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ORIGINAL ARTICLE

Diagnostic accuracy of CHROMagar™ COL-*APSE* medium for the detection of colistin resistant Gram- negative rods directly from positive blood cultures

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ABSTRACT

Background: A last-resort antibiotic for multidrug-resistant (MDR) Gram-negative rod (GNR) infections is colistin (polymyxin E). It causes cell lysis in Gram-negative bacteria by rupturing their outer membrane. The objective of this study is to determine the diagnostic accuracy of CHROMagar™ COL-*APSE* medium for the detection of colistin resistant Gram-negative rods by direct inoculation from positive blood cultures, keeping the standard blood culture and sensitivity testing method as the reference standard.

Material and Methods: This clinical diagnostic accuracy study was conducted at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi from September 2024 to March 2025. The ability COL-*APSE* to directly identify colistin-resistant Gram-negative rods from positive blood cultures was evaluated. Following inoculation, samples were cultured for 18 to 24 hours at 37°C, and the outcomes were compared to CLSI 2024 criteria. Analysis of colony development and morphology showed how well the medium performed as a diagnostic tool in comparison to conventional blood culture and sensitivity testing techniques.

Results: When it came to identifying colistin-resistant Gram-negative rods, the COL-*APSE* medium showed 82.86% sensitivity, 52.99% specificity, and 69.26% diagnostic accuracy. Although it was able to detect, its ROC AUC of 0.679 (95% CI: 0.612–0.746) indicated a modest level of overall diagnostic performance but with low precision and limited identification power.

Conclusion: CHROMagar™ COL-*APSE* medium demonstrates restricted validity for direct detection of colistin-resistant Gram-negative rods from blood culture samples because of specific diagnostic optimization constraints.

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

BACKGROUND

Colistin (polymyxin E) is a last-resort antibiotic used to combat infections with multidrug-resistant (MDR) Gram Negative Rods (GNR). Colistin kills Gram-negative bacteria by permeabilizing the outer membrane, causing cell lysis. Resistance to colistin generally develops via outer membrane modifications leading to a reduced binding affinity for colistin.[1] It was found that the prevalence of colistin resistance in carbapenem resistant *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* blood isolates in an academic institution in Oman were 24.2% and 4.8% respectively.[2] A high prevalence of colistin resistance in Enterobacterales has been reported with rates reaching 22.2% in China.[3] A study conducted in Karachi, Pakistan, reported a 16% prevalence of colistin resistance, while a 5.8% prevalence was identified in Rawalpindi among carbapenem-resistant Enterobacterales causing bloodstream infections.[4-5] This highlights the growing concern of colistin resistance in clinical settings. Infections due to colistin resistant gram-negative rods (CR-GNR) have restricted treatment options which are associated with adverse clinical outcomes.[6]

Timely and precise identification of colistin resistant pathogens is important for proper antimicrobial management of patients and correct antibiotic stewardship practice.[7] The CLSI 2024 recommends broth microdilution, broth disk elution and agar dilution-MIC methods for the detection of CR-GNR which requires a lot of time and consequently delays the initiation of suitable treatment. CHROMagar™ COL-

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APSE is a better method of detection than traditional methods in terms of its chromogenic media system that allows in order to quickly identify pathogens resistant to colistin from positive blood cultures.[8] Optimizing the medium allows researchers to differentiate colistin resistant bacteria by using selective agents and chromogenic substrates, making laboratory procedure easier and shortening the diagnostic periods.[9-10]

The purpose of this study is to evaluate the diagnostic accuracy of COL- *APSE* medium for the detection of CR-GNR by direct inoculation from positive blood cultures, keeping the standard blood culture and sensitivity testing method as the reference standard.

