Verification of direct methods for detection of carbapenemase producing Enterobacteriaceae from blood cultures

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Background: The aim of the study was to evaluate blood modified carbapenem inactivation method (mCIM) and automated susceptibility via VITEK2 compact system directly from positive blood culture bottles.

Methods: The study was carried out using 54 strains positive for carbapenemase genes (OXA-48 type, KPC-type, NDM-type, and VIM-type) by multiplex PCR and 30 strains negative for these genes as controls, these strains were inoculated into blood culture bottles and then tested phenotypically for carbapenem resistance by mCIM and Vitek2 AST directly from the blood culture bottle.

Results: MCIM was positive for all tested CPE strains and negative for the control strains with a 100% sensitivity, specificity and agreement with the PCR results. While vitek2 system had a sensitivity of 96%.

Conclusion: Both methods save time when performed directly from the blood culture bottle that should guide appropriate administration of antimicrobials targeting bloodstream infections caused by carbapenemase producing Enterobacteriaceae. The mCIM is superior in being inexpensive requires basic reagents available in all microbiology laboratories with minimal processing compared to other tests and gives positive results with different carbapenemase classes.

Introduction

Carbapenem resistance is one of the most concerning forms of antimicrobial resistance in members of the Enterobacteriaceae family. These are defined by the Centers for Disease Control and Prevention (CDC) as Enterobacteriaceae that test resistant to at least one of the carbapenem antibiotics (ertapenem, meropenem, doripenem, or imipenem) or produce a carbapenemase and should be known as carbapenem resistant Enterobacteriaceae (CRE) [1]. Also according to the Clinical & Laboratory Standards Institute (CLSI), Enterobacterales are suspected as carbapenemase producers when imipenem/meropenem have MIC value of 2-4 ug/ml or Ertapenam has MIC of 2 ug/ml requiring further use of a confirmatory test [2].

Carbapenem resistant Enterobacteriaceae continue to spread worldwide and in Egypt including our institute the “Alexandria university hospital” and since carbapenems are considered among the main lines of defense for life threatening infections, especially among intensive care unit (ICU) patients, where bloodstream infections due to carbapenemase-producing Enterobacteriaceae (CPE) are associated with poor outcomes compared to non-carbapenemase-producing-carbapenem-resistant (NCPE) ones [3]. Further, delayed treatment with effective antimicrobial agents in
patients with sepsis is associated with a decrease in survival for every hour that therapy is delayed. Phenotypic resistance to carbapenems is usually conferred by carbapenemases [4].

Therefore, accurate and steadfast methods to detect carbapenemase activity directly from positive blood culture are important for assisting physicians with antimicrobial therapy selection to treat bloodstream infections due to these organisms [5,6]. Furthermore, rapid detection and characterization of all types of carbapenemases would lead to improved patient care and guide the implementation of infection control measures [7].

The present study aimed to verify the performance of blood modified carbapenem inactivation method (mCIM) and VITEK2 compact system for the accurate, economic and rapid detection of CPEs with different classes of carbapenemases directly from positive blood culture samples to help guide carbapenem therapy in patients with bloodstream infections due to Enterobacteriaceae in a timely and effective manner.

Methods

Selection of strains to be included in the study
Out of Enterobacteriaceae clinical strains recovered, identified and tested by disc diffusion [2,8] through the Microbiology Laboratory of the Main University Hospital over a 3 months period; 84 strains identification was confirmed by VITEK 2 GN ID (bioMérieux, France), then they were genetically characterized to be included in the study based on multiplex-PCR results for carbapenemase production according to the method of Doyel et al. [9] strains were characterized according to carbapenemase type produced (OXA-48 type, KPC-type, NDM-type, and VIM-type) into: 54 CPE and 30 NCPE strains to be included in the study. Both the CPE and NCPE were subjected to the following:

Seeding of blood culture bottles
BACT/ALERT FA Plus (bioMérieux, France) aerobic blood culture bottles (inoculated with 5 ml human blood) were seeded with one ml of ≈ 1.5 x 10^3 CFU/ml of the suspension of each of the study isolates, this was done by using fresh bacterial colonies to prepare a 0.5 McFarland suspension (=1.5 x 10^8 cfu/ml) that was further serially diluted to reach the required count [7]. All bottles were processed in BACT/ALERT 3D blood culture system as per manufacturer’s recommendations.

