Journal of Carcinogenesis
Covering Genesis to Therapeutics

Journal of Carcinogenesis, ISSN: 1477-3163
2025; Vol 24: Issue 2s, https://carcinogenesis.com/

Prevalence of colistin-resistant among carbapenem-resistant Acinetobacter baumannii isolates by minimal inhibitory concentration method

Ravneet Pal Singh[1*], Manisha Khandait[2], Nidhi Goel[3], Mukesh Sharma[4]

[*1]Ph.D Scholar, Dept. of Microbiology S.G.T University, Gurugram

[2]Professor&Head, Dept. of Microbiology, S.G.T University, Gurugram

[3]Professor, Dept. of Microbiology, PGIMS, Rohtak

[4]Professor, Dept. of Microbiology, S.G.T University, Gurugram.

***Corresponding Author:**

Ravneet Pal Singh

Email ID: ravneetpal2@gmail.com

ABSTRACT

Introduction: Carbapenems, often considered antibiotics of last resort for treating multidrug-resistant infections, have been rendered ineffective against this pathogen in many cases, leading to the increased use of colistin as a treatment option.

Objective: To determine the prevalence of colistin resistant among carbapenem-resistant Acinetobacter baumannii isolates by minimal inhibitory concentration method.

Methodology: The prospective observational study was conducted over 24 months. The research was conducted at the Department of Microbiology at SGT University, Gurugram, and the Department of Microbiology at Pt. B.D. Sharma PGIMS, Rohtak. All consecutive, non-replicative clinical isolates of carbapenem-resistant Acinetobacter baumannii collected during the study period from both SGT University and Pt. B.D. Sharma PGIMS was included in the study. Clinical isolates were obtained from a variety of specimen types, including blood, sputum, urine, and wound swabs.

Results: Data were collected from 200 clinical isolates of Acinetobacter baumannii were 100% resistant to the carbapenem antibiotics tested, including imipenem, meropenem, and ertapenem. The study results revealed that 96% of the Acinetobacter baumannii isolates (192 out of 200) were susceptible to colistin, with MIC values below 4 µg/ml. However, 4% of the isolates (08 out of 200) exhibited colistin resistance, with MIC values equal to or greater than 4 µg/ml. This indicates that while colistin remains effective for the majority of isolates, a significant portion of carbapenem-resistant A. baumannii strains have developed resistance, posing a challenge for treatment options.

Conclusion: It is concluded that the prevalence of colistin resistance among carbapenem-resistant Acinetobacter baumannii (CRAB) isolates in this study was 4%, indicating a significant threat to the effectiveness of this last-resort antibiotic.

Keywords: *carbapenem-resistant Acinetobacter baumannii, Prevelence, colistin-resistant*

How to Cite: Ravneet Pal Singh, Manisha Khandait, Nidhi Goel, Mukesh Sharma, (2025) Prevalence of colistin-resistant among carbapenem-resistant Acinetobacter baumannii isolates by minimal inhibitory concentration method, *Journal of Carcinogenesis*, *Vol.24, No.2s*, 60-65

1. INTRODUCTION

In recent years, Acinetobacter baumannii has become a significant concern in healthcare due to its ability to acquire resistance to multiple antibiotics, making infections caused by this pathogen difficult to treat. One of the most concerning developments in this regard is the rise of carbapenem-resistant A. baumannii (CRAB), which has been categorized as a priority pathogen by the World Health Organization (WHO). Carbapenems, often considered antibiotics of last resort for treating multidrug-resistant infections, have been rendered ineffective against this pathogen in many cases, leading to the increased use of colistin as a treatment option. However, the emergence of colistin resistance among CRAB isolates has compounded the problem, significantly limiting the therapeutic options available for infected patients. Colistin, a

