Laboratory and Clinical Evaluation of Screening Agar Plates for the Detection of Carbapenem-Resistant Enterobacteriaceae from Surveillance Rectal Swabs

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ABSTRACT

The increased world-wide spread of carbapenem-resistant *Enterobacteriaceae* (CREs) emphasizes the need for a sensitive screening procedure to identify these microorganisms. Gastrointestinal carriers may serve as the reservoir for cross-transmission in the healthcare setting and thus active surveillance is a key part in preventing the spread of such strains. Three agar-based methods for direct CRE detection from rectal swabs were compared: CHROMagar-KPC (Chrom); MacConkey agar with imipenem1µg/mL (MacI); MacConkey plates with imipenem, meropenem and ertapenem disks (MacD).

First, we compared the level of detection (LOD) of 10 molecularly characterized carbapenemase-producing *Enterobacteriaceae* strains by the three methods. Second, we compared their performance in a surveillance study using rectal swabs (n=139). The LOD of carbapenemase-producing *Enterobacteriaceae* strains was influenced by their MIC to carbapenems and was best for MacI followed by Chrom. The MacD method was able to detect only the strains exhibiting MIC of \( \geq 32 \) µg/mL to at least ertapenem.

In the surveillance study both Chrom and MacI had superior sensitivity (85%) as compared to MacD (76%). However, MacI was the most specific method. In conclusion, MacI appears to be most appropriate medium for the detection of CRE in settings in which multiclonal CRE strains with varying MICs to carbapenems are circulating.
INTRODUCTION

Carbapenem-resistant *Enterobacteriaceae* (CRE) had emerged globally and have become a major threat to public health (1, 17). Carbapenem resistance may be caused by a variety of mechanisms and have been identified in a variety of *Enterobacteriaceae* species (1, 20). In 2006, an epidemic strain of KPC-3-producing *Klebsiella pneumoniae*, exhibiting resistance to nearly all antimicrobial agents, has spread in all major Israeli hospitals (11, 16). This strain, identified as Sequence Type (ST) 258, is identical to the epidemic strain that had spread across the USA (9, 12) and is characterized by high minimal inhibitory concentration (MIC) values of carbapenem antibiotics (16).

As gastrointestinal carriage may serve as a reservoir for CRE cross-transmission in healthcare settings, active surveillance among high risk patients has been deemed important for controlling this epidemic in acute care facilities (2, 23). The implementation of a reliable and sensitive method for detection of this strain, as well as other CRE is therefore critical to the success of infection control measures. Although PCR-based methods have been proven to be highly sensitive and reliable for rapid diagnosis (8, 22), these methods require expertise that are not readily available in many centers. Moreover, as emergence and spread of other types of CRE is increasingly reported (7, 19), culture-based methods are still essential for the initial detection of these strains.

In our center, we have been using MacConkey agar supplemented with imipenem 1 µg/mL (HyLabs, Rehovot, Israel) as the main agar-screening plate for the detection of CREs from rectal swabs. In the present study, we compared this method to two other culture-based methods, namely CHROMagar-KPC (HyLabs, Rehovot, Israel).
and MacConkey plates with imipenem, meropenem and ertapenem disks. This paper reports the laboratory and clinical evaluation of these screening media.
MATERIALS AND METHODS

Setting, patient selection, and collection of surveillance specimens

The study was conducted as part of an ongoing surveillance program that had been implemented at the Tel-Aviv Sourasky Medical Center, a 1,200-bed tertiary care hospital in Tel-Aviv, Israel. From August 2008 through April 2009, rectal specimens were collected from known CRE carriers and from contacts of patients newly discovered to be harboring CRE as previously described (22). A nylon flocked swab system with liquid Amies medium was used according to manufacturer’s instructions (Eswab, Copan, Brescia, Italy) and immediately transferred to the laboratory following sampling and processed.

