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New Delhi Metallo-β-lactamase (NDM)-mediated Carbapenem-Resistant *Pseudomonas aeruginosa* Clinical Isolates in Sudan

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Authors' contributions

This work was carried out in collaboration between all authors. Author SERM designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author AA supervised the study and author MIS managed the practical experiments of the study. Author WMH oversaw the literature searches. All authors read and approved the final manuscript.

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Short Research Article

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ABSTRACT

Carbapenem resistance mediated by NDM is particularly gruesome as this carbapenemase can hydrolyze a wide range of β-lactam antibiotics.

Aim: This study aims to detect NDM mediated carbapenem resistance in clinical isolates of *Pseudomonas aeruginosa*.

Materials and Methods: 50 multi-drug resistant clinical urinary isolates of *Pseudomonas aeruginosa* from three major hospitals in Khartoum state Sudan; Khartoum Teaching Hospital, Medical Army Hospital and Omdurman teaching hospital, in period from July 2016 to September 2017, were investigated for carbapenem resistance using standard disc diffusion method and underwent real-time PCR to detect carbapenem resistance gene bla_{NDM}. Data were analyzed using

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IBM SPSS.
Results: 60% were positive for the *bla*$_{NDM}$, 82% were resistant to Imipenem and 75% of the samples were resistant to Meropenem.
Conclusion: The emergence of carbapenem resistance is a global problem that requires earnest attention. To make the suitable preventive measures, the emergence of these genes must be monitored closely. Our findings revealed that carbapenem-resistant due to the gene *bla*$_{NDM}$ is accounted for 60% of the cases, and due to lack of proper 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$_{NDM}$.

1. INTRODUCTION

Pseudomonas aeruginosa is an opportunistic organism that can cause serious infections in immunocompromised and hospitalized patients. Being genetically designed to resist antibiotics, clinical isolates of *Pseudomonas* are usually difficult to treat. Furthermore, this organism can acquire and disseminate resistance genes to other microorganisms in the environment, making it the major cause of nosocomial infections in a hospital setting [1].

Carbapenems are strong antibiotics and it's considered the last resort of treatment for patients with various bacterial infections. The first carbapenem resistance was detected in 1997 and started to spread worldwide, posing a risk on the availability of an active antibiotic against resistant bacteria [2].

bla$_{NDM}$ is the gene encoding for class B β-lactamase enzyme NDM, it was first discovered in 2008 by Swedish scientists treating a patient of Indian descent who travelled to India [3,4]. Thereafter, reports of NDM resistance was registered worldwide, in the united states, South Africa and Kenya as well as East Africa [2,5-7].

bla$_{NDM}$ mediated resistance has not been reported in Sudan, therefore, this paper reports the first carbapenem resistance gene *bla*$_{NDM}$ in carbapenem-resistant clinical isolates of *Pseudomonas aeruginosa* in Khartoum state Sudan.

2. MATERIALS AND METHODS

Fifty multi-drug resistant clinical urinary isolates of *Pseudomonas aeruginosa* from three major hospitals in Khartoum state Sudan; Khartoum Teaching Hospital, Medical Army Hospital and Omdurman teaching hospital, in period from July 2016 to September 2017, were investigated for carbapenem resistance using standard disc diffusion method, implementing Imipenem and Meropenem permeated antibiotic discs (HiMedia, India) in a Muller-Hinton agar (HiMedia, India). Using primers sequence adapted from a study published by Monteiro *et al* Monteiro, Widen et al. 2012 [Table 1]. real-time PCR was performed to determine the presence of the *bla*$_{NDM}$ 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 a Sacycler-96 instrument (Sacacae biotechnology, Italy). The real-time PCR conditions were as follows: 94 Ċ/10 min; 40 cycles of 94 Ċ/40 s, 55 Ċ/45 s and 72 Ċ/50 s and a final elongation step at 72 Ċ/10 min. The Sacycler-96 instrument automatically calculated the derivatives of fluorescence measured at 533 nm. Data were analyzed using IBM SPSS.

