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ARTICLE

Laboratory evaluation of different agar media for isolation of carbapenem-resistant *Acinetobacter* spp.

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Abstract The optimal method for surveillance of carbapenem-resistant *Acinetobacter* spp. (CRAB) is unknown. A collection of CRAB strains (n=42), carbapenem-susceptible strains (CSAB), and non-*Acinetobacter* strains (n=18) was used to evaluate six laboratory surveillance methods: MacConkey (MAC), MAC+1 μg/ml imipenem (MAC-IPM), minimal salts agar+1 % acetate (MSA), MSA with IPM disk (MSA-IPM), CHROMagarKPC, and CHROMagar Acinetobacter with CR102 (CHROMAcineto). CHROMAcineto was 100 % sensitive and specific. CHROMagarKPC and MAC-IPM were highly sensitive (>95 %), but their specificity was substantially hampered by the breakthrough growth of CSAB. MSA was unsuitable for CRAB detection. CHROMAcineto is a promising medium for CRAB detection and warrants further clinical evaluation.

Introduction

Carbapenem-resistant *Acinetobacter spp.* (CRAB) is a significant nosocomial pathogen worldwide [1]. Detection of patient colonization by CRAB may be important in order to prevent nosocomial cross-transmission and subsequent invasive infections. This can be achieved by microbiological surveillance cultures, especially in endemic healthcare settings, since CRAB colonization commonly involves nonsterile body sites, such as the skin and rectum [2].

The optimal culture method for microbiological surveillance of CRAB has not yet been defined. Various culture media, featuring selective properties achieved by nutrient composition or antimicrobial agents, or differential properties based on colony morphology and color, have been utilized in past. These include MacConkey-based agar plates, minimal salts agar, and more recently various chromogenic media.

One notable limitation of previous studies is that the evaluation of the medium of interest included only a few comparators, whereas a comprehensive comparison of several available methods had not been carried out. The goal of this study was therefore to perform a laboratory evaluation of several different culture media in parallel, all of which have been considered in the past for CRAB surveillance.

Materials and methods

In the current study, a laboratory evaluation involving six types of agar media has been performed in order to provide a valid comparison between methods. The following agar media were studied:

1. Standard MacConkey plates (MAC), which served as growth control
2. MacConkey plates supplemented with 1 μg/ml of imipenem (MAC-IPM), a medium that has been successful in the isolation of carbapenem-resistant *Enterobacteriaceae* [3]
3. Minimal salts agar with 1 % acetate supplmentation (MSA), which has been used in the past for selective isolation of *Acinetobacter* spp. [4]

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4. Minimal salt acetate agar with standard imipenem discs (MSA-IPM)
5. CHROMagarKPC™
6. CHROMagar Acinetobacter™ (CRHOMAcineto) with CR102 (MDR supplement)

All plates were purchased from a single manufacturer/distributor (Hy-Labs, Rehovot, Israel) and kept refrigerated (4 °C) and protected from light until use. Plates were processed using similar conditions to those of previously published studies. Quality control was ensured using *E. coli* ATCC 25388, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 13883, *K. pneumoniae* ATCC 700603, and *A. baumannii* 19606. The latter is the recommended QC strain for CHROMAcineto by the manufacturer. MSA was also subject to quality control of salt and acetate concentrations by the manufacturer. A bla_{KPC-3}-producing carbapenem-resistant *K. pneumoniae* ST258 was used as an additional positive growth control for MAC-IPM on top of the manufacturer's QC protocols.

The following strain groups were used for the study:

1. Non-duplicate unrelated clinical CRAB strains exhibiting MIC_{50} and MIC_{90}>32 μg/ml (n=42)
2. Non-duplicate unrelated clinical carbapenem-susceptible *Acinetobacter* spp. (CSAB) exhibiting MIC_{50} and MIC_{90} of 0.25 μg/ml and 1 μg/ml respectively (n=11)
3. Other Gram-positive, Gram-negative, and yeast reference strains (n=18).

Clinical CRAB and CSAB strains were identified according to standard microbiological procedures and using the VITEK-2 GN-ID card (bioMerieux, Marcy l'Etoile, France) according to the manufacturer's instructions. Susceptibility testing to imipenem and meropenem was performed using the VITEK GN-059 card and confirmed by the E-test (bioMerieux, Marcy l'Etoile, France). Results were interpreted according to CLSI breakpoints [5]. All study strains belong to the strain repository of the National Center for Infection Control, Ministry of Health, Israel.

After overnight incubation on Mueller–Hinton plates, isolates were suspended in sterile saline to the turbidity of 0.5 McFarland using a spectrophotometer. The same procedure was followed for all plates. Quality control of spectrophotometry was performed using live counts of bacterial suspensions performed by the same operator. Suspensions were further diluted 1:10 and 1:100 in sterile saline (high and low inocula respectively) and 10 μl of each suspension was then plated onto test agar plates [6]. Each suspension was simultaneously inoculated on all plate types in duplicate. Following 18–20 h of incubation, plates were read by two experienced microbiologists. Growth was assessed semi-quantitatively using a growth index of 0–4 (0, no growth; 1, 1–10 colonies; 2, 10–50 colonies; 3, semi-confluent growth; 4, confluent growth). Confluent growth was defined as a growth index of 3 or 4 and scant growth as a growth index of 1 or 2. Colony color was assessed according to the manufacturer's instructions [7].

