DETECTION OF TIGECYCLINE RESISTANCE IN ACINETOBACTER BAUMANNII ISOLATES FROM INTENSIVE CARE UNIT PATIENTS

Zainab Tufail,[1] Mahnoor Chaudhry,[2] Sana Mustafa,[3] Kokab Jabeen,[4] Junaid Azmat,[5] Mamoona Waseem[6]

ABSTRACT

Background and Objective: Acinetobacter baumannii is a nosocomial pathogen that is becoming more resistant to antibiotics nowadays. The study aims to isolate Acinetobacter baumannii from different clinical specimens of intensive care unit (ICU) patients and to determine the antimicrobial activity of tigecycline against these isolates.

Methods: A descriptive cross-sectional study was conducted in Microbiology laboratory, at Lahore General Hospital. The Kirby Bauer disc diffusion assay was used to test Acinetobacter baumannii's susceptibility to antibiotics, and the results were reported in compliance with the Clinical and Laboratory Standards Institute (CLSI 2023). The British Society for Antimicrobial Chemotherapy's (BSAC 2021) standards were followed while reporting tigecycline's activity.

Results: A total of 195 isolates of Acinetobacter baumannii were recovered from patients admitted to the Lahore General Hospital's Intensive Care Unit. 9% of them were resistance to tigecycline.

Conclusion: One of the common nosocomial pathogen among the most frequently isolated organism from ICU patients is Acinetobacter baumannii. It has been concluded that this new drug has developed resistance. This is an alarming situation which calls for precautions to be taken against misuse and overuse of antibiotics.

Key Words: Acinetobacter baumannii, Tigecycline, Intensive Care Unit, antimicrobial resistance.

How to cite: *Tufail Z, Chaudhry M, Mustafa S, Jabeen K, Azmat J, Waseem M. Detection of tigecycline resistance in Acinetobacter baumannii isolates from Intensive Care Unit patients. JAIMC.2025;23(1):12-17*

In nature, non-fermenting bacteria are abundant, particularly in soil and water. Acinetobacter baumannii is considered as a nosocomial pathogen that is predominantly linked to diseases.[1] However, the skin of patients and staff members serves as a reservoir for Acinetobacter spp. in hospitals and is the likely cause for the majority of outbreaks of hospital infection.[2] It is a member of the bacterial genus known as ESKAPE, which also contains Staphylococcus aureus, Pseudomonas aeruginosa Enterococcus faecium, Klebsiella pneumoniae, Acinetobacter baumannii and Enterobacter species.[3] A. baumannii, is capable of acquiring genes that confer resistance to antibiotics or developing defense mechanisms against antibacterial medications.[4] Because of the organism's resilience to adverse circumstances, A. baumannii, has been able to maintain its presence in nosocomial environments for a significant amount of time. A. baumannii, has an advantage over other pathogens when, it comes to thriving in these environments.[5]

Antimicrobial resistance has emerged as a major global health threat which has led to rising costs for medical care in various countries

1,5-6. Department of Pathology, Sialkot Medical College, Sialkot.
2. Department of Pathology, Shalamar Medical and Dental College, Lahore.
3. Department of Microbiology, Lahore General Hospital/ Post-Graduate Medical Institute, Lahore.
4. Department of Pathology, Sahiwal Medical College, Sahiwal.

Correspondence:
Dr Mahnoor Chaudhry, *Department of Pathology, Shalamar Medical and Dental College, Lahore.*
Email: *mahnoorchaudhry474@gmail.com*

