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Detection of the bla_{VIM-2} Gene in Carbapenem-Resistant *Acinetobacter baumannii* Clinical Isolates in Sudan

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Abstract

Background: *Acinetobacter baumannii* is a *pleomorphic aerobic* Gram-negative bacillus that is notorious for having multidrug resistance traits. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a global concern due to its ability to retain and disseminate resistance genes, which poses a threat to the spread of resistance among bacterial communities in hospital settings. The aim of our study was to evaluate the existence of the bla_{VIM-2} gene in carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Sudanese hospitals.

Methods and Results: Forty clinical isolates of *Acinetobacter baumannii* were collected from June 2021 to April 2022. All isolates of *Acinetobacter baumannii* were identified via BioMérieux's Vitek-2 automated system (Marcy l'Étoile, France) and evaluated for phenotypic resistance to carbapenem, using imipenem and meropenem. The enzymatic mode of resistance was assessed by the modified Hodge test (MHT). A real-time PCR was used to detect the presence of the bla_{VIM-2} gene.

Of 40 isolates, 32(80%) were resistant to imipenem, 4(10%) were moderately resistant, and 4(10%) were susceptible to imipenem; 24(60%) were resistant to meropenem, 10(25%) were moderately resistant, and 6(15%) were susceptible to meropenem. MHT was 70% positive with imipenem use and 55% positive with meropenem use. Real-time PCR revealed that only 30% of the samples were positive for the bla_{VIM-2} gene. All bla_{VIM-2}-positive isolates were resistant to both imipenem and meropenem.

Conclusion: Resistance to carbapenem poses a serious threat, denying patients treatment options. It is essential to make continuous surveillance of these strains to prevent the development of resistant strains.(**International Journal of Biomedicine. 2022;12(4):636-639.**).

Keywords: carbapenem-resistant *Acinetobacter baumann* • bla_{VIM-2} gene • carbapenemases

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Abbreviations

CRAB, carbapenem-resistant *Acinetobacter baumannii*; **MBLs**, metallo-β-lactamases; **MHT**, modified Hodge test; **VIM**, Verona integron-borne metallo-β-lactamase.

Introduction

Acinetobacter baumannii is a pleomorphic aerobic Gram-negative bacillus that is notorious for having multidrug resistance traits. Carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a global concern due to its ability to retain and disseminate resistance genes, which poses a threat to the spread of resistance among bacterial communities in hospital settings.[(1)] CRAB can render carbapenems ineffective using various enzymes known as carbapenemases.[(2)] The carbapenems are β-lactam

antimicrobial agents with an exceptionally broad spectrum of activity. The emergence of metallo-β-lactamase (MBL)-producing bacilli that are resistant to carbapenems is becoming a severe therapeutic problem.[3] Two types of MBLs, IMP and VIM, have been reported.[4] Strains producing VIM-type MBLs were originally reported in European countries.[5,6] VIM enzymes confer resistance not only to carbapenems, but to virtually all β-lactam antibiotics,[7,8] including penicillins, cephalosporins, cephamycins, and carbapenems, but not monobactams (aztreonam). Their activity is zinc dependent and is inhibited by EDTA.[6]

Currently, the VIM-type enzymes constitute the second most dominant group of ß-lactamases[9] and have been reported in different species from 23 countries worldwide.[7,10] The first metallo-β-lactamase VIM-2 in clinical isolates of *Pseudomonas aeruginosa* was collected in 1995 in Portugal.[11,12] The carbapenem-hydrolyzing β-lactamase VIM-2 shared 90% amino acid identity with VIM-1.[6] VIM-2 has been obtained from an isolate from the French Riviera region(Marseilles), 300 km from Verona, where VIM-1 had been isolated.[5] VIM-2 can be classified in the protein sequence-based subclass B1 of MBLs.[13] VIM-2 has been reported frequently from different isolates including *Acinetobacter baumannii* in Europe, United States, Latin America, and Asia.[14-16]

VIM MBLs are often encoded by mobile gene cassettes inserted into integrons,[5,6] which are sometimes located on plasmids. Most of these integrons belong to class 1, but their structures vary among isolates. The bla_{VIM-2} gene was first described in a *Pseudomonas aeruginosa*[6] isolate in France.

The aim of our study was to evaluate the existence of the bla_{VIM-2} gene in carbapenem-resistant *Acinetobacter baumannii* clinical isolates from Sudanese hospitals.

Materials and Methods

Forty clinical isolates of *Acinetobacter baumannii* were collected from June 2021 to April 2022. In this study, forty isolates of *Acinetobacter baumannii* were identified via BioMérieux's Vitek-2 automated system (Marcy l'Étoile, France) and evaluated for phenotypic resistance to carbapenem, using imipenem and meropenem. The enzymatic mode of resistance was assessed by the modified Hodge test (MHT). The primer sequence was adapted from a study by Monteiro et al.[17] (Table 1). A real-time PCR was used to detect the presence of the bla_{VIM-2} gene. The experiment was optimized and performed using a previously published protocol[18] as follows: a 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 18.7 µL sterile water to complete the reaction volume. The real-time PCR run was performed using the Sacycler-96 instrument (Sacacae biotechnology, Italy), which automatically calculated the derivatives of fluorescence measured at 533 nm. The real-time PCR conditions were as follows: 94°C for 10 minutes; forty cycles of 94°C for 40 seconds, 55°C for 45 seconds, 72°C for 50 seconds, and a final elongation step at 72°C for 10 minutes.

