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ORIGINAL ARTICLE

Detection Of New Delhi Metallo Beta Lactamase-1 (Bla_{NDM-1}) Gene among A Group of Carbapenem Resistant *Acinetobacter Baumannii* Isolates.

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Key words:

Acinetobacter baumanii; Carbapenem resistance; bla_{NDM-1} gene

ABSTRACT

Background: *A variety of carbapenemases have been reported in Acinetobacter species. One of the most clinically significant carbapenemases is the recently described New Delhi metallo-β-lactamase (bla_{NDM-1}).* ***Objective:*** *This study aimed to determine the presence of bla_{NDM-1} gene among a group of carbapenem resistant Acinetobacter baumanii isolates from hospitalized patients.* ***Methodology:*** *Thirty carbapenem resistant Acinetobacter baumanii isolates from different sites of nosocomial infections from ICU patients were included in the study. They were tested by real time PCR for the presence of bla_{NDM-1} gene.* ***Results:*** *None of the tested isolates in this study was positive for this gene.* ***Conclusion:*** *Further studies on larger numbers should be done to confirm or rule out the role of the bla_{NDM-1} gene in carbapenem resistance of Acinetobacter baumanii in nosocomial infections.*

INTRODUCTION

Acinetobacter spp. is one of the most difficult pathogens to treat. The species *Acinetobacter baumannii (A. baumannii)*, largely unknown 30 years ago, has risen to prominence.While community-acquired *Acinetobacter* infections are rare, hospital-acquired infections are far more common and of a greater concern. *Acinetobacter* infections are associated with immunocompromised patients with infection rates often being highest in intensive care units (ICUs) and Surgical Wards. Commonly the organisms cause pneumonia, particularly associated with mechanical ventilation, and bloodstream infections following invasive procedures[1].

The rise in the number of infections caused by *A. baumannii* over the recent decades is of great concern due to the difficulties that are faced in administrating effective antimicrobial treatment. Intrinsic properties of the species such as chromosomally-encoded -lactamases, an effective permeability barrier, and the ability to acquire and maintain resistance determinants on mobile genetic elements, have severely reduced the number of effective antibiotics that can be used against some isolates, in a few cases to zero[2].

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The rapid accumulation of resistance determinants to multiple classes of antibiotics has resulted in the elimination of the penicillins, cephalosporins, aminoglycosides, quinolones and tetracyclines as effective treatment options for many *A. baumannii* isolates. This left the carbapenems as the only major sustainable group of antibiotics to treat infections with *A. baumannii*, due to their good activity and low toxicity[3].

During the last decade, the emergence of carbapenemase-producing strains among Enterobacteriaceae, *Pseudomonas* spp., and *Acinetobacter baumannii* is remarkable. A variety of carbapenemases have been reported in *Acinetobacter* species, such as GES-type, and the Ambler class D carbapenemases of the OXA-23, OXA-24/-40, OXA-58, and OXA-143 types[4]. One of the most clinically significant carbapenemases is the New Delhi metallo-*β*-lactamase (bla_{NDM-1}). This carbapenemase belongs to the class B of Ambler *β*-lactamases classification that includes the metallo-*β*-lactamases (MBLs) [5].

The emergence of the New Delhi metallo-*β*-lactamase (bla_{NDM-1}) constitutes a critical medical issue. A quite systematic association with other antibiotic resistance determinants is observed in almost all bla_{NDM-1} producers (Enterobacteriaceae, Acinetobacter, *and* Pseudomonas*)*.[6] Those associated resistance determinants include the Amp C cephalosporinases, clavulanic acid inhibited expanded-spectrum *β*-lactamases (ESBLs), other types of carbapenemases (OXA-48-, VIM-, and KPC-types), resistance to aminoglycosides (16S RNA methylases), to quinolones

(Qnr), to macrolides (esterases), to rifampicin (rifampicin-modifying enzymes), to chloramphenicol, and to sulfamethoxazole. Consequently, most of the bla_{NDM-1} producers remain susceptible only to two bactericidal antibiotics (colistin and fosfomycin) and a single bacteriostatic antibiotic (tigecycline). [7,8]

$Bla_{NDM-1}$was first identified in 2008 in a *K. pneumoniae* isolate recovered from a Swedish patient who has been previously hospitalized in New Delhi, India[9]. In July 2010, isolation of three cases of *Acinetobacter baumanii* bearing bla_{NDM-1} from the intensive care unit of a hospital in Chennai, India, was reported by a team in New Delhi.[10]

A second reservoir of bla_{NDM-1} producers was then highlighted through several studies reporting patients colonized or infected with bla_{NDM-1} producers originating from the Balkan states[11,12]. Bla_{NDM-1} producing *Acinetobacter* has also been recovered from environmental and clinical samples in China.[4,13] Several reports also suggested that the Middle East might be an additional reservoir of bla_{NDM-1} producers [14-17].

