ABSTRACT

Background and Objectives: Acinetobacter baumannii is an opportunistic pathogen that affects different groups of people, especially intensive care unit (ICU) patients. The prevalence of infections caused by this bacterium is very high. Today, prevalence of infections caused by multidrug-resistant (MDR) and extreme-drug resistant (XDR) strains is increasing. This study aimed to determine the antibiotic susceptibility pattern of A. baumannii isolates from ICU patients.

Methods: This cross-sectional study was conducted from October 2014 to March 2015 on patients admitted to ICU of Imam Khomeini hospital in Tehran, Iran. Clinical samples of various sources were collected from patients. Isolates were detected and identified via microbiological and biochemical tests as well as PCR amplification of the blaOxa51 gene. Then, susceptibility testing was performed using the Kirby-Bauer disk diffusion test. Statistical analysis was performed with SPSS (version 22, Chicago, IL, USA) using Chi-square and Fisher’s exact tests.

Results: Of the total of 62 clinical samples, 24 (39%) were respiratory samples and only three (6%) were cerebrospinal fluid samples. Most MDR and XDR strains were isolated from respiratory samples. The highest resistance rate was against ceftriaxone, ticarcillin and erythromycin (100%), while the lowest resistance rate was against minocycline (20%).

Conclusion: Owing to detection of high multi-drug resistance isolates in the present study, and importance of multi-drug resistance in A. baumannii, the identification of multi-drug resistance genes and their reporting to health care/treatment centers is important. Thus, it is recommended to perform susceptibility testing to help determine the most effective antibiotic(s) for the treatment of infections in ICU patients.

Keywords: Acinetobacter baumannii, MDR, XDR, ICU.
INTRODUCTION

According to the Infectious Diseases Society of America, *Acinetobacter baumannii* is among the six top priority dangerous drug-resistant organisms (1). *A. baumannii* is an opportunistic gram-negative pathogen that affects different groups of people, especially intensive care unit (ICU) patients (2). The bacterium can cause various infections including urinary tract infection, wound infection, meningitis, endocarditis, peritonitis and skin and soft tissue infections (3). Prolonged length of hospital stay, immunodeficiency, surgery, burns, aging, antimicrobial agents and invasive devices are among the predisposing risk factors for development of infections caused by this bacterium (2). The infections are typically treated with beta-lactams and fluoroquinolones. In recent years, the increased use of antibiotics has led to the emergence of resistant strains (4). Antibiotic resistance in *A. baumannii* may be acquired through intrinsic mechanisms, such as enzymes, mutations in target genes, permeability of the outer membrane and increased expression of efflux pumps (4, 5). This has caused difficulties in treatment of infections caused by *A. baumannii*, which may lead to increased length of stay, increased health care costs, an unfavorable prognosis and increased risk of mortality (3, 6).

Carbapenems, a class of beta-lactam antibiotics with broad antimicrobial activity, are used as antibiotic of choice for treatment of infections caused by resistant strains (6, 7). Considering the rising prevalence of multidrug-resistant (MDR) *A. baumannii* strains that are also resistant to carbapenems, colistin and tigecycline have been recommended (6). Studies on different *Acinetobacter* species have shown that antibiotic resistance is more common among *A. baumannii* compared to other *Acinetobacter* species (8, 9). Identification of antibiotic resistance patterns in different regions is necessary for selecting the treatment of choice and determining appropriate policies to prevent spread of antibiotic resistance. Therefore, this study aimed to determine antibiotic susceptibility pattern of *A. baumannii* strains isolated from ICU patients.

MATERIAL AND METHODS

This cross-sectional study was conducted over a period of 10 months (January 2015 to October 2016) on patients in ICU of Imam Khomeini hospital in Tehran, Iran. Clinical samples (blood, urine, cerebrospinal fluid, wound and respiratory) were sent to a medical laboratory, and isolates were identified by morphological and biochemical tests, including oxidase, citrate, urease, TSI and growth at 42 °C. PCR amplification of *blaOXA* gene was performed to confirm the presence of *A. baumannii* in the clinical samples (10). For this purpose, specific primers were designed using the Genrunner software and then synthesized by Takapouzist Co., Iran. Sequence of the primers used in the polymerase chain reaction (PCR) process is shown in table 1. Cycling conditions were as follows: initial denaturation at 94 °C for 3 min (1 cycle), 35 cycles of denaturation at 94 °C for 45 s, annealing at 60 °C for 1 min, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method (11) according to the Clinical and Laboratory Standards Institute guidelines (No: M2-A9). First, bacterial suspensions with turbidity equivalent to 0.5 McFarland standard (approximately 1-2 × 10⁸ CFU/mL) were prepared and spread onto Mueller Hinton agar. Antibiotic disks were placed on the medium and the plate was incubated overnight. After assessing bacterial growth around each disk, growth inhibition zone was measured and compared with the CLSI table (12). The antibiotic disks (purchased from MAST Company) used in this study included: polymyxin B, cefepime, erythromycin, piperacillin-tazobactam, ampicillin-sulbactam, ticarcillin-clavulanic acid, minocycline, doxycycline, tigecycline, rifampicin, netilmicin, kanamycin, colistin, ceftazidime-clavulanic acid, doripenem, imipenem, tobramycin, ceftriaxone, ceftazidime, amikacin, tetracycline, ciprofloxacin, gentamicin and imipenem. Results of the antibiotic susceptibility testing were reported as susceptible, resistant and intermediate. *Escherichia coli* ATCC 25922 and *A. baumannii* ATCC 19606 were used as the...
negative control and the positive control, respectively (13). The isolates were categorized into three groups of MDR, non-MDR and extensively drug-resistant (XDR) strains. A. baumannii strains resistant to three current classes of antibiotics were identified as MDR, while A. baumannii strains resistant to three common classes of antibiotics in addition to imipenem were identified as XDR (7).

