INTERNATIONAL RESEARCH JOURNAL OF PHARMACY

www.irjponline.com

ISSN 2230 – 8407

Research Article

PREVALENCE OF CARBAPENEMASES AMONG IMIPENEM RESISTANT *ACINETOBACTER BAUMANNII* ISOLATES IN TANTA, EGYPT

Fatma Ibrahim Sonbol, Tarek El-Said El-Banna and Noha Abdallah Attia *

Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Tanta University, Tanta, 31611, Egypt

*Corresponding Author Email: noha.atiya@pharm.tanta.edu.eg

Article Received on: 05/08/19 Approved for publication: 02/12/19

DOI: 10.7897/2230-8407.1012332

ABSTRACT

The capability of carbapenemase production is one of the chief mechanisms for the development of multidrug-resistance (MDR) and extensively drug-resistance (XDR) in *Acinetobacter baumannii*. The aim of this study was to detect the prevalence of different types of carbapenemases produced by carbapenem-resistant *Acinetobacter baumannii* in Tanta region. Out of 1223 clinical samples, collected from Tanta University teaching hospitals, a total of 69 (5.6%) *Acinetobacter baumannii* isolates were recovered as identified by standard biochemical tests as well as polymerase chain reaction (PCR) technique that detect the intrinsic *bla*$_{\text{OXA-51-like}}$ gene. Susceptibility to various antimicrobials was performed by disc diffusion method and revealed multiple (1-15) drug resistance of all isolates. Nevertheless, colistin was effective against all tested isolates. A total of 31 out of 69 (44.9%) isolates were imipenem resistant. These isolates, which included 6 (19.4%) MDR and 25 (80.6%) XDR, were selected for further study. Detection of carbapenemases production by both phenotypic and genotypic techniques was performed and all the imipenem resistant *Acinetobacter baumannii* isolates (IMRAb) were found carbapenemases producers. These carbapenemases included metallo-β-lactamases, *bla*$_{\text{OXA-23-type}}$ and *bla*$_{\text{OXA-58-type}}$ carbapenemases detected in 100% (31/31), 71% (22/31) and 13% (4/31) of these isolates, respectively. Our findings highly recommend careful monitoring for the presence of carbapenem-resistant MDR or XDR *Acinetobacter baumannii* among hospitalized patients especially because some of the detected resistance genes are known to be located on a plasmid which could facilitate resistance dissemination.

Keywords: *Acinetobacter baumannii*; carbapenemases; IMRAb; XDR

INTRODUCTION

Acinetobacter baumannii has become a significant and worrying pathogen in hospitals worldwide, and nosocomial outbreaks have been reported due to multidrug-resistant strains, mostly in the intensive care units and burn units[1]. *Acinetobacter baumannii* is often related to nosocomial pneumonia, bloodstream, urinary tract, nosocomial meningitis, and wound infections[2]. Carbapenems are powerful broad-spectrum β-lactam antibiotics that are widely regarded by clinicians as "last-line" antibiotics, particularly for the management of critically ill patients and/or those with antimicrobial-resistant *A. baumannii* infections[3]. Unfortunately, the increased clinical use of these drugs has led to the evolution of resistant strains[4]. Carbapenem-resistant *A. baumannii* has become a problematic nosocomial pathogen globally[5]. If one of these resistant isolates gained access to the hospital, it is difficult to eliminate, as the hospital environment favors its growth and transmission either by colonizing or infecting healthcare workers or living on surfaces within bacterial biofilms guarding it against the effects of common disinfectants[6]. Different mechanisms including loss of outer membrane protein, change of penicillin-binding protein, carbapenem-hydrolyzing enzymes and efflux pump are involved in carbapenem resistance in *A. baumannii*[7]. However, production of carbapenem-inactivating enzymes belonging to metallo-beta-lactamases (MBLs) and the Class D carbapenemases oxacillinases (OXA enzymes) are the main mechanisms of resistance in *A. baumannii* worldwide[8]. Our study focused on the assessment of the antimicrobial resistance profile and prevalence of different types of carbapenemases enzymes in *A. baumannii* isolates from hospitalized patients in Tanta region.

MATERIALS AND METHODS

Ethics statement

All experiments were conducted according to national and international ethical standards. The experimental protocols, including sample collection from Tanta University teaching hospitals, and consent forms, were revised and approved by the Research Ethics Committee, Faculty of Pharmacy, Tanta University, Egypt.