Submission Date: 19-02-2025
1st Revision Date: 28-02-2025
Acceptance Date: 15-03-2025

throughout the world. In recent decades, multicenter studies have revealed that the prevalence of antimicrobial resistance (AMR) is on the rise for nosocomial as well as community-acquired infections.[6,7] In order to combat carbapenem-resistant A. baumannii (CRAB), the World Health Organization (WHO) conducted research and development of medicines in 2018. Resistance to a large number of different antibiotic families usually develops concurrently with resistance to carbapenems.[8] Tigecycline, the first member of glycylcycline was created to overcome the tetracycline resistance. The binding affinity of tigecycline is five times higher than other tetracyclines. Tigecycline was formulated by addition of a glycyclamide group to the 9th spot on minocycline to avoid the leading molecular mechanism contributing to tetracycline resistance. Tigecycline is majorly one of the end line treatment regimes for multidrug resistant (MDR) and extensively drug resistant (XDR) organisms.[9] Therefore, only a limited antibiotics are available for tigecycline resistant A. baumannii, contributing to the rise in the cost of medical care, morbidity and mortality.[10] The study aims to isolate Acinetobacter baumannii from clinical samples and to determine the activity of Tigecycline against them.

METHODS

A descriptive cross-sectional study was conducted at Microbiology department, Lahore General Hospital, Lahore. The sample size of 195 was calculated keeping the expected proportion of *A. baumannii* as 0.15,[11] 95% confidence interval and margin of error taken as 5%, using WHO sample size calculator. Non-probability convenient sampling technique was used. Ethical approval was taken from research and ethical committee, PGMI/ AMC/ LGH Lahore with reference number: UHS/Education /126-23/1120.The samples (blood, urine, CSF, sputum, pus, wound swab, tracheal secretions, external ventricular drain tips, fluids and tissues) received in the microbiology laboratory from July 2023 to June 2024 were included. Repeated samples from the same patients in the same course of illness were excluded. In the data collection procedure, clinical isolates growing non-fermenting gram-negative rods (NFGNRs) were collected from LGH, Lahore. Organism identification was done by carefully observing colony morphology, gram staining, oxidase test and analytical profile index-20 non-enterobacteriaceae system. According to the clinical laboratory standards institute (CLSI 2023), antimicrobial susceptibility testing was carried out using Mueller-Hinton agar (MHA) and the Kirby Bauer Disc Diffusion technique. Additionally, the breakpoints listed in CLSI 2023 were used to interpret the zones of inhibition. E-strip was used to determine the tigecycline minimum inhibitory concentrations (MIC) in accordance with the manufacturer's instructions. 0.5 McFarland's standard was used to modify the bacterial solution. A sterile cotton swab was used to inoculate the surface of the Muller Hinton agar plate with isolates. At -20°C, e-strips were kept in storage before usage and applying to the agar surface. The strip was placed slowly and carefully so that it fully touched the agar surface after the lowest concentration was first placed at the agar plate. Plates were incubated aerobically at 37°C for 18 to 24 hours after the E-strips were placed. BSAC 2021 was used to interpret MIC data.[9] MIC >2 mg/l for tigecycline was considered resistant. The Statistical Package for Social Sciences (SPSS; Version 26.0) was used to enter and analyze the data. Categorical descriptive variables like gender, and Tigecycline resistance, were presented as frequencies and percentages. Continuous numerical variables like age were described as mean and standard deviation.

RESULTS

To ascertain the frequency of antibiotic resistance in Acinetobacter baumannii, a gram-negative non-fermenter, clinical specimens from intensive care unit (ICU) patients, comprising both

Table 1: Frequency of A. baumannii in clinical isolates from hospitalized ICU samples.

S. No.	Specimen	Frequency n=195	Percent
1	Sputum	4	2.1
2	Urine	50	25.6
3	Blood	26	13.3
4	Pus	61	31.3
5	Tracheal aspirate	32	16.4
6	Biopsy	2	1
7	CSF	10	5.1
8	Wound Swab	3	1.5
9	CVP tip	2	1
10	EVD tip	5	2.6
Total Specimens		195	100

male and female patients, the samples were examined. Frequency of clinical isolates from different samples is illustrated in table 1.

Moreover, upon age-wise grouping, the age group (41-50 years) showed 12.3% prevalence, 31-40 years showed 13.8% prevalence, less than 18 years showed 15.9% prevalence, above 50 years showed 22.1% prevalence and age group (18-30 years) appeared with highest frequency (35.9%) of having A. baumannii infections. Upon gender distribution male population showed more frequent infections from A. baumannii (n=124) and females showed (n=71) less infection rate.

