are clustered on their resistance islands where Insertion sequences called *IS Abal* present in its genome is responsible for several key resistance mechanisms. *Acinetobacter* exerts its antibiotic resistance by expression of beta-lactamases in which group-1 AmpC beta-lactamases are chromosomally encoded and are cephalosporinases responsible for hydrolysis of penicillins and 1<sup>st</sup>, 2<sup>nd</sup> & 3<sup>rd</sup> generation cephalosporins like cefazidime, cefotaxime and ceftriaxone<sup>[8]</sup>.

Emergence and rapid spread of *Acinetobacter* species with group-2be Ambler class A Extended-spectrum  $\beta$ -lactamases (ESBL's) lead to global spread of Multidrug Resistant (MDR) *Acinetobacter* species which hydrolyse penicillins and all cephalosporins which lead to the usage of carbapenems. However, resistance to carbapenems has increased in recent years due to acquisition of group 2d Ambler class D oxacillinases through Insertion sequence *IS Abal* resulted in increase in carbapenem resistance successfully spreading the International Clonal Complexes (ICC's)<sup>[9]</sup>.

*Acinetobacter* also expresses resistance through efflux pumps AdeABC which are chromosomal and in the Resistance-Nodulation-Division (RND) which are chromosomal encodes resistance to antibiotics like  $\beta$ -lactams, chloramphenicol, macrolides, tetracyclines, tigecycline, aminoglycosides, polymyxins etc.<sup>[10]</sup>

Other mechanisms of resistance include expression of porins ( $\beta$ -lactams and carbapenems), mutations (fluoroquinolones, polymyxins)<sup>[11,12]</sup>.

Carbapenem Resistant *Acinetobacter baumannii* is now a global health threat where therapeutic options available for infections with carbapenem resistant strains are limited. Minocycline, which is a second generation tetracycline introduced in 1960's is proven to have antibacterial activity against *Acinetobacter baumannii* which is available as oral and IV formulation. It acts by inhibiting bacterial protein synthesis by binding to 30s ribosomal subunit of bacteria which inhibits the tRNA to ribosomal acceptor site thus preventing peptide chain elongation leading to inhibition of protein synthesis<sup>[13]</sup>.

Minocycline is more lipophilic counterpart of tetracycline which will help the drug for increased tissue penetration, enhanced antibacterial activity, longer half-life and increase the spectrum of activity against many bacteria including *Acinetobacter baumannii*<sup>[14]</sup>.

Resistance to these tetracyclines is mediated by a variety of mechanisms like efflux pumps, ribosomal protection proteins, target modification etc.; where nearly 20 efflux pump mechanisms are identified in Gram-negative organisms in which common is *Tet A* which leads to decreased intracellular concentration by exchanging a proton for the tetracycline cation complex. In vitro efflux pumps present in *Acinetobacter* species are effective in transporting out other drugs in this group but not Minocycline<sup>[15]</sup>.

## Materials & Methods

This study was a cross sectional observational study conducted between February 2024 to January 2025 (1 year) in the department of microbiology, Dr Pinnamaneni Siddhartha institute of medical sciences and research foundation, Andhra Pradesh which is a tertiary care centre after obtaining approval from institutional ethics committee (IEC) – Reg no: faculty/1028/23 dated 01/12/2023 (CDSCO Reg no- ECR/804/Inst/AP/2016-RR-19 and DHR Reg no- EC/NEW/INST/2021/2140.

**Inclusion criteria:** All the culture specimens sent to microbiology laboratory from various OPD's and IPD's during the study period showing the growth of *Acinetobacter baumannii* and were resistant to carbapenems (Imipenem and Meropenem) were included in the study.

**Exclusion criteria:** Repetitive samples and samples showing growth of bacterial isolates other than *Acine-*

*tobacter baumannii* and *Acinetobacter baumannii* isolates which were sensitive to carbapenems were excluded in the study.

**Sample design:** A total of 50 non-duplicate strains of *Acinetobacter baumannii* isolates from various samples like pus, urine, wound swabs, ET secretions, central line tips etc. which were identified by Gram's stain (Gram negative coccobacilli), catalase test (positive), oxidase (negative), motility (non-motile) and other conventional biochemicals and sugar fermentation tests were collected and further processed by antibiotic susceptibility testing by Kirby Bauer disc diffusion test<sup>[16,17]</sup> on Mueller Hinton agar (MHA) by routine antibiotics recommended by CLSI<sup>[16]</sup> and the strains which were resistant to imipenem and meropenem in disc diffusion method were considered as carbapenem resistant *Acinetobacter baumannii* and these were further subjected to E test on Mueller Hinton agar and were interpreted as sensitive (S) ( $\leq 4\mu\text{g/ml}$ ), intermediate (I) ( $8\mu\text{g/ml}$ ) and resistant (R) ( $\geq 16\mu\text{g/ml}$ ) according to CLSI guidelines<sup>[16]</sup>.