MATERIAL AND METHODS

This clinical diagnostic accuracy study was conducted at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi from September 2024 to March 2025 following approval by the institutional ethical review vide letter number FC-MIC23-08/READ-IRB/24/3648. After a thorough literature search, we calculated a sample size of 257 using the WHO calculator, keeping sensitivity 82.05%, specificity 66.67%[11] precision 10%, confidence level at 95% and prevalence of colistin resistance at 22.2% respectively.[12]

Blood culture specimens from suspected bacteremia patients of both genders which are flagged positive by the BacT/Alert 3D™ automatic blood culture system yielding gram negative rods (GNR) on microscopy were included in the study. To confirm that the GNR were true pathogens, isolates were correlated with clinical findings and only non-duplicate, clinically significant samples were selected. Additionally, confirmation was done by subculturing onto appropriate medias (eg: Blood agar and MacConkey agar) followed by biochemical identification. Quality control procedures including control strains and equipment checks were followed to ensure reliable results.

BacT /Alert 3D™ flagged positive blood cultures yielding no organism on gram stain, gram-positive organisms on gram stain, mixed gram-positive and gram-negative organisms on gram stain and duplicate sample from same patient were all excluded from the study.

All participants provided written agreement before the enrolment and their confidentiality was maintained at all levels. All sets of positive blood cultures recovered during the study period from nonduplicate patients that are flagged positive by the BacT/Alert 3D™ automated blood culture system (bioMérieux) were selected. Aseptically, a drop of broth from each positive blood culture bottle was used to prepare a thin smear on a clean glass slide which was then air dried, heat fixed and subjected to gram staining by using the conventional four-step method. Crystal violet was applied as the primary stain for one minute and rinsed with distilled water. This was followed by Gram's iodine, applied for one minute to act as a mordant and again rinsed. Decolorization was carried out using acetone-alcohol for few seconds then rinsed. The smear was covered with Safranin for one minute, then washed and air dried. All reagents used were commercially prepared, within expiry and stored under recommended conditions. To ensure staining accuracy and reliability, internal quality control was performed daily using reference strains of gram-positive bacteria (*Staphylococcus aureus* ATCC25923) and gram-negative bacteria (*Escherichia coli* ATCC25922). Slides were then examined under 100X oil immersion lens using a bright field microscope. The presence of GNR was confirmed based on consistent morphology observed in multiple fields. Smears were considered positive for GNR if organisms were visualized in at least 5 to 10 fields with a typical count of five or more organisms per field, indicating a significant bacterial load and minimizing the possibility of contamination or false positives. All the samples found to have GNR on gram staining were processed further for routine bacterial identification and sensitivity testing as well as were subjected to primary colistin susceptibility testing on COL- *APSE* medium. One drop (10 µl) of specimen from the positive blood culture bottles was directly plated onto the freshly prepared COL- *APSE* medium agar plate and incubated at 37°C under aerobic conditions for a duration of 18–24 hours. The results were recorded by observing different colored colonies using the manufacturer's interpretation criteria for identifying CR-GNR i.e. colistin-resistant *E. coli* appeared as dark-pink to reddish, colistin resistant coliforms as metallic blue, colistin resistant Pseudomonas spp as translucent or natural pigmentation cream to green, colistin resistant Acinetobacter spp as cream or opaque colonies and other gram-negative colistin-resistant species as colorless colonies. The growth of colistin sensitive GNR in this medium was inhibited.[11] Quality control was performed using

Escherichia coli NCTC 13846 as colistin resistant strain and *Escherichia coli* ATCC25922 as colistin susceptible strain. The positive blood culture bottles were simultaneously dealt with the inhouse blood culture & sensitivity testing procedure including subculture on Blood agar, MacConkey agar (Condalab, Spain) and incubation at 37°C under aerobic conditions for a duration of 18-24 hours followed by identification of the colonies using gram stain, biochemical tests like oxidase test, motility test etc and final identification using Vitek-2 automated bacterial identification system. Colistin susceptibility of the isolates was tested by colistin agar test (CAT) medium prepared by adding 4µg/ml of colistin sulphate in Mueller Hinton agar as recommended by CLSI guidelines 2024. One drop (10µl) of 1/10 dilution of 0.5 McFarland suspension of the isolate was inoculated on CAT medium and incubated for further 24 hrs at 37°C under aerobic conditions. The results were then recorded by observing the presence and absence of bacterial colonies on CAT medium. The presence of bacterial growth on CAT medium indicated the presence of CR-GNR and absence of growth indicated the absence of CR-GNR in positive blood culture bottle after 48hrs. To ensure accuracy and reproducibility, quality control was performed using *Escherichia coli* NCTC13846 (colistin- resistant control) along with *Escherichia coli* ATCC25922 (colistin-susceptible control). Quality control strains were tested in parallel with clinical isolates to validate the performance of the CAT medium. The performance of COL- *APSE* medium directly from positive blood cultures was then compared to the blood culture and sensitivity testing method for the detection of CR-GNR. The data was analyzed using computer software "Statistical Package for Social Sciences, SPSS version 26.0. Shapiro-Wilk test was used to check the normality of data. Age was found non-normally distributed as p value < 0.05. The qualitative variables like gender, isolates, colistin sensitivity were presented as frequencies and percentages. While median and IQR were calculated for quantitative variables like age. To assess the diagnostic accuracy of the COL-*APSE* medium compared to the gold standard blood culture and sensitivity method sensitivity, specificity, PPV, NPV and accuracy were computed. ROC was also plotted and AUC was calculated. The p-value <0.05 was considered significant for all the analysis.

