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Comparative evaluation of a new commercial media, the CHROMAgar™ mSuperCARBA™, for the detection of carbapenemase-producing Enterobacteriaceae

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ABSTRACT

A new chromogenic-based medium (mSuperCARBA™) was tested for screening carbapenemase-producing Enterobacteriaceae (CPE). mSuperCARBA™ was more sensitive (83%) in detecting CPE isolates ($n = 69$, including KPC, NDM, OXA-48, VIM, and IMI) compared with CHROMAgar™-KPC (65%) and MacConkey agar with Imipenem (69%) with comparable specificity for non carbapenemase-producing, carbapenem-resistant Enterobacteriaceae ($n = 29$).

The carbapenemase-producing Enterobacteriaceae (CPE) epidemic in Israel, Italy, and the USA was initially caused mainly by KPC-producing *Klebsiella pneumoniae* (KPC-KP) (Tzouvelekis et al., 2012). An essential part of the intervention that had led to the containment of the epidemic in Israel (Schwaber and Carmeli, 2014) and had since been recommended in other countries (Wilson et al., 2016) has been the isolation of carriers, by the widespread use of rectal surveillance cultures. In recent years, new types of CPEs, mostly NDM- and OXA-48-producing Enterobacteriaceae, have disseminated into countries where KPC-KP were previously endemic, e.g., Israel (Adler et al., 2015) or the USA (Lascols et al., 2012). Thus, surveillance media that were adequate for the detection of KPC-KP, such as the CHROMAgar™-KPC (ChromKPC), MacConkey agar with Imipenem 1 mg/L (MAC/IMI) or with carbapenem disks (Adler et al., 2011) may no longer be adequate. Hence, in order to detect these types of CPEs (e.g., OXA-48 producers), there was a need for more sensitive types of surveillance media that also retained similar degree of specificity regarding non-CPE organisms. The SUPERCARBA media was initially developed as a non-commercial media by the Nordmann and Poirel group in order to meet these challenges and was indeed found to be more sensitive compared to media such as the CHROMAgar™-KPC (Girlich et al., 2013; Nordmann et al., 2012). However, the SUPERCARBA is not commercially manufactured, thus making it de-facto unavailable for most clinical laboratories. The CHROMagar mSuperCARBA™ agar is a chromogenic variation of the SUPERCARBA formulation published by Nordmann et al. (personal communication). The objective of this study was to evaluate the performance the new CHROMAgar™ mSuperCARBA™ media, for the detection of CPEs.

The study compared the in-vitro performance of three media: 1) ChromKPC; 2) MAC/IMI; and the 3) mSuperCARBA™ (all manufactured with license by Hylabs, Rehovot, Israel). The study used a collection of previously characterized 98 carbapenem-resistant Enterobacteriaceae (CRE) strains (Lifshitz et al., 2016), that included 69 CPEs with various mechanisms, including KPC, NDM, OXA-48, VIM, and IMI (Table 1). Isolates were suspended in NaCl 0.9% solution, and growth was tested with inoculum of 10^1 to 10^3 cfu. Differentiation of suspicious colonies was done based on the manufacturer's instructions. The sensitivity was calculated by three parameters, including 1) the growth of CPEs at the 10^1 inoculum; 2) sensitivity score-growth at 10^1,10^2, and 10^3 inoculum was credited 2, 1, and 0.5 points, respectively (hence, the maximal score was twice the total number of strains); and by an 3) adjusted sensitivity [Σ(CPE gene-specific sensitivity X institutional proportion of that gene)]. The latter was added in order to adjust for the contribution of each CPE gene to the overall sensitivity in accordance with their actual proportions (KPC-58%, OXA-48-25%, NDM-16%, VIM-1%) at our institution (Tel-Aviv Sourasky Medical Center), which are not significantly different from their overall proportions in Europe, with the exception of higher proportion of VIM compared with NDM (Grundmann et al., 2016). For specificity, we tested the ability of the media to prevent the growth of 29 non-carbapenemase-producing (NCP) CREs (Lifshitz et al., 2016), since the main epidemiological goal (In Israel), has been to detect and cohort CPE but not NCP-CRE carriers (Schwaber and Carmeli, 2014). In addition, the three media were tested with a set of twelve non-CRE organisms, including ESBL- and AmpC-producing Enterobacteriaceae, carbapenem-sensitive and

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Table 1
Analytical performance of three selective media for the detection of carbapenemase-producing Enterobacteriaceae.