3. RESULTS

Real-time PCR revealed that 60% of the samples were *bla*$_{NDM}$ gene positive [Fig. 1]. 82% were resistant to Imipenem, 10% were intermediately resistant and 8% were sensitive to Imipenem. 75% were resistant to Meropenem, 20% were intermediately resistant and 5% were sensitive to Meropenem
Fig. **2**. Among positive *bla*$_{NDM}$, 70% were resistant to Imipenem, 25% were intermediately resistant and 5% were sensitive to Imipenem. 61% were resistant to Meropenem, 29% were intermediately resistant and 10% were sensitive to Meropenem
Fig. **3**.

4. DISCUSSION

Pseudomonas aeruginosa is an environmental opportunistic organism, capable of causing a variety of infections including nosocomial

Table 1. Primer sequence

	Primer	Sequence	GC%	TmC°	M.W µg/µmol	Final Con µM	Amplicon size	Reference
NDM	NDM-F	5'-GGTTTGGCGATCTGGTTTTC-3'	55.6	54.9	5463.6	0.2	82 bp	[8]
	NDM-R	5'-CGGAATGGCTCATCACGATC-3'	47.6	55.6	5463.6	0.2		

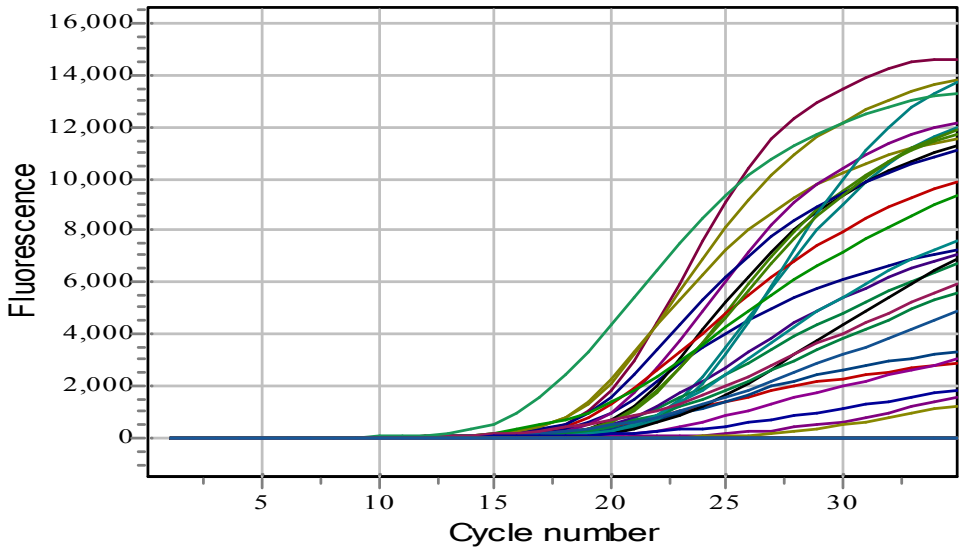

Fig.1. Real-time PCR

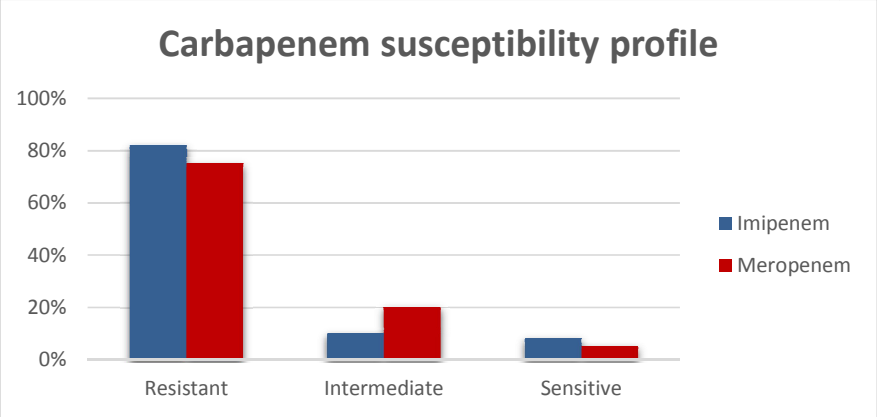

Fig. 2. Carbapenem susceptibility profile

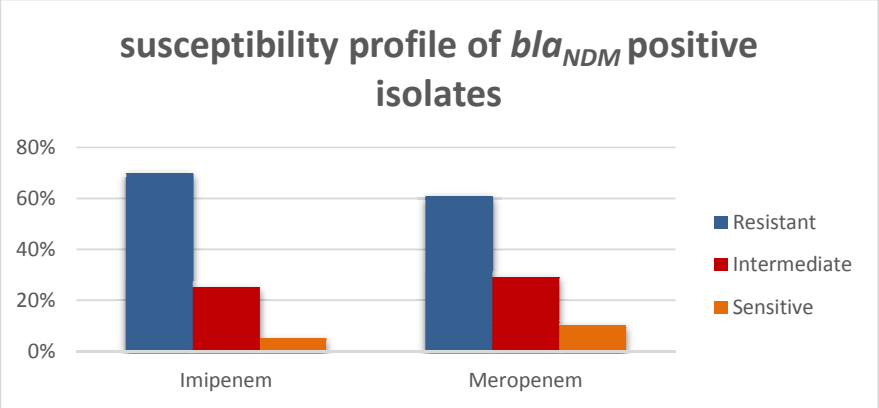

Fig. 3. Susceptibility profile of bla_{NDM} positive isolates

infections, it's genetically designed to surpass the environmental challenges, these features allowed it to be the leading agent in causing the most resistant infections among the Gram-negative bacteria.

The NDM carbapenemase has become one of the most widespread Carbapenemases and is found in Gram-negative pathogens including *A. baumannii*, *K. pneumoniae* and *E. coli* throughout the world. New Delhi Metallo-β-lactamase (NDM) can hydrolyze all beta-lactam antimicrobials except for monobactam, a transferable molecular class B β-lactamase gene that confers resistance to a variety of antibiotics like aminoglycosides, fluoroquinolones, macrolides