Blood-modified carbapenem inactivation method (Blood mCIM) [5]
Upon positive signal, one ml of the positive blood culture broth was transferred to a 1.5 ml Eppendorf tubes. A meropenem disc (10 ug) (Oxoid, UK) was added and fully immersed in the blood culture broth. The tubes were incubated at 37 °C without agitation for 2 hours. The meropenem discs were then removed using a 10 ul disposable loop and applied to Mueller-Hinton agar plates (Oxoid, UK) inoculated with 0.5 McFarland suspension of the reference strain carbapenem susceptible E. coli ATCC 25922 (Becton Dickinson (BD), USA). The plates were incubated overnight at 37 °C. Results were interpreted as per CLSI guidelines [2]. A meropenem zone diameter of 6-15 mm or pinpoint colonies within 16-18 mm inhibition zone was interpreted as positive carbapenemase test.

VITEK-2 direct carbapenem antimicrobial susceptibility testing from positive blood cultures
A 6 ml sample from positive blood culture bottles was centrifuged at 3000 rpm for 3 min to pellet blood cells. The resulting supernatant was centrifuged at 6000 rpm for 15 min to pellet bacteria. The resulting pellet was used to inoculate 0.45% sodium chloride and used to adjust the turbidity of the bacterial suspension by VITEK Densichek (bioMérieux) to match 0.5 McFarland standard [10]. VITEK-2 GN and GN 222 cards were used for identification and antimicrobial susceptibility using VITEK-2 Compact (bioMérieux, France) as recommended by the manufacturer. Ertapenem MIC was done manually according to CLSI broth dilution guidelines as it is not included in the card available in our setting [11].

Statistical analysis
Data were analyzed using the SPSS 22.0 statistical package (Chicago, Illinois). Results of Blood mCIM and direct VITEK2 antimicrobial susceptibility were evaluated with reference to the carbapenemase positive and negative isolates.

Results
Fifty four PCR positive strains for the tested carbapenem genes (CPE) were used to perform this study and 30 Enterobacteriaceae strains, negative for carbapenemase genes (NCPE) were used as control.

The 54 (CPE) strains found positive for the tested genes were 42 klebsiella pneumoniae, 4 klebsiella aerogenus and 8 Escherichia coli, while the NCPE strains used as controls included, 12...
Klebsiella pneumoniae, 15 Escherichia coli, 2 Citrobacter freundii and one Proteus vulgaris strain.

Carbapenemase genes detected for the CPE strains are shown in Table (1). More than one gene were detected in 67% of klebsiella pneumoniae strains (Class B and D in 57.2%, and class B in 9.5%) the most common combination was NDM+OXA (18 strains, 43%), while the remaining 33% had only one gene detected, similarly all E.coli strains and 2 K.aerogenes strains had one gene detected. The most singly detected gene was OXA, found singly in 22(41%) of the tested strains, while none of the strains tested were positive for the KPC gene. The metalo-beta-lactamase (MBL) Class B enzymes were detected in 30 strains of the 54 CPE whether singly or in combination. MBL only producing strains included 2 (4.8%) strains of K.pneumoniae that only produced NDM and 4 strains (9.5%) of the same organism produced MBLs in combination (NDM+IMP+VIM).

Blood-mCIM was assessed on the of 84 Enterobacteriaceae strains. All 54 CPE strains were found positive for the Blood mCIM while the 30 NCPE strains were all negative giving a 100% for; sensitivity (95% CI, 93.4% to 100%), specificity (95%CI, 88.4% to 100%) and overall agreement with PCR results. The reference strain E. coli ATCC 25922 (Becton Dickinson (BD), USA) used showed growth till the edge of the meropenem disc as shown in figure (1) for all CPE strains, while for the NCPE strains the zone exceeded 19 mm for all.

As for VITEK-2 direct carbapenem antimicrobial susceptibility testing from positive blood cultures (Figure 2), 52 strains were considered CRE based on meropenem and imipenem MICs. Isolates of Enterobacteriaceae are suspicious for carbapenemases production based on imipenem or meropenem MICs of 2–4 μg/mL [2]: 2 CPE strains were not reported as such, both these strains were E.coli, they both had MICs less than 2 ug/ml for both drugs, another 2 strains one E.coli and the other K.pneumoniae both were sensitive to imipenem and resistant to meropenem by VITEK-2 and were suspected as carbapenemase producer as per CDC definition. The used VITEK-2 card doesn’t include ertapenem so Ertapenem MIC was done manually and the above 4 isolates had MIC of 2ug/ml or more indicating resistance. While none of the NCPE strains were resistant to imipenem and meropenem by VITEK-2 and were considered NCRE. Giving a sensitivity of 96.3% (95% CI,87.2% to 99.5%) and specificity of 100% (95%CI, 88.4% to 100%), with an overall agreement of 97.6%.