	MIC (mg/L); range, median			Isolates detected at ~10^1 cfu (n)			Detection score		
Carbapenemase type (n)	Meropenem	Imipenem	Ertapenem	MAC/IMI	mSuperCARBA	ChromKPC	MAC/IMI	mSuperCARBA	ChromKPC
KPC (11)	4–16, 8	4–16, 8	8–16, 16	8	9	9	16	19	19
NDM (19)	2–16, 4	2–16, 4	2–16, 8	13	12	11	28.5	26.5	24.5
OXA-48 (19)	0.5–8, 2	0.5–16, 4	4–16, 8	5	14	6	15	32	13
VIM (15)	0.5–16, 4	0.5–16, 4	0.5–16, 4	14	14	10	28	28.5	21
IMI (5)	1–16, 8	1–16, 8	4–16, 8	5	3	4	10	6	9
CPE[a], total (69)				45	52	40	97.5	112	86.5
Sensitivity[c] (%)				65	75	58	71	81	63
95% C.I.				53–75	64–84	46–69	63–78	74–87	54–70
Adjusted sensitivity				60	77	65	65	83	69
NCP- CRE[b] (29)	0.5–16, 4	0.5–16, 4	0.5–16, 4	14	15	18	32	32.5	37
Specificity[c]				52	48	38	45	44	36

[a] CPE-carbapenemase producing *Enterobacteriaceae*.
[b] NCP-CRE- non-carbapenemase producing, carbapenem-resistant *Enterobacteriaceae*.
[c] The sensitivity and specificity were calculated according to the ability of the media to detect CPE vs. NCP-CRE.

resistant non-fermentors (*Acinetobacter baumannii* and *Pseudomonas aeruginosa*), Gram-positive bacteria (methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus*), and *Candida* spp.

The sensitivity and specificity of the three media in detecting CPEs versus NCP-CREs are presented in Table 1. The mSuperCARBA™ was the most sensitive media by all three parameters, especially in detecting OXA-48 CPEs. The mSuperCARBA™ was less sensitive in detecting two rare types of CPE, the IMI-producing *Enterobacter cloacae* and the NDM-producing *Providencia rettgeri*.The MAC/IMI media was the second most sensitive by the non-adjusted parameters but scored below the ChromKPC media by the prevalence-adjusted parameter. All three media were able to efficiently select or differentiate CRE from non-CRE organisms: Carbapenem-resistant *A. baumannii and P. aeruginosa* grew on the plates but were easily differentiated from CREs and the other non-CRE isolates did not grow. The specificity values for detecting CPE vs. NCP-CRE were relatively low in all three media, with the MAC/IMI being slightly better compared with the mSuperCARBA™ media.

In this study, we have validated the analytical performances of the new commercial media, the mSuperCARBA™ in comparison with two other commonly used selective media (Adler et al., 2011). As previously reported for the non-commercial SUPERCARBA media (Girlich et al., 2013; Nordmann et al., 2012), the mSuperCARBA™ media was more sensitive in detecting CPE compared with ChromKPC as well as MAC/IMI. The increased sensitivity was mostly thanks to its ability to better detect OXA-48 CPEs and was comparable with KPC-, and VIM-, and NDM-CPEs as was also reported in the recent publication describing the mSuperCARBA™ media (García-Fernández et al., 2017). Of note, the mSuperCARBA™ was less sensitive in detecting NDM-producing *Providencia rettgeri*, that is typically isolated from anatomical sites other than the rectum (e.g., blood) (Gefen-Halevi et al., 2013) and is thus less relevant as a target for surveillance cultures. Since NDM-CPEs were not included in the analytic part of the study by García-Fernández et al., this specific feature cannot be compared between the studies.

The sensitivity of the mSuperCARBA™ was lower compared with the non-commercial SUPERCARBA media (Girlich et al., 2013; Nordmann et al., 2012). This was probably caused due to the different definitions used to calculate the sensitivity and due to difference in strain selection, which can limit the general applicability of such studies. Since the selection of strain in in-vitro studies tends to include relatively rare CPEs (e.g., IMI-producing *Enterobacter cloacae*), we applied a new concept of adjusting the sensitivity values to the actual prevalence of the different CPEs. The adjustment increased the sensitivity of the ChromKPC compared with the MAC/IMI media. Still, even after adjustment the sensitivity values remained 83% or lower, highlighting the imperfection of surveillance cultures and the need to retest high-risk patients (Feldman et al., 2013).

Regarding specificity, this parameter was not tested the analytic part of the study by García-Fernández et al. but the values in our study were lower compared with previous studies (Girlich et al., 2013; Nordmann et al., 2012), due to the fact that we included only NCP-CRE rather than non-CRE bacteria in our analysis. However, considering that the proportion of NCP-CRE among all *K. pneumoniae* isolates in our institution is about 2% (Adler et al., 2012), the actual specificity in real-life surveillance culture is likely to be over 95%. Together, this highlights the limits of comparing analytical parameters between invitro studies and the importance of including surveillance samples as well. However, thanks to the containment of the CPE epidemic in our institution, it had become almost impossible to include enough CPE carriers that would allow meaningful comparison. Notwithstanding this limitation, our study provides a valuable data in validating a much-needed novel surveillance media that would be instrumental for better detection of non-KPC CPEs that are now becoming more prevalent worldwide.

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