RESULTS

A total of 257 participants were included in this study. Out of the total, 165 (64.2%) were male with the median age of 30.00 (16.00-55.00) years and 92 (35.8%) were female with the median age of 34.50 (19.25-62.25) years. The gender distribution is shown in Figure -I. The demographic and microbiological characteristics of study population are shown in Table-I. Frequency of gram-negative bacterial isolates in study samples is shown in Figure-II.

Table-II presents the diagnostic performance of COL-*APSE* compared to the standard blood culture method. COL-*APSE* demonstrates a sensitivity of 82.86%, specificity of 52.99%, positive predictive value (PPV) of 67.84%, negative predictive value (NPV) of 72.09%, and an overall diagnostic accuracy of 69.26%. The p-value of <0.001 indicates a statistically significant difference in performance between the two methods.

Receiver Operating Characteristic (ROC) Curve for COL-*APSE* in comparison with standard Blood Culture Method is shown in Figure-3. The area under the ROC curve (AUC) was 0.679 (95% CI: 0.612–0.746), indicating poor diagnostic ability of the COL-*APSE* medium

Table-I: Demographic and microbiological characteristics of study participants (n=257).

Variables		Median, IQR
Age (Years)		32.00 (17.00-56.00)
		n (%)
COL-*APSE*	Positive	171 (66.5%)
	Negative	86 (33.5%)
Blood C/S	Positive	140 (54.5%)
	Negative	117 (45.5%)

Table-II: Diagnostic performance of COL-*APSE* in comparison with standard blood culture sensitivity (n=257).

COL- *APSE*	Standard Blood C/S Method		p-value
	Positive	Negative	
Positive	116 (TP)	55 (FP)	<0.001
Negative	24 (FN)	62 (TN)	

Sensitivity= TP/(TP+FN) = 116/(116+24)*100=82.86 %; **Specificity=** TN/(TN+FP) = 62/(62+55)*100=52.99%; **Positive Predictive Value=** TP/(TP+FP)*100= 116/(116+55)= 67.84%; **Negative Predictive Value=** TN/(TN+FN)*100=62/(62+24)= 72.09%; **Diagnostic Accuracy**=(TP+TN)/All patients*100 = (116+62)/257=69.26%

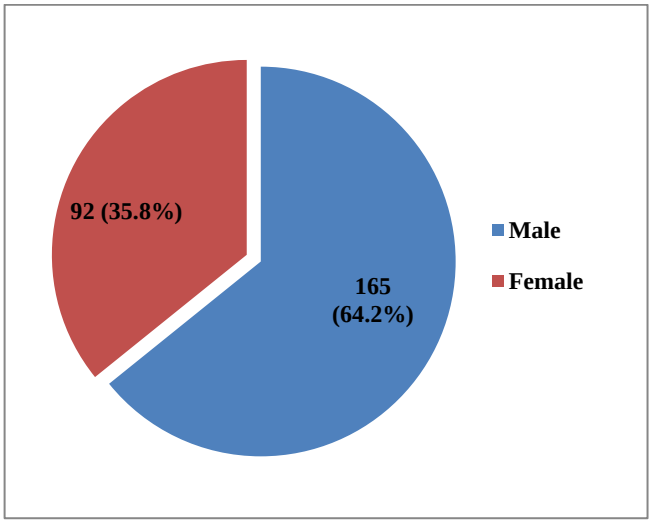

Figure-1: Gender distribution in study population (n=257).

