Detection of Carbenem resistance genes among selected Gram Negative bacteria isolated from patients in-Khartoum State, Sudan

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Background: Carbapenem-resistant Gram-negative rods (CR-GNR) are gaining increasing importance in healthcare settings, especially in high-dependency units and among critically ill patients. These bacteria are frequently resistant to all antibiotics except colistin, some aminoglycosides and variably tigecycline, posing a serious challenge for treatment. CR-GNR cause infections associated with significant morbidity and mortality. Data on the prevalence of carbapenem resistant genes in Sudan is limited. This study, aimed to determine the prevalence of CR-GNR isolated from clinical specimens in Khartoum, Sudan during January 2015 to August 2015.

Methods: A total of 83 Carbapenem resistant clinical isolates (Klebsiella pneumoniae n=21, Escherichia coli n=7, Pseudomonas aeruginosa n=15, Citrobacter n=2, Proteus n=1 and Acinetobacter baumannii n=37) were screened for the presence of carbapenemases (blaTEM, blaVIM, blaIMP, blaSHV, blaCTX and blaKPC genes) by using Multiplex PCR.

Results: Out of 83 isolates, 68 were Tem gene positive, while 50 isolates were Vim gene positive, Imp gene was present in 42 isolates, Kpc gene in 41 isolates, Ctx gene present in 40 isolates and Shv gene in 15 isolates. TEM gene was the predominant gene among the positive (antibiotic resistant) species.

Conclusion: Detection of the genes related with carbapenemase production indicated widespread prevalence and multiplicity of these genes in carbapenem resistant clinical isolates. The results also showed that the multiplex PCR as reliable, fast method for the detection of these genes.

Keywords: Tem, Vim, Kpc, Imp, Shv, Ctx, Carbapenemases, Multiplex PCR.

Carbapenemase-producing (GNR) have been associated with increasing mortality and with serious hospital outbreaks that present major therapeutic and infection control challenges [4]. CR-GNR has been associated with the use of medical devices such as: intravenous catheters, ventilators, urinary catheters, and through wounds caused by injury or surgery [15]. Detection of Carbapenemases resistance genes producing bacteria may be difficult based on routine antibiotic susceptibility testing. Therefore it is essential to implement efficient infection control actions to limit the spread of these pathogens [18]. Tests based on molecular techniques are considered the standard tests for the identification of carbapenemase genes [5]. Considering the fact that information on the subject is limited in our country, utilization of a suitable method for determination of the carbapenemase production is important in the microbiology laboratory [19]. Therefore the aim of the present study was to test the feasibility of nucleic acid extraction and a multiplex PCR amplification assay for identification of blaTEM, blaSHV, blaIMP, blaKPC, blaVIM and blaCTX genes in a series of clinical isolates of (CR-GNR). Such data serve an important role in understanding the spread of carbapenem-resistant gram-negative pathogens.
Methods

Bacterial Isolates:

A total number of 83 CR-GNR isolates including (Pseudomonas aeruginosa, Acinetobacter baumannii, Klebsiella pneumonia, Escherichia coli, Citobacter freundii and Proteus mirabilis) and 12 negative controls isolated from three private hospitals, during January till August 2015, Khartoum, Sudan were investigated using multiplex PCR. The isolates were identified using microbiological and biochemical methods.

Antibiotic Resistance Investigation:

Measurement of antibiotic resistance was conducted by disk diffusion method for the following antibiotics: Ceftazidime (CAZ), Cefteriaxon (CRO), Cefotaxime (CTX), Cephalexin (CN30), Cefixime (CFM), Penicillin (P), Cefuroxime (CXM), Meropenem (MRP) and Imipenem (IMP) according to the standards of Clinical and Laboratory Standards Institute (CLSI).

