ORIGINAL ARTICLE

Diagnostic accuracy of colistin agar test medium for the detection of colistin resistant gram-negative rods directly from positive blood cultures

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ABSTRACT

Background: Colistin (polymyxin E) is an important treatment choice against infections with multidrug-resistant (MDR) Gram-negative rods (GNRs). The mechanism of its activity is its bactericidal action, which involves disrupting the integrity of the bacterial outer membrane. Resistance is usually caused by changes in the lipid A portion of lipopolysaccharides, which reduce the affinity of colistin for binding, leading to a loss of antimicrobial activity.

Material and Methods: Positive blood culture bottles containing Gram-negative rods after Gram staining were directly inoculated onto CAT medium supplemented with 4 µg/mL colistin. Growth after 18–24 hours of incubation at 37 °C indicated colistin resistance. The outcomes were compared with conventional culture and susceptibility testing (Vitek-2, CLSI 2024). Species that are intrinsically resistant were excluded. Known colistin-resistant and -susceptible bacterial strains were used for quality control.

Results: The CAT medium was 85.13% sensitive, 83.81% specific, and 84.66% diagnostically accurate in identifying colistin-resistant Gram-negative rods. With an ROC AUC of 0.845 (95% CI: 0.795–0.895), it demonstrated good overall diagnostic performance, indicating its reliability, precision, and high discriminatory power compared with conventional methods.

Conclusion: The CAT medium has good diagnostic performance for the direct detection of colistin-resistant Gram-negative rods in positive blood cultures.

Keywords: Colistin, Gram-negative bacteria, Gram-negative rods**.**

BACKGROUND

The rise and transmission of antimicrobial resistance (AMR) is a critical global health concern, and multidrug-resistant Gram-negative bacteria (GNB) are responsible for a significant number of morbidities and deaths, especially in intensive care units.[1] MDR bacteria are estimated to cause over 700,000 fatalities annually worldwide, with projections of up to 10 million deaths by 2050, along with a substantial economic burden.[2] For medical practitioners, the increase in MDR-GNB cases has become a major challenge. Overuse of antibiotics is one of the main factors accelerating the spread of antibiotic resistance, particularly the use of antibiotics without a prescription.[3]

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Colistin, also known as polymyxin E, is a polycationic antibiotic known for its effectiveness against Enterobacteriaceae and other Gram-negative bacteria. Chromosomal mutations that alter the lipid A component of bacterial lipopolysaccharide and reduce its interaction with colistin through cationic substitutions are the main cause of colistin resistance.[4] Bacteria employ various mechanisms to develop resistance to colistin, such as modifying LPS to decrease its negative charge, overproducing efflux pumps, and forming capsules.[5] Colistin-resistant strains were reported in Europe as early as 2000, with 43% of carbapenemase-producing, carbapenem-resistant *K. pneumoniae* isolates also being colistin-resistant.[6]

In a prospective study conducted in Sindh province, the overall prevalence of colistin resistance in carbapenem-resistant GNR bloodstream infections was 0.96%, 3.8% *in A. baumannii*, and 5.2% in *Klebsiella* spp.[7] Timely and accurate identification of colistin-resistant pathogens is crucial for appropriate antimicrobial management of patients and effective antibiotic stewardship.[8] Traditional techniques such as broth microdilution (BMD) remain the gold standard but are time-consuming and not widely available in resource-limited settings.[9] The colistin agar test (CAT) is a

simpler and more practical method for routine clinical application and was approved by CLSI in 2020.[10]

The objective of this study was to determine the diagnostic accuracy of the colistin agar test medium in detecting colistin-resistant Gram-negative rods directly from positive blood culture bottles, with emphasis on early identification of resistance, prevention of treatment failure, and assessment of the cost-effectiveness and feasibility of the test in resource-constrained laboratories.

MATERIAL AND METHODS

This diagnostic accuracy study was conducted at the Microbiology Department of the Armed Forces Institute of Pathology (AFIP), Rawalpindi, from 1st September 2024 to 28th February 2025, over a period of six months, following approval by the institutional ethical review committee (Approval number: FC-MIC23-08/READ-IRB/24/3647). After a thorough literature review, a sample size of 56 was calculated using the WHO sample size calculator, assuming a sensitivity of 100% and specificity of 85.88% for the colistin agar test, with 10% precision, a 95% confidence level, and an estimated prevalence of colistin resistance of 16%.[11] A maximum number of participants (300 patients) were included in the study using non-probability consecutive sampling.

