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bla$_{OXA-48}$ 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.

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Short Research Article

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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 blaoxa-48 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*$_{oxa-48}$, 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_{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; blaOXA-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 non-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 pneumonae* 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 alsoreports of the enzyme producing organism in Senegal and South Africa [9-11].

In this study, we report the detection of bla_{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_{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_{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_{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

	Primer	Sequence	GC%	TmCº	M.W µg/µmol	Final con µM	Amplicon size	Reference
bla_{OXA-48}	OXA-48-F	5'-GCGTGGTTAAGGATGAACAC-3'	42.1	50.8	5855.9	0.2	177 bp	[12] Monteiro
	OXA-48-R	5'-CATCAAGTTCAACCCAACCG-3'	42.1	50.8	5865.5	0.2		et al

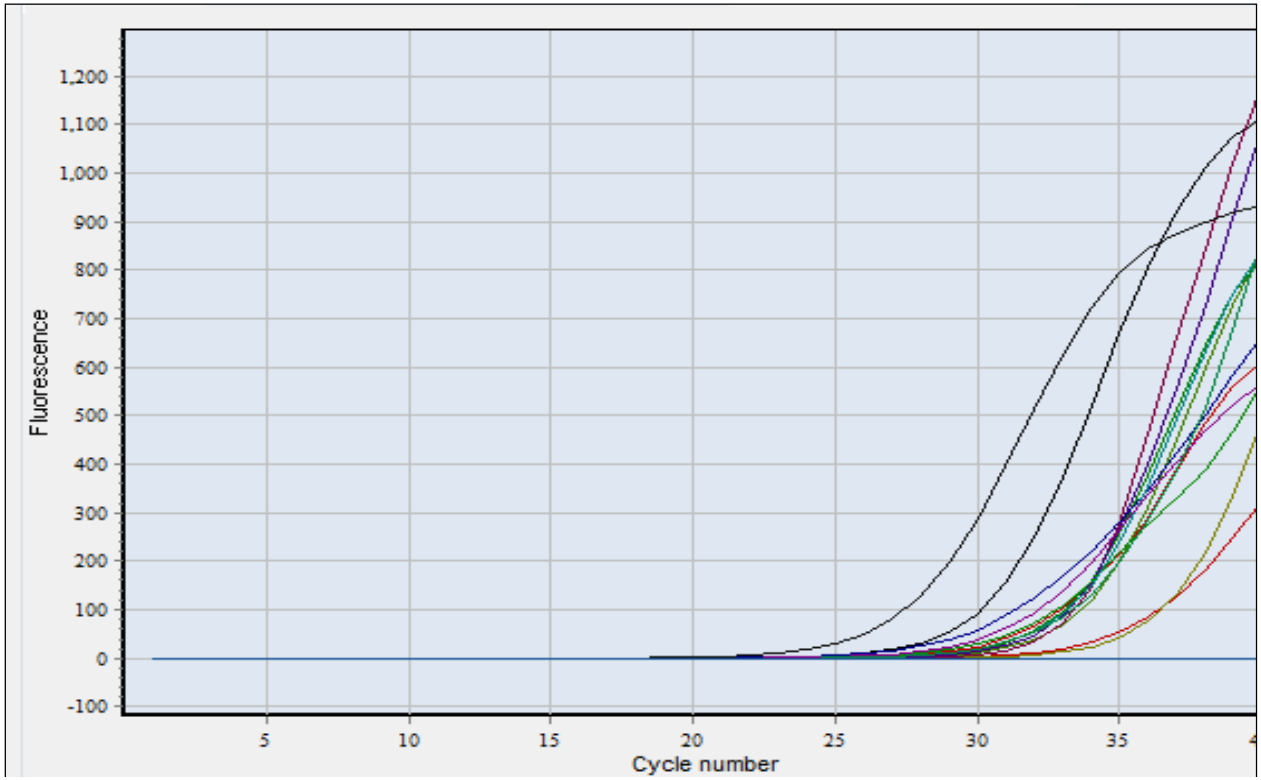

Fig. 1. Real time PCR

Table 2. Carbapenem sensitivity profile

	Resistant	Intermediate	Sensitive
Imipenem	46.3%	13.4%	40.3%
Meropenem	32%	11.9%	56.1%