and sulfonamides, leaving few treatment options available [9, 10]. The spread of NDM has been underpinned by the extreme mobility of the bla_{NDM} gene itself, which is mediated by an ISAba125 element upstream of the NDM gene rather than by expansion of an epidemic clone of bacteria or plasmid, as seen with the other carbapenem resistance genes such as KPC. NDM-producing isolates have been found in many species: the genes are located both on plasmids and on the host chromosome and able to move between the two at high frequency. Having 60% of the isolates positive to this insidious gene considering its ease of transferability triggers an alarm that outbreaks may occur and there should be a pivotal measure to contain and control this resistance.

Antimicrobial resistance has been reported worldwide yet reports of NDM mediated resistance in Northern Africa region and Sudan's neighbouring countries were in form of single cases reported in Egypt, Libya and Tunisia, outbreaks due to NDM mediated resistant were registered in Morocco and Algeria. Due to improper reporting of resistance genes in Sudan, there were no reports about the presence

bla_{NDM} gene in *Pseudomonas aeruginosa* in Sudan.

5. CONCLUSION

Our findings revealed that carbapenem-resistant due to the gene *bla_{NDM}* is accounted for 60% of the investigated cases, considering the possibility of the presence carbapenem resistance genes as well as setting surveillance programs for monitoring of emerging resistance, will assist in adjusting the treatment plan and improve the patient's outcomes.

ETHICAL APPROVAL

This work has been approved by Alneelain University, Sudan. All Authors declare no conflict of interest.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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