ORIGINAL ARTICLE

Rapid Screening for Colistin Resistant Bacteria by Chromogenic Agar

[1]Mohammed Elshaer*, [2]Lyna Zekri, [1]Ahmed Elewa, [3]Yasmin Nabiel, [4]Ahmed Elghrieb, [1]Heba Elshahawy

[1]Clinical Pathology Department, Faculty of Medicine, Mansoura University, Egypt
[2]M.B.B. Ch. Faculty of Medicine, Mansoura University, Egypt
[3]Microbiology Department, Faculty of Medicine, Mansoura University, Egypt
[4]General Surgery Department, Faculty of Medicine, Mansoura University, Egypt

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Corresponding Author:
Mohammed Elshaer. Department of Clinical Pathology, Faculty of Medicine, Mansoura University, Mansoura, Egypt
Tel.: +201020480202
Melshaer85@mans.edu.eg.
ORCID: 0000-0002-0179-2869

ABSTRACT

Background*: The growing incidence of infections caused by Colistin resistant gram-negative bacteria is seen as a serious public health concern. The CHROMID Colistin R agar is the first selective chromogenic agar to be validated for the screening of Colistin resistant microorganisms in clinical samples.* ***Objective:*** *The aim of the study was to assess the diagnostic performance of CHROMID Colistin R agar in detection of Colistin resistant gram-negative bacteria based on the different color of colonies.* ***Methodology:*** *This study was carried out between January 2020 and January 2021. Clinical samples were isolated from patients admitted to Mansoura university hospitals with different signs and symptoms of infection. The colony morphology, Gram stain, biochemical reactions, and Vitek-2 System were used to identify isolated colonies. Antibiotic susceptibility testing was performed by Vitek-2 System. Colistin resistant bacteria were further sub-cultured on CHROMID Colistin R Agar.* ***Results:*** *Out of 4800 collected samples, bacterial growth was detected in 15%. The colistin-resistant strains were collected from 51 patients. The most frequently isolated organisms were Acinetobacter baumanii (29.4%) followed by Pseudomonas (21.6%), Klebsiella pneumoniae (21.6%), and Escherichia coli (19.6%). There was a very good agreement between the two techniques in the detection of Colistin resistant Gram-negative bacteria (Kappa= 0.921, P-Value = 0.001). CHROMID Colistin R Agar had an overall sensitivity of 92.2%, 100% specificity, 100% PPV, and 99.9 % NPV.* ***Conclusion:*** *We concluded that CHROMID Colistin R agar is a reliable culture medium that can be used effectively for rapid screening of Colistin resistant Gram-negative bacteria.*

INTRODUCTION

The growing incidence of infections caused by Colistin-resistant Gram-negative bacteria is seen as a serious public health concern [1]. This is mainly caused by the increased use of Colistin in the recent years [2]. Polymyxins, such as Colistin, can bind lipopolysaccharides and disrupt the outer membrane; however, their toxicity limits their use [3]. Aside from the intrinsic and chromosomal mutation-derived mechanisms of Colistin resistance, a horizontally transferable plasmid-borne Colistin resistance MCR-1 gene has recently been identified in human [4].

Several techniques for detecting Colistin resistance have developed in recent years directly from samples and colonies, such as culture-based methods like Super Polymyxin medium and CHROMagar [5-6]. In addition to molecular testing such as loop-mediated isothermal amplification. Rapid Polymyxin NP test or a microarray method including the CT103XL array, can also confirm resistance from colonies [7].

Chromogenic media are culture media used to isolate and identify certain microorganisms from a heterogeneous population. The medium contains chromogenic substrate, which the microorganisms use to produce colorful colonies that are unique to each organism. The presence or absence of microorganisms is identified and reliably distinguished from others based on the color of the colony [8].

The CHROMID Colistin R agar is the first selective chromogenic agar to be validated for the screening of Colistin-resistant microorganisms in clinical samples within 18-24 hours which can provide targeted, adjusted, and specific therapies to improve the patient outcome, while limiting the spread of antibiotic resistance globally [9]. The present study was done to assess the diagnostic performance of CHROMID Colistin R agar in detection of Colistin resistant gram-negative bacteria based on the different color of colonies.