Results

The growth performance of media for CRAB strains is summarized in Table 1. CRAB strains grew well on MAC with and without imipenem supplementation, as well as on both types of CHROMagar media at both test dilutions. Growing colonies almost invariably yielded the expected colony colors and morphology; all 42 CRAB strains grew as creamy red colonies on CHROMAcineto, whereas 41 (97.6 %) grew as white creamy colonies on CHROMagarKPC (one isolate exhibited a white–greenish color). Growth on MSA was poor, especially with lower inocula and in the presence of imipenem discs.

The growth performance of media for the 11 CSAB strains is summarized in Table 2. All strains grew on control MAC plates. Breakthrough growth was noted on MAC-IPM for three strains (27.2 %) at a higher inoculum. All strains grew on MSA, but none grew on MSA-IPM. Breakthrough growth was also noted for six strains (54.5 %) on CHROMagarKPC at a low inoculum and for seven strains (63.6 %) at a high inoculum, but no growth was evident on CHROMAcineto. The ATCC19606 CSAB grew similarly on CHROMagarKPC, but not CHROMAcineto.

The growth performance of media with non-*Acinetobacter* strains is shown in Table 3. Of the Gram-negative control strains, carbapenem-resistant *P. aeruginosa* and carbapenem-resistant *K. pneumoniae* grew on all MAC- and CHROMagar-based media. With chromogenic media, the carbapenem-resistant *P. aeruginosa* colony's morphology and color were indistinguishable from CRAB, whereas carbapenem-resistant *K. pneumoniae* was easily detected by its different color: blue on CHROMagarKPC (vs white for CRAB) and purple with a blue halo on CHROMAcineto (vs red for CRAB). CHROMagarKPC also showed growth of a susceptible *P. aeruginosa*. While MAC-based plates efficiently prevented fungal growth, all three *Candida* species grew on CHROMagarKPC and two of the three on CHROMAcineto, with distinct, small, white-creamy colonies that were easily differentiated from CRAB. Gram-positive growth was not evident with all media.

Discussion

Clinical laboratories worldwide face the challenge of accurate detection of CRAB strains in clinical and surveillance specimens. Laboratory detection is complicated by varying

Table 1 Growth characteristics of carbapenem-resistant *Acinetobacter* spp. strains (n=42)

Medium	Inoculum dilution	CRAB growth index (%)					Colony morphology
		0 No growth	1 Scant growth	2	3 Confluent growth	4	
MAC	1:10	0	0	0	7.1	92.9	Typical
	1:100	0	0	0	9.5	90.5	
MAC-IPM	1:10	0	0	0	19	81	Typical
	1:100	0	0	2.3	16.7	81	
MSA	1:10	19	9.5	28.6	31	11.9	Minute, white, opaque non-distinct colonies
	1:100	28.6	28.6	26.2	16.6	0	
MSA-IPM	1:10	90.5	9.5	0	0	0	Minute, white, opaque non-distinct colonies
	1:100	92.9	7.1	0	0	0	
CHROM KPC	1:10	0	0	0	0	100	White, creamy, opaque colonies
	1:100	0	0	0	2.4	97.6	
CHROMAcineto	1:10	0	0	0	0	100	Creamy red colonies
	1:100	0	0	0	100	0	

carbapenem MIC values, the diversity of resistance gene mechanisms involved, and the low yield of various surveillance sampling sites. An appropriate medium having selective and differential properties is thus of great relevance for both the screening of suspected isolated CRAB colonies and as a primary isolation medium for surveillance cultures. An optimal medium should identify CRAB with sufficient accuracy to facilitate treatment decisions and infection control measures.

This study has evaluated several different agar-based methods for selective detection of CRAB. MSA has been shown in the past to be highly selective for the isolation of *Acinetobacter* spp. from the skin of healthy subjects [4]. Consequently, this medium has been recommended for use during outbreak situations [8], but has not been further evaluated in comparative studies. Of note is that MSA is expected to select for *Acinetobacter* spp., regardless of carbapenem MIC, and therefore we attempted to increase its selectivity by the addition of an imipenem disk. In our hands, a substantial proportion of CRAB strains failed to grow on MSA and the addition of an imipenem disk to MSA inhibited the growth of almost all CRAB strains. The performance of MSA with CSAB was similar. Therefore, MSA is in our opinion not suitable for CRAB isolation and detection.

