Microbes and Infectious Diseases 2023; 4(2): 522-529

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Rapid detection of carbapenemases producing *Enterobacteriaceae* (CPE) by Chromatic ™ CPE media in intensive care units in Ain Shams University Hospitals

Amira G Elshenawy [1], Shereen El-Bendary [2], Yasmeen M Mahmood [3], Fatma M Mahmoud [*2]

1- Medical Microbiology and Immunology Department, Faculty of Medicine, 6 October University, Cairo, Egypt.
2- Medical Microbiology & Immunology Department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.
3- Clinical pathology department, Faculty of Medicine, Ain Shams University, Cairo, Egypt.

ARTICLE INFO

Article history:
Received 25 March 2023
Received in revised form 11 April 2023
Accepted 11 April 2023

Keywords:
CPE
Chromatic ™ CPE Media
ICU

ABSTRACT

Background: The evolution and springing up of carbapenemase-producing *Enterobacteriaceae* (CPE) threatens worldwide health. The capability of carbapenemases to degrade all β-lactam antibiotics leads to less antibiotics retaining activity against infections caused by CPE that are associated with high mortality and bad prognosis. There is an urgent need to define robust standardized screening methods for the effective detection of CPE in order to control their spread. This study aimed to detect the efficiency of Chromatic ™ CPE medium in identifying CRE directly from ICU clinical samples. **Methods:** A Cross section study was conducted at intensive care units and Medical Microbiology and Immunology laboratory, Faculty of medicine, Ain Shams university. The sample was directly inoculated on CRE chromagar plate and incubated at 35°C aerobically. Isolation and identification of CPE was performed according to Manual of Clinical Microbiology 2019 then antimicrobial susceptibility test by Kirby-Baur disc diffusion. The sensitivity and specificity of CRE screening by CRE chromogenic media were detected using Modified carbapenemase inactivation method (mCIM) as the standard method. **Results:** One hundred and fifty-eight isolates were obtained from hospitalized ICU patients in Ain shams university hospital between April to November 2022. There were different types of samples, the most common one was sputum. Among 71 isolates grew on blood and MacConkey agar media, 59 (83.15%) showed growth on chromogenic media while 12 (16.9%) showed no growth on chromogenic media. Fifty-four (76.1%) showed MDR *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Among 54 MDR isolates, 50 (92.6%) were positive carbapenemase production, while 4 (7.4%) were negative carbapenemase production using mCIM test as confirmatory test. The sensitivity of chromogenic media was 98%, specificity was 50% in all isolates and the accuracy of the test was 94.4%. While the sensitivity of chromogenic media in all *Enterobacteriaceae* was 96.9%, specificity was 33.3%. **Conclusion:** This study revealed that CRE chromogenic is highly sensitive in the screening of CRE detection. It efficiently saves more time in identifying CRE as compared to mCIM method. The use of a single chromogenic medium can reduce the cost of sample processing and be an effective tool for rapid detection of CRE. Accurate and fast detection of patients colonized by CPEs is clinically important in applying proper infection control precautions.

DOI: 10.21608/MID.2023.202163.1493
* *Corresponding author:* Fatma Mostafa Mahmoud
E-mail address: fatma_mostafa@med.asu.edu.eg

Introduction

Infections caused by multidrug-resistant (MDR) organisms among patients in intensive care units (ICUs) are associated with high mortality [1]. There is a rapidly expanding antimicrobial resistance in regions with bad hygiene and unrestrained usage of antimicrobials. The distribution of MDR differs across the world being more in the low economic countries than the high economic ones. Patients in intensive care units (ICUs) are considered a crucial targeted population for nosocomial infectious pathogens. Attributable to low immunity status, broad-spectrum antibiotic usage, and invasive interventions [2], therefore monitoring ICU infectious microorganisms is vital step to implement the effective organization of measures related to preventive infection control measures, and therapeutic actions [3]. Carbapenem-resistant *Enterobacteriaceae* (CRE) are considered a significant public health threatening remark. Infections occur due to those organisms are related to notable morbidity and mortality. There are multiple mechanisms of drug resistance in Gram-negative bacteria (GNB); the principal one for the ultimate spread of antibiotic-resistant GNB worldwide are β-lactamase genes which are present on mobile genetic elements. Detection of carbapenem-resistance in *Enterobacteriaceae* has been known for the last 2 decades, but worldwide dispersal of carbapenemase-producing *Enterobacteriaceae* (CPE) is a more contemporary concern that, once started, has been occurring at an alarming pace [4]