Bacterial isolates

During the period from April 2014 to July 2017, sixty-nine *A. baumannii* isolates were recovered from various clinical samples obtained from 1223 hospitalized patients admitted to different departments of Tanta University teaching hospitals.

Bacterial identification

Our isolates were primarily identified at the genus level by conventional biochemical tests including oxidase, catalase, indole, motility, and sugar fermentation tests followed by using API™ 20 NE identification system (Bio Mérieux, France). Identification of *A. baumannii* isolates at the species level has been based on polymerase chain reaction (PCR) amplification of the intrinsic *bla*$_{\text{OXA-51-like}}$ gene, a 353-base-pair (bp) amplicon. Genomic DNA for PCR was extracted by the boiling method as previously described[9]. The used primers (Thermo Scientific, USA) were Forward 5`-TAA TGC TTT GAT CGG CCT TG-3`and Reverse 5`-TGG ATT GCA CTT CAT CTT GG-3`. The

amplification reaction was carried out in a final volume of 25 µl with 12.5 µl of 2X PCR master mix, 2 µl of genomic DNA extract, 1 µl of each primer, and 8.5 µl of nuclease-free water. PCR conditions were programmed as follows: initial denaturation at 94°C for 5 minutes, followed by 33 cycles of denaturation at 94°C for 25 seconds, annealing at 55°C for 40 seconds and extension at 72°C for 50 seconds, and a final extension at 72°C for 6 minutes. Storage temperature was 4°C. The PCR products were separated by electrophoresis using 1.5% agarose gel then visualized under UV gel documentation system after staining with midori green dye[10].

Anti-microbial susceptibility testing

Testing of susceptibility to various antimicrobial agents was performed by disc diffusion in Mueller-Hinton agar (MHA)[11] and results were interpreted according to guidelines of the Clinical Laboratory Standard Institute (CLSI 2016). Bacterial suspension at a turbidity equivalent to 0.5-McFarland standard was inoculated on a MHA plate. The used antimicrobial discs (Oxoid, England) included: piperacillin 100 µg, piperacillin-tazobactam 100/10 µg, ampicillin-sulbactam 10/10 µg, cefepime 30 µg, ceftazidime 30 µg, ceftriaxone 30 µg, cefotaxime 30 µg, imipenem 10 µg, tobramycin 10 µg, amikacin 30 µg, tetracycline 30 µg, ciprofloxacin 5 µg, levofloxacin 5 µg, gatifloxacin 5 µg, trimethoprim/sulfamethoxazole 1.25/23.75 µg, and colistin 10 µg.

Phenotypic screening of carbapenemases production

All imipenem resistant *A. baumannii* (IMRAb) isolates were screened for carbapenemases production by modified Hodge test (MHT)[12]. The surface of a MHA plate was inoculated with a lawn of carbapenem susceptible *Escherichia coli* strain (ATCC 25922) using a sterile cotton swab. After drying, a 10-µg imipenem disc was placed at the center of the plate according to the standard disc diffusion method. In a straight line, tested isolates were streaked from the edge of the disc to the periphery of the plate and incubated at 35 ± 2°C for 16-24 hours. Up to four isolates can be tested on the same plate with one drug disc. After incubation, plates were examined for a characteristic clover leaf-like indentation (positive test) of the *E. coli* (ATCC 25922) growing along with the tested isolate growth streak within the disc diffusion zone.

Phenotypic screening of metallo-β-lactamases production

Ethylene diamine tetraacetic acid-combined disc test (EDTA-CDT) was performed for detection of MBLs production among all carbapenemase producing *A. baumannii* isolates**.** Briefly, an overnight culture suspension of the test isolate equivalent to a 0.5 McFarland was inoculated on a MHA plate. Two 10 µg imipenem discs were positioned within a center-to-center distance of 4-5 cm on a dried agar plate and 10 µl of 0.5 M EDTA was added to one of the discs. Following overnight incubation at 37°C, the inhibition zone diameters were determined and if the diameter of the zone around the disc to which the EDTA was added increased by ≥ 7 mm in diameter compared to the inhibition zone around the imipenem disc, this was interpreted as a positive test result[13].