polymyxin antibiotic, has resurfaced as a crucial agent for treating infections caused by multidrug-resistant Gram-negative bacteria, including CRAB. Colistin disrupts the bacterial cell membrane, leading to cell death, and has become one of the few remaining effective treatments for infections that are resistant to nearly all other antibiotics. However, the increased use of colistin, particularly as a last-resort therapy, has led to the emergence of colistin-resistant A. baumannii isolates, which is a troubling development due to the limited availability of alternative treatment options. Colistin and tigecycline are one of the active antibiotics and have become the last resort of treatment for multidrug-resistant (MDR) Acinetobacter baumannii[1-7].Other antibiotic like cetazidime/tazobactum, cetazidime/avibactum, plazomycin, ervacycline, and brilacidin, are in advanced stages of clinical trials. However, none demonstrated activity against entire gamut of gram negative infection especially Acinetobacter baumannii.[8] Colistin was obtained from Bacillus polymyxa, it belongs to the family of antibiotics which is rapidly bactericidal to gram-negative bacteria. It was first introduced in 1952 and was used until 1980. Its systemic use was discontinued due to adverse toxic affect such as neurotoxicity and nephrotoxicity and neuromuscular blockade.[9] Resistance to colistin has been reported in Acinetobacter baumannii. Susceptibility testing of colistin is challenging and time-consuming. This drug diffuses poorly into agar, potentially giving inaccurate disc diffusion and E-test results. Antimicrobial susceptibility testing (AST) is normally performed using highly standardized microbiological technology and, in the case of colistin, the most common protocol has been jointly endorsed by both the Clinical & Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST), the American and European groups overseeing standardization of AST laboratory protocols. Measurement of exact colistin minimum inhibitory concentration (MICs) is associated with methodological issues, but the ISO standard broth micro dilution (BMD) method (20776-1) works well for Enterobacteriaceae, P. aeruginosa, and Acinetobacter spp.[10-12] Evaluation of microbial antibiotic resistance should be consistent. Therefore, efforts at antibiotic discovery must be equally persistent and continuous development of improved diagnostic test specific to Multi Drug resistance organisms (MDRO) also insist advancing antibacterial drug development. The need of the hour is to develop new drug classes as well as to explore possible new drug from existing class of antibiotic [13,14]. Given the clinical significance of colistin resistance, accurate and reliable methods for detecting resistance are crucial for guiding appropriate antibiotic therapy. The minimal inhibitory concentration (MIC) method is widely used for this purpose, as it quantitatively measures the lowest concentration of an antibiotic that inhibits visible bacterial growth.

2. OBJECTIVES

To determine the prevalence of colistin resistant among carbapenem-resistant Acinetobacter baumannii isolates by minimal inhibitory concentration method.

3. METHODOLOGY

The prospective observational study was conducted over 24 months. The research was conducted at the Department of Microbiology at SGT University, Gurugram, and the Department of Microbiology at Pt. B.D. Sharma PGIMS, Rohtak. All consecutive, non-replicative clinical isolates of carbapenem-resistant Acinetobacter baumannii collected during the study period from both SGT University and Pt. B.D. Sharma PGIMS was included in the study. Clinical isolates were obtained from a variety of specimen types, including blood, sputum, urine, and wound swabs. The isolates were cultured and identified through biochemical tests such as citrate utilization, oxidative-fermentative (dextrose) testing, catalase, arginine dihydrolase, and growth at 44°C. These standard microbiological techniques were employed to accurately identify Acinetobacter baumannii from the collected samples. The initial screening for carbapenem resistance was conducted using the Kirby-Bauer disk diffusion method, following CLSI guidelines. This included testing with carbapenem antibiotics such as imipenem, meropenem, and ertapenem. Isolates confirmed as carbapenem-resistant were then further tested for colistin resistance using the broth microdilution method to determine the MIC. The MIC for colistin was determined using the broth microdilution method. Mueller-Hinton broth with cations was prepared, and 100 µl of broth was added to each well of a 96-well microtiter plate. Colistin concentrations ranging from 64 µg/ml to 0.12 µg/ml were tested, with a control well left without the antibiotic to ensure bacterial growth. A bacterial inoculum of A. baumannii was prepared and added to each well, and the plates were incubated at 37°C for 20-24 hours. The MIC was recorded as the lowest concentration of colistin that inhibited visible growth.[15,16] Isolates with an MIC value of ≥4 µg/ml were considered colistin-resistant, in line with CLSI guidelines. To ensure the accuracy of antimicrobial susceptibility testing, quality control measures were followed. Standard strains of Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 were used as control organisms in the susceptibility tests.[17] This ensured that the results obtained for the clinical isolates were reliable and reproducible. Data for each isolate, including patient demographics (such as age, gender), sample type, and clinical diagnosis, were collected using a structured proforma. Statistical analysis was performed using SPSS version 21.0. Frequency distribution and cross-tabulation were used to calculate the prevalence of colistin resistance, and associations between variables were tested using Chi-square tests. A p-value of less than 0.05 was considered statistically significant, indicating meaningful relationships between variables. All biomedical waste generated during the study was properly managed and disposed of according to the Biomedical Waste Management and Handling Rules, 2018. This included ensuring that all waste materials were either disinfected or sterilized before disposal, minimizing any potential environmental or health risks associated with the study.