It is considered an important clinical issue to identify patients colonized by CPEs in order to apply proper infection prevention and control precautions. It helps to target the antibiotic chemotherapy, especially in intensive care units which characterized by high mortality rates. There are different methods for identification the activity of carbapenemase enzymes such as carbapenem inactivation method (CIM), rapid colorimetric methods, immunochromatographic (IC) assays, and molecular-based methods [5]. The carbapenem inactivation method (CIM), is considered simple to be done and had a high sensitivity in the detection of carbapenemases. In 2017, based on the CIM method, clinical and laboratory standard institute (CLSI) recommended the modified carbapenem inactivation method (mCIM) for carbapenemase enzyme detection [6].

Rapid colorimetric methods usage nowadays is considered important because it is an easy identification way as targeted colonies of specific microorganisms can be identified by their color and no specific machine or skill are required. Moreover, chromogenic media is more time saving as it eliminates many sample processing steps, so the results can be obtained within 24 hours. Accurate and rapid diagnosis not only guarantee a best result for the patients but also aids in the infections prevention and control. Unfortunately, on the other hand, the chromogenic media is considered to be an expensive alternative [7].

Material and methods

One hundred and fifty-eight isolates were gathered from each patient and transported to the microbiology laboratory for further processing without delay. The sample was inoculated directly on Chromatic ™ CPE medium plate and incubated at 35°C aerobically. Examination of the plates were done at 24 hours for the color of the colonies to reach species level identification of *Enterobacteriaceae* as per the manufacturer's instructions.

The rest of sample was cultured on blood and MacConkey's agar and exposed for further identification as well as determination of carbapenem resistance according to Manual of Clinical Microbiology 2019 and Clinical and Laboratory Standards Institute (CLSI 2022). Carbapenemase inactivation method (mCIM) was done for CRE isolates as confirmatory test of Carbapenemase production. The sensitivity, specificity, negative, and positive predictive values of CRE screening by CRE chromogenic agar were set on using Modified carbapenemase inactivation method (mCIM) as the standard method.

Ethical approval

The current study was approved by the ethical committee of college of medicine, Ain Shams university in Cairo (IRB: FWA000017585).

Results

One hundred and fifty-eight isolates were obtained from hospitalized ICU patients in Ain Shams University Hospital between April to November 2022. Male patients represent only 36.1% while female patients represent 63.9% with mean age 42.39 ± 23.5 ranged from 8 months to 72 years.

Isolates were isolated from different clinical samples including sputum, drain wound,

endotracheal tube, and pleural fluid. The majority of them were isolated from sputum 109 (69.0%) and wound 37 (23.3%) **(Table 1)**.

Out of 158 isolates, 87 (55.1%) showed no growth on blood and MacConkey agar media while 71 (44.9%) showed growth of Gram-negative isolates.

Among 71 isolates grew on blood and MacConkey agar media, 59 (83.15%) showed growth on chromogenic media while 12 (16.9%) showed no growth on chromogenic media **(Table 2) (Figure 1).**

According to the result of antimicrobial susceptibility test done by disk diffusion methods according to CLSI 2022, out 71 isolates, 17 (23.9%) showed normal flora or isolates sensitive to carbapenems while 54 (76.1%) showed MDR *Enterobacteriaceae, Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The distribution of CRE isolates was *Klebsiella pneumonia* 30 (42.3%) followed *Pseudomonas aeruginosa* 11(15.5%), then *Acinetobacter baumannii* 7 (9.9%), while *E. coli* and *proteus* were the least 5 (7%) and 1 (1.4%) respectively **(Table 3) (Figure 2).**

As a confirmatory test for carbapenem resist isolates, mCIM test done. Among 54 MDR isolates, 50 (92.6%) were positive carbapenemase production, while 4 (7.4%) were negative carbapenemase production **(Table 4) (Figure 3).**

Regarding detection of carbapenemase producing Gram-negative bacilli, **Table 5** shows a fair agreement (94.4%) in all Gram-negative bacilli isolates as (kappa value = 0.542) when comparing the results of the chromogenic media to the mCIM, and poor agreement (91.7%) in case of *Enterobacteriaceae* as (kappa value = 0.357) and excellent agreement (100%) (kappa value = 1.00) in *Pseudomonas aeruginosa*. The application of kappa agreement measures in *Acinetobacter baumannii* was not applicable as there were no negative cases in mCIM.

