Scientific Research Publishing

Open Journal of Medical Microbiology, 2022, 12, 38-48
https://www.scirp.org/journal/ojmm
ISSN Online: 2165-3380
ISSN Print: 2165-3372

Molecular Detection of Carbapenemase Genes in Extensive Drug Resistant *Acinetobacter baumannii* Clinical Isolates from ICU Patients, Khartoum

Shirehan M. Ibrahim[1*], Elamin M. Ibrahim[2], Omer A. Ibrahim[3], Omnia M. Hamid[4], Hassan A. Alaziz[5]

[1]Department of Medical Microbiology, Faculty of Medical Laboratories, Ibn Sina University, Khartoum, Sudan
[2]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, University of Khartoum, Khartoum, Sudan
[3]Department of Medical Microbiology, Faculty of Medical Laboratory Sciences, National Ribbat University, Khartoum, Sudan
[4]Department of Medical Microbiology, Faculty of Medical Laboratory sciences, University of Medical Sciences & Technology, UMST-Khartoum, Sudan
[5]Faculty of Medicine, National Ribbat University, Khartoum, Sudan
Email: *shirehanmohammed66@hotmail.com

How to cite this paper: Ibrahim, S.M., Ibrahim, E.M., Ibrahim, O.A., Hamid, O.M. and Alaziz, H.A. (2022) Molecular Detection of Carbapenemase Genes in Extensive Drug Resistant *Acinetobacter baumannii* Clinical Isolates from ICU Patients, Khartoum. *Open Journal of Medical Microbiology*, **12**, 38-48.
https://doi.org/10.4236/ojmm.2022.121004

Received: February 6, 2022
Accepted: March 21, 2022
Published: March 24, 2022

Abstract

Background: The emergence of carbapenemase producing *Acinetobacter baumannii* is increasingly reported nowadays and constitutes a major problem to the intensive care unit (ICU) patients with notable extensive-drug resistance ability. The study investigates carbapenemase producing *A. baumannii* strains exhibiting an extensively drug-resistant (XDR) phenotype, isolated from ICU patients in Khartoum. **Methods:** A total of 100 nonduplicate Gram-negative coccobacilli strains were obtained from microbiology laboratory of ICU patients' clinical isolates. Molecular identification of *A. baumannii* was performed by targeting 16S rRNA gene using specifically designed primers. Then, XDR strains were determined by susceptibility testing (disc diffusion). For detection of carbapenemase genes Polymerase chain reaction (PCR) was carried out. **Result:** Of 100 ICU clinical isolates, 38 (38.0%) was confirmed *A. baumannii* strains, those strains showed 100% carbapenem resistance and 60.5% extensive drug resistance to the antibiotics tested. The frequency of carbapenemase producer was 57.9% (22/38) of carbapenem resistance *A. baumannii* (CRAB). The most common carbapenemase associated with resistance was blaOXA gene followed by blaNDM and blaGES *A. baumannii* isolates. The co-occurrence of blaOXA-48-like and blaNDM, blaOXA-23-like and blaOXA-51, and blaNDM-1 and blaOXA-51 was detected in 22.7%, 18.2% strains and 4.5% respectively. A unique characteristic of our

findings was the coharbouring of the genes blaNDM-1, blaOXA-23-like, blaOXA-51 and blaOXA-143 in 9.1% strains (2/22), and this was the first report in the Khartoum city, Sudan. **Conclusion:** We have demonstrated for the first time a high prevalence of XDR-carbapenemase producing *A. baumannii* clinical isolates from ICU patients in Khartoum. Also an emergent blaOXA-143 was reported as High-Risk Clones. This highlights the routine mentoring of XDR-carbapenemase producing *A. baumannii* to avoid clone dissemination in our region hospitals.

