Evaluation of Chromogenic Medium for the Detection and Isolation of Carbapenem-Resistant Organisms from Perirectal Swabs

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Abstract

Background: Multidrug-resistant (MDRO) organisms are a major concern within a healthcare setting particularly the increasing number of carbapenem-resistant organisms (CRO). It has been shown that MDROs can colonize the intestinal tract and can be part of the normal intestinal microbiota of healthy individuals. The prevalence within hospital settings is increasing and is most often associated with medical travel from countries outside the US, long term use of antibiotics, and older adults being admitted from long term care facilities with the following risk factors: chronic wounds, medical devices, dementia and increased age. Intervention strategies are needed to help reduce the transmission of CRO in colonized patients admitted to a hospital. CRO surveillance programs can be labor intensive and costly, requiring rapid and sensitive methods. In this evaluation, we studied the utility of chromogenic media, CHROMagar™ mSuperCARBA™, for the detection of CRO in a high-risk patient population being admitted to our medical center.

Materials/methods: Remnant perirectal swab specimens obtained from patients undergoing surveillance cultures for vancomycin-resistant Enterococci surveillance (VRE) were used for the study. The specimens were collected using an Eswab® transport system consisting of a flocked swab with 1mL of liquid Amies media. Swabs were transported to the laboratory at room temperature for processing. Remnant specimens from the Eswab were used to inoculate CHROMagar™ mSuperCARBA™ media. The inoculated plates were incubated at 35ºC in ambient air. The chromogenic cultures were examined for growth after 24 hours of incubation. Carbapenem-resistant organisms were identified based on the manufacturer's recommended typical colony appearance. Further carbapenem-resistant bacterial characterization was performed using an alternative method to confirm carbapenemase-producing (CPO) versus non-carbapenemase-producing bacteria. **Results:** A total of 75 remnant perirectal swabs submitted for VRE surveillance and 5 axilla/groin Eswabs submitted for *C. auris* PCR were processed for this study. Twenty-nine (38.7%) out of the 75 swabs evaluated grew one or more confirmed CRO. Of the 29 CRO isolated, 24 isolates were confirmed to be CRO by susceptibility testing but a carbapenemase gene was not detected. Five isolates were found to be carbapenemase producing organisms with an *E. coli* confirmed to harbor an OXA-48 carbapenemase gene. **Conclusions:** CHROMagar™ mSuperCARBA™ media was able to directly detect high-risk patient colonization with carbapenem-resistant organisms, including OXA-48 carbapenemase-producers, helping in the prevention and control of CROs within the hospital setting.

Background

Carbapenem resistant organisms can be defined as a member of the Enterobacterales group, *Pseudomonas aeruginosa* and *Acinetobacter baumannii complex* that has resistance to any of the carbapenem class of antibiotics. Carbapenemase resistant organisms (CROs) are classified as an urgent and critical public health threat not only in the United States of America but worldwide. Their emergence has limited a gap in appropriate treatment options, leading to significant rates of morbidity and mortality and healthcare costs attributed to prolonged hospital stays[1]. CROs can be further classified based on the resistance mechanism attributed to its phenotypic pattern. Carbapenem resistance can be attributed to diverse resistance pathways that indiscriminately divides them into two differential groups: cabapenemase-producing organisms (CPOs) or non-carbapenemase producing organisms (Non-CPOs). CPOs acquired genes enable them to produce carbapenemase enzymes that directly break down carbapenem antibiotics, ie. Meropenem, Ertapenem or Imipenem, essentially rendering them inactive[2]. Usually, these genes are located on plasmids making them mobile and easily transferable between different bacterial species. The ease of transmissibility is a significant risk factor for widespread dissemination within a hospital setting. Conversely, non-CPOs develop carbapenem resistance through mechanisms that do not involve the production of enzymes but rather a combination of genetic alterations such as porin mutations and loss, overexpression of efflux pumps, or overproduction of other beta (β)-lactamases (AmpC β-lactamases)[3]. Epidemiologically, it is critical to identify CPOs with a robust multi-drug-resistant organism (MDRO) surveillance program. This would help with early detection of a colonized patient which can lead to appropriate containment and outbreak management.

MDRO surveillance programs have shown to be effective in preventing the spread of CPOs and other resistant organisms like *Candida auris*. The main limiting factor for surveillance implementation common to all healthcare facilities is the cost associated with the labor and reagents. In this study, we evaluated an approach to implementing a CRO surveillance workflow that would be cost-effective and sensitive to recover CROs. The CHROMagar mSuperCARBA™ media is specifically designed for the qualitative direct detection of gastrointestinal colonization by carbapenemase-producing Gram-negative bacteria providing a method to detect a broad range of CPOs including the most common types(Figure 1).