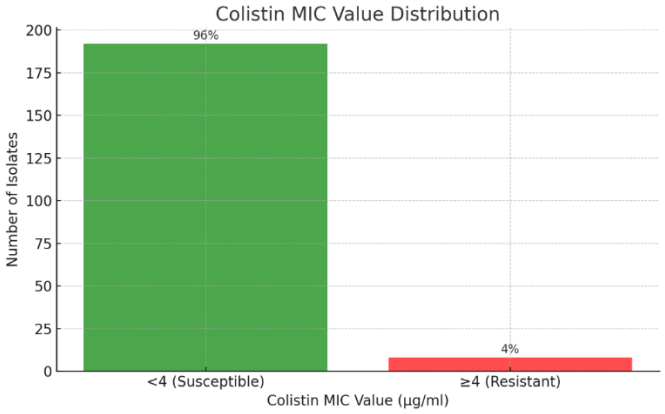
4. RESULTS

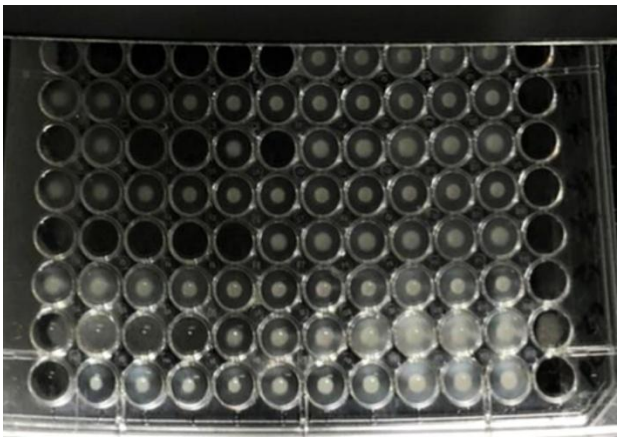
Data were collected from 200 clinical isolates of *Acinetobacter baumannii* were 100% resistant to the carbapenem antibiotics tested, including imipenem, meropenem, and ertapenem.

Table 1: Colistin Resistance Based on MIC Method

Colistin MIC Value (µg/ml)	Number of Isolates	Percentage (%)
<4 (Susceptible)	192	96%
≥4 (Resistant)	8	4%
Total	**200**	**100%**

The study results revealed that 96% of the ***Acinetobacter baumannii*** isolates (192 out of 200) were susceptible to colistin, with MIC values below 4 µg/ml. However, 4% of the isolates (8 out of 200) exhibited colistin resistance, with MIC values equal to or greater than 4 µg/ml. This indicates that while colistin remains effective for the majority of isolates, a significant portion of carbapenem-resistant ***A. baumannii*** strains have developed resistance, posing a challenge for treatment options.