Results

Of 40 isolates, 32(80%) were resistant to imipenem, 4(10%) were moderately resistant, and 4(10%) were susceptible to imipenem; 24(60%) were resistant to meropenem, 10(25%) were moderately resistant, and 6(15%) were susceptible to meropenem (Figure 1). MHT was 70% positive with imipenem use and 55% positive with meropenem use (Figure 2). Real-time PCR revealed that only 30% of the samples were positive for the bla_{VIM-2} gene (Figure 3). All bla_{VIM-2}-positive isolates were resistant to both imipenem and meropenem.

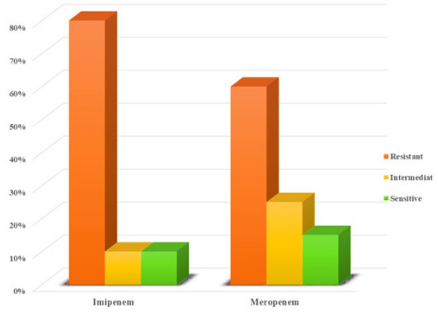

Fig. 1. *Carbapenems Susceptibility Profiles.*

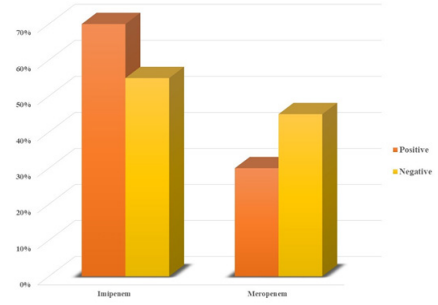

Fig. 2. *Modified Hodge test (MHT) results.*

Table 1.
Primer sequence.

	Primer	Sequence	GC%	T, C°	M.W, µg/µmol	Final Con., µM	Amp size, bp
bla_{VIM-2}	VIM-F	5'-GATGGTGTTTGGTCGCATA-3'	50.0	55.4	6024.0	0.2	382
	VIM-R	5'-CGAATGCGCAGCACCAG-3'	65.0	57.4	6160.1	0.2	

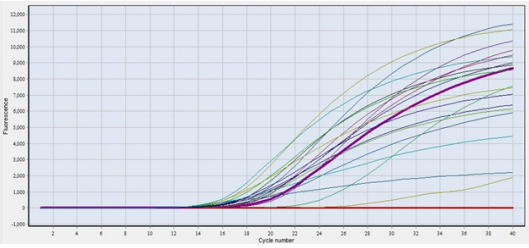

Fig. 3. *Real-time PCR detection of* bla_{VIM-2}

Discussion

Acinetobacter baumannii can cause serious, life-threatening infections, and the number of resistant strains of *Acinetobacter baumannii* isolates has increased worldwide over the last decade. WHO has declared *Acinetobacter baumannii* one of the priority pathogens.[19] All isolates in this study were resistant to one or both carbapenem antibiotics - imipenem and meropenem. In addition to structural barriers, such as cell wall permeability and lack of porins, organisms become resistant to antibiotics due to mechanical barriers, such as efflux pumps or lack or alteration of target sites, as well as by the emergence of MBL-producing bacilli.

Carbapenems are considered as last-resort antibiotics for the treatment of infections caused by multidrug-resistant, Gram-negative bacteria.[20] Susceptibility to carbapenem is typically tested using one of the antibiotics from the carbapenem family, usually imipenem or meropenem. Based on our research, we found that almost one-third of imipenem-resistant isolates were moderately resistant or sensitive to meropenem. Using only imipenem may not detect a substantial percentage of carbapenem-resistant organisms. In addition, a meropenem-sensitive isolate may carry carbapenem resistance genes that may go unnoticed, leading to further resistance spread.

Carbapenemases are groups of β-lactamases that confer resistance on carbapenem antibiotics and in some cases resistance on other classes of antibiotics, like aminoglycosides and fluoroquinolones. Enzymatic resistance is one of the most effective ways for bacteria to resist antibiotics, the evolution of enzymatic resistance is continuous with the diversity of antibiotics used for treatment.

One of the most widely used methods to detect enzyme-mediated resistance is the MHT. The principle of MHT is based on the ability of susceptible organisms to grow in the presence of an antibiotic that is enzymatically hydrolyzed by the test organism, resulting in a growth characteristic, clover-shaped zone of inhibition. In this study, MHT results also showed variation between imipenem and meropenem, with imipenem showing 70% positive MHT, suggesting that these isolates produce carbapenemase enzymes that can successfully hydrolyze imipenem antibiotics. About 45% of the isolates were MHT-positive when meropenem was used, reflecting the potency of meropenem, with a minority of isolates able to hydrolyze meropenem successfully.

Only 30% of the isolates carried the bla_{VIM-2} gene, suggesting that the bla_{VIM-2} negative isolates may have different resistance mechanisms. However, bla_{VIM-2} resides on a mobile gene cassette that is transduced by integrins with broad substrate specificity, making it a dangerous resistance element.

In conclusion, resistance to carbapenem poses a serious threat, denying patients treatment options. It is essential to make continuous surveillance of these strains to prevent the development of resistant strains.

Acknowledgments

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Competing Interests

The authors declare that they have no competing interests.

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