Original Research Article

Detection of NDM and OXA-48 Resistant Genes in *Acinetobacter baumannii* Isolated from Intensive care units' patients Clinical Samples in Khartoum State.

Abstract

Objectives: *Acinetobacter baumannii* is an opportunistic bacterial pathogen with intrinsic and acquired resistance to many antibiotics, resulting in high morbidity and mortality. This study aimed to detect MDR *Acinetobacter baumannii* and its resistant genes (bla_{NDM}, bla_{OXA48}) from clinical isolates in Khartoum state.

Methodology: A cross-sectional hospital-based study was conducted during the period fromApril 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.

Result: Of the 50 isolates examined, 47 (94%) were confirmed to *be A. baumannii*, and 3 (6%) were other species. Moreover, the 47 *A. baumannii* isolates were furtherly examined for the presences 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 *A. baumannii* ICUs isolates. However, continuous regional monitoring of antimicrobial resistance and improvement of infection control measures are required in the intensive care units of Khartoum hospitals to prevent further spread.

Key word: *A. baumanni*, NDM gene, OXA-48 Resistant.

Introduction

The genus *Acinetobacter* is belonging to the family *Moraxellaceae* in the order *Pseudomonadales*. More than 25 species have been described within the genus *Acinetobacter*. The most important species of this genus is *Acinetobacter baumannii* which causes 2-10% of all Gram-negative infections in the United States and Europe. It does not pose a significant risk to healthy individuals, but generally causes infections in people with weakened immune systems. Specifically, the intensive care unit (ICU) [1].

The Genus *Acinetobacter* contains Gram-negative cocco-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 difficult to distinguish by phenotypic characteristics. *A. baumannii* is the most common species isolated from human clinical specimens, followed by such species as *A. luffy, 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% to 54%. 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 Band 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. ND 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

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.

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 clinical isolates obtained from ICU patient samples and containing *Acinetobacter baumanii* resistant to commonly used antibacterial agent (including Ceftriaxone, ceftazidime, colistin, Imipenem, Meropenem and cefotaxime) were included in this study. Patients who had not started treatment for at least 48 days were included in this study. However, *Acinetobacter baumanii* isolated from other hospital departments were excluded from this study. Any patient under treatment at the time of sample collection was excluded from this study.

Specimen Collection and Processing

Samples were cultured and *A. baumannii* was isolated, identified and antibiogram was done to the MDR *Acinetobacter spp*. The resistant isolates were processed by PCR to confirm the identification of *A. baumannii* and to determine the presence of the NDM and OXA-48 genes.

Preservation of the isolates:

Bacterial isolates from clinical samples were identified and preserved using a sterile loop in 15% glycerol brain heart infusion, charcoal in cryo tubes and placed in freezer in -20 °C until use. Each bacterial isolate carries a specific number that is assigned to it for later handling. to restore bacteria from preservative media; Cooling tubes were left to thaw at room temperature, and a sterile full-loop ring was used to line the Muller-Hinton suspension at 37 °C for 24 h.

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 was performed for the identification of *Acinetobacter baumannii* and for the detection of the following genes (NDM, OXA48) using a Maxime PCR master tube. Primers were designed to amplify the inner fragment with a product size of 791 bp for *A. baumannii* housekeeping gene (-F 5'-AGAGTTTGATCCTGGCTCAG-3' and R5'-TACCAGGTATCTAATCCTGTT-3') and 597 bp for OXA48 gene (AACGGGCGAACCAAGCATTTT – 3'and R5' - TGAGCACTTCTTTTGTGATGGCT – 3'), and 380 bp for NDM gene (-F 5'-ATGACCAGACCGCCCAGAT-3' and R5'-CAAGTCGCTCGGCAATCTC-3').

Conventional PCR procedure

The reaction mixture was amplified at the following temperature: initial denaturation at 94° for 2 minutes, 35 cycles of denaturation at 94° for 30 s, annealing at 56° for 50 s and extension at 72° for 50 s. Final extension at 72 degrees for 50 seconds. The product was then subjected to gel electrophoresis to detect the 791 bp, 597 bp, and 380 ppb band size of *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 *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 two isolates were obtained from urine culture (4%). and one isolates (2%). was originated from CSF, catheter tip and body fluid culture respectively [Table 1].

PCR confirmation of isolates illustrate that frequency of *A. baumannii* *was* found to be 47 (94%) while 3 (6%) were negative (other *Acinetobacter* spp) [Fig. 1].NDM gene was detected in 2 (4.3%) out of 47 *A. baumannii* PCR confirmed clinical isolates, the two isolates were obtained from sputum and wound swab samples [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 [Fig. 3].