Genotypic detection of class d β-lactamases using duplex pcr

Amplification of the resistant determinants $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$ genes encoding OXA-23 and OXA-58 carbapenemases was performed using total DNA extract (boiled suspension of bacterial cells) of each tested carbapenemase producing *A. baumannii* isolates using duplex polymerase chain reaction (dPCR) technique. The primers sequences (Thermo Scientific, USA) were OXA-23-F (5′-GAT GTG TCATAG TAT TCG TCG-3′) and OXA-23 R (5′-TCA CAA CAA CTA AAA GCA CGT-3′) and OXA-58 F (5′-CGA TCA GAA TGT TCA AGC GC-3′) and OXA-58 R (5′-ACG ATT CTC CCC TCT GCG C-3′). The amplification reaction was carried out in a final volume of 25 µl with 12.5 µl of 2X PCR master mix, 2 µl of total DNA extract, 1 µl of each forward primer, 1 µl of each reverse primer and 6.5 µl of nuclease-free water. Amplification was performed using the following thermocycler conditions: initial denaturation at 94°C for 5 minutes, followed by 30 cycles of denaturation at 94°C for 25 seconds, annealing at 52°C for 40 seconds and elongation at 72°C for 50 seconds, and a final extension at 72°C for 6 minutes[14]. Storage temperature was 4°C. PCR products were separated with electrophoresis in 1.5% agarose gel and visualized under UV gel documentation system after staining with midori green dye.

RESULTS AND DISCUSSION

A total of 69 *A. baumannii* isolates were recovered from different clinical samples obtained from 1223 hospitalized patients admitted to different departments of Tanta University teaching hospitals. The isolates which were morphologically consistent with *A. baumannii* (i.e. oxidase-negative, catalase-positive, indole-negative, nonmotile, and glucose and lactose non-fermenting when inoculated onto Kligler Iron Agar) were further confirmed using API™ 20 NE identification system[15,16]. All isolates were positive for $bla_{\text{OXA-51-like}}$ gene, an intrinsic component of the species[10]. Susceptibility testing to different antimicrobials revealed 100% resistance to piperacillin, 88.4% (61/69) to 3rd generation cephalosporins (ceftriaxone and cefotaxime), 87% (60/69) to ceftazidime, 85.5% (59/69) to cefepime, 76.8% (53/69) to piperacillin/tazobactam, 73.9% (51/69) to ampicillin/sulbactam and 44.9% (31/69) to imipenem. A relatively high resistance rate to fluoroquinolones was observed, where resistance rate to ciprofloxacin, levofloxacin, and gatifloxacin was exhibited by 92.8% (64/69), 85.5% (59/69) and 71% (49/69) of the isolates, respectively. Regarding the aminoglycosides, the resistance rates to amikacin and tobramycin were 69.6% (48/69) and 31.9% (22/69), respectively. The resistance rate to trimethoprim/sulfamethoxazole and tetracycline was 75.4% (52/69) and 27.5% (19/69), respectively. However, no colistin resistant isolate was detected. Heterogeneous resistance patterns were observed, and all isolates exhibited resistance to 1-15 of the tested antimicrobials. As reported by Magiorakos *et al*. (2012), isolate that showed resistance to at least one agent in three or more antimicrobial categories was considered multi-drug resistant (MDR). The isolate that showed resistance to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) were considered extensively drug-resistant (XDR)[17]. Accordingly, out of 69 tested, 35 (50.7%) and 27 (39.1%) isolates exhibited MDR and XDR profiles, respectively. Only the 31 IMRAb isolates were selected for further study. These isolates included 6 (19.4%) and 25 (80.6%) isolates that exhibited MDR and XDR profiles, respectively. Basustaoglu *et al*. (2001) in Pakistan; found that MDR and XDR profiles were exhibited by 82.4% and 65.0% of tested *A. baumannii* isolates, respectively[18]. All 31 (100%) IMRAb isolates evidenced phenotypic carbapenemases activity using MHT. Metallo β-lactamases production was detected in 100% of IMRAb isolates using EDTA-CDT. Previous reports stated that Carbapenemases and MBLs production among *A. baumannii* ranged from 49% up to 99%[19-21]. Among 31carbapenemase-producing *A. baumannii* isolates, 22 (71%) and 4 (13%) were harboring $bla_{\text{OXA-23-like}}$ and $bla_{\text{OXA-58-like}}$, respectively as detected by dPCR technique (Figure 1).