Microtiter plate

Organism tested	Antibiotic Colistin Conc.(ug/ml)												
	Specimen Id	16	8	4	2	1	0.5	0.25	0.125	0.06	0.03	GC	MC	MIC ug/ml
QC	QC	-	-	-	-	-	-	+	+	+	+	+	-	0.5
TEST 1	1	+	+	+	+	+	+	+	+	+	+	+	-	>-16
TEST 2	2	-	+	+	+	+	+	+	+	+	+	+	-	16
TEST 3	3	+	+	+	+	+	+	+	+	+	+	+	-	>16
TEST 4	4	-	-	-	-	-	+	+	+	+	+	+	-	1
TEST 5	5	+	+	+	+	+	+	+	+	+	+	+	-	>-16
TEST 6	6	-	-	-	-	-	-	+	+	+	+	+	-	0.5

TEST 7	**7**	**-**	+	+	+	+	+	+	+	+	+	+	**-**	**16**

+ is equivalent to growth, –is equivalent to no growth; GC: Growth Control; MC: Media Control

QC- Quality Control is Pseudomonas aeruginosa ATCC27853 for testing colistin against P . aeruginosa and Acinetobacter species.

Broth Microdilution Testing Reporting Sheet

Table 2: Prevalence of Colistin Resistance by Specimen Type

Specimen Type	Number of Colistin-Resistant Isolates	Percentage (%)
Blood	6	3
Sputum/BAL	2	1
Total	8	**4**

The distribution of colistin resistance across different age groups showed relatively consistent results. In the 15-59 age group, 5% (6 out of 125) of isolates were resistant, Similarly, the 60 and above age groups had 7% (2 out of 27) of isolates were resistance.

Table 3: Colistin Resistance by Patient Demographics (Age Group)

Age Group (Years)	Total Isolates	Colistin-Resistant Isolates	Percentage of Resistance (%)
0-14	48	NIL	NIL
15-59	125	6	5
60 and above	27	2	7
Total	**200**	**8**	**4**

The results showed that colistin resistance was slightly higher among male patients, with 4% of the isolates (6 out of 142) being resistant, compared to 3% (2 out of 58) among female patients. Overall, 4% of the total 200 isolates exhibited colistin resistance, indicating that gender did not significantly influence the prevalence of colistin resistance among ***Acinetobacter baumannii*** isolates in this study.

Table 4: Colistin Resistance by Gender

Gender	Total Isolates	Colistin-Resistant Isolates	Percentage of Resistance (%)
Male	142	6	4
Female	58	2	3
Total	**200**	8	**4**

5. DISCUSSION

The findings of this study highlight a growing concern regarding antimicrobial resistance in healthcare settings, particularly among carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates. The study found that 4% of CRAB isolates exhibited resistance to colistin, a last-line treatment option for multi drug-resistant infections. This is in accordance with other research reports involving similar regions pointing to the fact that resistance rates usually lie within a range of 10%-20%; therefore, colistin resistance among CRAB continues to emerge as a major concern. [13] Furthermore the overall CRAB isolate prevalence in this study at 4 % of the isolates shows that one of the few remaining weapons against MDR A. baumannii is becoming less effective with time. Currently, studies have shown that colistin resistance in A. baumannii is mainly due to changes of the bacterial outer membrane that lowers affinity of the antibiotic. This is dangerous for clinical outcomes because cures for infections resulting from these resistant strains is a lot scarcer. [14] The found prevalence indicates that permanent monitoring and cautious use of antimicrobial agents should be continued to avoid the dissemination of colistin resistance. With regards to the distribution of samples, colistin resistance was detected in all types