Table 3. Resistance profile of *bla*$_{OXA-48}$ positive isolates

	Resistant	Intermediate	Sensitive
Imipenem	73.3%	13.3%	13.3
Meropenem	40%	33.3%	26.7%

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 *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 *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 Senigal[15]; 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 *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

1. Chang HH, Cohen T, Grad YH, Hanage WP, O'Brien TF, Lipsitch M. Origin and proliferation of multiple-drug resistance in bacterial pathogens. Microbiology and Molecular Biology Reviews. 2015;79(1): 101-16.
2. Chalhoub H, Sáenz Y, Rodriguez-Villalobos H, et al. High-level resistance to meropenem in clinical isolates of Pseudomonas aeruginosa in the absence of carbapenemases: Role of active efflux and porin alterations. International Journal of Antimicrobial Agents. 2016;48(6):740-3.
3. Poirel L, Nordmann P. Acquired carbapenem-hydrolyzing β-lactamases and their genetic support-an update. Medicinal Chemistry Reviews-Online. 2005;2(3):183-95.

4. Giuliani F, Docquier JD, Riccio ML, Pagani L, Rossolini GM. OXA-46, a new class D β-lactamase of narrow substrate specificity encoded by a blaVIM-1-containing integron from a Pseudomonas aeruginosa clinical isolate. Antimicrobial Agents and Chemotherapy. 2005;49(5):1973-80.
5. Jayol A, Poirel L, Dortet L, Nordmann P. National survey of colistin resistance among carbapenemase-producing Enterobacteriaceae and outbreak caused by colistin-resistant OXA- 48- producing Klebsiella pneumoniae, France. 2014. Eurosurveillance. 2016;21(37).
6. Drawz SM, Bethel CR, Doppalapudi VR, et al. Penicillin sulfone inhibitors of class D β-lactamases. Antimicrobial Agents and Chemotherapy. 2010;54(4):1414-24.
7. Aktaş Z, Kayacan ÇB, Schneider I, Can B, Midilli K, Bauernfeind A. Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiella pneumoniae in Istanbul, Turkey. Chemotherapy. 2008;54(2):101-6.
8. Livermore DM, Woodford N. The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends in Microbiology. 2006;14(9):413-20.
9. Pasteran F, Tijet N, Melano RG, Corso A. Simplified protocol for carba NP test for enhanced detection of carbapenemase producers directly from bacterial cultures. Journal of Clinical Microbiology. 2015; 53(12):3908-11.
10. Lafeuille E, Decré D, Mahjoub-Messai F, Bidet P, Arlet G, Bingen E. OXA-48 carbapenemase-producing Klebsiella pneumoniae isolated from Libyan patients. Microbial Drug Resistance. 2013;19(6): 491-7.
11. Nasr AB, Decré D, Compain F, Genel N, Barguellil F, Arlet G. Emergence of NDM-1 in association with OXA-48 in a Klebsiella pneumoniae from Tunisia. Antimicrobial Agents and Chemotherapy. AAC. 2013; 00536-13.
12. Monteiro J, Widen RH, Pignatari AC, Kubasek C, Silbert S. Rapid detection of carbapenemase genes by multiplex real-time PCR. Journal of Antimicrobial Chemotherapy. 2012;67(4):906-9.
13. Héritier C, Poirel L, Nordmann P. Genetic and biochemical characterization of a chromosome-encoded carbapenem-hydrolyzing Ambler class D β-lactamase from Shewanella algae. Antimicrobial Agents and Chemotherapy 2004;48(5): 1670-5.
14. Potron A, Poirel L, Nordmann P. Origin of OXA-181, an emerging carbapenem-hydrolyzing oxacillinase, as a chromosomal gene in Shewanella xiamenensis. Antimicrobial Agents and Chemotherapy. 2011;55(9):4405-7.
15. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases. 2011;17(10):1791.

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