The sensitivity of chromogenic media to detect true positive results in all isolates was 98%, specificity to detect true negative results was 50% and the accuracy of the test was 94.4%. While the sensitivity of chromogenic media to detect true positive results in all Enterobacteriaceae was 96.9%, specificity to detect true negative results was 33.3% **(Table 5).**

Table 1. Demographic data and sample type for the study group.

(N= 158)		**Mean**	**SD / %**
Age		42.39	23.52
Sex	**Male**	57	36.1%
	Female	101	63.9%
Type of samples	**Sputum**	109	69.0%
	Drain	5	3.2%
	Endotracheal tube	5	3.2%
	Pleural fluid	2	1.3%
	Wound	37	23.3%

SD: Standard deviation

Table 2. Culture growth for the study group on conventional and chromogenic media.

		N	**%**		
Culture growth	**No growth**	87	55.1%		
	Growth	71	44.9%		
Growth on chromogenic media			**Negative**	12	16.9%
			Positive	59	83.1%

Table 3. Antibiotic susceptibility for isolated microorganisms, detected by conventional methods.

			N	%
Antibiotic susceptibility (N= 71)	**Normal flora and sensitiveto carbapenems (N= 17)**	Normal flora	6	8.5%
		Pesudomonas / Klebsiella	1	1.4%
		Burkhulderia	1	1.4%
		Candida	1	1.4%
		E. coli	2	2.8%
		Pseudomonas	6	8.5%
	MDR (N= 54)	*Acinetobacter baumannii*	7	9.9%
		E. coli	5	7.0%
		Klebsiella pneumoniae	30	42.3%
		Proteus mirabilis	1	1.4%
		Pseudomonas aeruginosa	11	15.5%

MDR: multi drug resistance

Table 4. Carbapenemase inactivation method results for CRE isolates.

		N	%
mCIM	**Negative**	4	7.4%
	Positive	50	92.6%

mCIM : Modified carbapenemase inactivation method

Table 5. Agreement between growth on chromogenic media and mCIM.

Growth on chromogenic		**mCIM**		**Agreement %**	**Kappa value *p*-value)**
		Negative	Positive		
		N (%)	N (%)		
All organisms	Negative	2 (50%)	1 (2%)	94.4%	0.542 (<0.001)
	Positive	2 (50%)	49 (98%)		
Enterobacteriacea	Negative	1 (33.33%)	1 (3.03%)	91.7%	0.357 (0.028)
	Positive	2 (66.67%)	32 (96.97%)		
Pseudomonas aeruginosa	Negative	1 (100%)	0 (0%)	100%	1.00 (0.001)
	Positive	0 (0%)	10 (100%)		
Acinetobacter baumannii	Negative	0 (0%)	0 (0%)	100%	———
	Positive	0 (0%)	7 (100%)		—

Table 6. Sensitivity and specificity of chromogenic media.

	TP	**TN**	**FP**	**FN**	**Sensitivity**	**Specificity**	**PPV**	**NPV**
All organisms	49	2	2	1	98.0%	50.0%	96.1%	66.7%
Enterobacteriacea	32	1	2	1	96.9%	33.3%	94.1%	50.0%
Pseudomonas aeruginosa	10	1	0	0	100.0%	100.0%	100.0%	100.0%
Acinetobacter baumannii	7	0	0	0	———	———	———	———
					———	———	———	———
					—	—	———	———

Figure 1. Culture growth for the study group on conventional and chromogenic media.

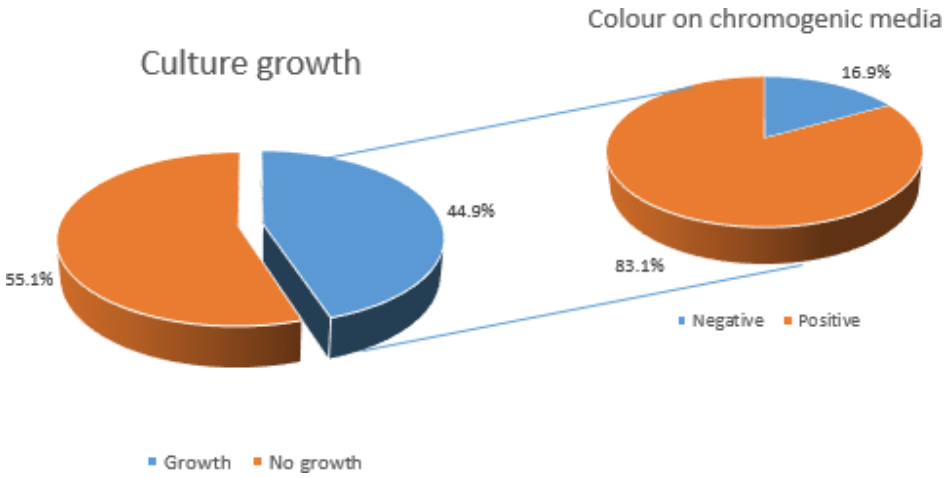


Figure 2. Carbapenem resistant isolate's distribution.