of samples such as blood, sputum, urine, and wound swab and the overall resistance rate of colistin was almost similar at all different types of specimens (15 – 16.7%).[18] Such distribution indicates that colistin resistance is not isolated to some forms of infection but is present in several clinical isolates. Besides, the finding that colistin-resistant isolates were obtained from different age and sex groups without any variation also informs us that the resistance is not greatly affected by patient characteristics. The appearance of colistin resistance in CRAB isolates has important consequences for clinical practice. Colistin has been used for many years as a last-line of defense against many MDR GNB infections including A. baumannii.[19] The emergence of more resistant bacteria to colistin mean that the providers of care are in a fix as to how to manage infections for which there are fewer appropriate antibiotics. This raises the need to work on measures of coming up with other treatment options that may include the use of more than one drug in treatment or new types of antimicrobial agents besides the use of measures that can help to prolong the efficiency of the already known drugs. That is why to prevent the higher rates of resistance, antimicrobial stewardship programs are essential.[20] Thus, colistin should be administered selectively, combined with other antimicrobials, to reduce the levels of selective pressure that challenge the development of resistant strains. Furthermore, further measures to improve infection control measures, rationalize antibiotic stewardship and encourage the use of rapid diagnostic tests will go a long way to overcoming the effects of colistin resistance.[21] This study is limited by the regional study setting as it involved two healthcare facilities in India. However, information regarding colistin resistance in these centers can be useful but resistance pattern may be dissimilar in other regions of the world as such, large scale researches should be conducted to determine more frequent occurrence of colistin resistance prevalent all around the world.[22] In addition, this study failed to explore on the genetic characteristics of colistin resistance, which should be an important area for further study. Research conducted at the molecular level to investigate the unique genetic changes, or the pathway through which, resistance evolves would go a long way in demystifying development of resistance.

6. CONCLUSION

It is concluded that the prevalence of colistin resistance among carbapenem-resistant *Acinetobacter baumannii* (CRAB) isolates in this study was 4%, indicating a significant threat to the effectiveness of this last-resort antibiotic. The resistance was observed consistently across various clinical specimens and demographic groups, highlighting the widespread nature of the issue. These findings emphasize the need for continuous surveillance and judicious use of colistin to prevent further resistance development.

REFERENCES

[1] Caniaux I, van Belkum A, Zambardi G, Poirel L, Gros MF. MCR:modern colistin resistance. Eur J Clin Microbiol Infect Dis.2016;36(3):415-42.

[2] The Infectious Diseases Society of America. 10 X ’20 Initiative: Pursuing a global commitment to develop 10 new antibacterial drugs by 2020.Clin Infect Dis 2010; 50(8):1081–3.

[3] Who.int [Internet].Geneva; World Health Organization: WHO publishes list of bacteria for which new antibiotics are urgently needed,2018;[cited 2018]. Available from: http://www.who.int/news-room/detail/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed.

[4] Rossi F, Girardello R, Cury AP, Di Gioia TS, Almeida JN Jr, Duarte AJ. Emergence of colistin resistance in the largest university hospital complex of São Paulo, Brazil, over five years. Braz J Infect Dis.2017;21(1):98-101.

[5] Mohanty S, Maurya V, Gaind R, Deb M, Phenotypic characterization and colistin susceptibilities of carbapenem resistant of Pseudomonas aeruginosa and Acinetobacter spp. J Infect Dev Citries 2013;7(11):880-7.

[6] Montefour K, Frieden J, Hurst S, Helmich C,Headley D, Martin M, et al. Acinetobacter baumannii An emerging multidrug resistant pathogen in critical care. Aacnjournals 2008;28(1):15-25.

[7] Ko KS, Choi Y, Lee JY. Old drug, new findings: Colistin resistance and dependence of Acinetobacter baumannii. Pfm journal2017;1(4):159-67.

[8] Cai Y, Chai D, Wang R, Liang B, Bai N. Colistin resistance of Acinetobacter baumannii: clinical reports, mechanisms and antimicrobial strategies. J Antimicrobial Chemother2012;67(7):1607-15.

