Detection of NDM and OXA-48 Resistant Genes in Acinetobacter Baumannii Isolated From Intensive Care Units’ Patients Clinical Samples in Khartoum State

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Research

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Abstract

Background: Acinetobacter baumannii is an opportunistic bacterial pathogen with intrinsic and acquired resistance to many antibiotics causing high rates of morbidity and mortality. This study was aimed to detect MDR Acinetobacter baumannii and its resistant genes (bla<sub>NDM</sub>, bla<sub>OXA48</sub>) from clinical isolates in Khartoum state.

Method: A cross sectional hospital-based study was done during the period from April to July 2019. A total of 50 clinical isolates were obtained from samples of patients in intensive care units (ICUs) for the purpose of molecular confirming of A. baumannii and detecting NDM and OXA-48 resistance genes by using conventional PCR.

Results: Out of 50 isolates investigated PCR was confirmed 47 (94%) as A. baumannii isolates, while 3 (6%) isolates were appeared to be other species. Moreover, the 47 A. baumannii isolates were examined for the presence of resistant genes and the result showed that NDM gene was detected in 2 isolates (4.3%) and OXA-48 gene was detected in only one isolate (2.1%).

Conclusion: There is low prevalence of NDM and OXA-48 Resistant Genes among ICUs A. baumannii isolates. However, continuous regional antimicrobial resistance surveillance and improved infection control measures are required in Khartoum hospitals ICUs to prevent further dissemination.

Background

Genus Acinetobacter is a belong to the family Moraxellaceae in the order Pseudomonadales. More than 25 species within the genus Acinetobacter have been described. The most important species of this genus is Acinetobacter baumannii which causes 2–10% of all Gram-negative infections in the Unites State and Europe. It possesses little risk to healthy individuals, but generally causes infections in those with weakened immune systems specifically, the intensive care unit (ICU) [1].

Genus Acinetobacter contains Gram-negative coco-bacilli which are aerobic, non-fermentative, non-motile, catalase positive, oxidase negative and with a G + C content of 39–47%. Four species of Acinetobacter including A. calcoaceticus, A. baumannii, A. pittii and A. nosocomialis are similar to each other and it is difficult to distinguish them by phenotypic characteristics. A. baumannii is the commonest species isolated from human clinical specimens, followed by such species as A. luffý, A. pittii, A. nosocomialis, A. haemolyticus and A. johnsonii [2, 3].

The clinical impact of Acinetobacter infection in terms of morbidity and mortality has been discussed widely in which the mortality rates range from 19–54%. The infections caused by A. baumannii are often treated with cephalosporins including ceftazidime and ceftriaxone, aminoglycosides such as tobramycin and amikacin, carbapenems, and tetracycline. However, to date, most strains of A. baumannii have become increasingly resistant to all these currently available antibacterial agents. The clinical significance of A. baumannii has grown significantly over the last few decades mainly due to the fact...
that this species possesses a variety of antibiotic resistance genes on plasmids, transposons and integrons and innate antimicrobial resistance mechanisms such as cell surface structures that prevent the influx of antibiotics which lead to failure of treatment [1].

Polymyxins are well-established antibiotics that have recently regained significant interest as a consequence of the increasing incidence of infections due to multidrug-resistant gram-negative bacteria. Polymyxin B and Colistin are being seriously reconsidered as last-resort antibiotics in many areas where multidrug resistance is observed in clinical medicine. In parallel, the heavy use of polymyxins in veterinary medicine is currently being reconsidered due to increased reports of polymyxin-resistant bacteria. Susceptibility testing is challenging with polymyxins [4].

In 2009, a novel MBL (metalo-beta-lactam), the New Delhi MBL (NDM), was described. NDM was first recognized in a *K. pneumonia* isolate from a Swedish patient who had received medical care in India and was soon recognized as an emerging mechanism of resistance in multiple species of *Enterobacteriaceae* in the United Kingdom [5]. However, OXA-48-type carbapenem-hydrolyzing class D β-lactamases are increasingly reported in enterobacterial species. To date, there are six OXA-48-like variants have been recognized, with OXA-48 being the most widespread [6].