Investigation of carbapenem resistance genes by PCR:

Carbapenem resistant isolates were used to investigate bla-VIM, bla-IMP, blaKPC, blaSHV,blaKPC and blaCTX genes by PCR. For DNA extraction the boiling method was applied [13]. Firstly, three to five colonies were picked from fresh culture medium and then a suspension was prepared using 200 ml of distilled water boiled at 100 °C for 30 minutes. The suspension was then centrifuged at 12000 rpm for 30 minutes, and the supernatant containing DNA was transferred to new Eppendorf tubes for PCR in order to amplify the genes, (bla-VIM, bla-IMP, blaKPC, blaTEM and bla-CTX). The primers used to amplify the genes are shown in the table below:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Sequence (5′- 3′)</th>
<th>Product size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>KPC FP</td>
<td>TCGAACAGGACCTTGGCG</td>
<td>201</td>
<td>6</td>
</tr>
<tr>
<td>KPC RP</td>
<td>GGAACCGGCGCATTTTGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IMP FP</td>
<td>GAAAGGCTTTATGCTCATAC</td>
<td>587</td>
<td>6</td>
</tr>
<tr>
<td>IMP RP</td>
<td>GTAAGTTGCAAGTGGATGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VIM FP</td>
<td>GTTTGTCGATATCGCAAC</td>
<td>382</td>
<td>6</td>
</tr>
<tr>
<td>VIM RP</td>
<td>AATGCCGACCGCCAGGATAG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TEM FP</td>
<td>TCGCGGCATACATATTCCTAGA</td>
<td>445</td>
<td>12</td>
</tr>
<tr>
<td>TEM RP</td>
<td>ACGTCACGGCTCCAGTATAAT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHV FP</td>
<td>ATGCCATATTCGCTTGTG</td>
<td>747</td>
<td>7</td>
</tr>
<tr>
<td>SHV RP</td>
<td>TGCTTTGTTATCGGGCCAA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTX FP</td>
<td>ATGCAGYACCAGTAARGTATGCG</td>
<td>593</td>
<td>8</td>
</tr>
<tr>
<td>CTX RP</td>
<td>GGTrAARTGTSACCAGAAYCAGCG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table1: Primer sequences of 6 target genes (blaKPC, blaIMP, blaVIM blaTEM , blaSHV and blaCTX genes) for Multiplex PCR.

To perform PCR, 2μl from the primers, 5 μl of the extracted DNA, 13 μl of distilled water was added to the PCR Master Mix (Maxime PCR Permix Kit, Korea) with a final volume of 20 μl. Thermal cycling (Aeris Machine Pelter technology Thermo Assist) for 30 cycles was done at 94 °C for 1 min, 60 °C for 1 min and 72 °C for one and half min. The final extension step was performed for 5 min at 72 °C. The PCR products were applied and electrophoresed in 2% agarose gel along with ladder DNA and then stained using ethidium bromide. The result was observed by transilluminator system (Biometer an analytical Jena company).

Figure 1:  Agrose gel result of Shv, Tem and Ctx. (lane 1: 100bp ladder; lane 2: positive control; lane 3: Ctx positive sample; lane 4: Tem and Ctx positive sample; lane 5: negative control).

Results

The results of the Multiplex PCR for the six target genes are shown in Table 2.

Total of 99 clinical isolates which successfully grew, 83 were identified to be resistant to Meropenem, while 12 isolates were susceptible to Meropenem.