**Study variables:** The MIC (Minimum Inhibitory Concentration) of minocycline against carbapenem resistant *Acinetobacter baumannii* (CRAB) isolates cut-off range is based on CLSI guidelines and interpreted as sensitive ( $\leq 4\mu\text{g/ml}$ ), intermediate ( $8\mu\text{g/ml}$ ) and resistant ( $\geq 16\mu\text{g/ml}$ )<sup>[16]</sup>.

MIC was marked at the point where ellipse intersects the scale and the MIC value at complete inhibition of all growth was also marked. If there is any presence of variation in the intersect on either side of the strip, the greater value of MIC was marked and if any growth was observed at the edge of the strip, it was ignored.

**Study procedure:** A total of 50 non-duplicate strains of *Acinetobacter baumannii* isolated from different samples like pus, urine, wound swabs, ET-secretions, central-line tips etc which were identified by standard phenotypical tests like Gram's stain, catalase test, oxidase test, motility test, Indole test, Nitrate test and other standard biochemical and sugar fermentation tests as per CLSI guidelines<sup>[16-18]</sup> were included in the study and these strains were subjected to antibiotic susceptibility testing by Kirby Bauer disc diffusion method on Mueller Hinton agar (MHA) by using antibiotic discs (Himedia Labs, Mumbai) of Ampicillin/Sulbactam ( $10/10\mu\text{g}$ ), Piperacillin/Tazobactam ( $100/10\mu\text{g}$ ), Cef-

tazidime ( $30\mu\text{g}$ ), Ceftriaxone ( $30\mu\text{g}$ ), Ciprofloxacin ( $5\mu\text{g}$ ), Imipenem ( $10\mu\text{g}$ ), Meropenem ( $10\mu\text{g}$ ), Cotrimoxazole ( $1.25/23.75\mu\text{g}$ ), Amikacin ( $30\mu\text{g}$ ), Gentamicin ( $10\mu\text{g}$ ), Tigecycline ( $15\mu\text{g}$ ), Doxycycline ( $30\mu\text{g}$ ) and interpreted as Sensitive(S), Intermediate (I) and Resistant(R) according to zone diameter as per CLSI guidelines<sup>[16]</sup>.

The strains which were resistant to imipenem, meropenem were considered as carbapenem resistant strains and were subjected to susceptibility testing by minocycline ( $30\mu\text{g}$ ) disc (Himedia Labs, Mumbai) by Kirby-Bauer Disc diffusion test using Mueller-Hinton agar (MHA) and were interpreted according to zone diameter as Sensitive ( $\geq 16\text{mm}$ ), Intermediate ( $13-15\text{mm}$ ), Resistant ( $\leq 12\text{mm}$ )<sup>[16]</sup>. All these strains were also subjected to Epsilometer (E-test) on Mueller-Hinton agar plate by using E-strips of minocycline (Himedia Labs, Mumbai) with MIC's ranging from  $0.016$  to  $256\text{mcg/ml}$  according to CLSI guidelines and manufacture recommendation<sup>[16]</sup>.

The inoculum was prepared using 3-4 test strain colonies which were already subcultured from original plates to sheep blood agar and Mac Conkey agar to obtain pure colonies and were emulsified in a test tube containing normal saline and turbidity was compared to that of 0.5 McFarland standards and the turbidity was adjusted accordingly and inoculation was done on Mueller Hinton agar (MHA) by using a sterile cotton swab dipped into the inoculum and the swab was streaked over the entire surface of Mueller Hinton agar and then E strips of minocycline were applied onto the agar surface and the plates were incubated at  $35-37^\circ\text{C}$  for 18-24 hrs<sup>[19]</sup> and were then interpreted as Sensitive ( $\leq 4\mu\text{g/ml}$ ), Intermediate ( $8\mu\text{g/ml}$ ) and Resistant ( $\geq 16\mu\text{g/ml}$ ) according to CLSI guidelines<sup>[16]</sup>. The entire procedure was carried out for Quality control strain (*Acinetobacter baumannii* - 19606).

