

Phenotypic Detection of Colistin Resistance Among Multidrug-Resistant Gram-Negative Bacilli

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Abstract

Background: Increasing colistin resistance among multidrug-resistant Gram-negative bacilli is a major concern because colistin remains an important therapeutic option for difficult-to-treat infections. Reliable phenotypic detection is essential for appropriate antimicrobial therapy and surveillance. The objective is to determine the phenotypic profile of colistin resistance among MDR Gram-negative bacilli using broth microdilution and to evaluate feasible phenotypic approaches for its detection. **Material and Methods:** This prospective, hospital-based, cross-sectional study included 150 non-duplicate MDR Gram-negative bacterial isolates obtained from clinically significant specimens. Organisms were identified using standard microbiological methods, and routine antimicrobial susceptibility testing was performed. Colistin susceptibility was determined using broth microdilution (BMD) as the reference phenotypic method. Colistin resistance was analysed according to bacterial species and clinical specimen. The proposed phenotypic screening method, COL-AS, was designed for comparison with BMD using sensitivity, specificity, predictive values, accuracy and agreement. **Results:** Of the 150 MDR Gram-negative isolates, *K. pneumoniae* was the most common (36.0%), followed by *E. coli* (30.0%), *A. baumannii* (18.0%) and *P. aeruginosa* (12.0%). BMD identified 24 (16.0%) isolates as colistin resistant. Resistance was highest among *A. baumannii* (25.9%), followed by *K. pneumoniae* (20.4%), *P. aeruginosa* (16.7%) and *E. coli* (6.7%). The association between bacterial species and colistin resistance was statistically significant ($p=0.041$). **Conclusion:** A considerable proportion of MDR Gram-negative bacilli demonstrated phenotypic colistin resistance, particularly *A. baumannii* and *K. pneumoniae*. Reliable phenotypic testing using BMD remains important for accurate detection and surveillance of colistin resistance.

Keywords: Colistin resistance, multidrug-resistant Gram-negative bacilli, broth microdilution, phenotypic detection, antimicrobial susceptibility testing, *Acinetobacter baumannii*, *Klebsiella pneumoniae*.

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INTRODUCTION

The emergence of colistin resistance among multidrug-resistant (MDR) Gram-negative bacilli has become an important clinical concern because colistin remains an important therapeutic option for infections caused by organisms with limited treatment options. Recent studies have demonstrated the occurrence of colistin resistance among clinically important Gram-negative bacilli, emphasizing the increasing challenge posed by antimicrobial resistance in healthcare settings.^[1] The development of colistin resistance further limits available therapeutic options and highlights the need for accurate and timely laboratory detection.^[2]

The increasing occurrence of colistin resistance among Gram-negative organisms has been documented across different geographical regions and bacterial species.^[3] Indian studies have also reported colistin resistance among MDR *Escherichia coli* and *Klebsiella* spp., demonstrating that this problem is relevant to routine clinical microbiology practice.^[4] Similar trends in the prevalence of colistin resistance have been reported internationally, indicating the need for continued laboratory surveillance.^[5] Accurate phenotypic detection of colistin resistance is

particularly important because conventional antimicrobial susceptibility methods may not provide reliable results for colistin. Broth microdilution (BMD) is regarded as the reference phenotypic method for determination of the colistin minimum inhibitory concentration (MIC). However, its routine implementation may be relatively laborious, creating a need to evaluate simpler and feasible phenotypic methods. Comparative studies have demonstrated variation in the performance of different phenotypic colistin susceptibility-testing methods when assessed against BMD.^[6]

Therefore, the present study was undertaken to determine the phenotypic profile of colistin resistance among MDR Gram-negative bacilli and to compare feasible phenotypic methods for

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detection of colistin resistance, using broth microdilution as the reference method.

MATERIALS AND METHODS

Study Design and Setting: The present study was designed as a prospective, hospital-based, cross-sectional study conducted in the Department of Microbiology of a tertiary-care teaching hospital. Consecutive, non-duplicate Gram-negative bacilli isolated from clinically significant specimens submitted to the microbiology laboratory during the predefined study period were screened for multidrug resistance. Isolates fulfilling the predefined MDR criteria were subsequently evaluated for colistin susceptibility using phenotypic methods.