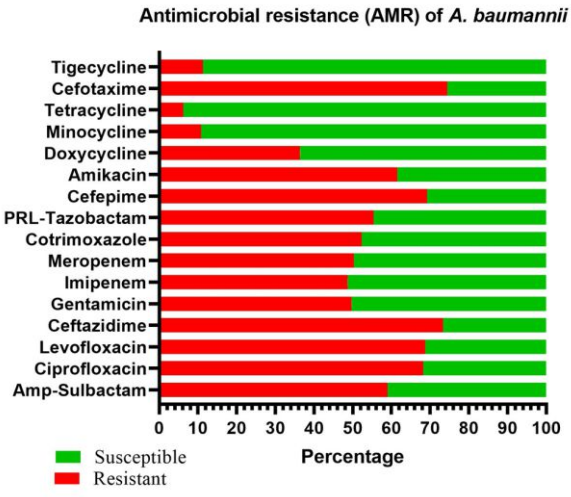

Figure-1: *Antimicrobial resistance and susceptibility of isolates.*

The in-vitro activity of Tigecycline (TGC) compared with 13 different antibiotics (Ciprofloxacin, Levofloxacin, Ceftazidime, Gentamicin, Imipenem, Meropenem, Cotrimoxazole, Cefepime, Amikacin, Doxycycline, Minocycline, Tetracycline, and Cefotaxime) and combination such as Amp-Sulbactam and Piperacillin-Tazobactam. The raw data of above-mentioned antibiotic susceptibility of A. baumannii obtained from clinical isolates of ICU patients are shown in figure 1.

The zones of inhibition for Kirby-Bauer tigecycline susceptibility test were found as ≥18mm for susceptible categories and ≤15mm for resistant categories. Disk diffusion was performed for 195 specimens (clinical isolates of A. baumannii) among hospitalised ICU patients. For tigecycline 22 specimens were formed resisent. For further confirmation Tigecycline MIC strip was applied on these 22 resistant strains (i.e those found to be resistant on disk diffusion method). Out of these 22 strains 100% were resistant with MIC >2mg/l. (Table 2)

Table 2: The frequency of Tigecycline (MIC) susceptibility of A. baumannii obtained from clinical isolates of ICU patients.

Anti-microbial activity of Tigecycline (MIC) n=195	Sensitive	Resistant
Frequency	173	22
Percentage	88.70%	11.30%

DISCUSSION

Hospital-acquired infections caused by Acinetobacter have become increasingly common, especially in intensive care and burn units, and Acinetobacter baumannii is now recognised as a primary contributor to these infections as well as to the antibiotic resistance crisis. According to our study, 15.9% of patients were less than 18 years, 35.9% of patients between 18-30 years, 13.8% of patients were in the ages of 31-40 years, 12.3% of patients were 41-50 years, 22.1% of patients were above 50 years. In comparison to another study, which was done in Morocco 2022, the median age was found to be 48.2 years, and the ages of those

surveyed ranged from 19 to 72 years old.[12]

This study revealed the frequency of A. baumannii in clinical specimens obtained from intensive care unit (ICU) patients, its comparative in-vitro activity of Tigecycline against A. baumannii. We observed the highest frequency of A. baumannii (31.3%) in pus clinical specimens obtained from ICU patients and highest frequency of this gram-negative bacteria (35.9%) in age groups (18-30 years). By comparing the in-vitro activity of Tigecycline (TGC) compared with 13 different antibiotics and combination such as Amp-Sulbactam and Piperacillin-Tazobactam against A. baumannii, highest resistance to Cefotaxime (74.4%) and Ceftazidime (73.3%) and lowest resistance to Minocycline (10.8%) and to Tigecycline (analogue of Minocycline) (11.3%) was observed. Minocycline was found to be most active antibiotic tested (89.2%), followed by tigecycline in the clinical isolates of this study. A. baumannii isolates found to be highly susceptible to tigecycline (88.7%) with a MIC $\leq$ 1 µg/ml and showed low resistance to tigecycline (11.3%) with a MIC $\geq$ 2 µg/ml.