Keywords

Carbapenem Producing *Acinetobacter baumannii* (CPAB), Intensive Care Unit (ICU) Patients, Extensive Drug-Resistant (XDR), Colistin

1. Introduction

Acinetobacter baumannii is a major cause of hospital acquire infections mainly among patients admitted at intensive care units (ICU) in many hospitals [1], regarding its ability to survive for long periods and could easily spread in hospital environment beside developing resistance to multiple antimicrobial agents leading to serious therapeutic problems [2] [3]. These traits could define its propensity for causing extended outbreaks [2]. Throughout last decades, mortality ranging from 5% in general wards to 54% in intensive care units (ICUs) is associated with *A. baumannii* infections [3] [4]. Carbapenems have been used as the most appropriate choice for treatment of infections due to MDR strains of *A. baumannii*. Unfortunately, extensive administration of broad-spectrum cephalosporins and/or carbapenems is a significant risk factor for development of colonization or infection with carbapenemase-producing *A. baumannii* [2] [5]. CPAB in hospital settings ranged from 2.3% to 67.7% in North Africa and from 9% to 60% in sub-Saharan Africa and the major bla genes were OXA-23-like, OXA-58-like, OXA-48-like, NDM-1 and VIM-2 associated with *A. baumannii* isolates of hospitalized patient years between (2010-2018) [3] [6]. More alarmingly; there was record of extensive drug resistance *A. baumannii* with intermediate resistance to colistin. This situation leads to limited options for treatment [7]. The lack of systematically collected data on the Sudan area contributes to a poor understanding of antimicrobial resistance and limits an effective response to the problem. The present study was aimed to investigate frequency of carbapenemase producing *A. baumannii* strains exhibiting extensive drug-resistant (XDR) profile isolated from ICU patients in two large hospitals at Khartoum city of Sudan.

2. Materials and Methods

2.1. Study Design and Setting

This cross-sectional, descriptive study was conducted in Royal Care Internation-

al Hospital (RCIH) and National Ribat Hospital (NRH) in Khartoum state, in Sudan between 2017 and 2019, from previous isolated Gram-negative coccobacilli isolates of various clinical from intensive care unit (ICU) patients. Those hospitals serve an average of 30 patients per day in ICU as well as the referral hospitals for the Khartoum city.

2.2. Clinical Isolates

A total of 100 nonduplicate Gram-negative coccobacilli strains were purposively collected regarding availability at study time. The nonduplicate Gram-negative coccobacilli obtained from various cultures of sputum, blood, urine, wound swabs, central-line catheter and tips of ICU patients at the RCHI and NRH in the Microbiology Laboratory. This strains were preliminarily re-identified phenotypically using standard microbiological procedures: growth characteristics and Gram-negative identification biochemical set and confirmed by restriction analysis of the 16 s - 23 s using polymerase chain reaction (PCR) amplification of *A. baumannii* (**Table 1**). All of the strains were stored in skim milk with 15% glycerol at −80˚C until further use. Before performing the tests strains were cultured on Brain Heart Infusion (BHI) agar plates at 37˚C for 24 h.

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility test of confirmed *A. baumannii* isolates was performed by disc diffusion method as per the (CLSI) guidelines [8], on Muller-Hinton agar (Hi-Media, Mumbai) using gentamicin (10μg), amikacin (30 μg), Co-trimoxazole (25 μg), ceftriaxone (30 μg), cefixime (30 μg), ceftazidime (30 μg), cefuroxime (30 μg), ciprofloxacin (5 μg), amoxycillin/clavulanic acid (30 μg), Imipenem (10 μg), meropenem (10 μg), tetraycline (10 μg) and colistin (10 μg) (bioanalyse, Turkey and Hi-Media, Mumbai). The diameter of inhibition zones was measured and reported as susceptible or resistant. For quality control of the disks were checked by using reference strain *A. baumannii* strain ATCC 17,978 was used. Interpretation of Multidrug-resistant (MDR) and extensively drug-resistant (XDR) profiles were defined using previously established criteria [9].

2.4. Detection Carbapenemase-Encoding Blagenes

The deoxyribonucleic acid (DNA) was extracted by boiling technique as follow; a loopful of each *A. baumannii* isolate was emulsified in 200μl of distilled water then boiled for 15 min and centrifugation at 13,000 rpm for 10 min. Supernatant was used for PCR amplification. *A. baumannii* isolates were screened for 6 common carbapenemase-encoding genes including bla_{NDM} [10], bla_{IMP}, bla_{KPC}, bla_{VIM}, $bla_{OXA\text{-}48\text{-}like}$, bla_{GES} [11] and for OXA-type carbapenemase-encoding genes including $bla_{OXA\text{-}23\text{-}like}$, $bla_{OXA\text{-}24\text{-}like}$, $bla_{OXA\text{-}143\text{-}like}$ *and* $bla_{OXA\text{-}51\text{-}like}$ [12] by PCR amplification with specific sets of primers (**Table 1**). All PCR-Reaction conditions were prepared by using ready master mix (APSLABS, India), 0.5 μl of each primer

Table 1. List of primers used for PCR amplification with sequence and amplicon size (bp).

