**bla**<sub>OXA-48</sub> Carbapenem Resistant *Pseudomonas aeruginosa* Clinical Isolates in Sudan

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**Authors’ contributions**

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

**Article Information**

DOI: 10.9734/JAMB/2018/34964

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Complete Peer review History: [http://www.sciencedomain.org/review-history/24849](http://www.sciencedomain.org/review-history/24849)

**ABSTRACT**

Carbapenems are the last resort of antimicrobial therapy, resistance to antibiotics specially carbapenems poses a global problem that may have devastating consequences on the community. We investigated 67 multidrug resistant clinical isolates of *Pseudomonas aeruginosa* for the presence of the *bla*<sub>OXA-48</sub> gene using real time PCR.
The isolates were obtained from Three major hospitals in Khartoum state Sudan; Army hospital, Khartoum hospital and the national Ribat university teaching hospital, from December 2015 to January 2017.
Out of 67 samples, 22.4% were positive for the *bla*<sub>OXA-48</sub>, 46.3% were resistant to Imipenem and 32.8% of the samples were resistant to Meropenem.
The emergence of carbapenem resistance possesses an imminent threat, leaving patients with no choice of treatment. Monitoring the emergence of resistant strains will allow taking suitable preventive measures to take place. Our findings revealed that carbapenem resistant due to the gene

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bla\textsubscript{OXA-48} is accounted for 22.4\% of the cases, and due to poor data documentation about the emergence of this gene in Sudan, these cases to the best of our knowledge are the first to be reported in Sudan.

Keywords: Pseudomonas aeruginosa; antimicrobial resistance; carbapenem resistance; bla\textsubscript{OXA-48}.

1. INTRODUCTION

Antimicrobial resistance is one of the prime public health crisis worldwide. Several factors contributed to the emergence of the resistant bacteria, the most important factor is the irrational use of antibiotics, incomplete dosage of treatment and the use of antibiotics in agricultural settings and animal farming.

*Pseudomonas aeruginosa* is a gram-negative bacterium notorious for causing opportunistic infections that can be more severe in immunocompromised patients. The increased isolation of *P. aeruginosa* strains not susceptible to most anti-pseudomonal agents is due to number of factors, including the presence of both intrinsic resistance and acquired resistance [1, 2].

The enzyme OXA-48 is a class D β-lactamase that possesses the ability to hydrolyze carbapenems as well as Oxacillin, Cloxacillin and Penicillin [3-5]. OXA-48 is not inhibited by clavulanic acid and EDTA, some studies showed that this class D enzymes can be inhibited by JDB/LN-1-255 compounds [6].

The first case of resistance mediated by OXA-48 was identified in 2001 from a multi-drug resistant *Klebsiella pneumoniae* in Istanbul Turkey [7]. Reports of OXA-48 producing organisms have followed. Several cases of OXA-48 producing organisms like *Pseudomonas*, *E. coli* and *citrobacter* were reported in patients from or have a relation with Turkey [8]. Outbreaks were reported worldwide, disseminating from Middle Eastern countries and North Africa. Sporadic cases have been reported in Lebanon, Oman, Saudi Arabia and Kuwait. In Africa, mostly in northern African countries of Egypt, Tunisia, Libya and Morocco. There were also reports of the enzyme producing organism in Senegal and South Africa [9-11].

In this study, we report the detection of *bla\textsubscript{OXA-48}* gene in multidrug-resistant *Pseudomonas aeruginosa* clinical isolates in period from December 2015 to January 2017 from three major hospitals in Khartoum state Sudan; Army hospital, Khartoum hospital and the national Ribat university teaching hospital.

2. MATERIALS AND METHODS

Sixty-seven clinical isolates of *Pseudomonas* were phenotypically tested for carbapenem resistance, using Imipenem and Meropenem. Using primers sequence adapted from a study published by Monteiro et al. [Table 1] [12]. Real-time PCR was implemented to determine the presence of *bla\textsubscript{OXA-48}* gene. Amplifications were performed in 25 µL reaction volume containing 5 µL of 5× FIREPol PCR Master Mix premixed (Solis BioDyne, Estonia), 1 µL optimized primers at a final concentration of 0.2 mM, 0.3 µL of the DNA template and a sufficient quantity of sterile water. The PCR run was performed using Sacycler-96 instrument (Sacacae biotechnology, Italy). The real-time PCR conditions were as follows: 94°C for 10 min; 40 cycles of 94°C for 40 s, 55°C for 45s and 72°C for 50s and a final elongation step at 72°C for 10 min. The Sacycler-96 instrument automatically calculated the derivatives of fluorescence measured at 533 nm.