Several studies show that A. baumannii strains are becoming more and more resistant to carbapenems as well as to expanded-spectrum cephalosporins. This looks like that it will soon be the norm for these types. In Pakistan, the rate of A. baumannii resistance to cephalosporins and carbapenems is significant (90%), according to previous study.[13]

In-vitro activity of TGC in clinical isolates from South Lebanon[14] had reported that majority of A. baumannii infections had a respiratory tract source (80.2%) but highest frequency of A. baumannii was found in pus (31.3%) followed by urine (25.6%) and tracheal aspirate (16.4%) in the clinical specimens from ICU patients of this study. Hence, pus and urine specimens have become the main source of infections by A. baumannii in our population. Moreover, same study reported that all isolates of A. baumannii from Lebanese were sensitive to TGC in comparison to the susceptibility of A. baumannii (88.7%) in the clinical isolates of this study.[14]

A recent study of Turkish[15] patients showed that 94% of the A. baumannii isolates were found susceptible to TGC (according to EUCAST criteria), approximately similar to the susceptibility rate of TGC against A. baumannii (88.7%) in our clinical isolates.[15] Another study has reported an important aspect, by considering five Iranian patients' A. baumannii isolates (non-susceptible to tigecycline), that the TGC resistant isolates were found resistant to all antimicrobials tested excluding minocycline with susceptibility rate of 40%.[16] It is interesting to know that Minocycline was found to be most active antibiotic tested (89.2%), followed by tigecycline (88.7%) in comparison to in vitro activity of 13 different antibiotics and two combination regimens (Amp-Sulbactam and Piperacillin-Tazobactam) in the clinical isolates of this study. The great deal of regional variations has been reported with regards to the comparative activity of the TGC against A. baumannii.

The overall susceptibility rate (80.9%) to tigecycline against A. baumannii isolates from the 19 hospitals in Taiwan [17] has been reported which is almost similar to the susceptibility rate of TGC (88.7%) against A. baumannii here in our clinical isolates. Tigecycline is still effective against isolates of A. baumannii from Africa/Middle East, Asia/South Pacific, Europe, Latin America, and North America, including isolates that are resistant to antibiotics, according to a global study. In a study, tigecycline had the lowest rate of resistance and was effective against isolates of A. baumannii (MIC values of 1 or 2 mg/L) and these results were consistent with our results for tigecycline susceptibility with a MIC $\leq$ 1 µg/ml.[18] This had been observed that TGC had a higher in vitro sensitivity (42.7%) against clinical isolates from India that were resistant to multiple drugs. This highlights TGC's potential as a solution to the problem of antibiotic resistance.[19]

Tigecycline is the only treatment option available in oral form and is now the treatment of choice for uncomplicated cases of Acinetobacter baumannii, which caused extended outbreaks, primarily located in intensive care units. However, alarmingly in our study, 22 (9% of the isolates) in our analysis showed disc diffusion resistance to tigecycline. Then, following a conventional methodology, those isolates were examined using

the E-test method. Using the E-test method, 22 (100%) of them were resistant to tigecycline. By BSAC 2022 guidelines, a value greater than 2 mg/l was deemed resistant.

Our study had certain limitations. Sample size was limited, and the study was conducted on clinical isolates from different samples of ICU patients and did not collect data of antibiotics consumption in those patients. This data solely showed that Acinetobacter baumannii was resistant to tigecycline in the context of Lahore General Hospital.

In future, studies must be conducted on broader sample avoiding limitations as well as genetic mechanisms conveying resistance in these multidrug resistance pathogens.