Aim of the work

The aim of this study was to determine the presence of bla_{NDM-1} gene among a group of carbapenem resistant *Acinetobacter baumanii*isolates from cases with nosocomial infection in the ICU of Al Zaitoun Specialized Hospital in Egypt.

METHODOLOGY

Collection and identification of isolates:

The study was conducted on 30 clinical isolates of carbapenem resistant *A. baumannii* isolated from patients with nosocomial infection (sputum samples from ventilated patients, pus from bedsores and central venous catheter tips). Patients were admitted to the ICU at Al Zaitoun Specialized Hospital (A 200 bed tertiary medical center in Cairo, Egypt), during the period from October 2014 to November 2015.

Identification of the isolates was done based on colony morphology, microscopic examination of Gram stained films and conventional biochemical reaction of the isolated organisms[oxidase negative, catalase positive, citrate positive, strict aerobes (non-fermenting) with aerobic acidification of glucose (oxidation-fermentation test), and growth at 44°C].[18,1]

API 20E Test:

The identification of isolates was then confirmed by Analytical Profile index 20 E (API 20E) test (BioMérieux, Marcy L'Etoile, France), performed according to manufacturer's protocol. The results were read as 7 digit number and identified by API 20 analytical index.

Detection of carbapenem resistance:

A. baumannii isolates were tested for carbapenem resistance using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar plates (BD Diagnostics, Heidelberg, Germany) using doripenem (10 µg), meropenem (10 µg), and imipenem (10 µg) (Oxoid, United Kingdom). Interpretation was carried out using CLSI guidelines[20].For imepenem (> 22mm indicated sensitivity, <18mm indicated resistance and 19-21 mm indicated intermediate sensitivity), for meropenem and doripenem (>18mm indicated sensitivity, < 14mm indicated resistance and 15-17 mm indicated intermediate sensitivity). Carbapenem resistance was defined by being non-susceptible to at least 1 of the 3 carbapenems tested.

Detection of the bla_{NDM-1} gene by real time PCR:

DNA extraction:

DNA extraction for all *A. baumannii* strains was performed using the QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany) as per the manufacturer's protocol for isolation of bacterial whole cell nucleic acid. Briefly, a 2-McFarland-standard bacterial suspension was prepared in saline, and bacterial DNA was extracted from 200µl ($1.2X10^8$ CFU) of the suspension. Extracted bacterial DNA was eluted from the columns in 100µl elution buffer and stored at -20°C.

DNA amplification by real time PCR:

Real-time PCR assay was performed using the primers provided by (Roche Diagnostics, Germany). Primers' sequences were derived from the Genbank sequence AB571289. The forward primer sequence F-(5'-GCT GGC GGT GGT GAC TC-3'), and the reverse primer sequence R-(5'-GGC AAG CTG GTT CGA CAA C-3') which are specific for the detection of bla_{NDM-1} gene [21]. The assay contained 5 µl (10 ng) of whole cell DNA, 0.5 µL of each primer, 0.5 µL dNTPs mix and 0.5 µL SYBR green I dye. The PCR cycles were performed; running 10 min denaturation at 95°C followed by 45 cycles each at 10 s at 95°C, 1 min at 58°C and 1 s at 72°C [21] in a Light Cycler 2.0 System (Roche Diagnostics, Germany) for DNA amplification using SYBR Green I dye as detection format. The bla_{NDM-1}-positive *A. baumannii* ABCA207 strain was included as a positive control. Melting Curve analysis for the amplicon was done. The amplification curves, melting curves and melting peaks were recorded by the instrument. The expected product size was 475 bp. A sample was considered positive when giving a melting peak at 64ºC.

RESULTS

This study was conducted on 30 carbapenem resistant *A. baumanii* clinical isolates. they were isolated from 13 sputum samples from ventilated patients, 9 pus samples from bed sores and 8 samples from central venous catheter tips)

The isolates were identified by conventional methods and confirmed by API 20E system. **Fig. 1**

Figure 1: Multi-test system for identifying of *A. baumanii*isolates. Very good identification level of *A. baumannii* by API 20E system with 7 digit number (0004042) according the analytical profile index.

The 30*Acinetobacter baumanii* carbapenem resistant clinical isolates were tested for the presence of bla_{NDM-1} gene using real time PCR. None of the isolates was positive for this gene.

DISCUSSION

Among the 30*Acinetobacterbaumanii* isolates in this study, bla_{NDM-1} gene was not detected in any isolate using real time PCR.

Though the first reported case of *A. baumanii* carrying the bla_{NDM-1} gene had a history of Indian origin, its exact geographical origin, however, has not been conclusively verified. Moreover, bacteria carrying the bla_{NDM-1}gene has been reported from patients from different countries also, suggesting its wide dissemination[22]. There are previous reports of carbapenem resistant *Acinetobacter baumanii* expressing the bla_{NDM-1} gene in North Africa and the Middle East area.