PCR name	Sequence (5'-3')	Amplicon size (bp)
(recA) specific primers of *A. baumannii*	F-CCTGAATCTTCTGGTAAAAC R-GTTTCTGGGCTGCCAAACATTAC	500
Multiplex-1		
bla$_{VIM}$	F-GATGGTGTTTGGTCGCATA R-CGAATGCGCAGCCCAG	390
bla$_{IMP}$	F-TTGACACTCCATTTACDG R-GATYGAGAATTAAGCCACYCT	139
bla$_{KPC}$	F-CATTCAAGGGCTTTCTTGCTGC R-ACGACGGCATAGTCATTTGC	538
Multiplex-2		
bla$_{GES}$	F-AGTCGGCTAGACCGGAAAG R-TTTGTCCGTGCTCAGGAT	399
bla$_{OXA\text{-}48\text{-}like}$	F-GCTTGATCGCCCTCGATT R-GATTTGCTCCGTGGCCGAAA	281
bla$_{NDM\text{-}1}$	F-ATGGAATTGCCCAATATTATGCAC R-TCAGCGCAGCTTGTCGGC	813
Multiplex-3		
bla$_{OXA\text{-}51\text{-}like}$	F-TAA TGC TTT GATCGG CCT TG R-TGG ATT GCA CTT CAT CTT GG	353
bla$_{OXA\text{-}23\text{-}like}$	F-GAT CGG ATT GGA GAA CCA GA R-ATT TCT GAC CGC ATT TCC AT	501
bla$_{OXA\text{-}143\text{-}like}$	F-TGGCACTTTCAGCAGTTCCT R-TAATCTTGAGGGGGCCAACC	180
bla$_{OXA\text{-}24\text{-}like}$	F-GGT TAG TTG GCC CCC TTA AA R-AGT TGA GCG AAA AGG GGA TT	246
bla$_{OXA\text{-}58\text{-}like}$	F-AAG TAT TGG GGC TTG TGC TG R-CCCCTCTGCGCTCTACATAC	599

and 1 µl of template DNA (about 10 ng) in a total 25 µl. The PCR cycling conditions were as follows [10] [11] [12]: PCR products were assessed by electrophoresis using 1.5% (w/v) agarose gel and visualized by using an ultraviolet (UV) transilluminator.

2.5. Statistical Analysis

All data were analysed using the Statistical Package for the Social sciences for Windows software package version 21.0 (SPSS-IBM, Armonk, NY). Results were presented using frequency and percentages for qualitative variables. Categorical variables were compared by Chi-square test and all tests were two-sided, and differences with P-value < 0.05 were considered statistically significant.

3. Results

3.1. Detection of *A. baumannii*

A total of 38 *A. baumannii* were identified out of 100 gram-negative coccobacilli isolates using PCR, from different clinical specimens. The demographic characteristics of the patients with *A. baumannii* infection investigated in our study presented a slight male preponderance of 63.2% females versus 36.8% males). The age of the overall patients ranged between 28 - 73 years (mean 55.6 years, SD ± 11.4 years). *A. baumannii* were isolated highest from sputum (n = 28), urine (n = 2), blood, central line and tip (n = 3) for each, wound and bed sore (n = 1) for each, collected from the microbiology laboratory at Royal Care International Hospital (RCIH) and National Ribat Hospital (NRH) were included in the study.

3.2. Antimicrobial Susceptibility

All strains of *A. baumannii* (n = 38) showed resistance for all antimicrobials tested except 39.5% (15/38) of the isolates were susceptible to colistin. However, according to the susceptibility testing results (Table 2). Besides, 60.5% of *A. baumannii* isolated strains were categorized as XDR strains.

Table 2. Antimicrobial susceptibilities profiles of 38 *A. baumannii* strains.