CONCLUSION

Acinetobacter baumannii, a nosocomial pathogen was isolated frequently from different clinical samples. Tigecycline and minocycline were found to be the most active antibiotic tested against A. baumannii in comparative in vitro activity of 13 different antibiotics and with lowest frequency of resistance even in comparison to two different combination regimens including amp-sulbactam and piperacillin-tazobactam.

Ethical Approval:

Approval was obtained from institutional ethical review committee of PGMI/Ameer-ud-Din Medical College Lahore vide reference no. PGMI/AMC/UHS/126-23/1120.

Conflict of Interest: *None*

Funding Source: *None*

Author's Contribution

Conceptualization study design	ZT, MC
Data Acquisition	ZT, MC, SM
Data Analysis/ interpretation	ZT, MC, KJ
Manuscript drafting	ZT, MC, KJ, MW
Manuscript review	ZT, MC, JA, MW

All authors read and approved the final draft.

REFERENCES

1. Hua X, He J, Wang J, Zhang L, Zhang L, Xu Q, et al. Novel tigecycline resistance mechanisms in Acinetobacter baumannii mediated by mutations in adeS, rpoB and rrf. Emerg Microbes Infect. 2021;10(1):1404-1417. doi: 10.1080/22221751-.2021.1948804. PMID: 34170209; PMCID: PMC8274536.
2. Person, Itzchak E, Levi R. Acinetobacter infections - overview of clinical features: 5 : Acineto [Internet]. Taylor & Francis; 2020 [cited 2025 Jun 3]. Available from: https://www.taylorfrancis.-com/chapters/edit/10.1201/9781003069263-5/acinetobacter-infections-overview-clinical-features-itzchak-levi-ethan-rubinstein
3. Jo J, Ko KS. Tigecycline Heteroresistance and Resistance Mechanism in Clinical Isolates of Acinetobacter baumannii. Microbiol Spectr. 2021 ;9(2):e0101021. doi: 10.1128/Spectrum.01010-21. Epub 2021 Sep 15. PMID: 34523993; PMCID: PMC8557860.
4. Ardehali SH, Azimi T, Fallah F, Owrang M, Aghamohammadi N, Azimi L. Role of efflux pumps in reduced susceptibility to tigecycline in Acinetobacter baumannii. New Microbes New Infect. 2019;30(1):100547. doi: 10.1016/j.-nmni.2019.100547. PMID: 31193724; PMCID: PMC6541740.
5. Tiku V, Kofoed EM, Yan D, Kang J, Xu M, Reichelt M, et al. Outer membrane vesicles containing OmpA induce mitochondrial fragmentation to promote pathogenesis of Acinetobacter baumannii. Sci Rep. 2021;11(1):618.doi:10.1038/s41598-020-79966-9. PMID: 33436835; PMCID: PMC7804284.
6. Ayoub Moubareck C, Hammoudi Halat D. Insights into Acinetobacter baumannii: A Review of Microbiological, Virulence, and Resistance Traits in a Threatening Nosocomial Pathogen. Antibiotics (Basel). 2020;9(3):119.doi:10.3390/-antibiotics-9030119. PMID: 32178356; PMCID: PMC7148516.
7. Vrancianu CO, Gheorghe I, Czobor IB, Chifiriuc MC. Antibiotic Resistance Profiles, Molecular Mechanisms and Innovative Treatment Strategies of Acinetobacter baumannii. Microorganisms. 2020;8(6):935.doi: 10.3390/-microorganisms8060935. PMID: 32575913; PMCID: PMC7355832.
8. Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018 ;18(3):318-327. doi: 10.1016/S1473-3099(17)30753-3. Epub 2017 Dec 21. PMID: 29276051.

9. Yaghoubi S, Zekiy AO, Krutova M, Gholami M, Kouhsari E, Sholeh M, et al. Tigecycline antibacterial activity, clinical effectiveness, and mechanisms and epidemiology of resistance: narrative review. Eur J Clin Microbiol Infect Dis. 2022 ;41(7):1003-1022. doi: 10.1007/s10096-020-04121-1. Epub 2021 Jan 5. PMID: 33403565; PMCID: PMC7785128.