Sample Size: The sample size was estimated using the formula for a single proportion:

$$n = Z^2pq/d^2$$

where $Z = 1.96$ at a 95% confidence level, $p =$ anticipated prevalence of colistin resistance (10%), $q = 1 - p$ (90%), and $d = 5\%$ absolute precision.

$$n = (1.96)^2 \times 0.10 \times 0.90 / (0.05)^2 \approx 138$$

After allowing approximately 10% for non-evaluable isolates or unsuccessful/repeat phenotypic testing, the final sample size was rounded to 150 MDR Gram-negative bacterial isolates.

Clinical Specimens and Isolation of Gram-Negative Bacilli: Clinical specimens including urine, blood, pus/wound specimens, respiratory specimens, sterile body fluids and other clinically relevant samples were processed according to standard laboratory procedures. Specimens were inoculated onto appropriate primary culture media and incubated under suitable conditions. Plates were examined for significant bacterial growth, and pure cultures of Gram-negative bacilli were obtained for further testing.

Only the first clinically significant isolate of a particular organism from each patient was included to avoid duplication.

Identification of Bacterial Isolates: Gram-negative isolates were identified to the species level using conventional microbiological methods. Identification included Gram staining, colony morphology, oxidase test, catalase where applicable, indole, citrate, urease, triple sugar iron agar reaction, motility and other relevant biochemical tests, according to the organism isolated.

Inclusion Criteria: Non-duplicate MDR Gram-negative bacilli isolated from clinically significant specimens were included. MDR was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. Only isolates with adequate growth and complete susceptibility-testing results were evaluated.

Exclusion Criteria: Gram-positive organisms, duplicate isolates from the same patient, colonizers or contaminants without clinical significance, isolates not fulfilling the predefined MDR criteria, and isolates with inadequate growth or incomplete antimicrobial susceptibility results were excluded.

Routine Antimicrobial Susceptibility Testing: Routine

antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method according to the current methodology adopted by the laboratory. Depending upon the bacterial species, susceptibility to relevant antimicrobial agents such as ampicillin, amoxicillin-clavulanate, cefotaxime/ceftriaxone, ceftazidime, cefepime, piperacillin-tazobactam, gentamicin, amikacin, ciprofloxacin, meropenem and imipenem was recorded.

Routine disk diffusion was not used for determination of colistin susceptibility. Based on the routine susceptibility profile, isolates satisfying the MDR criteria were selected for phenotypic colistin susceptibility testing.

Phenotypic Detection of Colistin Resistance: Two phenotypic approaches were used for evaluation of colistin susceptibility:

Broth Microdilution (BMD) – Reference Method: Colistin susceptibility was determined using broth microdilution (BMD) as the reference phenotypic method for determining the colistin minimum inhibitory concentration (MIC). A validated commercial BMD system or standardized manual broth microdilution method available in the laboratory was used consistently throughout the study.

A fresh pure culture was selected and a standardized bacterial suspension was prepared. The final inoculum was prepared according to the validated BMD protocol and inoculated into wells containing appropriate concentrations of colistin. Growth and sterility controls were included. Following incubation according to the validated protocol, the wells were examined for visible bacterial growth.

The MIC was recorded as the lowest concentration of colistin that inhibited visible bacterial growth. The MIC result was interpreted according to the applicable susceptibility criteria used during the study period.

Colistin Agar Spot Test (COL-AS) – Index Method: The Colistin Agar Spot Test (COL-AS) was evaluated as a simple phenotypic screening method for detecting colistin resistance. Mueller–Hinton agar containing a validated screening concentration of colistin was prepared. A standardized bacterial suspension was spot-inoculated onto the colistin-containing medium and incubated according to the validated COL-AS protocol.

After incubation, plates were examined for bacterial growth. No visible growth was interpreted as COL-AS negative, whereas definite visible growth was interpreted as COL-AS positive. Doubtful or contaminated results were repeated before final classification.

Repeat Testing of Discordant Isolates: Isolates showing discordant findings between COL-AS and BMD were subjected to repeat testing using both methods. This included isolates that were BMD susceptible but COL-AS positive and those that were BMD resistant but COL-AS negative. A separate record of discordant isolates was maintained for final analysis.

Quality Control: Quality-control testing was performed according to the applicable laboratory recommendations and manufacturers' instructions. The QC organism, expected and observed results, date of testing, media and colistin lot details, operator and test method were documented. Results from clinical isolates were accepted only when the corresponding quality-control results were satisfactory.

Outcome Measures: The primary outcome was the proportion

of MDR Gram-negative bacilli demonstrating phenotypic colistin resistance by the reference BMD method.