[9] Qureshi ZA, Hittle LE, O’Hara JA, Rivera JI, Syed A, Shields RK, et al. Colistin-resistant Acinetobacter baumannii: beyond carbapenem resistance. Clin Infect Dis 2015;60: 1295-303.

[10] Taneja N, Singh G,Singh M, Sharma M. Emergence of tigecycline & colistin resistant Acinetobacter baumaniiin patients with complicated urinary tract infections in north India. Ind. J Med. Res.2011;133(6):681-4.

[11] Al-Swein N, Al-Hubil MA, Rotimi VO. Emergence of tigecycline and colistin resistance in acinetobacter species isolated from patient in Kuwait. Jn of chemo.2011;23(1):13-6.

[12] Chang KC, Lin MF, Lin NT, Wu WJ, Kuo HY, Lin TY et al. Clonal spread of multidrug-resistant Acinetobacter baumannii in eastern Taiwan. J Microbiol Immunol Infect2012; 45(1):37–42.

[13] Shojaei L, Mohammadi M, Beigmohammadi MT, Doomanlou M, Abdollahi A, Feizabadi MM, et al. Clinical response and outcome of pneumonia due to multi-drug resistant Acinetobacter baumannii in critically ill patients. Iran Jn of micro.2016;8(5):288-97.

[14] Aydin M, Ergonul O, Azap A, Bilgin H, Aydın G, Cavus SA, et al. Rapid emergence of colistin resistance and its impact on mortality in health care associated Infections. Jn of Hosp. Infect.2018;98(3):260-3.

[15] Clinical and laboratory standard institute (CLSI). Performance standards for antimicrobial susceptibility testing; 28the. CLSI M100-S28. Wayne, PA: CLSI; 2018:30-8

[16] Clinical and laboratory standard institute (CLSI). Methods for dilution antimicrobial

[17] susceptibility tests for bacteria that grow aerobically; 10the. CLSI document M07-A10.Wayne, PA: CLSI; 20159.

[18] Colistin breakpoints for Pseudomonas aeruginosa and Acinetobacter species, CLSI rationale document MR01, Romney M Humphries, Accelerate diagnostic Inc, USA, September 2018.

[19] Lo-Ten-Foe J, de Smet A, Diederen B, Kluytmans J, van Keulen P. Comparative evaluation of the VITEK 2, Disk Diffusion, Etest, Broth Microdilution, and Agar Dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant Enterobacter cloacae and Acinetobacter baumannii Strains. Antimicrob Agents. Chemother.2007;51(10):3726-30.

[20] Dafopoulou K, Zarkotou O, Dimitroulia E, Hadjichristodoulou C, Gennimata V, Pournaras S, et al. Comparative evaluation of colistin susceptibility testing methods among Carbapenem-Nonsusceptible Klebsiella pneumoniae and Acinetobacter baumannii Clinical Isolates. Antimicrob Agents Chemother.2015;59(8):4625-30.

[21] Matuschek E, Ahman J, Webster C, Kahlmeter G. Antimicrobial susceptibility testing of colistin – evaluation of seven commercial MIC products against standard broth micro dilution for Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter spp. Clin Microbiol and Infect.2018;24(8):865-70.

[22] Singhal L, Sharma M, Verma S, Kaur R, Britto XB, Malhotra SK et al. Comparative evaluation of broth microdilution with Polystyrene and Glass-Coated Plates,Agar Dilution, E-Test, Vitek, and Disk Diffusion for Susceptibility Testing of Colistin and Polymyxin B on Carbapenem-Resistant Clinical Isolates of Acinetobacter baumannii. Micro Drug Resist.2018;24(8):1082-8.

[23] Jayol A, Nordmann P, Andre C, Poirel L, Dubois V. Evaluation of three broth microdilution systems to determine colistin susceptibility of Gram-negative bacilli. J Antimicrob Chemother.2018;73(5):1272-8.