Antibiotic disc (µg/ml)	Resistance Frequency (%)
Ciprofloxacin	38 (100%)
Cefixime/Clavulanic acid	38 (100%)
Ceftazidime	38 (100%)
Ceftriaxone	38 (100%)
Cefuroxime	38 (100%)
Amoxicillin/Clavulanic Acid	38 (100%)
Amikacin	38 (100%)
Gentamycin	38 (100%)
Meropenem	38 (100%)
Imipenem	38 (100%)
Co-trimoxazole	38 (100%)
Tetraycline	38 (100%)
Colistin	23 (60.5%)

3.3. Carbapenemase-Encoding Genes in *A. baumannii* Isolates

57.9% (22/38) of XDR *A. baumannii* were positive for one or more carbapenemase blagenes. The most prevalent single blagenes detected were $bla_{OXA\text{-}48\text{-}like}$ (n = 5) in Figure 1, followed by bla_{NDM} (n = 4) and bla_{GES} (n = 1). Whereas twelve *A. baumannii* isolates were co-produced carbapenemase blagenes $bla_{NDM+OXA\text{-}48\text{-}like}$ (n = 5), $bla_{OXA\text{-}23\text{-}like/OXA\text{-}51\text{-}like}$ (n = 4) in Figure 2(a), $bla_{NDM\text{-}1+OXA\text{-}23/51/143\text{-}likes}$ (n = 2),

while *bla*$_{NDM\text{-}1+\ blaOXA\text{-}51\text{-}like}$ (Figure 2(b)) was detected in only one (n = 1) isolate (Table 3).

Table 3. Distribution of carbapenemase (blagenes) in 38 XDR *A. baumannii* strains.

Carbapenemases-encoding blagenes	Frequency (%)
bla$_{OXA\ -48like}$	5 (22.7%)
bla$_{NDM}$	4 (18.2%)
bla$_{GES}$	1 (4.5%)
bla$_{NDM}$ and *bla*$_{OXA\text{-}48\text{-}like}$	5 (22.7%)
bla$_{OXA\text{-}51\text{-}like}$ and *bla*$_{NDM\text{-}1}$	1 (4.5%)
bla$_{OXA\text{-}51\text{-}like}$ and *bla*$_{OXA\text{-}23\text{-}like}$	4 (18.2%)
bla$_{OXA\text{-}51\text{-}like}$, *bla*$_{OXA\text{-}23\text{-}like}$, *bla*$_{OXA\text{-}143\text{-}like}$ and *bla*$_{NDM\text{-}1}$,	2 (9.1%)
Total CPAB	**22 (57.9%)**

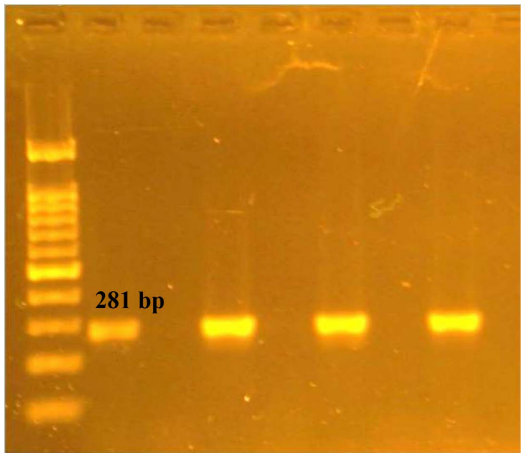

Figure 1. Gel-electrophoresis result of *bla*$_{OXA\text{-}48\text{-}like}$ amplification: lane 1: DNA ladder 100 bp; lane 2: bla$_{OXA\text{-}48\text{-}like}$ positive control 281 bp; lane 3: negative control; lanes 4, 6 & 8: Positive bla$_{OXA\text{-}48\text{-}like}$ *A. baumannii* isolates.