Analysis of the level of detection of the CRE screening plates

To analyze the limit of detection (LOD) of CREs, we compared the three type of screening plates used in our study for their ability to detect 10 distinct well-characterized strains (Table 1). Strains were stored in LB broth with 25% glycerol at -80°C, thawed and sub-cultured onto MacConkey agar plates before use. All strains were isolated in Israel, except for strains 2565 and 2577 that were isolated in Europe (see in 'Acknowledgement'). Isolates were suspended in saline to the density of 0.5 McFarland, followed by serial 10-fold dilutions. An aliquot of 100 µl from each dilution (0.5 McFarland:10 and lower) of each study strain was plated on each of the three screening plates evaluated in this study, as well as on Muller-Hinton agar for performing viable colony counts. The following selective agar plates were used: 1. CHROMagar-KPC (Chrom); 2. MacConkey agar with imipenem 1 µg/mL (MacI); 3. MacConkey agar plates with standard imipenem, meropenem and ertapenem 10 µg paper disks (Oxoid), sterilely applied at the 4-, 8-, and 12-o’clock positions (MacD).
Plates were incubated over-night at 35°C in ambient air and then read. The LOD was determined based on the minimal colony count allowing detection on the respective screening plate. The cost of each method per one sample was as following: Chrom-7.27 NIS; MacI-2.1 NIS; MacD-1.95 NIS (1 USD= 3.6 NIS).

Detection and identification of CRE from rectal swabs

We compared the three agar-based methods for the detection of CRE directly from rectal swabs. Swabs were vortexed for 10 seconds and 100 µl aliquots were plated onto the three different selective agar plates in parallel. Plates were then processed as described above. Following incubation plates were visualized for suspected CRE growth by two different observers.

CRE colonies on Chrom were identified according to the manufacturer's instructions (Klebsiella & Enterobacter species-medium-size dark metallic blue colonies; E. coli – medium-to-large size pink/dark rose colonies). CRE colonies on MacI and MacD were identified as any typical growth of lactose-fermenting pink colonies on the plate or within a 21mm diameter of at least one of the carbapenem disks, respectively. Suspected CRE colonies were sub-cultured from the respective screening plate onto standard MacConkey plates. Identification and antimicrobial susceptibility testing (AST) of bacterial strains was then performed by the VITEK-2 system using GN-ID and GN09 cards (bioMerieux, Marcy l’Etoile, France). Imipenem and meropenem MIC was verified by the E-test (AB Biodisk, Solna, Sweden). Susceptibility was determined using MIC breakpoints of the Clinical and Laboratory Standards Institute (CLSI) 2010 criteria (4).

Isolates non-susceptible to either imipenem or meropenem were defined as CRE positive and were subject to PCR for the bla_{KPC} gene according to a previously
described protocol (22). PCR-negative isolates were further tested by the modified Hodge test (MHT) according to CLSI recommendations (4).

The following were calculated for each of the methods: sensitivity, specificity, positive-predictive value (PPV), negative-predictive value (NPV) and overall accuracy. A true positive was defined as growth with phenotypic features compatible with CRE diagnosed as CRE by confirmatory testing. False positive was defined as growth with phenotypic features compatible with CRE diagnosed as non-CRE by confirmatory testing. We also calculated the turnaround time (TAT) as time elapsed from sample receipt in the laboratory to communication of final result to the clinician.
RESULTS

Analytical sensitivity of the 3 screening plates for identification of different CRE strains

The reference strains used, MIC of different carbapenems and growth performance with studied screening methods are summarized in Table 1. Strains exhibited varied MICs to different carbapenems. Any growth was detected in 10/10 on MacI, 9/10 on Chrom and 4/10 on MacD. With MacI, the LOD for detected strains was <10⁴ cfu/mL in 8/10. The respective rates were 2/4 for MacD and 7/9 for Chrom. All 3 CRE screening plates detected successfully all the strains with MIC of 32 µg/mL or higher for all carbapenems. These included the epidemic K. pneumoniae ST 258 (strain 490), strains 14 and 2577 (Table 1). These strains were detected at a lower inoculum by Chrom and MacI, but only at a higher inoculum (10-fold) by MacD. For the 9 strains that grew on both MacI and Chrom, the LOD was lower with MacI for 3 and similar with both media for 6. The colony morphology and color of the various K. pneumoniae strains were indistinguishable.