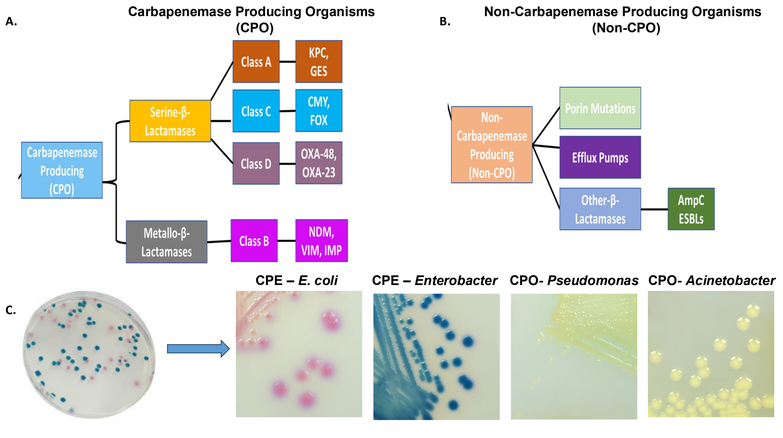

Figure 1. Carbapenemase Resistant Organisms: (A) Carbapenemase-producing organisms that contain mobile genes that produce enzymes that break down carbapenem antibiotics rendering them inactive.(B) Non-carbapenemase-producing organisms involve a combination of genomic alternations such as point mutations, efflux pumps and the over expression of β-lactamases. (C) Colony growth on mSuperCARBA CHROMagar™

Materials and Method

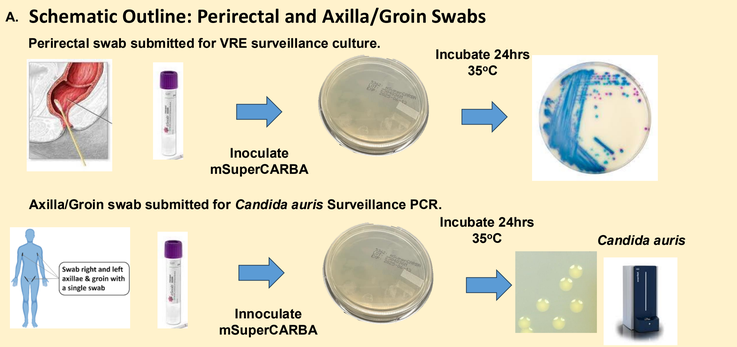

Figure 2. Schematic Workflow Evaluation of mSuperCARBA. Illustration of the evaluation of both perirectal and axilla groin collected and previously tested eSwabs for the evaluation of the CHROMagar mSuperCARBA™ agar.
Note: preliminary small sample size for the *C. auris* swabs.

Figure 3. Downstream Analysis. As recommended by the manufacturer, confirmation studies are needed for both identification and phenotypic studies.

Results - Performance

Table 1. Previously Characterized and Confirmed Carbapenem-resistant Organisms Included in the Performance Study of the CHROMagar mSuperCARBA™

Isolate No.	ID by MALDI	Phenotypic MIC Interpretation					CPO	CPO Gene	Growth mSuperCARBA	
		MEM	ETP	CAZ	CRO	FEP			Yes	No
ISO 1	*Klebsiella pneumoniae*	S	S	R	R	R	No	NA		✓
ISO 2	*Escherichia coli*	R	R	R	R	R	Yes	NDM	✓	
ISO 3	*Escherichia coli*	S	S	S	S	S	No	NA		✓
ISO 4	*Acinetobacter baumannii complex*	R	-	-	-	-	No	OXA-23	✓	
ISO 5	*Acinetobacter baumannii complex*	R	-	-	-	-	No	OXA-23	✓	
ISO 6	*Escherichia coli*	R	S	R	R	R	No	NA	✓	
ISO 7	*Klebsiella pneumoniae*	R	R	R	R	R	Yes	KPC	✓	
ISO 8	*Enterobacter cloacae complex*	S	R	R	R	R	No	NA	✓	
ISO 9	*Citrobacter freundii complex*	S	R	R	R	R	No	NA	✓	
ISO 10	*Enterobacter cloacae complex*	S	S	S	S	S	No	NA		✓
ISO 11	*Klebsiella (Enterobacter) aerogenes*	S	R	R	R	R	No	NA	✓	
ISO 12	*Pseudomonas aeruginosa*	R	-	R	R	R	No	NA	✓	
ISO 13	*Klebsiella pneumoniae*	S	S	S	S	S	No	NA		✓
ISO 14	*Enterobacter cloacae complex*	S	R	R	R	R	No	NA	✓	
ISO 15	Acinetobacter baumannii complex	S	-	-	-	-	No	NA		✓
ISO 16	*Escherichia coli*	S	S	R	R	R	No	NA		✓
ISO 17	*Klebsiella pneumoniae*	S	R	R	R	R	No	NA		✓
ISO 18	*Escherichia coli*	R	R	R	R	R	Yes	OXA-48	✓	
ISO 19	*Klebsiella (Enterobacter) aerogenes*	S	R	R	R	R	No	NA	✓	
ISO 20	*Escherichia coli*	R	R	R	R	R	Yes	NDM	✓	