3. RESULTS

Real time PCR revealed that only 22.4\% of the samples were *bla\textsubscript{OXA-48}* gene positive [Fig. 1]. 46.3\% were resistant to Imipenem, 13.4\% were intermediately resistant and 40.3\% were sensitive to Imipenem. 32\% were resistant to Meropenem, 11.9\% were intermediately resistant and 56.1\% were sensitive to Meropenem [Table 2]. Among positive *bla\textsubscript{OXA-48* 73.3% were resistant to Imipenem, 13.3% were intermediately resistant and 13.3% were sensitive to Imipenem, 40.0% were resistant to Meropenem, 33.3% were intermediately resistant and 26.7% were sensitive to Meropenem.
Table 1. Primer sequence

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>GC%</th>
<th>TmC°</th>
<th>M.W µg/µmol</th>
<th>Final con µM</th>
<th>Amplicon size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla_{OXA-48} F</td>
<td>5'-GCGTGGTTAAGGATGAACAC-3'</td>
<td>42.1</td>
<td>50.8</td>
<td>5855.9</td>
<td>0.2</td>
<td>177 bp</td>
<td>[12] Monteiro et al</td>
</tr>
<tr>
<td>OXA-48-R</td>
<td>5'-CATCAAGTCTCAACCCAACCG-3'</td>
<td>42.1</td>
<td>50.8</td>
<td>5865.5</td>
<td>0.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. Real time PCR
Table 2. Carbapenem sensitivity profile

<table>
<thead>
<tr>
<th></th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>46.3%</td>
<td>13.4%</td>
<td>40.3%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>32%</td>
<td>11.9%</td>
<td>56.1%</td>
</tr>
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</table>

Table 3. Resistance profile of \( \text{bla}_{OXA-48} \) positive isolates

<table>
<thead>
<tr>
<th></th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imipenem</td>
<td>73.3%</td>
<td>13.3%</td>
<td>13.3%</td>
</tr>
<tr>
<td>Meropenem</td>
<td>40%</td>
<td>33.3%</td>
<td>26.7%</td>
</tr>
</tbody>
</table>

4. DISCUSSION

*Pseudomonas aeruginosa* is an environmental opportunistic organism notorious for being a multi-resistant. Its genetically designed to elude antimicrobials agents by implementing many mechanisms such as efflux pumps, loss of porins and abridged cell permeability as well as chromosomally encoded enzyme mediated resistance, also this organism can acquire and disseminate resistance via motile genetic elements such as plasmids. While only 22.4% of the organisms were positive for \( \text{bla}_{OXA-48} \) gene it's possible that other means of resistance and or presence of other resistance genes are responsible for the presence of resistance in the \( \text{bla}_{OXA-48} \) gene negative yet resistant organisms.

Carbapenems are the last resort of antibiotics, OXA-48 is a multi-substrate enzyme with activity not only restricted to carbapenems antibiotics but similarly it can induce resistance to Oxacillin Cloxacillin and penicillin as well which result in limited treatment options for patient. Before the identification of OXA enzymes in clinical samples, the origin of these enzymes was found to be descending from the waterborne species Shewanella [13]. The fact that these genes are present in an environmental organism opens a valid speculation that other organisms found in the same ecological niches can contract the gene and express it [14].

Antimicrobial resistance has been reported in all part of the world, OXA-48 reports in neighboring countries were in form of single care in Egypt, Algeria and Tunisia, and outbreaks in Morocco and Senegal15; yet there were no proper case reports about the presence of carbapenem resistant *Pseudomonas* in Sudan. The dissemination of resistant organisms became faster and can spread in large geographical regions, and for that proper monitoring and surveillance should be implemented.

5. CONCLUSION

Our findings revealed that carbapenem resistant due to the gene \( \text{bla}_{OXA-48} \) is accounted for 22.4% of the investigated cases, considering the possibility of carbapenem resistance genes presence will assist in adjusting the treatment plan and improve the patient's outcomes.

The emergence of carbapenem resistance possesses an imminent threat that may sanction the antibiotic era to an impending end. Identification of the resistant organisms and setting surveillance programs for monitoring of resistance will help in disentanglement of this problem and improve the recovery chances of patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