### Methodology flow Chart

Samples - urine, pus, wound swabs, ET secretions, central line tips etc → Gram's stain → Inoculation onto sheep blood agar and Mac Conkey agar → Incubation at  $35-37^\circ\text{C}$  for 18-24 hrs → Sheep blood agar - Non hemolytic colonies, Mac Conkey agar - Non lactose fermenting colonies → Gram's stain → Gram Negative Coccobacilli → Subculture onto sheep Blood agar and Mac Conkey agar → Incubate at  $35-37^\circ\text{C}$  for 18-24hrs → Gram's stain → Gram Negative Coccobacilli → Catalase test (Positive) → Oxidase test (Negative) → Motility (Non motile) →

Triple sugar iron test (K/K with no gas, no H<sub>2</sub>S) → Mannitol motility test (Non fermented / Non motile) → Indole test (Negative) → Nitrate test (Negative) → Glucose oxidation (OF medium) (Positive) → Lysine decarboxylase (Negative) → Growth at 42°C (Positive) → *A. baumannii* → Antibiotic susceptibility testing by Kirby Bauer disc diffusion test using MHA with routine antibiotics recommended by CLSI → *A. baumannii* strains resistant to imipenem and meropenem in Kirby Bauer disc diffusion test → Carbapenem resistant *A. baumannii* → Susceptibility testing by minocycline (30µg) disc by Kirby Bauer disc diffusion method using MHA → interpreted as Sensitive (≥16mm), Intermediate (13-15mm) and Resistant (≤12mm) according to CLSI guidelines → All strains subjected to Epsilometer test (E test) by E strips MIC's ranging from 0.016 to 256mcg/ml and interpreted as Sensitive (≤4µg/ml), Intermediate ( 8µg/ml) and Resistant (≥16 µg/ml) as per CLSI guidelines → Data was collected and tabulated in Microsoft excel sheet → Descriptive statistics were used to represent the variables → Proportion of isolates which were sensitive, intermediate or resistant to minocycline were expressed as numbers and percentages.

### Statistical Analysis

The collected data was tabulated in Microsoft excel sheet and analyzed. Descriptive statistics were used to represent the variables. The proportion of isolates which were sensitive, intermediate or resistant to minocycline was expressed as numbers and percentages.

### Results

**Table 1: Shows susceptibility of Carbapenem Resistant *A. baumannii* strains to Doxycycline and Minocycline based on Disc diffusion method**

S. no	Antimicrobial agents	Susceptible	Resistant	Intermediate
1.	Doxycycline	19 (38%)	18(36%)	13(26%)
2.	Minocycline	40(80%)	6(12%)	4(8%)

### Discussion

The prevalence of various infections caused by Multidrug Resistant organisms (MDR) has been increasing over past decade due to acquisition of various resistance mechanisms which have made treating infections with such organisms more challenging to clinicians. Treatment of infections with MDR pathogens hence require careful evaluation

**Table 2: Shows susceptibility of Carbapenem resistant *A. baumannii* strains based on E-test to Polymyxin-B and Minocycline**

S. no	Antimicrobial agents	Susceptible	Resistant	Intermediate
1.	Polymyxin B	50(100%)	-	-
2.	Minocycline	43(86%)	6(12%)	1(2%)

**Table 3: Shows susceptibility of Carbapenem Resistant *A. baumannii* strains based on testing by Disc diffusion method and E-test to Minocycline**

S. no	Antimicrobial agent	Disc diffusion method			E- test		
		S	R	I	S	R	I
1.	Minocycline	40 (80%)	6 (12%)	4 (8%)	43 (86%)	6 (12%)	1 (2%)

**Table 4: Shows comparison of Minocycline susceptibility to *A. baumannii* isolates based on testing methods**

S. no	Minocycline susceptibility based on testing methods	Interpretation	No. of isolates
1.	Disc diffusion method + E- test	Sensitive	40 (80%)
2.	Disc diffusion method + E- test	Resistant	6 (12%)
3.	Disc diffusion method + E- test	Intermediate	1 (2%)
4.	Disc diffusion method + E- test	Intermediate + Sensitive	3 (6%)
5.	Total	-	50 (100%)

of various factors including antibiotic susceptibility testing apart from evaluation of pharmacokinetics and pharmacodynamics of the drug well designed clinical trials and implementation of strict infection control practices in order to avoid horizontal spread of antibiotic resistance among pathogens<sup>[20,21]</sup>.