Performance of screening agar plates in recovery of CRE from rectal swabs

A total of 139 rectal swabs were collected; CRE was identified in 33 (24%) samples (31 patients) on at least one of the screening plates (figure 1). All isolates were K. pneumoniae, except for 2 (Klebsiella oxytoca and Enterobacter aerogenes). The MIC₁₀, MIC₅₀ and MIC₉₀ of the isolates, as determined by E-test, were as follows:

Imipenem - 6, >32, and >32 µg/mL; meropenem - 8, >32 and >32 µg/mL, respectively.

All isolates, but one, tested positive by bla_kPC PCR. The PCR-negative isolate was K. pneumoniae that tested negative by the MHT and had the lowest MIC values - 1 and 6 µg/mL to imipenem and meropenem, respectively.
The performance of different screening media is summarized in Table 2 and figure 1. Chrom and MacI detected 28 of 33 CRE strains while MacD detected only 25. Of CREs, 67% were detected by all media, 12% by two media and 21% by only one type of medium. There were 12, 6, and 11 samples in which growth on Chrom, MacI and MacD, respectively, was initially mistaken for CRE, resulting in unnecessary laboratory work-up. The implicated false positive isolates were mainly carbapenem-susceptible Enterobacteriaceae and also Acinetobacter baumannii. Chrom and MacI showed a similar sensitivity and negative predictive values, but MacI had superior specificity and positive predictive value and thus greater overall accuracy. The turnaround times were comparable. MacD had clearly inferior sensitivity and its specificity was similar to that of Chrom.
DISCUSSION

In this study, we compared the performance of three culture-based screening methods, for the detection of CRE from surveillance rectal swabs. In the clinical evaluation study, we found that both Chrom and MacI screening plates had a comparable sensitivity and negative-predictive value in detection of the highly-resistant \( \text{bla}_{\text{KPC}} \)-producing CRE that had spread in our hospital and exhibits high-level carbapenem resistance (11). MacI had higher specificity and positive-predictive value as compared to Chrom. Both screening plates were superior to MacD, in line with previous reports (21). Notably, MacI performed slightly better than Chrom during the laboratory evaluation and both were superior to MacD (Table 1). Only strains that were highly resistant to at least Ertapenem (Etest MIC value \( \geq 32 \) µg/mL) were detected by all 3 methods. Two strains with an Etest MIC of 12 µg/mL (1679 and 533) were not detected by the MacD method. This might be explained by the fact that we used inocula that were at least 10-fold lower than the standard 0.5 McFarland, that better resemble physiologic conditions. The difference in sensitivity between MacI and Chrom was not apparent during the surveillance study, as the dominant CRE at the time of study in our hospital was the \( \text{bla}_{\text{KPC}} \)-producing \( K. \text{pneumoniae} \) that is characterized by a high-level of resistance to carbapenems (11). However, with the increase in the diversity of CRE strains from different genera with variable MIC values (5-7, 14-15), the higher sensitivity of the MacI plates is likely to become important for adequate detection of CRE carriage.

MacI media had higher specificity, i.e. was the least likely to require additional work-up following growth of non-CRE strains (either carbapenem-susceptible \( \text{Enterobacteriaceae} \) or \( A. \text{baumannii} \)). This medium is considerably cheaper as compared to Chrom which had similar performance during clinical evaluation.
Combining these factors, in our hands, MacI appeared to be superior to the other two methods as the primary method for CRE surveillance.