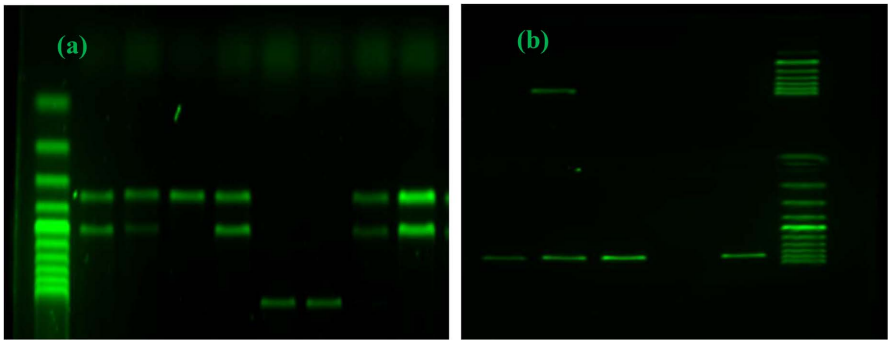

Figure 2. Gel-electrophoresis result of: (a) *bla*$_{OXA}$ specific amplification: lane 1: DNA ladder; lane 5 & 6: bla$_{OXA\text{-}143\text{-}like}$; lane 3: bla$_{OXA\text{-}51\text{-}like}$ enzymes; lane 1, 2, 4, 7 & 8: bla$_{OXA\text{-}51\text{-}like}$ and bla$_{OXA\text{-}23\text{-}like}$. The molecular size marker (lane 1) is a 123 bp ladder (Invitrogen, Paisley, UK) and (b) *bla*$_{NDM\text{-}1}$ amplification: lane 1: DNA ladder; lane 4: bla$_{OXA\text{-}51\text{-}like}$ & bla$_{NDM\text{-}1}$ positive.

4. Discussion

An emerging rise in the incidence of carbapenemase producing *A. baumannii* has been increasingly reported worldwide [6] and in Sudan as well [13] [14], leaving behind a significant challenging to treat infections. In our study, for a deeper understanding the molecular mechanisms underlying carbapenem resistance, a collection of accurately characterized 38 *A. baumannii* clinical isolates was screened for carbapenemase-coding genes by PCR. A total of 57.9% (22/38) *A. baumannii* isolates were confirmed by molecular amplification (PCR) to be carbapenemase positive in clinical samples recovered from ICU patients with severe infections. Among the source of the isolates in the study, the majority of CPAB were from respiratory specimens (72.7%) followed by tip, blood, central line and bed sore specimens, consistent with other studies [1] [14] [15]. Extensive drug resistant among CPAB infections has become a world-wide contest as this organism is resistant to cephalosporins, Monobactams, aminoglycosides, fluoroquinolones, cephalosporins, carbapenems, and now emergence of colistin resistance in this species is of significant concern, leaving restricted treatment options for ICU infections. In our region, lack of systematically identification of *A. baumannii* infection among ICU patients and environments in hospital contributes to a poor understanding of antimicrobial resistance and limits an effective response to the problem. In recent years, the global emerge of extensive drug resistant producing carbapenemase *A. baumannii,* the strains have significantly threatened public health and become a major problem in the intensive care unit (ICU) reported by [1] [4] [16]. The results of the present study show that there was an extreme increase in the resistance rate of *A. baumannii* to meropenem, from 89% in 2015 to 100% in 2019 [14] [17]. In addition, the resistance rate of *A. baumannii* to colistin was 60.5%, which is higher than in previous reports in Khartoum state and other studies [1] [4] [13] [14] [16]. The present study showed 100% resistant rates of the most clinically applicable antibiotics for the treatment of infections caused by *A. baumannii*, except for colistin, which may be used as the final options in the management of infections caused by this bacterium. In this study, the high resistance rate of *A. baumannii* against carbapenems may indicate the outcome of overuse and misuse of carbapenems in our hospital.

Overall, $bla_{OXA\text{-}51\text{-}like}$ genes were the most prevalent subgroup, which is consistent with the view that they are intrinsic to *A. baumannii* [5] [10] [14] [18]. These genes were detected in 7 of 12 isolates, irrespective of levels of carbapenem susceptibility or resistance, these alleles do not correlate with the level of carbapenem resistance of the host isolate. Thus, resistance to carbapenems cannot be inferred from detection of $bla_{OXA\text{-}51\text{-}like}$ alleles. In contrast, alleles encoding OXA-23-like, OXA-24-like and OXA-58-like enzymes were consistently associated with resistance or, at least, with reduced susceptibility. The $bla_{OXA\text{-}23\text{-}like}$ carbapenemase-producing *A. baumannii* is becoming widespread globally in Europe, South America, and Asia [19] [20]. In this study, $bla_{OXA\text{-}48\text{-}like}$ was the most common gene encountered in the study isolates as single carbapenemase