*Acinetobacter baumannii* has emerged as a major cause of healthcare-associated infections [1]. It commonly presents resistance to multiple antimicrobial agents, occasionally including carbapenems and polymyxins. Polymyxins are often last-line therapeutic agents used to treat infections caused by multidrug-resistant (MDR) *A. baumannii*. MDR *A. baumannii* is a rapidly emerging pathogen, especially in the intensive care setting, causing infections including bacteremia, pneumonia, meningitis, urinary tract infection and wound infection [4] Hence healthcare facilities services are poor in Sudan and there are no recent published studies performed concerning detection of MDR *Acinetobacter baumannii* resistant genes (NDM, OXA48) this study was performed.

**Methods**

**Study design and setting**

A cross sectional study was performed during the period from April to July, 2019, in eleven different hospitals in Khartoum, Sudan. 50 Acinetobacter *baumannii* Isolates obtained from clinical samples were confirmed using polymerase chain reaction (PCR) with a specific primer for *Acinetobacter baumannii* (Ac_bum) and for the resistant genes (NDM, OXA48).

**Inclusion and Exclusion Criteria**

All the clinical isolates obtained from ICU patients’ samples and contain *Acinetobacter baumannii* resistant to commonly used antibacterial were included in this study. However, *Acinetobacter baumannii* isolated from other hospital departments patients were excluded from this study.

**Specimen Collection and Processing**
Specimens were ready isolated, identified and antibiogram known MDR *Acinetobacter spp.* Theses isolates were processed through PCR in order to confirm identification of *A. baumannii* and to determine the presence of NDM and OXA-48 genes.

**Preservation of the isolates:**

The bacterial isolates from clinical samples were identified and preserved using a sterile loop in 15% glycerol brain heart infusion, charcoal in cryo tubes by placing 2 to 3 colonies and placed in freezer in -20 °C until use. Each bacterial isolate holds a specific number, which is assigned to handle later on. For bacterial recovery from the preservative media; cryo tubes were left to melt in room temperature, a loop full sterile loop is used to streak the suspension in a Muller and Hinton at 37°C for 24 hours.

**DNA Extraction:**

Whole-cell DNAs were extracted from clinical isolates and standard strains by boiling extract procedure, using a few colonies of each bacterial strain re-suspended in 100 μl of DEPC water. After heating at 100°C for 10 minutes, freezing at -80°C for 10 minutes and boiling for five additional minutes, the suspensions were centrifuged (5 min, 10,000 × g) and recovered supernatant was frozen at -20°C until use [7].

**Conventional PCR:**

Conventional PCR amplification for identification of *Acinetobacter baumannii* and for the detection of following genes (NDM, OXA48) was performed by using Maxime PCR premix master mix tube. The primers were designed to amplify internal fragment with product size 791bp (*A. baumannii*-F and R), 389bp (OXA48-F and R) and 597 bp (NDM-F and R) 380bp table (1)

**Conventional PCR procedure**

Reaction mixture amplified at the following temperature: initial denaturation at 94° for 2 minutes, 35 cycle of denaturation at 94° for 30 seconds, annealing for 56° for 50 seconds and extension at 72° for 50 seconds. The final extension at 72° for 50 seconds. Then product has been subjected to gel electrophoresis procedure to detect band size791bp, 597 bp and 380 pb for *A. baumannii*, OXA48 and NDM genes respectively.

**Statistical analysis**

Data obtained in this study was analyzed by using SPSS version 20, descriptive analysis were used to describe isolates distribution and frequency and percentage of resistant genes. result has been presented in form of tables and figure.

**Results**
In this study we include 50 isolates of \textit{A. baumannii} obtained from patients in different Khartoum state hospitals ICUs. The distribution of isolates according to the samples were 28 (56\%) from sputum samples, 6 (12\%) from Endo-tracheal tube samples, 5 (10\%) for blood and wound swabs samples. While only tow isolates were obtained from urine culture (4\%). And one isolates (2\%) was originated from CSF, catheter tip and body uid culture respectively [Table 2].