Table 1. The distribution of different carbapenemases genes in the study.

<table>
<thead>
<tr>
<th>Species</th>
<th>NDM (Class B)</th>
<th>OXA (Class D)</th>
<th>OXA+NDM (Class B &amp; D)</th>
<th>OXA+VIM (Class B &amp; D)</th>
<th>NDM+OXA+VIM (Class B &amp; D)</th>
<th>NDM+IMP+VIM (Class B)</th>
<th>OXA+NDM+IMP+VIM (Class B &amp; D)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K.pneumoniae</td>
<td>4.8%</td>
<td>28.5%</td>
<td>42.8%</td>
<td>4.8%</td>
<td>4.8%</td>
<td>9.5%</td>
<td>4.8%</td>
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<tr>
<td>42(78%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E.coli</td>
<td>8(15%)</td>
<td></td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>K.aerogenus</td>
<td>4(7%)</td>
<td>50%</td>
<td>50%</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4(7%)</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>54(100%)</td>
<td>3.7%</td>
<td>40.7%</td>
<td>37%</td>
<td>3.7%</td>
<td>3.7%</td>
<td>7.4%</td>
<td>3.7%</td>
</tr>
</tbody>
</table>
Figure 1. The results of mCIM for four CPE strains (1–4) showing no zones of inhibition around the meropenem disc (10 ug).

Figure 2. Shows the antibiotic susceptibility pattern for CPE strains.

Discussion

In the current study we aimed to assess the blood mCIM for implementation in our laboratory, this was carried out first by genotypically characterizing CP-CRE clinical strains to be used in the study, from which K. pneumoniae isolates compromised a majority of 78% as it is the most common CRE in Egypt as confirmed by others [12,13]. Also among the tested carbapenemases genes 57% of the K. pneumoniae isolates harbored combination of class B and D genes, the most common combination was NDM and OXA48 in our study and elsewhere [14] while the remaining isolates had only one gene detected.

Blood mCIM performance against our CPE strains was 100% sensitive and 100% specific saving valuable time in reporting carbapenem resistance results to clinicians on the next day from a positive signal bottle with a Gram negative bacilli. Adding to this the easy performance of the test directly on sample from positive signal bottle after performing Gram stain with no need of any prior processing steps as needed for the Carba-Np which requires steps for lysis of RBCs not to affect the test color, nor does the mCIM test require any specific reagents or equipment’s, on the contrary it is performed using accessible tools in any microbiology laboratory. Also quite the reverse to modified Hodge test, which is poor in detection of metallo beta lactamase and requires supplementation, the mCIM test was positive for class B-MBL only producing strains without the need for adding a chelating agent [15], the limitation here is that we only had 6 isolates falling in this category.

Regarding VITEK-2 system, the device doesn’t report strains as CRE instead we used the CLSI suggestion that CPE are suspected if...
imipenem/meropenem MIC value is 2-4 ug/ml or Ertapenem MIC is 2 ug/ml [2]. Thus, on evaluating the VITEK-2 system for detection of carbapenem resistance directly from positive blood culture bottle, the sensitivity was 96.3% and specificity of 100% using the GN 222 AST card available in our laboratory, which only tests for imipenem and meropenem, although after MIC was done manually for ertapenem the sensitivity reached 100% confirming that ertapenem is the carbapenem drug most sensitive to detection of CPE as confirmed by Mohapatra and Kapil [16] and with the increasing importance for the detection of CRE strains it is better to choose cards including the 3 carbapenems.

To conclude, blood mCIM proved to be an efficient, standardized, simple and cost-effective method for implementing in routine laboratory work to guide and expedite appropriate carbapenem therapy in cases with Enterobacteriaceae blood stream infection (BSI) as it yields results a day earlier since it is done directly from positive blood culture bottle rather than from the subcultured colony, which would definitely play a role in assisting infection control and surveillance without the need of any advanced equipment. Also VITEK system using card GN222 was of high sensitivity but should be supplemented with mCIM method to avoid false carbapenem sensitivity results in critical patients with BSI.

Ethical considerations
This study has been reviewed and approved by the Alexandria faculty of medicine Ethical committee (S.N:0305108 April 2021), and was conducted according to the principles of the Declaration of Helsinki. The Alexandria faculty of medicine Ethical committee did not request an informed consent as the study did not involve any patient contact and only used bacterial strains already isolated by the hospitals’ microbiology laboratory without the need for any patient related data and total anonymity to the subjects.

Conflict of interest: The authors declare absence of conflict of interest.

Financial disclosure: Non to disclose.

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