Blood culture specimens from suspected bacteremia patients of both genders that flagged positive on the BacT/Alert 3D™ automated blood culture system and yielded Gram-negative rods (GNR) on microscopy were included in the study. Blood cultures that flagged positive but showed no organisms on Gram stain, Gram-positive organisms, mixed Gram-positive and Gram-negative organisms, or duplicate samples from the same patient were excluded. Additionally, organisms intrinsically resistant to colistin (*Proteus* spp., *Serratia* spp., *Morganella morganii*, *Providencia* spp., and *Burkholderia cepacia*) were excluded. Species identification was performed using routine biochemical tests and confirmed with the Vitek-2 automated identification system.

Written informed consent was obtained from all participants before enrollment, and confidentiality was maintained throughout the study. All blood culture samples flagged positive by the BacT/Alert 3D™ system were first subjected to Gram staining. A drop of broth from each positive blood culture bottle was aseptically used to prepare a thin smear on a clean glass slide, air-dried, heat-fixed, and Gram-stained using the conventional four-step method. Crystal violet was applied as the primary stain for one minute and rinsed with distilled water, followed by Gram's iodine for one minute as a mordant. Decolorization was done with acetone-alcohol for a few seconds, followed by rinsing. Safranin was used as a counterstain for one minute, then the slide was washed and air-dried. All reagents were commercially prepared, within expiry, and stored as recommended. Internal quality control was performed daily using *Staphylococcus aureus* ATCC 25923 (Gram-positive control) and *Escherichia coli* ATCC 25922 (Gram-negative control). Slides were examined under a 100× oil immersion objective using a bright-field microscope. The presence of GNR was confirmed by observing consistent morphology in multiple fields. Smears were considered positive if organisms were visualized in at least 5–10 fields, with five or more organisms per field, indicating a significant bacterial load and reducing the likelihood of contamination or false positive.

Samples positive for GNR on Gram stain were processed for routine bacterial identification and antimicrobial susceptibility testing, as well as subjected to primary colistin susceptibility testing on colistin agar test (CAT) medium. The CAT medium was prepared in-house by adding 4 µg/mL colistin sulfate to Mueller-Hinton agar, according to CLSI 2024 guidelines. A 10 µL drop of broth from each positive blood culture bottle was directly inoculated onto freshly prepared CAT medium and incubated aerobically at 37 °C for 18–24 hours. Results were recorded based on the presence or absence of bacterial growth after 24 hours. Growth indicated colistin-resistant GNR (CR-GNR), whereas the growth of colistin-susceptible GNR was inhibited.[12]

Quality control was performed using *Escherichia coli* NCTC 13846 (colistin-resistant control strain) and *Escherichia coli* ATCC 25922 (colistin-susceptible control strain).

Simultaneously, the positive blood culture bottles were processed using the in-house culture and sensitivity protocol, including subculture on blood agar and MacConkey agar (Condalab, Spain) followed by aerobic incubation at 37 °C for 18–24 hours. Colonies were identified using Gram staining, biochemical tests (oxidase, motility, etc.), and final confirmation on the Vitek-2 automated identification system. Colistin susceptibility of the isolates was tested using CAT medium prepared as per CLSI 2024 guidelines. A 10 µL

inoculum from a 1/10 dilution of a 0.5 McFarland suspension of each isolate was inoculated onto CAT medium and incubated at 37 °C for 24 hours. Interpretation followed CLSI 2024 guidelines: isolates with MIC ≤ 2 µg/mL were considered susceptible, while those with MIC ≥ 4 µg/mL were considered resistant. The presence of growth on CAT medium confirmed CR-GNR, whereas absence of growth indicated susceptibility. Quality control was performed in parallel using *E. coli* NCTC 13846 and *E. coli* ATCC 25922 to validate CAT medium performance.