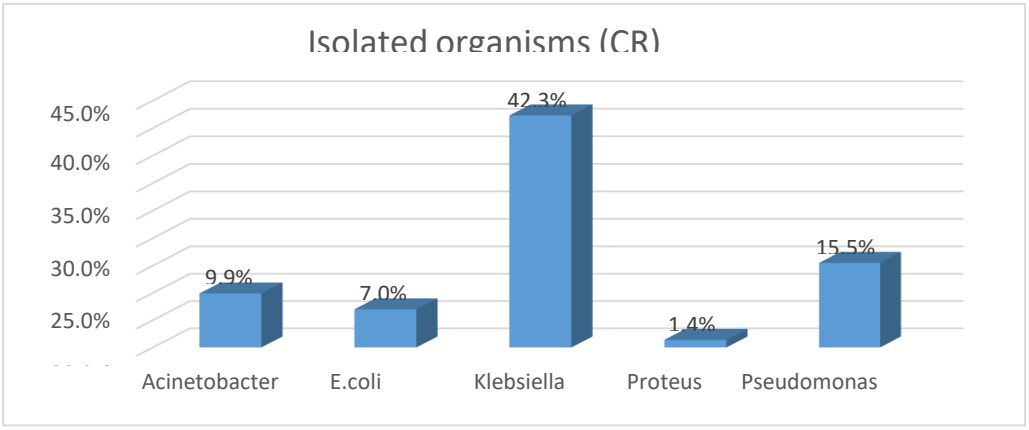


Figure 3. Carbapenemase inactivation method results for CRE isolates.. (A).

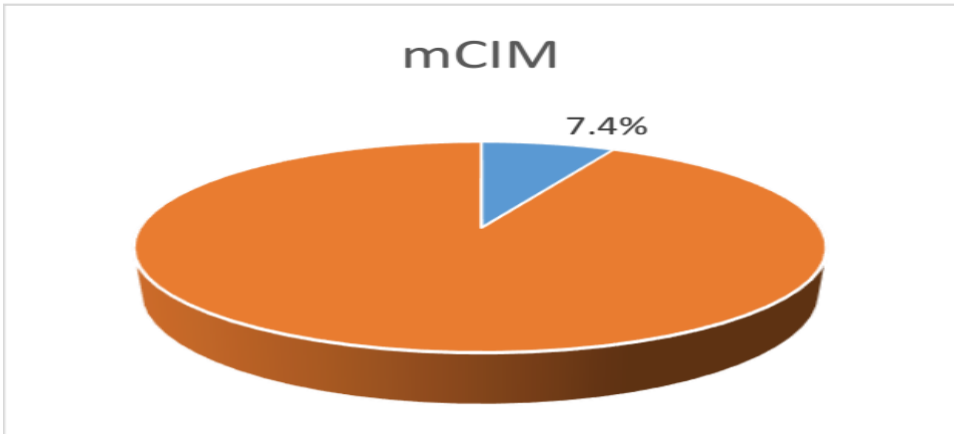
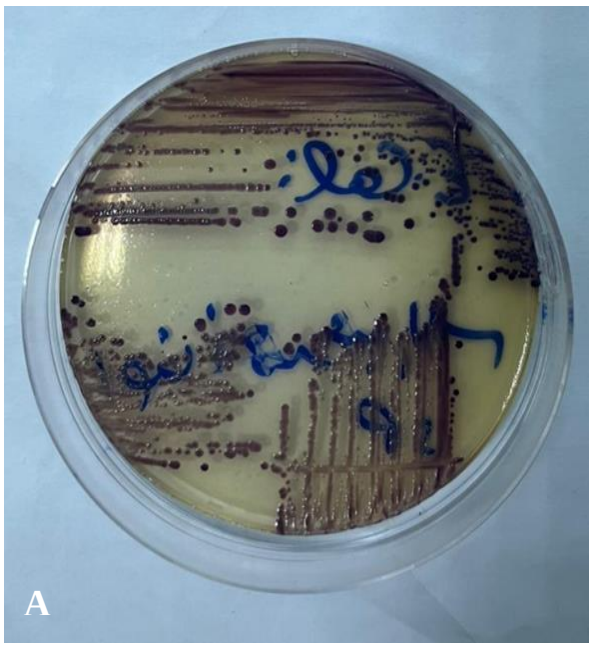


Figure 4. Chromogenic media plate showing red colonies of *E. coli* (A), and blue colonies of *Klebsiella* spp. (A).