Several studies have compared the performances of different culture-based methods, either to direct detection by PCR (8, 21-22) or to other culture-based methods (10, 13). A summary of these studies is presented in Table 3. The differences in studied populations, laboratory methods and study design make a head-to-head comparison difficult, but several conclusions can be made: 1. Among agar-based methods, the use of carbapenem disks on MacConkey plates appears to be the least sensitive; 2. Unlike screening for vancomycin-resistant enterococci (18), enrichment in broth was not superior to direct plating (13). Our study adds to the current literature the direct comparison of MacI with two previously described agar-based methods, along with an examination of the analytic sensitivity of these methods in detecting various types of CRE strains. Although direct detection by PCR has the advantage of rapid identification of CRE carriers, it is limited to the detection of CRE that harbor the target β-lactamase gene, e.g., bla\text{KPC}, and will inevitably miss non-carbapenemase producing CRE or those who carry other genes, such as \text{bla}_{\text{VIM-1}} or \text{bla}_{\text{NDM-1}} (New Delhi Metallo-β-lactamase-1). Moreover, the recovery of CRE strains is essential in order to perform molecular epidemiology studies (e.g., pulse-field gel electrophoresis), especially in an outbreak situation, in order to better direct infection control measures.

In conclusion, in geographic regions such as Israel, where CRE of various genera and with a wide range of MICs to carbapenems are being discovered (5-7, 14-15), the use of MacConkey agar supplemented with imipenem 1µg/mL is the most appropriate for detection of CRE carriage. This screening plate offers a sensitive, convenient and relatively low-cost method for identifying CRE species and it is able to detect even CRE species with relatively low carbapenem MICs.
Acknowledgements

We would like to acknowledge Prof. Marek Gniadkowski, Department of Molecular Microbiology, National Medicines Institute, Warsaw, Poland, for providing us with the metallo β-lactamase control strains characterized during the MOSAR project.

This work was supported in part by the European Commission Research grant FP7: SATURN - Impact of Specific Antibiotic Therapies on the Prevalence of Human Host Resistant Bacteria Grant No 241796.


Table 1. The level of detection of the three CRE screening plates for the detection of carbapenem-resistant *Enterobacteriaceae*

<table>
<thead>
<tr>
<th>Reference</th>
<th>Level of detection on the indicated screening plate (cfu/mL)</th>
<th>MIC µg/mL</th>
<th>bla type</th>
<th>Species</th>
<th>Bacterial Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chrom</td>
<td>MacD</td>
<td>MacI</td>
<td>Ertapenem</td>
<td>Meropenem</td>
</tr>
<tr>
<td>11</td>
<td>1.1x10²</td>
<td>1.1x10³</td>
<td>1.1x10³</td>
<td>64</td>
<td>32</td>
</tr>
<tr>
<td>16</td>
<td>4.1x10³</td>
<td>ND</td>
<td>4.1x10³</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>16</td>
<td>1x10²</td>
<td>1x10²</td>
<td>1x10²</td>
<td>&gt;32</td>
<td>&gt;32</td>
</tr>
<tr>
<td>3</td>
<td>1.1x10³</td>
<td>ND</td>
<td>1.1x10³</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>9.7x10³</td>
<td>ND</td>
<td>9.7x10³</td>
<td>2</td>
<td>1.5</td>
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<tr>
<td>6</td>
<td>6.5x10³</td>
<td>2x10⁶</td>
<td>6.5x10³</td>
<td>&gt;32</td>
<td>12</td>
</tr>
<tr>
<td>6</td>
<td>8.9x10³</td>
<td>ND</td>
<td>8.9x10³</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>ND</td>
<td>ND</td>
<td>8.3x10⁶</td>
<td>0.75</td>
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<tr>
<td>MOSAR1144</td>
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<td>ND</td>
<td>1.4x10³</td>
<td>4</td>
<td>32</td>
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<tr>
<td>MOSAR1156</td>
<td>1.1x10²</td>
<td>1.1x10³</td>
<td>1.1x10²</td>
<td>32</td>
<td>32</td>
</tr>
</tbody>
</table>

ND – no detection; Chrom- CHROMagar-KPC plates; MacI- MacConkey agar with imipenem 1 µg/mL; MacD-MacConkey agar plates with standard imipenem, meropenem and ertapenem 10 µg paper disks; CRE- carbapenem-resistant Enterobacteriaceae; ST- sequence type.
Table 2. Summary of the CRE screening plates performance.