The secondary outcomes included:

1. Organism-wise distribution of colistin resistance;
2. Specimen-wise distribution of colistin resistance;
3. Distribution of colistin MIC values;
4. Positivity of the Colistin Agar Spot Test;
5. Agreement between COL-AS and BMD; and
6. Diagnostic performance of COL-AS using BMD as the reference method.

Species-wise analyses were performed separately for relevant Enterobacterales, including *Escherichia coli* and *Klebsiella pneumoniae*, and non-fermenting Gram-negative bacilli, where applicable. Specimen-wise analysis included urine, blood, pus/wound specimens, respiratory specimens and sterile body fluids.

Comparison of Phenotypic Methods: BMD was considered the reference method, while COL-AS was considered the index test. COL-AS results were classified as true positive, false positive, false negative or true negative according to the corresponding BMD result.

The diagnostic performance of COL-AS was assessed by calculating sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), overall accuracy/agreement and appropriate error rates. Agreement between COL-AS and BMD was assessed using Cohen's kappa coefficient, with 95% confidence intervals where

applicable.

Statistical Analysis: Data were entered into Microsoft Excel and analysed using IBM SPSS Statistics. Categorical variables were summarized as frequencies and percentages, while continuous variables such as MIC values were summarized using appropriate descriptive statistics according to their distribution. Associations between categorical variables were evaluated using the Chi-square test or Fisher's exact test, as appropriate.

For comparison of COL-AS against the reference BMD method, sensitivity, specificity, PPV, NPV, diagnostic accuracy and 95% confidence intervals were calculated. Inter-method agreement was assessed using Cohen's kappa coefficient. A p -value <0.05 was considered statistically significant.

RESULTS

A total of 150 non-duplicate multidrug-resistant (MDR) Gram-negative bacterial isolates obtained from clinically significant specimens were included in the study. *Klebsiella pneumoniae* was the most frequently isolated organism, accounting for 54 (36.0%) isolates, followed by *Escherichia coli* 45 (30.0%), *Acinetobacter baumannii* 27 (18.0%), and *Pseudomonas aeruginosa* 18 (12.0%). Urine was the most frequent clinical specimen (34.7%), followed by respiratory specimens (24.0%), blood (20.0%), and pus/wound specimens (16.0%).

Table 1: Distribution of MDR Gram-negative isolates according to organism and clinical specimen (N=150)

Characteristic	Number (n)	Percentage (%)
Organism isolated		
<i>Klebsiella pneumoniae</i>	54	36.0
<i>Escherichia coli</i>	45	30.0
<i>Acinetobacter baumannii</i>	27	18.0
<i>Pseudomonas aeruginosa</i>	18	12.0
Other Gram-negative bacilli	6	4.0
Clinical specimen		
Urine	52	34.7
Respiratory specimens	36	24.0
Blood	30	20.0
Pus/wound specimens	24	16.0
Other specimens/body fluids	8	5.3
Total isolates	150	100.0

The organism and specimen distribution above is taken directly from the available study dataset.

Phenotypic colistin resistance by broth microdilution: Broth microdilution, used as the reference phenotypic

method, identified 24 (16.0%) isolates as colistin resistant, while 126 (84.0%) were non-resistant. The proportion of resistance differed among the bacterial species.

Table 2: Organism-wise distribution of colistin resistance by broth microdilution

Organism	Total isolates, n	Colistin resistant, n (%)	Colistin non-resistant, n (%)
<i>K. pneumoniae</i>	54	11 (20.4)	43 (79.6)
<i>E. coli</i>	45	3 (6.7)	42 (93.3)
<i>A. baumannii</i>	27	7 (25.9)	20 (74.1)
<i>P. aeruginosa</i>	18	3 (16.7)	15 (83.3)
Other Gram-negative bacilli	6	0 (0.0)	6 (100.0)
Total	150	24 (16.0)	126 (84.0)

$p=0.041$

The highest proportion of phenotypic colistin resistance was observed among *A. baumannii* (25.9%), followed by *K. pneumoniae* (20.4%) and *P. aeruginosa* (16.7%). Resistance

was comparatively less frequent among *E. coli* (6.7%). The association between bacterial species and phenotypic colistin resistance was statistically significant ($p=0.041$).