METHODOLOGY

A prospective study was carried out between January 2020 and January 2021. Clinical samples were isolated from patients admitted to Mansoura university hospitals with different signs and symptoms of infection.

Ethical Consideration

This study was approved by Mansoura University's Institutional review board. Informed consents were obtained from all participants prior to their inclusion, code number MS 19.01.451.R1.R1 in July 2019.

Collection of Clinical Samples

Samples were collected before antibiotic administration. If the patient was on antibiotic therapy, they were discontinued 48 hours before sample collection. The samples were collected under strict aseptic conditions.

Processing of Clinical Samples

Culture:

Samples were cultured on their appropriate culture media and incubated at 37°C for 48 hours. The colony morphology, Gram stain, biochemical reactions, and Vitek-2 System were used to identify isolated colonies. Vitek GN ID cards are based on established biochemical tests and newly developed substrates measuring carbon source utilization, enzymatic activities, and resistance. The cards were automatically filled with the standardized suspension, sealed, and incubated at 35.5ºC, and optical density was measured by the device every 15 minutes. Final results were analyzed and reported by Vitek 2 software within 18 hours. Antibiotic susceptibility testing was also done by Vitek-2 System using GN AST-222 cards. Isolates with a Colistin MIC ≤2 mg/L were reported to be susceptible whereas those with a Colistin MIC of ≥4 mg/L were reported to be resistant. The colistin-resistant bacteria were further sub-cultured on CHROMID Colistin R agar *(Biomérieux, Marcy-l'Étoile, France).*

The CHROMID Colistin R agar is composed of a nutritive base that includes a variety of peptones, chromogenic substrates, and a selective mixture that allows for the distinction of Colistin resistant and Colistin susceptible bacteria. It prevents the growth of most Gram-positive bacteria, as well as yeast and molds. The use of chromogenic substrates allows for the identification of the targeted organisms based on their colony color:

- *Escherichia coli*: range in color from pink to burgundy.
- *Klebsiella pneumoniae* and *Enterobacter spp*: blue-green colonies.
- *Salmonella spp, Acinetobacter baumannii* and *Pseudomonas*: white to colorless colonies.

Statistical analysis

All collected data were statistically analyzed using SPSS Statistics version 21.0 using appropriate statistical significance test. Kappa coefficient was run to assess the agreement between the chromogenic agar and the Vitek-2 system. The degree of agreement was very good if the Kappa value was between 0.81- 1.00, good if it was 0.61- 0.8, moderate if it was 0.41- 0.6, fair if it was 0.2 - 0.4 and poor if it was <0.2.

RESULTS

Out of 4800 collected samples, bacterial growth was detected in 15% (720 samples). The Gram's stain was used to classify isolated colonies into gram-negative bacteria (90%) Gram-positive bacteria (10%). Based on their biochemical reactions, colony morphology and with the help of Vitek-2 system, all isolates were identified to the species level and further subdivided into Colistin-sensitive and Colistin-resistant strains.

The Colistin-resistant strains were collected from 51 patients: 39 males and 12 females with median age of 55 years with an incidence of 1.1%. Forty samples were obtained from drains, (78.4%), 4 sputum samples (7.8%), 4 wound swabs (7.8%), and 3 urine samples (5.9%). The most frequently isolated organisms were *Acinetobacter baumanii* (29.4%) followed by *Pseudomonas* (21.6%), *Klebsiella pneumoniae* (21.6%), and *Escherichia coli* (19.6%).

In four samples, there was no growth after culture on chromogenic agar (7.8%). In terms of color, four samples showed no color, 11 sample showed green color 10 sample showed pink color and 26 sample showed white color**.**

Fourteen out of 18 samples previously identified as *Acinetobacter baumanii* by the Vitek-2 system, (77.8%) were confirmed by chromogenic agar (Color: white) (**Figure 1**), three out of the remaining four samples were identified as *Klebsiella pneumoniae* (16.7%) (green colored colonies instead of white colonies), with only one sample showed no growth (5.6 %). All *Escherichia coli* isolates identified by automated system gave a pink color when cultured on chromogenic media. Eight out of 11 *Klebsiella pneumoniae* samples were confirmed by chromogenic agar (72.7%) (Color: green) (**Figure 2**), one sample was identified as *Acinetobacter baumanii*, one sample was identified as *Pseudomonas* (9.1 % for each) whereas the remaining one exhibited no growth. Regarding *Pseudomonas* samples, 10 (83.3 %) of them were confirmed by chromogenic agar (Color: white) while the other two showed no growth (**Table 1).**