The diagnostic performance of CAT medium was then compared with the conventional blood culture and susceptibility testing method for detection of CR-GNR. Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 26.0. Normality of data was assessed using the Shapiro–Wilk test. Qualitative variables such as gender and colistin susceptibility were expressed as frequencies and percentages, while median and interquartile range (IQR) were calculated for quantitative variables such as age. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall diagnostic accuracy of CAT medium were computed against the reference method. Receiver operating characteristic (ROC) curves were plotted and the area under the curve (AUC) calculated. A p-value < 0.05 was considered statistically significant.

RESULTS

A total of 300 participants were included in this study. Of these, 181 (60.3%) were male with a median age of 28.00 years (IQR: 16.00–45.50), and 119 (39.7%) were female with a median age of 40.00 years (IQR: 22.00–60.00). The demographic and microbiological characteristics of the study population are summarized in Table-I. The distribution of Gram-negative bacterial isolates is shown in Figure-I.

Table-II presents the diagnostic performance of the CAT medium compared with the standard blood culture sensitivity method. The CAT medium demonstrated a sensitivity of 85.13%, specificity of 83.81%, positive predictive value (PPV) of 90.71%, negative predictive value (NPV) of 75.21%, and an overall diagnostic accuracy of 84.66%. The p-value of <0.001 indicates a statistically significant difference in diagnostic performance between the two methods.

The Receiver Operating Characteristic (ROC) curve for the CAT medium in comparison with the standard blood culture sensitivity method is shown in Figure 2. The area under the ROC curve (AUC) was 0.845 (95% CI: 0.795–0.895), indicating good diagnostic performance of the CAT medium.

Table-I: Demographic and Microbiological Characteristics of Study Participants.

Variables		Median (IQR)
Age (Years)		32.00 (17.00-56.00)
		n (%)
Gender	Male	181 (60.3%)
	Female	119 (39.7%)
CAT medium	Positive	183 (61.0%)
	Negative	117 (39.0%)
Blood C/S	Positive	195 (65.0%)
	Negative	105 (35.0%)

Table-II: Diagnostic performance of CAT medium compared with standard blood culture sensitivity method.

CAT Medium	Standard Blood C/S Method		p-Value
	Positive	Negative	
Positive	166 (TP)	17 (FP)	<0.001
Negative	29 (FN)	88 (TN)	

Sensitivity= TP/(TP+FN)= 166/(166+29)*100=85.13 %, **Specificity=** TN/(TN+FP)= 88/(88+17)*100=83.81%,
Positive Predictive Value= TP/(TP+FP)*100= 166/(166+17)= 90.71%, **Negative Predictive Value=** TN/(TN+FN)*100=88/(88+29)= 75.21%,
Diagnostic Accuracy=(TP+TN)/All patients*100 = (166+88)/300=84.66%

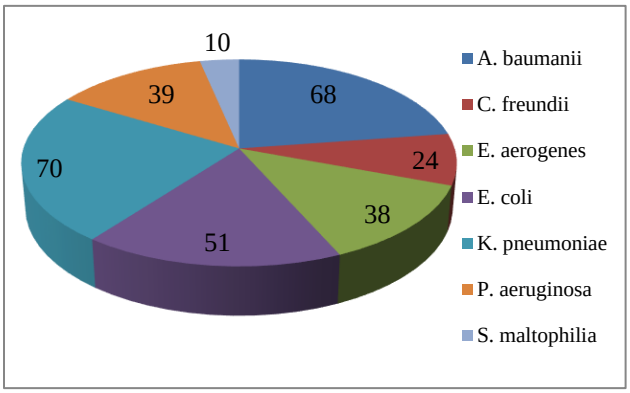

Figure-I: Frequency of Gram-negative bacterial isolates in study population (n=300)