Table 3: Overall phenotypic colistin susceptibility by broth microdilution

BMD result	Number (n)	Percentage (%)
Colistin resistant	24	16.0
Colistin non-resistant	126	84.0
Total	150	100.0

Thus, approximately one in six MDR Gram-negative isolates (16.0%) demonstrated phenotypic resistance to colistin by the reference BMD method. Species-wise variation was evident, with *A. baumannii* and *K. pneumoniae* accounting for most of the resistant isolates.

Comparison of BMD and Colistin Agar Spot Test:

According to the revised methodology, the Colistin Agar Spot Test (COL-AS) is to be evaluated against BMD as the reference method. The attached laboratory protocol requires

COL-AS results to be classified as true positive (TP), false positive (FP), false negative (FN), and true negative (TN), followed by calculation of sensitivity, specificity, PPV, NPV, accuracy and Cohen's kappa.

However, actual COL-AS observations are not provided in the available study dataset. Therefore, valid values for these diagnostic-performance parameters cannot presently be calculated without generating new data.

Table 4: Proposed comparison of COL-AS with broth microdilution

COL-AS result	BMD resistant	BMD non-resistant	Total
COL-AS positive	TP – data required	FP – data required	—
COL-AS negative	FN – data required	TN – data required	—
Total	24	126	150

Once the COL-AS results are available, the following parameters should be reported: sensitivity, specificity, PPV, NPV, overall diagnostic accuracy, 95% confidence intervals, and Cohen's kappa coefficient.

DISCUSSION

The present study demonstrated phenotypic colistin resistance in 24 (16.0%) of 150 MDR Gram-negative bacilli by broth microdilution (BMD). Resistance varied significantly according to bacterial species ($p=0.041$), being highest among *Acinetobacter baumannii* (25.9%), followed by *Klebsiella pneumoniae* (20.4%), *Pseudomonas aeruginosa* (16.7%), and *Escherichia coli* (6.7%).

The occurrence of colistin resistance among highly resistant Gram-negative organisms has increasingly been reported from Indian healthcare settings. Halder et al. documented colistin-resistant *Enterobacter cloacae* complex among ICU bloodstream isolates, highlighting the emergence of resistance even to last-line antimicrobial therapy.^[7] Similarly, the systematic review and meta-analysis by Dwibedy et al. demonstrated that colistin resistance is present among important Gram-negative nosocomial pathogens across India.^[8] These observations support the clinically relevant level of phenotypic resistance identified in the present study.

The mechanisms underlying colistin resistance are heterogeneous, as discussed by Yadav et al.^[9] Although molecular mechanisms were described in studies by Yadav et al. and Gürbüz et al.,^[10,11] the present study was intentionally restricted to phenotypic detection, as this directly determines the susceptibility profile available for routine clinical decision-making. Similarly, genomic investigations of highly resistant *K. pneumoniae* and *P. aeruginosa* have illustrated the complexity of resistance among clinically important Gram-negative pathogens.^[12]

The comparatively high resistance observed among *K. pneumoniae* and *A. baumannii* in the present study is

clinically important because these organisms are frequently associated with severe healthcare-associated infections. Diwane et al. also reported colistin-resistant Gram-negative organisms among hospitalized patients, with *K. pneumoniae* being the predominant organism in their study.^[13]

Accurate phenotypic detection is particularly important for colistin because susceptibility results may vary according to the testing method employed. Rout et al. evaluated different in-vitro colistin susceptibility-testing methods among carbapenem-resistant Gram-negative bacilli and emphasized the importance of appropriate methodology for reliable determination of colistin susceptibility.^[14] In the present study, BMD was therefore used as the reference phenotypic method. Evaluation of the Colistin Agar Spot Test against BMD may further determine whether it can provide a practical screening approach in laboratories where routine BMD testing is resource intensive. Overall, the findings demonstrate a considerable burden of phenotypic colistin resistance among MDR Gram-negative bacilli, particularly *A. baumannii* and *K. pneumoniae*. Continued phenotypic surveillance using validated susceptibility-testing methods is essential for accurate reporting and antimicrobial stewardship.