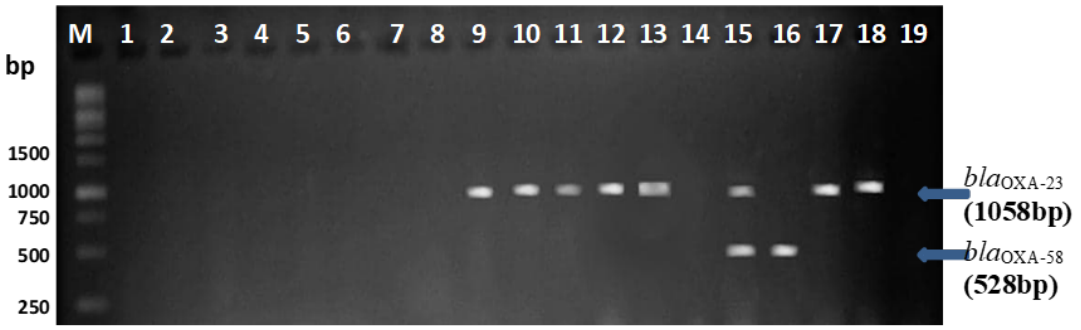

Figure 1: Caption

A representative electropherogram of PCR amplified products of *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$ genes extracted from carbapenemase-producing *Acinetobacter baumannii* isolates. First lane, M; 1Kbp DNA ladder. Lanes 1-19; amplified products of tested isolates. The amplicons of the genes are on the predicted size of *bla*$_{OXA-58-like}$ and *bla*$_{OXA-23-like}$ at approximately 528 and 1058 bp, respectively

Interestingly, either *bla*$_{OXA-23-like}$ or *bla*$_{OXA-58-like}$ carrying IMRAb isolates were XDR, with co-presence of these two resistance genes in one XDR isolate. On the other hand, *bla*$_{OXA-23-like}$ or *bla*$_{OXA-58-like}$ genes were totally undetectable in 6 IMRAb isolates which exhibited MDR profile. The OXA-23-type carbapenemase-producing *A. baumannii* are becoming increasingly widespread, with reports emerging from USA, Europe, Asia, Australia, Africa, and the Middle East[22]. In 2013, *bla*$_{OXA-23-like}$ and *bla*$_{OXA-58-like}$ were identified in 72% and 20% of studied carbapenem-resistant *A. baumannii* isolates in two Egyptian centers[23]. The emergence and subsequent dissemination of carbapenem-resistant *A. baumannii*, represent a global public health threat for which there is as yet no clear solution.

CONCLUSION

Acinetobacter baumannii isolates showed high resistance rate to most of the available antimicrobial agents. All IMRAb isolates exhibited MDR / XDR profile and were found to produce different carbapenemases including MBLs and OXA enzymes. These enzymes exhibit a wide spectrum of hydrolysis activity against various β-lactams. Therefore, careful monitoring for the presence of XDR *A. baumannii* among hospitalized patients is highly recommended to avoid wide dissemination of antibiotics resistance and to limit the indiscriminative use of cephalosporins and carbapenems in the hospitals. Empirical treatment of infections using carbapenems should be restricted and given only on the basis of clinical signs and laboratory parameters. Colistin should be reserved as the last choice to treat infections caused by such problematic pathogen.

ACKNOWLEDGEMENT

The authors wish to acknowledge the consistent and helpful support of the nursing staff at the Tanta University Hospitals in their providing of professional care to the subjects and implementation of proper research protocols during the study period.