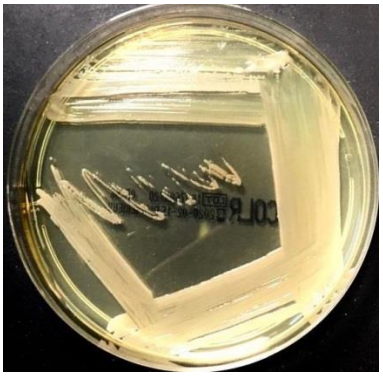

Fig. 1: White colonies of *Acinetobacter baumanii* on CHROMID Colistin R agar.

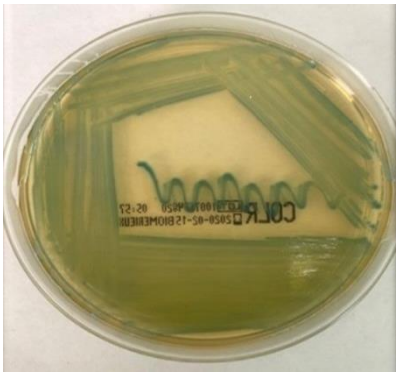

Fig. 2: Green colonies of *Klebsiella pneumonia* on CHROMID Colistin R agar.

Table 1: Identification of colistin resistant isolates by chromogenic agar compared to Vitek 2 system

Growth on chromogenic agar	Vitek 2 identification				
	Acinetobacter baumanii (n=18)	E. coli (n=10)	Klebsiella pneumoniae (n=11)	Pseudomonas aeruginosa (n=12)	**Total**
No growth	1	0	1	2	4
	5.6%	0.0%	9.1%	16.7%	(7.8%)
Acinetobacter baumannii **(white colonies)**	14	0	1	0	15
	77.8%	0.0%	9.1%	0.0%	(29.4%)
E. coli **(pink colonies)**	0	10	0	0	10
	0.0%	100.0%	0.0%	0.0%	(19.6%)
Klebsiella pneumoniae **(green colonies)**	3	0	8	0	11
	16.7%	0.0%	72.7%	0.0%	(21.6%)
Pseudomonas aeruginosa **(white colonies)**	0	0	1	10	11
	0.0%	0.0%	9.1%	83.3%	(21.6%)
Total	18 (35.3%)	10 (19.6%)	11 (21.6%)	12 (23.5%)	51

There was a very good agreement between the chromogenic agar and Vitek 2 system in the detection of Colistin resistant Gram-negative bacteria (Kappa= 0.921, P-Value = 0.001). CHROMID Colistin R Agar had an overall sensitivity of 92.2%, 100% specificity, 100% PPV and 99.9 % NPV **(Table 2).**

Table 2: The agreement between chromogenic agar and Vitek 2 system in the detection of colistin resistant bacteria

Chromogenic agar	**Automated identification by Vitek 2**		**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Kappa Value**
	Colistin-resistant (n=51)	Colistin-sensitive (n=51)					
Colistin-resistant	47 (92.2%)	0 (0%)	92.7%	100%	100%	99.9%	0.921
Colistin-sensitive	4 (7.8%)	51 (100%)					

P-Value = 0.001; **P** < 0.05**:** significant**; P** < 0.01: highly significant**.**
Kappa-Value: Strength of agreement Poor <0.2 Fair: 0.2-0.4 Moderate: 0.41-0.6 Good: 0.61-0.8 Very good: 0.81-1.0.

DISCUSSION

The spread of antibiotics resistance to a wide variety of antibiotics such as Beta-lactams, Aminoglycosides and Carbapenems is a major concern for health-care systems [10]. The problem of antimicrobial resistance is likely to be overshadowed by the ongoing COVID-19 pandemic for a while. Despite the World Health Organization's recommendations against its use, there are many reports of antibiotic overuse while treating COVID-19 patients, with up to 45 percent of patients on antibiotic therapy [11-13].