PCR confirmation of isolates illustrate that frequency of \textit{A. baumannii} was found to be 47 (94\%) while 3 (6\%) were negative (other \textit{Acinetobacter} spp) [Table 3] [Fig. 1]. NDM gene was detected in 2 (4.3\%) out of 47 \textit{A. baumannii} PCR confirmed clinical isolates, the two isolates were obtained from sputum and wound swab samples [Table 3] [ Fig. 2]. On the other hand, OXA-48 gene was detected in only one isolate (2.1\%) out of the 47 confirmed isolates, and this isolate was obtained from sputum sample [Table 3] [Fig. 3].

**Discussion**

Recently, \textit{A. baumannii} has become a major hospital pathogen especially in ICUs. Various factors, including poor immune system, consumption of antibiotics, clonal spread of resistant microorganisms, poor infection control and drug resistance mechanisms, result in the dissemination of highly resistant pathogens to commonly used antibiotics [1].

In the present study 50 clinical isolates were collected for the purpose of identifying \textit{A. baumannii} and detecting NDM and OXA-48 resistance genes by using PCR. In the present study, sputum showed the highest frequency among other type of samples with 28 (56\%), which is similar to the result found by Opazo \textit{et al.}, in 2018 found that respiratory tract samples were the most predominant type (26\%) containing \textit{A. baumannii} isolates [8]. On the other hand, Omer etal., in 2015 disagreed with our finding in that the greatest number of their isolates were recovered from sputum (61\%) [1] Also, Abdallah \textit{et al.}, finding in 2013 was disagreed with our finding, the found in a total of 150 \textit{A. baumannii} isolates, sputum showed the greatest frequency among other type of samples 77 (51.3\%) [9].

In term of molecular confirmation, the frequency of \textit{A. baumannii} was found in the present study was 47 (94\%) which is similar to the frequency rate found out by Falah etal., in 2019 whom confirmed 80 (97.56\%) of \textit{A. baumannii} isolates in a total of 82 [2]. While Marathe et al., in 2019 found results which were strongly disagreed with the frequency found in the present study they examined a total of 112 sample and found only 33(30\%) confirmed as \textit{A. baumannii} [10].

In this study, NDM gene was detected in only 2 isolates (4.3\%) out of 47, while Marathe et al., in 2019 detected 29 (87.8\%) NDM in a total of 33 \textit{Albuminoid}, which is dramatically high result compared to the frequency rate of NDM found in the present study. Also, Karaaslan \textit{et al.}, in 2016 found high frequency rate of NDM among \textit{A. baumannii} 22 (31\%) in a total of 72 samples [11]. Bakour et al., in 2015 showed frequency rate of NDM 10 (22.7 \%) in a total of 44 \textit{A. baumannii} which is also considered high according to the frequency of NDM found is the present study [12]. Khorsi etal., in 2015 found 10 (10.6 \%) NDM \textit{A. baumannii} in a total of 94 sample which is moderate results compared to the above results [13]. While Howard etal., in 2012 found 2 (1.85) NDM-1-producing \textit{Acinetobacter baumannii} isolates out of 108
sample which is lower frequency rate compared to the above results and approximately close to the frequency rate found in our study [14]. This discrepancies between our finding and the previous studies may be due to variation in sample size and study populations.

In this study OXA-48 gene was detected in only one sample (2.1%) out of 47 sample. Study of Robustillo-Rodela et al., in 2017 found that 13 patients were colonized or infected by OXA-48 out of 31 which disagreed with our finding. The cumulative incidence of OXA-48 was 3.48% which is considered high frequency rate compared to the findings of the present study [15]. While Bakour et al., in 2015 found no OXA-48 gene among 44 isolates which is closer to the results found in this study [12]. This discrepancies between our finding and the previous studies may be due to variation in sample size and study populations.

**Conclusion**

There was low prevalence of NDM and OXA-48 Resistant Genes among ICUs *A. baumannii* isolates. However, continuous regional antimicrobial resistance surveillance and improved infection control measures are required in Khartoum hospitals ICUs to prevent further dissemination.