Out of 83 CR-GNB 37 (44.5%) were A. baumanii as the most predominant species followed by K. pneumonia 22 (26.5%), P. aeruginosa 14 (16.8%), E. coli 7 (8.4%), C. freundii 2 (2.4%) and P. mirabilis 1 (1.2%). The prevalence of the resistance genes in the 83 resistant isolates were as follows TEM (81.9%) was the most predominant gene, VIM (56.6%), IMP (50.6%), KPC (45.7%), CTX (48.1%) and SHV (18.1%). A. baumanii, K. pneumoniae, P. aeruginosa, E. coli isolates harbored all six resistance genes (TEM, KPC, CTX, IMP, VIM, SHV), C. freundi contained five of the genes but lacked (SHV) gene, While P. mirabilis on the other hand contained only one gene (CTX).
Carbapenems and Beta lactam antibiotics are used considerably to treat infections due to multidrug-resistant Gram Negative bacteria. Resistance of Carbapenem agents is due to carbapenemase and presence of other resistance mechanisms, such as ESBLs, and porin mutations [9]. In the current study, these genes were mostly detected in a high percentage of antibiotic resistant isolates of A. baumannii, K. pneumoniae, P. aeruginosa, E. coli, C. freundii and P. mirabilis. Findings where TEM is reported as the commonest MBL to be found (81.9%), followed by VIM (56.6%), IMP (50.6%), KPC (45.7%), CTX (48.1%), while SHV (18.1%) had the lowest prevalence in the tested resistant isolates. Of 83 bacterial isolates detected of having carbapenemase genes, 70 had multiple genes coding for carbapenem resistance especially in A. baumannii and K. pneumoniae. The presence of multiple resistance genes in one strain provides selection advantage of these strains; such phenomenon has not been commonly detected in a large number of studies probably due to the limited number of genes studied since most of the studies research on one or two genes. Since various reports indicate the increased resistance of these bacteria against antibiotics (especially imipenem), proper use of these antibiotics and time-consuming identification of isolates generating MBL should be considered. This can lead to successful treatment and prevents propagation of resistant genes, which can be seriously harmful for societies [11] Testing of carbapenem resistant genes among MDR-GNB isolates should routinely be used to determine these species in clinical laboratories. In addition, increased effort at discovery of more effective antimicrobial compounds with new effecting mechanisms should be noted. Finally, as the most of MDR-GNB infection are hospital acquired every effort should be exerted to prevent spread of these bacteria in our health facilities.

Conclusion

Identification of carbapenem resistance and their resistance pattern of genes is necessary for the surveillance of their transmission in hospitals and to overcome the problems associated with G-ve carbapenem resistance. The multiplex PCR described here is a reliable and rapid method for detection of the most prevalent carbapenemase genes.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

SBS carried out the molecular genetic studies and drafted the manuscript. AIE conceived of the study and contributed to the conception and design of the study. AME, IME and MAA revised the manuscript. KAE contributed to the conception and design of the study and acquisition of funding. All authors read and approved the final manuscript.

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Discussion

Different antibiotics including beta lactamases, aminoglycosides and quinolones are applied to treat the infections caused by Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, E.coli, Proteus mirabilis and Citrobacter freundii. However, it is becoming a great challenge to treat infections caused by these bacteria due to its resistance against drugs and the rapid changes in the pattern of resistance. Additionally, the resistance of the bacteria against antibiotics fluctuates, especially regarding Imipenem. Carbapenems and Beta lactam antibiotics are being used increasingly to treat infections due to CR-GNR since they are resistant against most of the beta lactamases and have great membrane permeability [10].

Table 2: Results of Genotypic test (Multiplex PCR).

<table>
<thead>
<tr>
<th>Resistance gene</th>
<th>TEM</th>
<th>VIM</th>
<th>IMP</th>
<th>KPC</th>
<th>CTX</th>
<th>SHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.baumannii</td>
<td>31</td>
<td>25</td>
<td>17</td>
<td>19</td>
<td>16</td>
<td>6</td>
</tr>
<tr>
<td>K.pneumoniae</td>
<td>19</td>
<td>15</td>
<td>16</td>
<td>11</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>P.aeruginosa</td>
<td>10</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>7</td>
<td>1</td>
</tr>
<tr>
<td>E.coli</td>
<td>6</td>
<td>6</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>P.mirabilis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>C.freundii</td>
<td>2</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>68</td>
<td>50</td>
<td>42</td>
<td>41</td>
<td>40</td>
<td>15</td>
</tr>
<tr>
<td>%</td>
<td>0.819</td>
<td>0.566</td>
<td>0.42</td>
<td>0.457</td>
<td>0.481</td>
<td>0.181</td>
</tr>
</tbody>
</table>

Figure 2: Agarose gel result of IMP, VIM and KPC (Lane 1:100bp ladder; Lane 2: IMP Positive sample; Lane 3: VIM positive sample; Lane 4: KPC positive sample).
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