The first report of a bla_{NDM} gene in a clinical isolate originating from Egypt, with no obvious link with the Indian subcontinent was in 2011, reported by Kaase et al[17]. They isolated a strain of carbapenem-resistant *A. baumannii* from culture of a central venous line catheter (placed at an Egyptian hospital), at the first day after the patient was transferred to a hospital in Germany. PCRs for the metallobetalactamase genes gave a positive result for the bla_{NDM-1} gene and further sequencing revealed a substitution from C to G at position 82 from the start codon resulting in an amino acid substitution from proline to alanine at position 28 compared with bla_{NDM-1}. This new variant was assigned to bla_{NDM-2}. This was the first report of abla_{NDM-1} variant, although the ongoing spread of strains carrying the bla_{NDM-1} gene will enhance the likelihood of variants emerging. This is an important consideration when designing genetic tools to target carbapenem resistance genes.

This case report together with the isolation of bla_{NDM-1} producers in Iraq and the Sultanate of Oman,[23,24]suggested that bla_{NDM}producing isolates have already disseminated in the Middle East.

In the UAE, screening of 155 carbapenem resistant *Acinetobacter baumannii* strains that were recovered in Abu Dhabi hospitals, two metallo-ß-lactamase bla_{NDM}gene-carrying isolates were identified. They were isolated 4 months apart from the urine of a cancer patient previously treated in Egypt, Lebanon and in the United Arab Emirates. They were clonally related and carried the bla_{NDM-2} gene recently identified in *A. baumannii* in Egypt and Israel. Sequences surrounding the bla_{NDM-2} gene showed significant similarities with those associated with bla_{NDM-1} in Enterobacteriaceae and *A. baumannii*. Repeated isolation of bla_{NDM-2}positive *A. baumannii* in the Middle East raises the possibility of the local emergence and spread of a unique clone [14].

In a study by Boulanger et al [16],bla_{NDM-1} gene was identified in a clinical *A. baumannii* isolate from a patient admitted to the ICU in a hospital in Algeria, with no obvious link with the Indian subcontinent. They suggested that NDM-producing *A. baumannii* isolates might have spread already in North Africa.

In the study done by Bonninet al[25], they isolated 6 bla_{NDM-1} producing *A. baumannii* linked with North Africa from patients previously hospitalized in different cities in Algeria (including Algiers, Setif, Constantine, and Tlemcen), 1 from a patient previously hospitalized in Tunisia, and 1 from a patient previously hospitalized in Egypt. The identification of several clinical *A. baumannii* isolates that possessed the bla_{NDM-1} gene and originated from North Africa, with no obvious link to the Indian subcontinent, strongly suggested that the bla_{NDM-1} producing *A. baumannii* clone is probably widespread in North Africa and that it might act as a reservoir for bla_{NDM-1}.

The first epidemiological study that provided an explanation for the dissemination of the bla_{NDM-1} gene was performed by Bonninet al[26]. They analyzed a collection of five bla_{NDM-1} positive *A. baumanni* isolates recovered in four European countries. Genotyping was performed by pulsed-field gel electrophoresis, multiplex PCR sequence typing, and multilocus sequence typing. Three distinct sequence types (ST) were identified. All isolates harboured a chromosomally located blaNDM-1 gene within a Tn125-like transposon. They suggested that dissemination of bla_{NDM-1} in *A. baumannii* seems be due to different strains carrying Tn125 or derivatives of Tn125 rather than plasmid mediated or clonal dissemination.

While this study was in progress, two bla_{NDM-1}producing *A. baumannii* were isolated in Belgium and Czech Republic. The clinical isolate from the Czech

Republic belonged to the sequence type ST1, similar to isolate JH from Switzerland; whereas the clinical isolate from Belgium belonged to European clone II (which corresponds to ST2)[27,28]. These two reports reinforced the fact that the spread of the bla_{NDM-1} gene in *A. baumannii* is not linked to a clonal spread but to the spread of a genetic structure. The spread of transposon Tn*125* in *Acinetobacter* species harbouring bla_{NDM} genes mirrors what has been observed with the bla_{KPC} carbapenemase gene, which is associated with transposon Tn*4401* [29].

CONCLUSION

Despite the negative results of the present study, the role of the bla_{NDM-1} gene in the development of carbapenem resistant *Acinetobacter baumanii* in Egypt cannot be ruled out. A limitation in this study was the small sample size, so further studies on larger numbers are required.Understanding the molecular basis of multi-drug resistance in *Acinetobacter baumanii* is a great challenge. It will help in developing successful treatment regimens especially in critically ill patients.

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