10. Vázquez-López R, Solano-Gálvez SG, Juárez Vignon-Whaley JJ, Abello Vaamonde JA, Padró Alonzo LA, Rivera Reséndiz A, et al. Acinetobacter baumannii Resistance: A Real Challenge for Clinicians. Antibiotics (Basel). 2020 ;9(4):205. doi: 10.3390/antibiotics9040205. PMID: 32340386; PMCID: PMC7235888.

11. Zahra N, Zeshan B, Qadri MMA, Ishaq M, Afzal M, Ahmed N. Phenotypic and genotypic evaluation of antibiotic resistance of Acinetobacter baumannii bacteria isolated from surgical intensive care unit patients in Pakistan. Jundishapur J Microbiol. 2021;14(4):e113008. doi:10.5812/jjm.113008.

12. Rada R, Nakhli R, Khayati S, Said LA, Zahlane K. Epidemiological aspect and antibiotic resistance profile of Acinetobacter baumannii in the resuscitation department of Ibn Tofail Hospital CHU Med V. Haya Saudi J Life Sci. 2022;7(3):74–7. DOI: 10.36348/sjls.2022.v07i03.001

13. Khurshid M, Rasool MH, Ashfaq UA, Aslam B, Waseem M, Xu Q, et al. Dissemination of blaOXA-23-harbouring carbapenem-resistant Acinetobacter baumannii clones in Pakistan. J Glob Antimicrob Resist. 2020;21(2):357-362. doi: 10.1016/j.jgar-.2020.01.001. Epub 2020 Jan 30. PMID: 32006748.

14. Itani R, Khojah HMJ, Karout S, Rahme D, Hammoud L, Awad R, et al. Acinetobacter baumannii: assessing susceptibility patterns, management practices, and mortality predictors in a tertiary teaching hospital in Lebanon. Antimicrob Resist Infect Control. 2023 ;12(1):136. doi: 10.1186/s13756-023-01343-8. PMID: 38031181; PMCID: PMC10685635.

15. Çalışkan E, İnce N, Akar N, Öztürk CE. Investigation Of Tigecycline Susceptibility Of Multidrug-resistant Acinetobacter Baumannii Isolates By Disc Diffusion, Agar Gradient And Broth Microdilution Tests. Acta Clin Croat. 2022;61(1):46-51. doi: 10.20471/acc.2022.61-.01.06. PMID: 36398087; PMCID: PMC9616034.

16. Haeili M, Abdollahi A, Ahmadi A, Khoshbayan A. Molecular Characterization of Tigecycline Non-Susceptibility among Extensively Drug-Resistant Acinetobacter baumannii Isolates of Clinical Origin. Chemotherapy. 2021;66(3):99-106. doi: 10.1159/000515100. Epub 2021 Apr 6. PMID: 33823517.

17. Liao CH, Kung HC, Hsu GJ, Lu PL, Liu YC, Chen CM, et al. In-vitro activity of tigecycline against clinical isolates of Acinetobacter baumannii in Taiwan determined by the broth microdilution and disk diffusion methods. Int J Antimicrob Agents. 2008 ;32 Suppl 3:S192-6. doi: 10.1016/S0924-8579(08)70027-X. PMID: 19013354.

18. Seifert H, Blondeau J, Lucaßen K, Utt EA. Global update on the in vitro activity of tigecycline and comparators against isolates of Acinetobacter baumannii and rates of resistant phenotypes (2016-2018). J Glob Antimicrob Resist. 2022 ;31(1):82-89. doi: 10.1016/j.jgar.2022.08.002. Epub 2022 Aug 7. PMID: 35948242.

19. Wilson LA, Kuruvilla TS. Evaluation of in vitro activity of tigecycline against multidrug-resistant clinical isolates. APIC J Intern Med. 2023;11(3):150–3. doi:10.4103/ajim.ajim_53_22.