Discussion

Recently, *A. baumannii* has become one of the major pathogens in hospitals especially in intensive care units. Various factors, including a weakened immune system, consumption of antibiotics, the spread of relatively resistant microorganisms, poor infection control and drug resistance mechanisms, lead to the spread of pathogens that are highly resistant to commonly used antibiotics [1].

In the present study 50 clinical isolates were collected for the purpose of identifying *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*et al.,* in 2018 found that respiratory tract samples were the most predominant type (26%) containing *A. baumannii* isolates [8]. On the other hand, Omer et al., in 2015 disagreed with our finding in that the greatest number of their

isolates were recovered from sputum (61%) [1] Also, Abdallah *et al.*, finding in 2013 was disagreed with our finding, the found in a total of 150 *A. baumannii* isolates, sputum showed the greatest frequency among other type of samples 77 (51.3%) [9]. Regarding the frequency of the sputum sample, the results of Omer et al [1], and Abdallah et al, [9] are similar to our finding. However, Opazo et al are not [8].

In term of molecular confirmation, the frequency of *A. baumannii* was found in the present study was 47 (94%) which is similar to the frequency rate found out by Falah *et al.*, in 2019 whom confirmed 80 (97.56%) of *A. baumannii* isolates in a total of 82 [2]. While Maratheet 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 *A. baumannii* [10].

In this study, NDM gene was detected in only 2 isolates (4.3%) out of 47, while Maratheet al.,2019 detected 29 (87.8%) NDM in a total of 33 *A. baumannii*, which is dramatically high result compared to the frequency rate of NDM found in the present study. Also, Karaaslan *et al.*, in 2016found high frequency rate of NDM among *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 *A. baumannii* which is also considered high according to the frequency of NDM found is the present study [12]. Khorsi *etal.*, in 2015found 10 (10.6 %) NDM *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 *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]. Several other reports demonstrated prevalence higher than our finding [15-19]. 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 *etal.*,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 [20]. Other studies also report similar finding [21 – 24]. 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].These 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.

Ethics considerations

The study received approval from the ethics commission of University of Al Butana (**Number 2019/10 MLS**) and Khartoum hospital, and was conducted in accordance with the Declaration of Helsinki. Informed consent was taken from each participant.

Availability of data and materials

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

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Table (1) 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 %

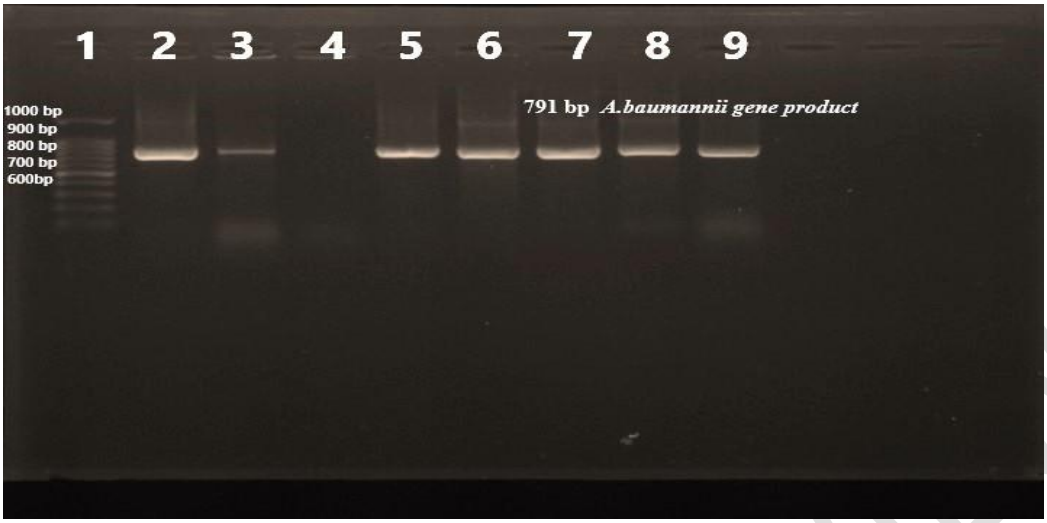

Fig1. Gel electrophoresis for detection of 791bp A. *baumannii isolate gene product. Lane 1. DNA ladder of 100bp. Lane2. Positive control of* 791 bp *A. baumannii gene product. Lane 3,5,6,7,8 and 9 are showed a typical positive isolate for band size of* 791 bp *A. baumannii gene product. Lane 4 was negative control.*

Fig 2. Gel electrophoresis for detection of 791bp A. *baumannii gene product and 380 bp NDM resistant gene product. Lane 1. DNA ladder of 100bp. For*A. *baumanniigene, 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.*

Fig 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.