We have previously shown that MacConkey agar supplemented by 1 μg/ml of imipenem was effective in the selective detection of carbapenem-resistant bacteria such as CRE [3]. A comparative study of media for CRAB isolation has utilized a similar approach using 6 μg/ml of imipenem [9]. In that study the sensitivity of MAC-IPM was 82 %, but strains that failed to grow exhibited low-to-intermediate MIC values. This drawback could be obviated by the use of 1 μg/ml of imipenem such as in our study. With this concentration virtually all CRAB strains demonstrated growth but the trade-off was

Table 2 Growth characteristics of carbapenem-susceptible *Acinetobacter* spp. strains (n=11)

Medium	Inoculum dilution	CSAB growth index (%)			Comments
		No growth	Scant growth	Confluent growth	
MAC	1:10	0	0	100	Typical
	1:100	0	0	100	
MAC-IPM	1:10	77.8	27.2	0	Typical
	1:100	100	0	0	
MSA	1:10	27.2	54.5	18.3	Minute, white, opaque, non-distinct colonies
	1:100	45.5	45.5	9	
MSA-IPM	1:10	0	0	0	Minute, white, opaque, non-distinct colonies
	1:100	0	0	0	
CHROM KPC	1:10	36.4	18.1	45.5	White, creamy, opaque colonies
	1:100	45.5	9	45.5	
CHROMAcineto	1:10	100	0	0	Creamy red colonies—not evident
	1:100	100	0	0	

Table 3 Growth characteristics of non-*Acinetobacter* spp. reference strains

Reference strains	MAC	MAC-IPM	MSA	MSA-IPM	CHROM KPC	CHROMAcineto
Gram-negative						
P. aeruginosa #27853	+	−	+	−	+	−
P. aeruginosa (blaVIM+)	+	+	−	−	+	+
E. coli #25922	+	−	−	−	−	−
E. coli #35218	+	−	−	−	−	−
K. oxytoca #700324	+	−	+	−	−	−
K. pneumoniae #700603	+	−	±	−	−	−
K. pneumoniae ST-258 (blaKPC-3 positive)	+	+	±	±	+	+
Salmonella enterica #8388	+	−	−	−	−	−
Yeasts						
C. albicans #90028	−	−	−	−	+	−
C. krusei #6258	−	−	−	−	+	+
C. parapsilosis #22019	−	−	−	−	+	+
Gram-positive						
S. aureus #43300	−	−	−	−	−	−
S. epidermidis #12228	−	−	−	−	−	−
E. faecalis #29212	−	−	−	−	−	−
B. subtilis #9372	−	−	−	−	−	−

growth of CSAB strains at a high inoculum (27.2 %). Therefore, the performance of MAC-IPM is largely dependent on the balance between imipenem concentration and the MIC of target strains at varying inocula. This implies that MAC-IPM may not be a robust method for CRAB screening. An advantage of MAC-IPM is the effective inhibition of yeasts and other types of bacteria that may be encountered in surveillance culture, except for other carbapenem-resistant Gram-negative rods.

The application of CHROMagar media for CRAB detection has evolved over recent years with the development of CHROMagarKPC and CHROMagar Acinetobacter. CHROMagar Acinetobacter has initially been shown to be sensitive and specific for the detection of multidrug-resistant *A. baumannii* [10]. This evaluation has been performed using clinical samples obtained from patients with known colonization and infection by a single CRAB clone. However, further clinical and laboratory evaluation of this medium has shown lack of specificity for antimicrobial-resistant strains, particularly CRAB, and difficulty in differentiating CRAB from other Gram-negative strains based on colony color and morphology [11]. These findings have been corroborated by additional studies [9].

Further improvement of the chromogenic features of CHROMagar Acinetobacter have resulted in improved performance based on preliminary laboratory data, especially when the KPC supplement of the CHROMagarKPC medium used for CRE detection was added to the CHROMAcineto medium [12]. A more extensive evaluation of this approach has shown this medium to be highly sensitive but only 20 % specific, owing to the growth of carbapenem-susceptible strains on the medium [13]. Incorporation of a more specific component for the selection of CRAB (CR102) by the manufacturer was expected to improve specificity even further [7]. Indeed, in a recent evaluation, addition of CR102 to the CHROMAcineto medium has resulted in improved performance (100 % sensitivity, >95 % specificity) [13].

Our evaluation utilized both the original CHROMagarKPC and CHROMAcineto containing CR102. CHROMagarKPC was 100 % sensitive, allowing the growth of all CRAB strains, and its specificity was very high in relation to colonial morphology. However, over half of the CSAB strains grew on this medium, rendering it nonspecific for CRAB. This phenomenon was not encountered when CHROMAcineto was used and this medium was 100 % sensitive and specific. Of note is that both chromogenic media exhibited growth of other carbapenem-resistant Gram-negative rods as well as yeasts, although these can be easily differentiated by either colony' color (yeasts and carbapenem-resistant *K. pneumoniae*) or by the simple oxidase test (carbapenem-resistant *P. aeruginosa*, data not shown). One limitation of our study is the small number of CSAB and non-CRAB, carbapenem-resistant, gram-negative rods. This needs to be addressed in future studies.

In conclusion, of several different types of growth media evaluated, including MacConkey-based, minimal salts-based, and chromogenic agars, CHROMagar Acinetobacter with supplemental CR102 appeared to be the most appropriate medium for CRAB screening. However, current performance data on this medium are restricted to laboratory evaluations and therefore further research is needed to elucidate the

performance of this medium under "real life" conditions, using spiked stool or patient samples, and to assess its impact on sample turnaround time, labor, and overall costs.

Conflict of interest The authors declare that they have no conflict of interest.

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