encoding gene followed by bla$_{NDM}$ and bla$_{GES}$. Various studies have noted that only bla$_{GES}$-type carbapenemase was reported in in an *Acinetobacter* isolate from Mediterranean countries and Kuwait [21] [22]. *bla*$_{OXA\text{-}23\text{-}like}$ carbapenemase was detected in 6 (15.4%) of the 38 carbapenem-resistant isolates and as in terms of carbapenem non-susceptibility, an alarmingly high rate of 75.0% over 2 years was detected, this high rate is similar to that reported by Perez *et al.* [19] [23]. This rate, however; is much higher than that reported for other African countries [3] revealing a worrisome situation in this country. Alleles encoding bla$_{OXA\text{-}24/40\text{-}like}$ enzymes were not detected in any of the *A. baumannii* ICU strains; these enzymes are reported in Portugal, Spain, Poland, Iran, the United States, Asia and Saudi Arabia [3] [5] [20]. In the current study we report *bla*$_{NDM\text{-}1}$- producing *A. baumannii* strains, in contrast to *bla*$_{NDM\text{-}1}$ was mostly carried by *Enterobacteriaceae*; all the *bla*$_{NDM\text{-}1}$-positive *A. baumannii* isolates, which suggests that this species, which has a robust survival capability, can easily acquire foreign resistance genes such as *bla*$_{NDM\text{-}1}$ [1] [14] [24]. The coexisted CP-AB was detected in 12 isolated CRAB with (5/22; 22.7%) *bla*$_{NDM}$ and *bla*$_{OXA\text{-}48\text{-}like}$, followed by (4/22; 18.2%) bla$_{OXA\text{-}51\text{-}like}$ and bla$_{OXA\text{-}23\text{-}like}$ and only two strains of *A. baumannii* produced *bla*$_{OXA\text{-}51}$, *bla*$_{OXA\text{-}23\text{-}like}$, *bla*$_{OXA\text{-}143\text{-}like}$ and *bla*$_{NDM\text{-}1}$, coexisted carbapenemase gens surveyed in this study as emergent detection in our region, mainly among ICU patients. As the *bla*$_{OXA\text{-}143\text{-}like}$ gene is frequently found in the Southeast region of Brazil, especially in the state of São Paulo. It is important to note that two new variants of this gene were recently described [12].

Here, we detected an emergent OXA subclass identified in two *A. baumannii* strains *bla*$_{OXA\text{-}143\text{-}like}$ which reported as High-Risk Clones among XDR *A. baumannii*, whereas *bla*$_{OXA\text{-}23}$, *bla*$_{OXA\text{-}51}$, *bla*$_{NDM\text{-}1}$ and *bla*$_{GES}$ producing in XDR *A. baumannii* strains were prevalent in the ICU. The coexisted genes (*bla*$_{OXA\text{-}51}$ and *bla*$_{OXA\text{-}23\text{-}like\ and}$ *bla*$_{OXA\text{-}51}$, *bla*$_{OXA\text{-}23\text{-}like}$, *bla*$_{OXA\text{-}143}$ and *bla*$_{NDM\text{-}1}$) were also associated with increased virulence as compared to other OXAs. Therefore, some infection control measures should be covered and implementation of whale genome sequence (WGS) as advanced molecular characterization of infectious agents could improve both, identification and genetic characterization including resistance profiles, facilitating outbreak investigations and molecular surveillance.

Acknowledgements

We are grateful to Royal Care International Hospital (RCIH) and National Ribat Hospital (NRH) for providing us the Gram negative coccobacilli isolates from ICU patients to perform this study. This work was partly of Ph.D. project under supervision of Prof. Hassan A. Alaziz and Prof. Elamin. M. Ibrahim, in the Alribat National University (Khartoum, Sudan).

Ethical Clearance

This study was approved by the ethics committee of Alribat National University-Graduate Collage. Bacterial isolates ethics approval and consent were not ap-

plicable as samples obtained from Microbiology Laboratory remaining samples and coded by Laboratory ID.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Sileem, A.E., Said, A.M. and Meleha, M.S. (2017) *Acinetobacter baumannii* in ICU Patients: A Prospective Study Highlighting Their Incidence, Antibiotic Sensitivity Pattern and Impact on ICU Stay and Mortality. *Egyptian Journal of Chest Diseases and Tuberculosis*, **66**, 693-698. https://doi.org/10.1016/j.ejcdt.2017.01.003