<table>
<thead>
<tr>
<th>Method</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
<th>Mean TAT (range, days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MacI</td>
<td>84.9%</td>
<td>94.3%</td>
<td>82.3%</td>
<td>95.2%</td>
<td>92.1%</td>
<td>2.8 (2-4)</td>
</tr>
<tr>
<td>MacD</td>
<td>75.8%</td>
<td>89.6%</td>
<td>69.5%</td>
<td>92.2%</td>
<td>86.3%</td>
<td>2.8 (2-4)</td>
</tr>
<tr>
<td>Chrom</td>
<td>84.9%</td>
<td>88.7%</td>
<td>70%</td>
<td>95%</td>
<td>87.8%</td>
<td>3.0 (2-4)</td>
</tr>
</tbody>
</table>

Chrom- CHROMagar-KPC plates; MacI- MacConkey agar with imipenem 1 µg/mL; MacD- MacConkey agar plates with standard imipenem, meropenem and ertapenem 10 µg paper disks; CRE- carbapenem-resistant *Enterobacteriaceae*; TAT- turnaround time.
<table>
<thead>
<tr>
<th>DESIGN</th>
<th>METHODS EVALUATED</th>
<th>RESULTS</th>
<th>STUDY</th>
</tr>
</thead>
</table>
| Surveillance rectal swabs  
(n=187) | MAC plate+ERT, IMI and MER disks, cutoff not stated  
 blaKPC qPCR from swabs following extraction method A  
 blaKPC qPCR from swabs following extraction method B | SN=87%, SP=100%  
 SN=100%, SP=95%  
 SN=97.9%, SP=96.4% | [8] |
| LOD analysis of CRE strains;  
 surveillance rectal swabs (n=51) | TSB broth + IMI disk, sub-cultured to MAC plates  
 TSB broth, sub-cultured to MAC plates + IMI disk,  
 cutoff<16mm | Comparable analytical LOD;  
 SN=100%  
 Comparable analytical LOD;  
 SN=50% | [10] |
| Surveillance rectal swabs  
(n=149) | TSB broth + IMI disk, sub-cultured to MAC plates  
 MAC plate+ERT disks, cutoff <27 mm | SN=65.6%, SP=49.6%  
 SN=97%, SP=90.5% | [13] |
| Surveillance rectal swabs  
(phenotypic methods compared to  
 blaKPC PCR; n=122) | MAC plate+ERT, IMI and MER disks, cutoff not stated  
 CHROMagar KPC | SN=92.7%, SP=95.9%  
 SN=100%, SP=98.4% | [21] |
| Surveillance rectal swabs  
(n=755) | Inoculated BHI broth subject to blaKPC PCR  
 MAC+IMI 1 µg/mL | SN=92.2%, SP=99.4%  
 SN=87.5%, SP=99.6% | [22] |
| Surveillance rectal swabs  
(n=139) | MAC plate+ERT, IMI and MER disks, cutoff <22mm  
 MAC+IMI 1 µg/mL  
 CHROMagar KPC | SN=75.8%, SP=89.6%  
 SN=84.9%, SP=94.3%  
 SN=84.9%, SP=88.3% | Current study |

TSB- tryptic soy broth; Md.- Method; IMI- imipenem; ERT- ertapenem; MER- meropenem; MAC- MacConkey; LOD- level of detection; SN- sensitivity; SP- specificity.
Figure 1. The recovery of carbapenem-resistant Enterobacteriaceae (CRE) from rectal swabs and performance of screening agar plates.

Numbers in brackets show percentage from total number of positive samples (n=33). MacI- MacConkey agar with imipenem (1 µg/mL plates); Chrom- CHROMagar-KPC plates; MacD- MacConkey agar plates with ertapenem, imipenem and meropenem disks; ND= not detected.