Table 2. Overall Essential Agreement of the CHROMagar mSuperCARBA™ media

	Non-CRO (No Growth)	CRO (Expected Growth)	Total
Positive Percent Agreement	0	13	100% (n=13)
Negative Percent Agreement	7	0	100% (n=7)

Results – Perirectal Swab Evaluation

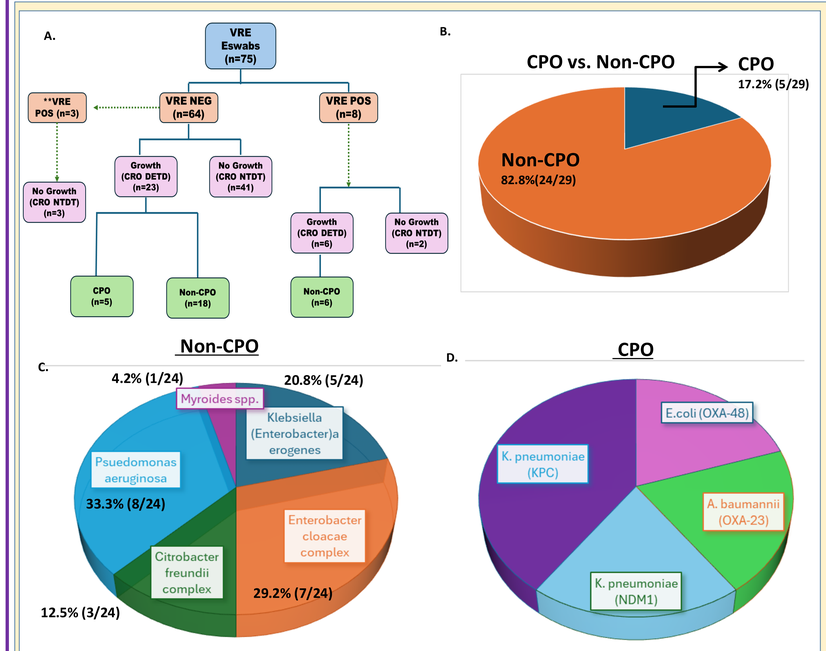

Figure 4. Perirectal Swab Findings. (A) Overall distribution of tested perirectal swabs. Note: there were two VRE tested negative by standard media but confirmed VRE positive on the mSuperCARBA™ media. (B) CPO vs non-CPO distribution (c) and (D) isolate distribution of non-CPO vs. CPO, respectively.

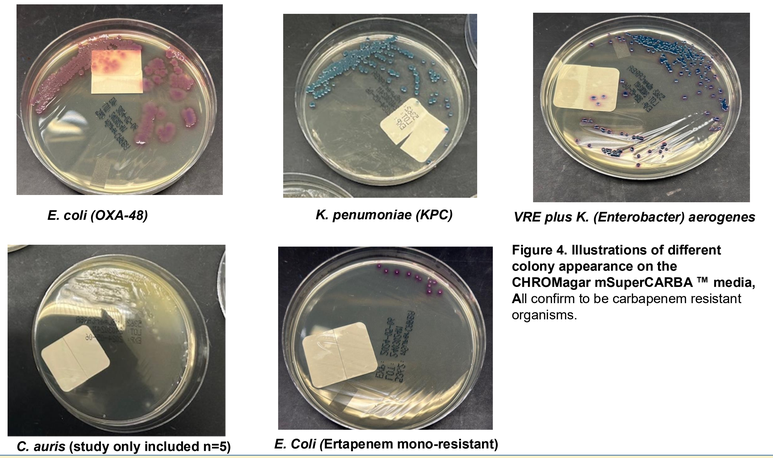

Figure 4. Illustrations of different colony appearance on the CHROMagar mSuperCARBA ™ media, All confirm to be carbapenem resistant organisms.

Conclusions

CHROMagar™mSuperCARBA™ media directly detected high-risk patient colonization with carbapenem-resistant organisms, including OXA-48 carbapenemase-producers. Surveillance cultures using mSuperCARBA™ medium could provide CRO detection in a program to prevent and control CROs within the hospital setting. A small sample size of 5 *C. auris showing growth on this medium suggests* the potential of multiple MDRO isolation from the same patient sample.

References

1. Aubron, C., Poirel, L., Ash, R. J., and Nordmann, P. (2005). Carbapenemase-producing Enterobacteriaceae, U.S. rivers. Emerg. Infect. Dis. 11, 260–264.
2. Hasman, H., Agersø, Y., Hendriksen, R., Cavaco, L.M., Guerra-Roman, B., 2015. Validation of Selective and Indicative Agar Plates for Monitoring of CarbapenemaseProducing E. coli.
3. CHROMagar mSuperCARBA media™ documents/package insert