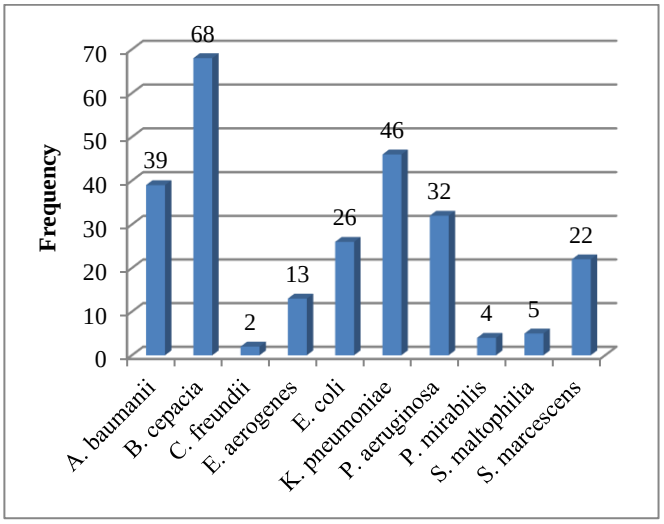

Figure-2: Frequency of gram-negative bacterial isolates in study samples (n=257)

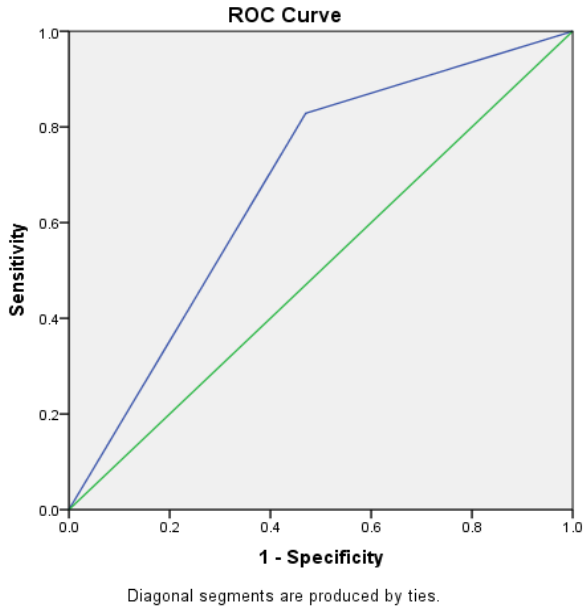

Figure-III: Receiver Operating Characteristic (ROC) curve for COL-*APSE* in comparison with standard blood culture method.

DISCUSSION

The study assessed CHROMagarTM COL-APSE's clinical usefulness in rapidly identifying pathogens in order to detect bloodstream infections. The findings demonstrated a moderate level of performance with a sensitivity of 82.86%, specificity of 52.99%, diagnostic accuracy of 69.26%, and AUC of 0.679. Colistin is vital in treating fatal infections brought on by carbapenem-resistant GNR, thus finding a reliable colistin diagnosis is crucial and could save lives. Still it is challenging to discover a diagnostic tool that can be integrated into the routine of conventional microbiology labs using the time-consuming BMD-recommended colistin sensitivity testing procedure.[13] To directly identify colistin from clinical samples or cultures, several rapid or non-rapid procedures have been developed and proposed; nevertheless, issues with diagnostic performance, TAT, necessary expertise, sample processing cost, etc, still exist.[14] The study evaluated the diagnostic capabilities of COL-*APSE* medium against existing blood culture methods while documenting patient and microbial findings from the population sample. These results enable critical assessment of COL-*APSE* method performance during clinical diagnostics evaluation.

The study enrolled 257 participants who belonged to the male group at 64.2% while females comprised the remaining 35.8%. Males in the study population reached their median age slightly earlier at 30 years as opposed to females who reached 34.5 years.

