Chromagar™ Col-Apse for Detection of Colistin Resistance in Clinical Isolates of Multidrug Resistant Gram Negative Bacilli

Saad Ali, Wajid Hussain, Farooq Ahmed, Raja Kamran Afzal, Irfan Mirza and Umer Khurshid

Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan

ABSTRACT:

Objective: To determine the diagnostic accuracy of CHROMagar™ COL-APSE, for detection of colistin resistance in clinical isolates of multi drug resistant (MDR) gram negative bacilli (GNB).

Study Design: Cross-sectional validation study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan from February 2019 to August 2019.

Methodology: Total 96 MDR-GNB in clinical isolates were included. Isolates were identified using gram stain, catalase, oxidase, API 20E and API 20NE. After taking approval from Institutional Ethical Review Committee, colistin susceptibility was determined simultaneously by CHROMagar™ COL-APSE (using 1×10^5 CFU/ml inoculum) and Broth Micro Dilution (BMD) Minimum Inhibitory Concentration (MIC) method as per CLSI. EUCAST guidelines were followed for interpretation of susceptibility profile. Results were validated with gold standard test, i.e. BMD.

Results: Out of 96 MDR clinical isolates, the distribution was *K. pneumoniae* n=63, *E. coli* n=18, *A. baumannii* n=11, *C. freundii* n=3, and *E. cloacae* n=1. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of CHROMagar™ COL-APSE for detection of colistin resistance, keeping BMD-MIC method as gold standard, was 97.96%, 97.87%, 97.96%, 97.87% and 97.92%, respectively.

Conclusion: CHROMagar™ COL-APSE can be used as screening agar by direct streaking of specimen as well as diagnostic for detection of colistin resistance by the use of standardised inoculum. It can be used as a critical time saving method for colistin resistance detection in absence of expertise or manpower required for BMD as well as the cost required for genetic sequencing to detect MCR genes.

Key Words: Multi drug resistant (MDR), Gram negative bacilli (GNB), Colistin resistance, CHROMagar™ COL-APSE.

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INTRODUCTION

Multi drug resistance (MDR) in bacteria not only poses a threat to patient, but also creates problems for treating physicians, especially in intensive care setups, where due to comorbid various options available for MDR organisms, may not be beneficial.¹ Gram negative bacilli (GNB) are among the common causes of healthcare as well as community acquired infections with a high propensity for antimicrobial resistance.² Alarming, the tap of antibiotic development has run dry with the last major antibiotic class discovery in 1990s.³

On the other hand, resistance to all currently available classes of antibiotics is rising exponentially, which has led to the use of older antibiotics like colistin and fosfomycin. Earlier, they were put out of use due to toxicity and lack of studies, as there were much safer options available. The combined effect of these two phenomena have brought colistin back as a crucial part of management against MDR-GNB and it is now being routinely advised as a last option.⁴ As a consequence, there has been a rapid rise in resistance to colistin as well, especially with the plasmid mediated carbapenem and colistin resistance. It leaves with no other option for management; and remarkably denotes the start of post-antibiotic era.⁵ According to a recent study conducted in Karachi, frequency of colistin resistance is found to be 15.9% in carbapenem resistant *enterobacterales* isolates.⁶

The solution to this crisis lies on accurate diagnostics, wise infectious disease management, and proper infection control measures.

There should be timely identification of colistin-resistant isolates and manage them efficiently.⁷ Due to the lack of

Correspondence to: Dr. Saad Ali, Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan
E-mail: alii.saad@live.com

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studies, a routine, quick and accurate method for colistin susceptibility is yet to be developed. Currently Broth Micro Dilution (BMD) is the recommended method for detection of Minimum Inhibitory Concentration (MIC); but there are issues, like adsorption to plastic and adjustment of cations.⁸ On the other hand, the diffusion of colistin molecule in agar is yet to be studied, due to which disk diffusion is not recommended method.⁹ BMD, apart from its limitations, is a long and critical procedure which requires expertise, long time procedure itself, as well as incubation with a difficult interpretation at the end, which may at times lead to false positive or negative due to observer error.¹⁰

Indicators have been used in agar medium for a long time; but in recent years, chromogenic media has provided a better and rapid approach in identifying as well as differentiating organisms on the basis of growth on agar.¹¹

CHROMagar™ COL-APSE was introduced in 2017 for the surveillance of colistin resistant bacterial pathogens with a lower limit of detection of 10 CFU/ml.¹ It yields different colored colonies specific to species. It is a very helpful aid for diagnosing critical colistin resistance by reducing time to detection with minimal expertise.

The aim of this study was to determine the diagnostic accuracy of CHROMagar™ COL-APSE for identification of colistin resistant GNB to help in early prevention of treatment failures in critical patients by rapid detection of colistin resistance. This will add to the ease of performance and cost effectiveness especially in resource limited setups of labs.

METHODOLOGY

This cross-sectional validation study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi from February 2019 to August 2019. Sample size of 96 was calculated, using sensitivity specificity calculator keeping prevalence of colistin resistance at 15.9%.^{6,12} Non-probability consecutive sampling was done. Permission of this study was obtained from Institutional Ethical Review Board.

To evaluate the diagnostic accuracy of the CHROMagar™ COL-APSE, inclusion criteria was set as MDR organisms having resistance to single agent of three or more antimicrobial classes.¹³ Same isolate from duplicate samples of same patients were excluded. These isolates were identified on the basis of colony morphology, gram stain, catalase, oxidase, API 20E, and API 20NE (BioMérieux).