Discussion

Relevant emergence of CRE is considered an important issue in ICU settings. Routine screening of CRE colonization in all ICU differ depending on epidemiology and resources of institutes [4]. Carbapenem-resistant *Enterobacterales* spreading, horizontal interspecies transfer, limited chemotherapeutic choices, and the ultimate increase in morbidity and mortality has taken utmost precedence in the last decade in the universe. As the treatment choices for CRE are limited; The rapid detection of CRE is an obligatory step to limit their spread. Laboratory surveillance of cultures to detect those organisms is crucial and prudent in areas where these strains are endemic. Chromogenic media are considered ideal for the preliminary detection of CRE. These media are considered a sensitive, appropriate, and economical way for detection of CRE species [8]

The Centers for Disease Control and Prevention (CDC) recommend routine surveillance of CRE infections in acute-care hospital settings and implementation of contact isolation precautions [7].

In the present study, out of 158 isolates, 54 (76.1%) showed MDR *Enterobacteriaceae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii.* Distribution of CRE isolates was *Klebsiella pneumonia* 30 (42.3%) followed *Pseudomonas aeruginosa* 11(15.5%), then *Acinetobacter baumannii* 7 (9.9%), while *E. coli* and *proteus* were the least 5 (7%) and 1 (1.4%) respectively.

Mahapatra et al. found that CRE was isolated from 49 swabs out of 140 rectal swabs, from those 49 CRE isolates, 33 were *E. coli* (33/140, 23.57%), 15 were *Klebsiella* species (15/140, 10.71%), and one was *Enterobacter* spp. (1/140, 0.71%). **Park** illustrated that the antimicrobial susceptibility test showed 14 CREs and 23 carbapenem-susceptible *Enterobacterales* (CSEs) out of a total of 45 rectal swab. Of all CREs, 57.1% (8/14) were CP CREs [7,9].

Demircan et al. found that 67 *Enterobacteriaceae* strains (30 *Escherichia coli*, 37 *Klebsiella pneumoniae*) detected by the susceptibility system were included. Disk diffusion of 67 isolated strains method and VITEK-2 automated system, it was determined that 28 (42%) were carbapenem sensitive and 39 (58%) were resistant to at least one carbapenem group antibiotic detected. **Gupta et al.** in their study, carbapenem resistance rate was 10.6% in *K. pneumoniae* isolates and *E. coli* isolates reported as 4.0% [11].

By using Kirby-Bauer disc diffusion approach, 54 CRE isolates were detected. On the other hand, 59 isolates of CRE were recovered from 158 sample using chromogenic agar. The cause of the disagreement of results in our study may be explained as lower concentrations of CRE in those 5

swabs that might have unable to grow in macConkey agar, but they could be grown on chromogenic agar [9].

By using mCIM as reference method, 50 isolates were cabapenemase producer while 4 were not carbapenemase producers. Growth of CRE was obtained in 49 samples (98 %) in both the methods. Concordant results in both the methods were observed in 51 samples, while discordant results were seen in 3 samples.

In a research done by **Errecalde et al.** two false-positive results had appeared on HiCrome KPC agar and the authors had recommended phenotypic confirmation of CRE isolates growing on HiCrome KPC agar. In our study, two CRE isolates recovered using chromogenic agar were negative by mCIM and hence were not carbapenemase producing-CRE [11].

In the present study taking mCIM as a reference method, the sensitivity of chromogenic media in all isolates was 98%, specificity was 50% and the accuracy of the test was 94.4%. While the sensitivity of chromogenic media to detect true positive results in all *Enterobacteriaceae* was 96.9%, specificity to detect true negative results was 33.3%.