CONCLUSION

Phenotypic colistin resistance was detected in 16.0% of MDR Gram-negative bacilli, with the highest resistance observed among *A. baumannii* (25.9%) and *K. pneumoniae* (20.4%). A significant association was observed between bacterial species and colistin resistance. These findings emphasize the importance of reliable phenotypic susceptibility testing, with broth microdilution serving as the reference method. Evaluation of simple phenotypic screening methods such as COL-AS against BMD may facilitate practical detection of colistin resistance in routine microbiology laboratories.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Jaglal P, Lowe M, Featherston J, Swe-Han KS, Velaphi S, Menezes C. Decoding genotypic antimicrobial resistance amongst colistin-resistant Gram-negative bloodstream isolates at a South African academic healthcare facility. *Diagn Microbiol Infect Dis*. 2026;115(3):117373. doi:10.1016/j.diagmicrobio.2026.117373.
2. Zeb S, Nazir A, Hameed MF, Ikram S, Naqvi SZH, Shoaib M, et al. Colistin resistance in Gram-negative bacteria: mechanisms, transmission, and novel intervention strategies. *Microorganisms*. 2026;14(1):173. doi:10.3390/microorganisms14010173.
3. Mehmood MS, Rupasinghe Koggala Marakkalage UK, Arif T, Javaid F, Abid MA, Shahid M, et al. mcr gene family evolution and structural mechanisms of colistin resistance: from mcr-1 to emerging variants. *Arch Microbiol*. 2026;208(4):186. doi:10.1007/s00203-026-04739-3.
4. Kumari P, Seth R, Nema S. Emergence of colistin resistance in multidrug-resistant *Escherichia coli* and *Klebsiella* species from Central India: a sentinel study with the detection of mcr-1 from blood isolates. *Cureus*. 2026;18(6):e111794. doi:10.7759/cureus.111794.
5. Azzam A, Salem H, Nazih M, et al. Prevalence, trends, and molecular insights into colistin resistance among Gram-negative bacteria in Egypt: a systematic review and meta-analysis. *Antimicrob Resist Infect Control*. 2025;24:32. doi:10.1186/s12941-025-00799-3.
6. Kazaz I, Cicek Karaman G, Karakus H, Kaya S, Hosbul T. Evaluation of colistin susceptibility by four phenotypic methods compared to broth microdilution in multidrug-resistant *Klebsiella pneumoniae*. *BMC Microbiol*. 2025;25(1):415. doi:10.1186/s12866-025-04121-1.
7. Halder G, Chaudhury BN, Denny P, Chakraborty M, Mandal S, Dutta S. Emergence of concurrently transmissible mcr-9 and carbapenemase genes in bloodborne colistin-resistant *Enterobacter cloacae* complex isolated from ICU patients in Kolkata, India. *Microbiol Spectr*. 2025;13(3):e0154224. doi:10.1128/spectrum.01542-24.
8. Dwibedy SK, Padhy I, Panda AK, Mohapatra SS. Colistin resistance among the Gram-negative nosocomial pathogens in India: a systematic review and meta-analysis. *J Chemother*. 2025;37(5):389-401. doi:10.1080/1120009X.2024.2405355.
9. Yadav KS, Datkhile K, Pawar S, Patil S. An overview of the genetic mechanisms of colistin-resistance in bacterial pathogens: an Indian perspective. *Cureus*. 2025;17(2):e78800. doi:10.7759/cureus.78800.
10. Yadav KS, Pawar S, Datkhile K, Patil SR. Study on the mobile colistin resistance (mcr-1) gene in Gram-negative bacilli in a rural tertiary care hospital in Western Maharashtra. *Cureus*. 2024;16(12):e75569. doi:10.7759/cureus.75569.
11. Gürbüz M, Türkeş Şen E, Şimşek M, Demir C. Investigation of plasmid-mediated colistin resistance genes (mcr-1-8) in Enterobacterales isolates. *Cureus*. 2024;16(6):e61538. doi:10.7759/cureus.61538.
12. Talat A, Khan F, Khan AU. Genome analyses of colistin-resistant high-risk blaNDM-5 producing *Klebsiella pneumoniae* ST147 and *Pseudomonas aeruginosa* ST235 and ST357 in clinical settings. *BMC Microbiol*. 2024;24(1):174. doi:10.1186/s12866-024-03306-4.
13. Diwane D, Rajhans PA, Jog SA, Dalvi M. Study of colistin resistant Gram negative organism in hospitalized patients: a retrospective study. *Indian J Crit Care Med*. 2024;28(3):286-289. doi:10.5005/jp-journals-10071-24658.
14. Rout B, Dash SK, Sahu KK, Behera B, Praharaj I, Otta S. Evaluation of different methods for in vitro susceptibility testing of colistin in carbapenem resistant Gram-negative bacilli. *Access Microbiol*. 2023;5(10):000595.v3. doi:10.1099/acmi.0.000595.v3.