Due to reported neurotoxicity and nephrotoxicity, Colistin has been deemed as the antibiotic of last resort for treatment of multi-drug resistance (MDR) bacteria[14]. Colistin resistance is the result of increased therapeutic use of Colistin, particularly in countries with high prevalence of Carbapenem-resistant Gram-negative bacilli [15]

Disc susceptibility testing methods are inexpensive and may be utilized as first screening tools in low-

income settings. However, some of the polymyxins' intrinsic characteristics make agar-based disc susceptibility testing challenging [16]. Polymyxins diffuse poorly on agar, resulting in narrow inhibitory zones. Consequently, categorical distinction of susceptible and resistant isolates is difficult. Using higher concentrations of Colistin in the discs does not seem to enhance test accuracy [17]. Therefore, in the current study, Vitek-2 system was used to identify all isolates to the species level and further categorize them into Colistin-sensitive or Colistin-resistant.

Previous studies have reported that the percentage of Colistin resistance ranged from (1.9%-3.3%) in 2007[18,19], 4.65% in 2014 [20], and 9.98% in 2016 [21]. Our study reported a lower incidence of 1.1%. Additionally, even higher rates have been reported in some studies, Colistin resistant bacteria were found in 70.8% of patients in an intensive care unit in Netherlands [22]. In another study by Ahmed and his colleagues, 156-Gram negative bacilli were isolated, of which 37 (23.7 %) were susceptible to Colistin and 119 (76.3 %) were resistant [15]. As a result, the problem of Colistin-resistant bacteria seems to be escalating in the next few years.

The emergence of Colistin resistance in Gram-negative bacteria is primarily caused by an adaptive or mutational mechanisms [23]. Mutations usually affect the outer membrane of Gram-negative bacteria, that's where Colistin acts[24]. Additionally, Colistin resistance mediated by plasmids has been developed both in animals and humans [4].

Acinetobacter baumanii was the commonest encountered organism (29.4%) followed by *Pseudomonas* (21.6%), *Klebsiella pneumoniae* (21.6%), and *Escherichia coli* (19.6%). In Europe-wide surveillance of Colistin-resistant *Enterobacteriaceae* from 37 European countries, 8.8% of *Klebsiella pneumoniae* isolates were found to be resistant to Colistin, with the majority originating from Greece, Italy, Romania, and Hungary, and 32% of Carbapenem resistant *Klebsiella pneumoniae* strains also being Colistin resistant [25].

Acinetobacter baumanii is considered an opportunistic pathogen and is often treated with Colistin if Carbapenem resistance is detected. Thus, the connection between Colistin resistance and resistance to other antimicrobials is particularly concerning. A study conducted on Carbapenem resistant *Acinetobacter baumanii* revealed substitutional mutations in the pmrA/B genes and subsequent Colistin resistance [26].

In the present study, E. coli was identified in 10 cases (19.6%). In 2016, Egypt identified the first Colistin resistant mcr-1 producing E. coli from a clinical setting. This strain co-produced the CTX-M-15 and had a sequence type of ST1011 which had previously been identified in an avian *Escherichia coli* strain from also from Egypt. This might be seen as a direct proof of zoonotic transfer of the mcr-1 gene from animals to humans [27].

Variation in colonies' color was observed in some organisms when grown on a chromogenic medium. Such discrepancy has not been previously described and should be examined further with a bigger sample size.

There was a very good agreement between the two techniques in the detection of Colistin resistant Gram-negative bacteria (Kappa= 0.921, P-Value = 0.001). The best sensitivity for chromogenic media was reported for *Escherichia coli*. When employed as screening tests, PCR and chromogenic media appear to have the same level of sensitivity. Additional criteria, such as the cost per test and the effect of turnaround time on patient care, may influence the decision to utilize chromogenic medium or molecular approach. Since there is no longer a choice, diagnostic algorithms that integrate two or more complementary tests have gain more popularity during the last decade [8].

There were some limitations to our study. First, this study has a small sample size and was conducted at a single center. Second, molecular testing for the Colistin resistance genes, as well as assessment of underlying risk factors for acquiring such infections, were not done, and should be considered in future studies.

CONCLUSION

From this study we concluded that CHROMID Colistin R agar is a reliable culture medium that can be used effectively for rapid screening of Colistin resistant Gram-negative bacteria.

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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