[2] Ibrahim, S., Al-Saryi, N., Al-Kadmy, I. and Aziz, S.N. (2021) Multidrug-Resistant *Acinetobacter baumannii* as an Emerging Concern in Hospitals. *Molecular Biology Reports*, **48**, 6987-6998. https://doi.org/10.1007/s11033-021-06690-6

[3] Manenzhe, R.I., Zar, H.J., Nicol, M.P. and Kaba, M. (2015) The Spread of Carbapenemase-Producing Bacteria in Africa: A Systematic Review. *Journal of Antimicrobial Chemotherapy*, **70**, 23-40. https://doi.org/10.1093/jac/dku356

[4] Mirzaei, B., Bazgir, Z.N., Goli, H.R., Iranpour, F., Mohammadi, F. and Babaei, R. (2020) Prevalence of Multi-Drug Resistant (MDR) and Extensively Drug-Resistant (XDR) Phenotypes of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* Isolated in Clinical Samples from Northeast of Iran. *BMC Research Notes*, **13**, Article No. 380. https://doi.org/10.1186/s13104-020-05224-w

[5] Al-Agamy, M.H., Khalaf, N.G., Tawfick, M.M., Shibl, A.M. and El Kholy, A. (2014) Molecular Characterization of Carbapenem-Insensitive *Acinetobacter baumannii* in Egypt. *International Journal of Infectious Diseases*, **22**, 49-54. https://doi.org/10.1016/j.ijid.2013.12.004

[6] Ayobami, O., Willrich, N., Harder, T., Okeke, I.N., Eckmanns, T. and Markwart, R. (2019) The Incidence and Prevalence of Hospital-Acquired (Carbapenem-Resistant) *Acinetobacter baumannii* in Europe, Eastern Mediterranean and Africa: A Systematic Review and Meta-Analysis. *Emerging microbes & infections*, **8**, 1747-1759. https://doi.org/10.1080/22221751.2019.1698273

[7] Karakonstantis, S., Kritsotakis, E.I. and Gikas, A. (2020) Pandrug-Resistant Gram-Negative Bacteria: A Systematic Review of Current Epidemiology, Prognosis and Treatment Options. *Journal of Antimicrobial Chemotherapy*, **75**, 271-282. https://doi.org/10.1093/jac/dkz401

[8] Patel, J.B., Cockerill, F. and Bradford, P.A. (2015) Performance Standards for Antimicrobial Susceptibility Testing: Twenty-Fifth Informational Supplement. Clinical and Laboratory Standards Institute, Wayne.

[9] Magiorakos, A.-P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M., Giske, C., *et al.* (2012) Multidrug-Resistant, Extensively Drug-Resistant and Pandrug-Resistant Bacteria: An International Expert Proposal for Interim Standard Definitions for Acquired Resistance. *Clinical Microbiology and Infection*, **18**, 268-281. https://doi.org/10.1111/j.1469-0691.2011.03570.x

[10] Hammoudi Halat, D. and Ayoub Moubareck, C. (2020) The Current Burden of Carbapenemases: Review of Significant Properties and Dissemination among Gram-Negative Bacteria. *Antibiotics*, **9**, Article No. 186. https://doi.org/10.3390/antibiotics9040186

[11] Dallenne, C., Da Costa, A., Decré, D., Favier, C. and Arlet, G. (2010) Development

of a Set of Multiplex PCR Assays for the Detection of Genes Encoding Important β-Lactamases in Enterobacteriaceae. *Journal of Antimicrobial Chemotherapy*, **65**, 490-495. https://doi.org/10.1093/jac/dkp498

[12] Higgins, P.G., Pérez-Llarena, F.J., Zander, E., Fernández, A., Bou, G. and Seifert, H. (2013) OXA-235, a Novel Class D β-Lactamase Involved in Resistance to Carbapenems in *Acinetobacter baumannii*. *Antimicrobial Agents and Chemotherapy*, **57**, 2121-2126. https://doi.org/10.1128/AAC.02413-12

[13] Satir, S., Elkhalifa, A., Ali, M., El Hussein, A. and Elkhidir, I. (2016) Detection of Carbepenem Resistance Genes among Selected Gram Negative Bacteria Isolated from Patients in-Khartoum State, Sudan. *Clin Microbiol*, **5**, Article No. 266. https://doi.org/10.4172/2327-5073.1000266