Clinical data show *B. cepacia* (26.5%) occurring most frequently while *K. pneumoniae* (17.9%) and *A.*

baumannii (15.2%) also appear among patients. The bloodstream infection epidemiology in this population includes two rare microorganisms named *C. freundii* and *P. mirabilis* which represent 0.8% and 1.6% of the total isolates respectively. These results emphasize why efficient diagnostic techniques need improvement to identify different types of microorganisms in patient infections. A study carried out at the Armed Forces Institute of Pathology in Rawalpindi, Pakistan, showed that CHROMagar™ COL-*APSE* accurately detects colistin resistance in multidrug-resistant Gram-negative bacilli. According to the study, the test was able to detect resistant organisms with a sensitivity of 97.96% and specificity of 97.87%.[15]

The COL-*APSE* test detected 82.86% of true positive results which proved its capability to identify accurate positive cases. Medical diagnosis benefits extensively from this high detection capability because it allows prompt accurate medical diagnosis followed by immediate treatment onset. The 52.99% specificity was observed the reason for this disparity might be because, whereas we assessed the media's performance using grown bacteria, the other study used serial dilutions in broth.[11] Sensitivity of Chromagar COL-*APSE* for the spread of colistin-resistant *E. coli* in healthy Vietnamese citizens was 100% in a study by Yoshimasa *et al*., 2019 but specificity was 68%, which is higher than this study.[16] In another study sensitivity and specificity of CHROMagar COL-*APSE* were 82.05% and 66.67% this trend was similar to our study.[17] Our results are in contradiction with the results of another study which found that CHROMagar had 100% specificity and sensitivity.[18]

Thiry et al., 2019 compared CHROM agar COL-*APSE* to CHROMID Colistin_R and agar disk diffusion. They found that growth on the media is associated with resistant disk diffusion findings, but not with the presence of mcr genes.[19] SuperPolymyxin's MCR-1 sensitivity was determined to be 86% (50/58) of MCR-1 positive *E. coli* in another investigation, which is lower than CHROMagar COL-*APSE*. However, additional evaluation tests are required to validate this finding. Super Polymyxin, in particular, suppressed colistin-susceptible *Salmonella spp.* more effectively than the other media. However, there aren't many studies comparing these two media, so more research is needed to determine which media is better at detecting MCR and colistin resistance, particularly to identify hetero resistant strains. But it's clear that the CHROMagar COL-*APSE*'s target spectrum is broader than SuperPolymyxin's.[13]

The chromogenic medium CHROMagar™ COL-*APSE* is influenced by the density of the inoculum. It was shown that CHROMagar™ COL-*APSE* was very sensitive and specific after standardizing the inoculum to 1 x 10^5CFU/ml.[20] Our results are consistent with those of another study that found CHROMagar ™ COL-*APSE* may be utilized in any normal laboratory with an incubator, despite being more expensive than the gold standard. However, it was not appropriate for routine usage in a diagnostic laboratory due to its low diagnostic performance.[11]

CONCLUSION

CHROMagar™ COL-*APSE* medium demonstrates restricted validity for direct detection of colistin-resistant Gram-negative rods from blood culture samples because of specific diagnostic optimization constraints. The evaluation results indicate the possibility of incorrect test outcomes that might cause unwarranted clinical actions. The research underscores the requirement to enhance the medium or establish different diagnostic methods which ensure dependable pathogen resistance detection.

CONFLICT OF INTEREST

None

GRANT SUPPORT & FINANCIAL DISCLOSURE

Declared none

AUTHOR CONTRIBUTION

Aleena Hussain Rana: Idealized and conceptualized the study, manuscript writing, final approval, agreement to be accountable for all aspects of the work

Sakeenah Hussain Naqvi: Subsequently critically reviewed and revised, final approval, agreement to be accountable for all aspects of the work

Raja Kamran Afzal: Statistical analysis, final approval, agreement to be accountable for all aspects of the work

Farooq Ahmad: Critical revisions, final approval, agreement to be accountable for all aspects of the work

Anam Imtiaz: Subsequently critically reviewed, final approval, agreement to be accountable for all aspects of the work

Aamir Hussain: Data collection, proofread, final approval, agreement to be accountable for all aspects of the work

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