REFERENCES

1. Wong TH, Tan BH, Ling ML, Song C. Multi-resistant *Acinetobacter baumannii* on a burns unit—clinical risk factors and prognosis. Burns 2002; 28(4): 349-57.
2. Kempf M, Rolain JM. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. International Journal of Antimicrobial agents 2012; 39(2): 105-14.
3. Papp-Wallace KM, Endimiani A, Taracila MA, Bonomo RA. Carbapenems: past, present, and future. Antimicrobial agents and chemotherapy 2011; 55(11): 4943-60.
4. Gao J, Zhao X, Bao Y, Ma R, Zhou Y, Li X, Chai T, Cai Y. Antibiotic resistance and OXA-type carbapenemases-encoding genes in airborne *Acinetobacter baumannii* isolated from burn wards. Burns 2014; 40(2): 295-9.
5. Irfan S, Turton JF, Mehraj J, Siddiqui SZ, Haider S, Zafar A, Memon B, Afzal O, Hasan R. Molecular and epidemiological characterization of clinical isolates of carbapenem-resistant *Acinetobacter baumannii* from public and private sector intensive care units in Karachi, Pakistan. Journal of Hospital Infection 2011; 78(2): 143-8.
6. Rolain JM, Loucif L, Al-Maslamani M, Elmagboul E, Al-Ansari N, Taj-Aldeen S, Shaukat A, Ahmedullah H, Hamed M. Emergence of multidrug-resistant *Acinetobacter baumannii* producing OXA-23 Carbapenemase in Qatar. New microbes and new infections 2016; 11: 47-51.
7. Huang L, Sun L, Xu G, Xia T. Differential susceptibility to carbapenems due to the AdeABC efflux pump among nosocomial outbreak isolates of *Acinetobacter baumannii* in a Chinese hospital. Diagnostic microbiology and infectious disease 2008; 62(3): 326-32.
8. Poirel L, Naas T, Nordmann P. Diversity, epidemiology, and genetics of class D β-lactamases. Antimicrobial agents and chemotherapy 2010; 54(1): 24-38.
9. Moore DD, Dowhan D. Preparation and analysis of DNA. In Ausubel FW, Brent R, Kingston RE, Moore DD, Seidman JG *et al*. (editors) Current Protocols in Molecular Biology New York, NY: Wiley; 1995. p. 2–11.
10. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. Journal of clinical microbiology 2006; 44(8): 2974-6.
11. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. American Journal of Clinical Pathology 1966; 45(4_ts): 493-6.
12. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of *Pseudomonas* and *Acinetobactet* species. Clinical microbiology and infection 2001; 7(2): 88-91.

13. Oh EJ, Lee S, Park YJ, Park JJ, Park K, Kim SI, Kang MW, Kim BK. Prevalence of metallo-β-lactamase among *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in a Korean university hospital and comparison of screening methods for detecting metallo-β-lactamase. Journal of Microbiological methods 2003; 54(3): 411-8.
14. Altun Ş, Tufan ZK, Altun B, Önde U, Kinikli S, Demiröz AP. Growing OXA-23 type strains among carbapenem-resistant *Acinetobacter baumannii* and tigecycline as an alternate combination therapy. Turkish Journal of Medical Sciences 2016; 46(6): 1894-9.
15. Mac Faddin JF. Biochemical tests for identification of medical bacteria 3rd edition lippincott Williams and Williams. USA; 2000.
16. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*: emergence of a successful pathogen. Clinical microbiology reviews 2008; 21(3): 538-82.
17. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG. 172 Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, 173 Selling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL 2012. Multidrug-174 resistant, extensively drug resistant and pan drug-resistant bacteria: an international expert; 175.
18. Basustaoglu AC, Kisa O, Sacilik SC, Ozyurt M, Yildiran ST. Epidemiological characterization of hospital-acquired *Acinetobacter baumannii* isolates from a 1500-bed teaching hospital by phenotypic and genotypic methods. Journal of Hospital Infection 2001; 47(3): 246-7.
19. Fallah F, Taherpour A, HakemiVala M, Hashemi A. Global spread of New Delhi metallo-beta-lactamase-1 (NDM-1). Iran J Clin Infect Dis 2011; 6(4): 171-77.
20. Peymani A, Nahaei MR, Farajnia S, Hasani A, Mirsalehian A, Sohrabi N, Abbasi L. High prevalence of metallo-b-lactamase-producing *Acinetobacter baumannii* in a teaching hospital in Tabriz, Iran. Jpn j infect dis. 2011; 64(1): 69-71.
21. Safari M, Nejad AS, Bahador A, Jafari R, Alikhani MY. Prevalence of ESBL and MBL encoding genes in *Acinetobacter baumannii* strains isolated from patients of intensive care units (ICU). Saudi Journal of Biological Sciences 2015; 22(4): 424-9.
22. Fouad M, Attia AS, Tawakkol WM, Hashem AM. Emergence of carbapenem-resistant *Acinetobacter baumannii* harboring the OXA-23 carbapenemase in intensive care units of Egyptian hospitals. International Journal of Infectious diseases 2013; 17(12): e1252-4.
23. Al-Hassan L, El Mehallawy H, Amyes SG. Diversity in *Acinetobacter baumannii* isolates from paediatric cancer patients in Egypt. Clinical Microbiology and Infection 2013; 19(11): 1082-8.

Cite this article as:

Fatma Ibrahim Sonbol *et al*. Prevalence of Carbapenemases among Imipenem resistant *Acinetobacter baumannii* isolates in Tanta, Egypt. Int. Res. J. Pharm. 2019;10(12):77-80 http://dx.doi.org/10.7897/2230-8407.1012332

Source of support: Nil, Conflict of interest: None Declared