Mahapatra et al. found that the sensitivity was (91.4–100), specificity (84.8–95.8),, and negative predictive values (70.9–91.4), and positive predictive values (95.9–100) of CRE screening by HiCrome KPC agar, taking the CDC recommended method as reference. The diagnostic accuracy of HiCrome KPC agar for CRE detection was calculated to be 94.2% (89.1–97) [9].

On the other hand, **Park** found that both chromID CARBA and CHROMagar KPC agar showed similarly high sensitivity for carpabenemase producing CRE detection in clinical specimens (100% vs. 100%). For CP CREs, chromID CARBA showed higher specificity (89.2%) as compared to CHROMagar KPC (70.3%). The sensitivity for detecting non-CP CREs on CHROMagar KPC was higher than that on chromID CARBA (100% vs. 50%) [7]. **Demircan et al.** illustrated that KDE detection of Chrom ID OXA-48 medium has a sensitivity of 75.8%, while other methods reported that the sensitivity was 57.6%.

Conclusion

In our study, we reported that CRE chromogenic showed high sensitivity in screening CRE in ICU settings. That efficiently reflected on patient outcome and implementation of infection prevention and control measures in ICU. As our sample size was limited, and the study was performed at one institution, further studies will be needed to establish the applicability CRE chromogenic of agar for detecting the predominant circulating carbapenemases in Egyptian setting.

Conflict of interest

The authors report no conflicts of interest in this work.

Financial disclosures: None.

References

1-**Tamma PD, Goodman KE, Harris AD, Tekle T, Roberts A, Taiwo A, et al.** Comparing the outcomes of patients with carbapenemase-producing and non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae bacteremia. Clin Infect Dis 2017; 64:257–64.

2-**Rezk AR, Bawady SA, Omar NN.** Incidence of emerging multidrug-resistant organisms and its impact on the outcome in the pediatric intensive care. Egypt Pediatric Association Gaz 2021 ;69, 25.

3-**Yetkin F, Yakupogullari Y, Kuzucu C, Ersoy Y, Otlu B, Colak C et al.** Pathogens of Intensive Care Unit-Acquired Infections and Their Antimicrobial Resistance: A 9-Year Analysis of Data from a University Hospital. Jundishapur J Microbiol 2018;11(10):e67716.

4-**Logan LK and Weinstein RA.** The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menaca J Infect Dis 2017; 215(Suppl 1): S28–S36

5-**Al-Zahrani IA.** Routine detection of carbapenem-resistant gram-negative bacilli in clinical laboratories. A review of current challenge. Saudi Med 2018 ;39(9):861-872.

6-**Jing X, Zhou H, Min X, Zhang X, Yang Q, Du S, et al.** The Simplified Carbapenem Inactivation Method (sCIM) for Simple and Accurate

Detection of Carbapenemase-Producing Gram-Negative Bacilli. Front Microbiol 2018;9:2391

7-**Chang-Hun Park** "A comparative evaluation of two chromogenic media for surveillance of carbapenem-resistant Enterobacterales with non-carbapenemase-producing or carbapenemase-producing strains other than bla_{KPC}: bla_{GES-5}, bla_{NDM-1} or bla_{VIM-2}" Journal of Laboratory Medicine 2019; 43: 3:173-176.

8-**Alizadeh N, Rezaee M A, Kafil H S**. Detection of carbapenem-resistant Enterobacteriaceae by chromogenic screening media. J Microbiol Methods 2018; 153:40–44

9-**Mahapatra A, Nikitha K, Rath S, Behera B, Gupta K.** Evaluation of HiCrome KPC Agar for the Screening of Carbapenem-Resistant Enterobacterales Colonization in the ICU Setting of a Tertiary Care Hospital. J Lab Physicians 2021;13(4):358-361.

10-**Demircan SA, Kılıç EK, Kınıklı S, Salih Cesur S, Yağcı S, Yücel M, et al.** Chromogenic media and Rapidec Carba NP evaluation of test performances for detecting carbapenemase-producing Enterobacteriaceae. J Med Palliat Care 2021; 2(1): 1-6.

11-**Gupta N, Limbago BM, Patel JB, Kallen A J.** Carbapenem-resistant Enterobacteriaceae: epidemiology and prevention. Clinical Infectious Diseases 2011;53(1):60–67.

12-**Errecalde L, Cogut A, Erbín M, Jordá Vargas L, Cattani E, Posse T, et al.** [CHROMagar KPC. Comparison with the method proposed by the Centers for Disease Control and Prevention (CDC, USA) for rectal screening and evaluation of false positive results] Rev Argent Microbiol 2012;44(2):89-93