Statistical analysis was performed with SPSS (version 22, Chicago, IL, USA) using Chi-square and Fisher’s exact tests. Of 10 imipenem-resistant isolates, only two isolates were resistant to minocycline. Based on the results of the susceptibility testing, 55%, 16% and 29% of A. baumannii strains isolated from ICU patients were MDR, XDR and non-MDR, respectively.

The result of PCR amplification of the \( \text{bla}_{\text{OXA-51}} \) gene is shown in figure 1.

**DISCUSSION**

A. baumannii is one of the main causes of hospital-acquired pneumonia, particularly in ICU patients (14). In our study, most MDR strains of A. baumannii were isolated from respiratory samples, and the prevalence of MDR strains was lowest in CSF samples. This finding is in line with findings of Zarifi et al. (15). Emergence and spread of MDR Acinetobacter strains is a growing global health problem (2, 4). In the present study, A. baumannii was highly resistant to most antibiotics, and 55% and 16% of the isolates were MDR and XDR, respectively. These findings are in agreement with findings of previous studies in Iran (16, 17).

The highest resistance rate was to ceftriaxone, ticarcillin and erythromycin (100%), while the lowest resistance rate was observed against minocycline (20%) and tigecycline (35%). A previous study also showed resistance of over 95% to imipenem, meropenem, ceftazidime, cefotaxime, cefuroxime, ceftriaxone, cefepime, ertapenem and ampicillin/sulbactam but not to colistin (15).
In the present study, of 10 imipenem-resistant isolates, only two isolates were resistant to colistin and sensitive to minocycline. In present study, the resistance rate to tigecycline was low. In line with this finding, some studies on Acinetobacter species reported sensitivity of 86.7-93.3% and resistance of 65.47% to tigecycline (18, 19). A similar study in Turkey also reported tigecycline as the most effective antibiotic against MDR Acinetobacter strains (21). Since most tigecycline-resistant strains are also resistant to other antibiotics including carbapenems (22), as shown in our findings, infections caused by tigecycline/imipenem-resistant strains can be treated with tobramycin. However, further studies are required to confirm these findings. We also observed that most MDR and XDR strains were related to respiratory samples, elucidating the importance of combination antibiotic therapy for treatment of respiratory infections caused by A. baumannii.

CONCLUSION
Considering the high prevalence of MDR A. baumannii isolates in our study population, it is recommend to perform susceptibility testing to help determine the most effective antibiotic(s) for the treatment of infections in ICU patients.

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CONFLICT OF INTEREST
All authors declare that there is no conflict of interest

REFERENCES
1. Talbot GH, Bradley J, Edwards JE, Gilbert D, Scheld M, Bartlett JG. Bad bugs need drugs: an update on the development pipeline from the Antimicrobial Availability Task Force of the Infectious Diseases Society of America. Clinical Infectious Diseases. 2006; 42(5): 657-68.
2. Fournier PE, Richet H, Weinstein RA. The epidemiology and control of Acinetobacter baumannii in health care facilities. Clinical infectious diseases. 2006; 42(5): 692-9.
3. Jeon B-C, Jeong SH, Bae IK, Kwon SB, Lee K, Young D, et al. Investigation of a nosocomial outbreak of imipenem-resistant Acinetobacter baumannii producing the OXA-23 β-lactamase in Korea. Journal of clinical microbiology. 2005; 43(5): 2241-5. DOI:10.1128/JCM.43.5.2241-2245.2005.
4. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clinical Microbiology and Infection. 2006; 12(9): 826-36. DOI:10.1111/j.1469-0691.2006.01456.x.
5. Quale J, Bratu S, Landman D, Heddamshetti R. Molecular epidemiology and mechanisms of carbapenem resistance in Acinetobacter baumannii endemic in New York City. Clinical infectious diseases. 2003; 37(2): 214-20.
6. Ernst EJ, Diekema DJ, BootsMiller BJ, Vaughn T, Yankey JW, Flach SD, et al. Are United States hospitals following national guidelines for the analysis and presentation of cumulative antimicrobial susceptibility data? Diagnostic microbiology and infectious disease. 2004; 49(2): 141-5. DOI:10.1016/j.diagmicrobio.2004.03.007.
7. Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection. 2012; 18(3): 268-81. doi: 10.1111/j.1469-0691.2011.03570.x.

