

The evaluation of the Liofilchem CRE and OXA-48 Chromatic agars for the isolation and presumptive identification of carbapenem resistant *Enterobacteriaceae*



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Objectives

The acquisition of the gene coding for carbapenemase is the main mechanism leading to carbapenem resistance in *Enterobacteriaceae*. The transfer of the resistance gene can readily occur between the members of the same species but also between different genera. This requires prompt identification of individuals colonized or infected with such bacteria so that necessary infection control measures can be taken. In our study we aimed to evaluate the performances of two chromogenic media developed for the presumptive identification of carbapenem resistant *Enterobacteriaceae* (CRE) from clinical specimens.

Methods

Klebsiella We used a large collection of CRE isolates to evaluate the performances of the media for the presumptive identification of CRE. We included 327 well characterized CRE isolates for testing (*Klebsiella pneumoniae*; n=282, *Escherichia coli*; n=45). All isolates were initially identified with MALDI-TOF MS instrument (Bruker Daltonics, Germany) and the antimicrobial susceptibility testing was performed using VITEK 2 instrument (bioMérieux, France). The carbapenemase genes IMP, VIM, KPC, NDM-1 and OXA-48 were investigated with PCR method. Isolates stored at -80°C in cryovials were retrieved and passaged twice using sheep blood agar before inoculating onto Chromatic™ CRE agar and Chromatic™ OXA-48 agar (Liofilchem, Italy). Chromatic™ CRE agar is developed for non-selective isolation of CRE from clinical specimens, however Chromatic™ OXA-48 agar is developed specifically to select OXA-48 harboring isolates from clinical specimens. The chromogenic media were inoculated with test strains, incubated overnight and then examined.

Results

On the Chromatic™ CRE agar only three *K. pneumoniae* and four *E. coli* isolates failed to grow, all OXA-48 positive. The described colony colors (blue-violet for *K. pneumoniae*, red for *E. coli*) were observed for 28% of the *K. pneumoniae* strains and 89% of the *E. coli* strains. On the Chromatic™ OXA-48 agar, of the 227 OXA-48 positive *K. pneumoniae* isolates, 24 (10.6%) failed to grow, whereas the remaining 203 OXA-48 positive *K. pneumoniae* isolates grew well. Only 55 of these revealed the expected colony color (blue-violet). The medium inhibited the isolates harboring the carbapenemase genes such as IMP (n=1), KPC (n=2), NDM-1 (46), however six NDM-1 positive strains grew on the medium. Of the 45 *E. coli* isolates tested, 40 were OXA-48 positive, 28 (70%) of these grew on the Chromatic™ OXA-48 agar, among which 12 exhibited the expected colony color (red) and 12 (30%) failed to grow. The medium successfully inhibited the growth of isolates harboring IMP (n=1) and NDM-1 (n=3) genes, however one NDM-1 positive isolate grew on the medium.

Conclusion

- Both agars performed satisfactorily to support or inhibit the growth of the targeted bacteria, however OXA-48 positive isolates which may exhibit low minimal inhibitory concentration values for carbapenems, present a challenge since the antimicrobials included in the media seem to also inhibit the growth of this organisms.
- Even though the colony colors were not helpful in the preliminary identification for most of the study isolates, for laboratories having an MALDI-TOF MS instrument the confirmation of the species identity can be easily accomplished.

Table 1. Growth and colony color characteristics of carbapenemase producing *K. pneumoniae* (n = 282) and *E. coli* (n = 45) on Chromatic CRE agar and Chromatic OXA-48 agar

Organism	Expected colony color	Chromatic CRE Agar		Chromatic OXA-48 Agar	
		Observed colony color	n	Observed colony color	n
<i>K. pneumoniae</i> (n = 282)	blue-violet	blue	65	blue	8
		blue-violet	1	blue-green	46
		violet	3	red-violet	1
		blue-green	16	green	142
		green	192	pink	3
		yellow	2	red	1
		no growth	3	pink-green	1
				yellow	1
				no growth of non-OXA-48	49
				no growth of OXA-48	24
		growth of non-OXA-48	6		
<i>E. coli</i> (n = 45)	red	red	4	red	9
		violet	17	red-violet	3
		pink	18	violet	1
		pink-violet	1	pink	15
		blue	1	no growth of non-OXA-48	4
		no growth	4	no growth of OXA-48	12
				growth of non-OXA-48	1

Color codes: ■ Appropriate colony color/growth characteristic
■ Inappropriate colony color/growth characteristic