DISCUSSION

This study evaluated the diagnostic accuracy of the Colistin Agar Test (CAT) medium for detecting colistin-resistant Gram-negative rods (CR-GNR) directly from positive blood culture bottles. The CAT medium demonstrated good diagnostic performance, with a sensitivity of 85.13%, specificity of 83.81%, positive predictive value (PPV) of 90.71%, and negative predictive value (NPV) of 75.21%. The overall diagnostic accuracy was 84.66%. The area under the ROC curve (AUC) was 0.845 (95% CI: 0.795–0.895), indicating strong discriminative ability. These findings support the reliability of CAT medium for timely identification of colistin-resistant Gram-negative infections. Our results are consistent with those of Kazaz et al. (2025) in *BMC Microbiology*, who reported sensitivity and specificity in the mid-to-high 80s for CAT compared with broth microdilution in *Klebsiella pneumoniae* isolates.[14] In contrast, Abu-El-Azayem et al. (2023) reported 100% sensitivity but reduced specificity (85.9%) and a very low PPV (25%) using agar dilution, suggesting an overestimation of colistin resistance compared to standard methods.[12] Similarly, Furqan et al. (2022) from Army Medical College, Rawalpindi, observed high agreement between CAT and the gold-standard broth microdilution method when assessing colistin susceptibility in carbapenem-resistant Enterobacterales.[15]

In our study, 300 participants were included, with a predominance of males (60.3%) and a median age of 32 years, representing a broad adult population. The prevalence of colistin resistance was high: CAT identified it in 61.0% of cases, while the standard method identified it in 65.0%. This is considerably higher than the 15% resistance rate reported by Alhamwi and Oksuz (2022) using broth microdilution.[16] Furthermore, a meta-analysis by Uzairu et al. (2022) reported colistin resistance rates of 12.9% in *Klebsiella pneumoniae* bloodstream infections in Pakistan and 48% in *Acinetobacter baumannii* globally, suggesting significant regional variation.[17]

The high PPV (90.71%) observed in our study indicates that a positive CAT result strongly correlates with true colistin resistance, making CAT a reliable first-line tool for rapid confirmation of resistance. However, the NPV (75.21%) was relatively lower, suggesting a moderate risk of false-negative results. Our PPV compares favorably with other agar-based methods. For instance, a study assessing CHROMID® Colistin R agar reported a PPV of 85.5% and an NPV of 98.9%, demonstrating better rule-out performance but slightly lower confirmation ability compared to our findings.[18]

The most common Gram-negative isolates identified in our study were *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*. This distribution is consistent with previous findings by Rana et al. (2025), who reported these species as the most frequent pathogens in bloodstream infections. Similarly, the WHO Global Antimicrobial Resistance Surveillance System (GLASS) 2022 report highlighted these pathogens as the leading causes of bloodstream infections worldwide. The rising resistance of these organisms to last-resort antibiotics such as colistin underscores the urgent need for rapid, reliable, and cost-effective diagnostic tools like CAT.[20]

Although CAT demonstrated good PPV, its relatively lower NPV necessitates careful interpretation of negative results, particularly in critically ill patients. In such cases, confirmatory testing using broth microdilution or other reference methods is recommended to avoid missed diagnoses of colistin resistance. Nevertheless, CAT remains a low-cost, easy-to-perform, and rapid screening method, making it well-suited for resource-limited laboratories.

CONCLUSION

This study concludes that the Colistin Agar Test (CAT) medium demonstrates high diagnostic sensitivity and specificity for the direct detection of colistin-resistant Gram-negative rods from positive blood cultures. Its high sensitivity, specificity, overall accuracy, and statistically significant AUC indicate that CAT is a reliable tool for the rapid identification of colistin-resistant strains. This can play a crucial role in the timely

and appropriate management of infections caused by colistin-resistant Gram-negative bacteria.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Aleena Hussain Rana: Study conception, acquisition, analysis and interpretation of data, manuscript drafting, final approval, accountable for all aspects of publication.

Sakeenah Hussain Naqvi: Study conception, critical revision, manuscript drafting, final approval, accountable for all aspects of publication.

Syed Adeel Hussain Gardezi: Interpretation of data, manuscript drafting, final approval, accountable for all aspects of publication.

Raja Kamran Afzal: Data analysis, results validation, manuscript review, final approval, accountable for all aspects of publication.

Farooq Ahmad: Critical revisions, data verification, final approval, accountable for all aspects of publication.

Aamir Hussain: Intellectual input, final review, final approval, accountable for all aspects of publication.

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