8. Valenzuela JK, Thomas L, Partridge SR, van der Reijden T, Dijkshoorn L, Iredell J. Horizontal gene transfer in a polyclonal outbreak of carbapenem-resistant Acinetobacter baumannii. Journal of clinical microbiology. 2007;45(2):453-60. DOI:10.1128/JCM.01971-06.
9. Turton J, Gabriel S, Valderrey C, Kaufmann M, Pitt T. Use of sequence-based typing and multiplex PCR to identify clonal lineages of outbreak strains of Acinetobacter baumannii. Clinical microbiology and infection. 2007; 13(8): 807-15. DOI:10.1111/j.1469-0691.2007.01759.x.
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. doi: 10.1128/JCM.01021-06.
11. Saghi H, Esmaeili D, Bahador A, Khaleedi A, Dastjerdi FA. Study of Expression of the Gene Alpha-6 in Multidrug-Resistant Acinetobacter Baumannii against Thyme Essence with Real Time Pcr. Iranian Journal of Public Health. 2014; 43(2): 36.
12. Wanger A. Disk diffusion test and gradient methodologies. Antimicrobial Susceptibility Testing Protocols. 2007:53-73. DOI: 10.1201/9781420014495.ch3.
13. Alipour M, Halvani M, Omri A, Sundres ZE. Antimicrobial effectiveness of liposomal polymyxin B against resistant Gram-negative bacterial strains. International Journal of Pharmaceutics. 2008; 355(1): 293-8. doi:10.1016/j.ijpharm.2007.11.035.
14. Khaleedi A, Elahifar O, Vazini H, Alikhani MY, Bahrami A, Esmaeili D, et al. Increasing Trend of Imipenem-Resistance Among Acinetobacter baumannii Isolated From Hospital Acquired Pneumonia in Northeast of Iran. Avicenna Journal of Clinical Microbiology and Infection. 2017;4(3): e45454. DOI: 10.5812/ajcmi.45454.
15. Zarifi E, Eslami G, Khaledi A, Vakili M, Vazini H, Zandi H. Prevalence of ESBLs in Acinetobacter baumannii isolated from intensive care unit (ICU) of Ghaem hospital, Mashhad, Iran. Journal of Pure and Applied Microbiology. 2017;11(2):811-9. DOI: 10.22207/JPAM.11.2.20.

16. Jain R, Danziger LH. Multidrug-resistant Acinetobacter infections: an emerging challenge to clinicians. Annals of Pharmacotherapy. 2004; 38(9): 1449-59. DOI:10.1345/aph.1D592.

17. Iglesias dSH, Mirón CJ, Fresnadirollo MM, Sáenz GM. Epidemiological study and effect on antimicrobial use in the genus Acinetobacter in a university hospital. Rev Esp Quimioter. 2004; 17(2): 177-83.

18. Morovat T, Bahram F, Mohammad E, Setareh S, Mohamad Mehd F. Distribution of different carbapenem resistant clones of Acinetobacter baumannii in Tehran hospitals. The new microbiologica. 2009; 32(3): 265.

19. Henwood CJ, Gatward T, Warner M, James D, Stockdale MW, Spence RP, et al. Antibiotic resistance among clinical isolates of Acinetobacter in the UK, and in vitro evaluation of tigecycline (GAR-936). Journal of Antimicrobial Chemotherapy. 2002; 49(3): 479-87.

20. Karmostaj A, Peerayeh SN, Salmanian AH. Emergence of Tigecycline resistant Acinetobacter baumannii from an intensive care unit (ICU) in Tehran. Jundishapur Journal of Microbiology. 2013; 6(3): 215-19.

21. Liu J-W, Wang L-S, Cheng Y-J, Hsu G-J, Lu P-L, Liu Y-C, et al. In-vitro activity of tigecycline against clinical isolates of Acinetobacter baumannii in Taiwan. Int J Antimicrob Agents. 2008; 32 Suppl 3:S192-6. doi: 10.1016/S0924-8579(08)70027-X.

22. Bouchillon SK, Hoban DJ, Johnson BM, Stevens TM, Dowzicky MJ, Wu DH, et al. In vitro evaluation of tigecycline and comparative agents in 3049 clinical isolates: 2001 to 2002. Diagnostic microbiology and infectious disease. 2005; 51(4): 291-5.