*Acinetobacter* species especially *Acinetobacter baumannii* is the most common pathogen associated with both hospital acquired, and community acquired infections which are associated with high mortality and morbidity. Carbapenems were drug of choice to treat MDR *Acinetobacter baumannii* infections but widespread use of these group of drugs in

recent years has led to increase in frequency of emergence of Carbapenem Resistant *Acinetobacter baumannii* (CRAB) strains which can be attributed to decrease permeability of outer membrane due to loss or modification of porins, modification of Penicillin binding proteins (PBP's), increased expression of efflux pumps and production of the enzyme carbapenemase which has led to decreased therapeutic effectiveness of these agents and has led to limitations in available treatment options of infections with such MDR *Acinetobacter baumannii* strains<sup>[22,23]</sup>.

Minocycline (both oral and intravenous forms) has been approved by US food and drug administration for treatment of infections caused by MDR *Acinetobacter baumannii* strains which are resistant to Carbapenems and / Polymyxin classes<sup>[24]</sup>.

Minocycline also has shown to have fewer side effects like dizziness, vertigo, nausea and diarrhea and has favorable pharmacodynamics and pharmacokinetic properties and is also stable to many tetracycline resistant mechanisms<sup>[25]</sup>.

The present study was conducted at our institution to evaluate the invitro Minocycline susceptibility against carbapenem resistant *Acinetobacter baumannii* isolates from various samples where a total of 50 CRAB isolates were collected and subjected to antibiotic susceptibility testing by Disc diffusion method and E-test and interpreted as Sensitive (S), Intermediate (I), Resistant (R) according to CLSI guidelines<sup>[16]</sup>. In our study 40 (80%) isolates were sensitive to Minocycline by Disc diffusion method and 43 (86%) were sensitive by E-test correlating with the findings of Pengwang et al 82.2%<sup>[24]</sup>, Gunasekaran Santhi et al 87.5%<sup>[26]</sup>, Shahram Shahraki Zahedani et al 81.5%<sup>[22]</sup>. But according to Anup R Warriar et al the sensitivity of CRAB isolates to Minocycline by E-test was low (50%)<sup>[20]</sup> and Asna Parveen et al showed 40.5 % sensitive<sup>[27]</sup> and Jia-ling Yang et al showed only 55% of isolates sensitive for Minocycline<sup>[28]</sup>.

## Conclusion

This study gives insight into invitro Minocycline susceptibility against Carbapenem Resistant *Acinetobacter baumannii* isolates from various clinical samples in a tertiary care hospital. In conclusion, the results of the present study have demonstrated that Minocycline is effective against Carbapenem Resistant *Acinetobacter baumannii* strains that were

included in the study. Since most of the CRAB isolates are susceptible to Colistin, Minocycline, Tetracycline and Doxycycline, these antibiotics or their combinations are recommended for treatment of infections caused by resistant strains.

Selection of appropriate antibiotic remains main key for treatment of infections caused by such CRAB strains and as Minocycline shows susceptibility against these strains it can be used as an alternative drug to Colistin. But some isolates show resistance to Tigecycline and Minocycline which is of great concern especially in hospital settings. In order to combat this problem, WHO recommended five infection control strategies namely hand hygiene, surveillance for carbapenem resistant organisms, contact precautions, environmental cleansing and patient isolation and these strategies should be monitored, audited and feedback to healthcare workers and management should be done from time to time.

## Limitations of the study

Inclusion of limited number of isolates may be considered as the drawback of the study. Comparison with more therapeutic agents might have provided a broader prospective of the effect of Minocycline for treatment of infections caused by carbapenem resistant *A. baumannii* and more multicenteric studies may be required in order to demonstrate pharmacodynamics, pharmacokinetics, drug interactions and clinical efficacy of Minocycline for better understanding of clinicians in order to implement Minocycline as a therapeutic agent in real world practice.

## Disclosure

**Funding:** None

**Conflict of Interest:** Nil

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**How to cite this article:** Poojita N, Pradeep MSS, Deepthi T. An Insight into *In vitro* Minocycline Susceptibility among Carbapenem Resistant *Acinetobacter baumannii*. *J Med Sci Health* 2026; 12(1):16-22

Date of submission: 10.05.2025  
Date of review: 17.05.2025  
Date of acceptance: 04.07.2025  
Date of publication: 01.12.2025