[14] Al-Hassan, L., Elbadawi, H., Osman, E., Ali, S., Elhag, K., Cantillon, D., *et al.* (0) Molecular Epidemiology of Carbapenem-Resistant *Acinetobacter baumannii* from Khartoum State, Sudan. *Frontiers in Microbiology*, **221**, Article ID: 628736. https://doi.org/10.3389/fmicb.2021.628736

[15] Reddy, D., Morrow, B.M. and Argent, A.C. (2015) *Acinetobacter baumannii* Infections in a South African Paediatric Intensive Care Unit. *Journal of Tropical Pediatrics*, **61**, 182-187. https://doi.org/10.1093/tropej/fmv017

[16] Carrasco, L.D.M., Dabul, A.N.G., Boralli, C.M.S., Righetto, G.M., Carvalho I.S., Dornelas, J.V., *et al.* (2021) Polymyxin Resistance among XDR ST1 Carbapenem-Resistant *Acinetobacter baumannii* Clone Expanding in a Teaching Hospital. *Frontiers in Microbiology*, **12**, Article ID: 622704. https://doi.org/10.3389/fmicb.2021.622704

[17] Omer, M.I., Gumaa, S.A., Hassan, A.A., Idris, K.H., Ali, O.A., Osman, M.M., *et al.* (2015) Prevalence and Resistance Profile of *Acinetobacter baumannii* Clinical Isolates from a Private Hospital in Khartoum, Sudan. *American Journal of Microbiological Research*, **3**, 76-79.

[18] Biglari, S., Alfizah, H., Ramliza, R. and Rahman, M.M. (2015) Molecular Characterization of Carbapenemase and Cephalosporinase Genes among Clinical Isolates of *Acinetobacter baumannii* in a Tertiary Medical Centre in Malaysia. *Journal of Medical Microbiology*, **64**, 53-58. https://doi.org/10.1099/jmm.0.082263-0

[19] Cieslinski, J.M., Arend, L., Tuon, F.F., Silva, E.P., Ekermann, R.G., Dalla-Costa, L.M., *et al.* (2013) Molecular Epidemiology Characterization of OXA-23 Carbapenemase-Producing *Acinetobacter baumannii* Isolated from 8 Brazilian Hospitals Using Repetitive Sequence-Based PCR. *Diagnostic Microbiology and Infectious Disease*, **77**, 337-340. https://doi.org/10.1016/j.diagmicrobio.2013.07.018

[20] Ibrahim, M.E. (2019) Prevalence of *Acinetobacter baumannii* in Saudi Arabia: Risk Factors, Antimicrobial Resistance Patterns and Mechanisms of Carbapenem Resistance. *Annals of Clinical Microbiology and Antimicrobials*, **18**, Article No. 1. https://doi.org/10.1186/s12941-018-0301-x

[21] Djahmi, N., Dunyach-Remy, C., Pantel, A., Dekhil, M., Sotto, A. and Lavigne, J.-P. (2014) Epidemiology of Carbapenemase-Producing *Enterobacteriaceae* and *Acinetobacter baumannii* in Mediterranean Countries. *BioMed Research International*, **2014**, Article ID: 305784. https://doi.org/10.1155/2014/305784

[22] Bonnin, R.A., Rotimi, V.O., Al Hubail, M., Gasiorowski, E., Al Sweih, N., Nordmann, P., *et al.* (2013) Wide Dissemination of GES-Type Carbapenemases in *Acinetobacter baumannii* Isolates in Kuwait. *Antimicrobial Agents and Chemotherapy*, **57**, 183-188. https://doi.org/10.1128/AAC.01384-12

[23] Correa, A., Del Campo, R., Escandón-Vargas, K., Perenguez, M., Rodríguez-Baños,

M., Hernández-Gómez, C., *et al.* (2018) Distinct Genetic Diversity of Carbapenem-Resistant *Acinetobacter baumannii* from Colombian Hospitals. *Microbial Drug Resistance*, **24**, 48-54. https://doi.org/10.1089/mdr.2016.0190

[24] Khan, A.U., Maryam, L. and Zarrilli, R. (2017) Structure, Genetics and Worldwide Spread of New Delhi Metallo-β-Lactamase (NDM): A Threat to Public Health. *BMC microbiology*, **17**, Article No. 101. https://doi.org/10.1186/s12866-017-1012-8