To determine colistin MICs for these isolates, reference BMD method in Cation-Adjusted Mueller Hinton broth (CAMB) as recommended by Clinical Laboratory Standard Institute (CLSI), 2019 guidelines was used.^{14,15} Isolates with colistin MICs ≤ 2 µg/mL were categorised as sensitive, and those with MICs ≥ 4 µg/mL as resistant as per EUCAST guidelines (2019).¹⁶ For BMD, 96 well-microtiter plates were inoculated with 10 isolates and 2 controls, both positive and negative for colistin resistance, on

each plate. Each plate had sterility and growth controls as well. Colistin Sulfate powder (Sigma-Aldrich) was used to make antimicrobial solution of 32 µg/ml concentration, which was diluted two-folds over 6 wells from 16 µg/ml to 0.5 µg/ml. Each well was inoculated with 0.05 ml of antimicrobial agent except growth control well. Isolate inoculum was prepared to a final density of 5×10^5 CFU/ml and 0.05 ml was dispensed in each well except sterility well. This inoculum was also applied on a quadrant of blood agar and MacConkey agar to rule out any contamination during this procedure.

CHROMagar™ COL-APSE plates were prepared, according to manufacturer's instructions, and stored at 2-8°C. These plates were allowed to warm at room temperature before inoculation. Selected MDR isolates were processed by direct streaking on the plates. The plates were incubated in aerobic conditions at 37°C for 18-24 hours. Any growth was considered as positive (Colistin resistant isolate) and no growth as negative (Colistin sensitive isolate). Initially on application of 0.5 McFarland standard inoculum (3×10^8 CFU/ml), it was observed that colistin susceptible isolates were able to grow on CHROMagar™ COL-APSE (Figure 1a).

Figure 1: (a) Before standardising inoculum (b) After standardising inoculum.

Inoculum was diluted to a density of 1×10^5 CFU/ml, following the principle of agar dilution as per CLSI guidelines, which led to growth concordant with results of BMD (Figure 1b). So for, in whole study a standard density inoculum of 1×10^5 CFU/ml was used.

The data obtained was entered in SPSS (version 25). Diagnostic parameters were calculated by analysis of descriptive data using bivariate cross tabulation and significance was ascertained by chi square test and Pearson equation.

RESULTS

Out of 96 isolates, the distribution was given in Figure 2. CHROMagar™ COL-APSE was able to identify all colistin resistant isolates as per product manual. Among these isolates, colistin resistance was found in 76% of *K. pneumoniae* while 0.9% in *A. baumannii*. Isolates of *E. Coli*, *C. freundii* and *E. cloacae* were all found to be sensitive to colistin in this study.

All the isolates were subjected to CHROMagar™ COL-APSE test and 48 were found to be true positive while one was false positive. Among 47, CHROMagar™ COL-APSE negative patients, one

(false negative) had colistin resistance on BMD method whereas 46 (true negative) had no colistin resistance on BMD method.

Figure 2: Distribution of clinical isolates.

Overall sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of CHROMagar™ COL-APSE, for detection of Colistin resistance in clinical isolates of multidrug resistant GNB, keeping BMD method as gold standard was 97.96%, 97.87%, 97.96%, 97.87% and 97.92%, respectively.

DISCUSSION

Since the resistance against colistin is at a rise, it is essential to formulate a method which is accurate as well as easy to perform for prompt diagnosis and management of such resistant bacteria.¹⁷ Chromogenic media has the ability to differentiate multiple species on the basis of colors simultaneously which could not be possible with indicators.

CHROMagar™ COL-APSE is a chromogenic media that is affected by the inoculum density. This was noted in this study as well, that on standardising the inoculum to 1×10^5 CFU/ml CHROMagar™ COL-APSE was highly sensitive and specific.

In a study conducted by Yoshimasa *et al.*, for dissemination colistin resistant *E. coli* in healthy residents of Vietnam sensitivity of CHROMagar™ COL-APSE was found to be 100%; however, specificity was low as compared to this study at 68%.¹⁸ This could be probably due to issue related to inoculum density as in this study all growth was taken as colistin resistant.

Momin *et al.* conducted a study for evaluation of CHROMagar™ COL-APSE for isolation and differentiation of GNB. In this study, inoculum was standardised to 10^5 CFU/ml and further 10 UL drop was inoculated on CHROMagar™ COL-APSE, which yielded sensitivity and specificity of 100%.¹⁹ It is comparable to the present study as with similar inoculum density similar results were achieved.

CHROMID® colistin R agar is another chromogenic media that has been available for detection of colistin resistant *enterobacteriales*; but its evaluation yielded a sensitivity of 88.1%, lower than that of CHROMagar COL-APSE™ that is 97.96% in this study; but with a higher specificity of 100%.²⁰

However, the limitations of CHROMID® colistin R agar are that it needs pre enrichment of 4-5 hours and is recommended to be used in stool and rectal swab specimens.

CONCLUSION

The diagnostic accuracy of CHROMagar™ COL-APSE, for detection of colistin resistance in clinical isolates of multidrug resistant, GNB is excellent. It can be used as diagnostic tool by inoculating standardised density of organism.

ETHICAL APPROVAL:

This study was approved by the Institutional Review Board of AFIP, Rawalpindi, Pakistan.

PATIENTS' CONSENTS:

Informed consents were taken from all the participants, whom samples were included in the study.

CONFLICT OF INTEREST:

The authors declared no conflict of interest.

AUTHORS' CONTRIBUTION:

SA: Microbiology research work, patient data and sample collection, article writing, statistics.

WH: Supervision of project, article writing, review of article.

FA: Data collection, microbiology work, article writing.

RKA: Article review, discussion writing.

IAM: Discussion review, article review.

UK: Statistics, variable correlation, review article.

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