**Abbreviations**

NDM
New Delhi metallo-β-lactamase
ICUs
Intensive care units.
CRAB
Carbapenem resistant *Acinetobacter baumannii*.
MDR
Multi-drug resistance.
ETT
Endo-tracheal tube.
PCR
Polymerase chain reaction

**Declarations**

**Ethical Approval and Consent to Participate**

Ethical approval was obtained from University of Medical Sciences and Technology (UMST), Khartoum ministry of health, research department and hospitals. All information obtained from this study were kept confidential at all levels and utilized only for the study. Positive findings were reported to the physicians attending in the ICU for the proper management of patients.
Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors received no fund for this study.

Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; took part in drafting the article and revising it critically for important intellectual content. All authors read and approved the final manuscript.

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Tables

Table (1) primers used in study
| Gene   | Primer type | Primer sequence                  | PCR product size | Reference |
|--------|-------------|----------------------------------|------------------|-----------|
| Ac_bum | Forward     | 5’ – AGAGTTTGATCCTGGCTCAG – 3’  | 791              | [24]      |
|        | Reverse     | 5’ – TACCAGGGTATCTAATCCTGTT – 3’|                  |           |
| OXA-48 | Forward     | 5’ - AACGGGCGAACCAAGCATTNTT – 3’| 597              | [25]      |
|        | Reverse     | 5’ - TGAGCACTTCTTTTGTGATGGCT – 3’|                  |           |
| NDM    | Forward     | 5’ – ATGACCAGACCGCCAGAT-3’       | 380              | [25]      |
|        | Reverse     | 5’ - CAAGTCGCTCGGCAATCTC – 3’   |                  |           |

Table (2) distribution of isolates according to the sample types

| Type of sample | No. of isolate | percent |
|----------------|----------------|---------|
| Blood          | 5              | 10.0 %  |
| Body fluid     | 1              | 2.0 %   |
| Catheter tip   | 1              | 2.0 %   |
| CSF            | 1              | 2.0 %   |
| ETT            | 6              | 12.0 %  |
| Pus            | 1              | 2.0 %   |
| Sputum         | 28             | 56.0 %  |
| Urine          | 2              | 4.0 %   |
| Wound swab     | 5              | 10.0 %  |
| Total          | 50             | 100.0 % |

Table (3) distribution of isolates according to PCR result (detection of *A. baumannii* NDM and OXA-48 Genes)
| Gene        | Result | Total |
|-------------|--------|-------|
| *A. baumannii* | Negative | 3     |
|             | Positive | 47    |
|             | Total    | 50    |
| NDM         | Negative | 48    |
|             | Positive | 2     |
|             | Total    | 50    |
| OXA-48      | Negative | 49    |
|             | Positive | 1     |
|             | Total    | 50    |

**Figures**

**Figure 1**

Gel electrophoresis for detection of 791 bp *A. baumannii* isolate gene product. Lane 1. DNA ladder of 100bp. Lane2. Positive control of 791 bp *A. baumannii* gene product. Lane 3,5,6,7,8 and 9 are showed a typical positive isolate for band size of 791 bp *A. baumannii* gene product. Lane 4 was negative control.
Figure 2

Gel electrophoresis for detection of 791 bp A. baumannii gene product and 380 bp NDM resistant gene product. Lane 1. DNA ladder of 100bp. For A. baumannii gene, Lane 4 is positive control. Lane 3,5-13 are typical positive isolates with band size of 791 bp A. baumannii gene product. Lane 2 is Negative control. For NDM gene, lane 4 is positive control contains A. baumannii with positive NDM resistant gene band size of 380 bp. Lane 3 and 13 contain atypical positive isolates with NDM resistant gene band size of 380 bp. Lane 5 to 12 is negative. Lane 2 is negative control.

Figure 3
Gel electrophoresis for detection of 597 bp OXA-48 gene product. Lane 1. DNA ladder of 100bp. Lane 2 is Negative control. lane 3,4,5,7,8,9,10,11,12,13 Negative isolates. Lane 6 is positive isolate showing band size of 597 bp of OXA-48 gene product.