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Integron types, gene cassettes and antimicrobial resistance profile of *Acinetobacter baumannii* isolated from BAL samples in Babol, north of Iran

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ABSTRACT

Multi-drug resistant isolates of *Acinetobacter baumannii* have created therapeutic problems worldwide. This current study was intended to determine the Integron types, gene cassettes and antimicrobial resistance profile of *A. baumannii* isolated from BAL samples in Babol, north of Iran. During a 15-month period, 35 *A. baumannii* isolates were studied. Different classes of antimicrobial agents were used to determine the resistance ratios. Multiplex-PCR was used to detect different types of integrons and associated gene cassettes. The resistance rates to GM, FEP, AK, TOB, CP, PIP, SAM, IPM, SXT, CTX, CAZ, CL, TIM, MEM, and TZP were 85.7%, 100%, 91.4%, 68.5%, 94.3%, 88.5%, 97.1%, 94.3%, 100%, 100%, 100%, 0.0%, 91.4%, 94.3% and 91.4%, respectively. The distribution analysis of *int* genes showed that 25.7%, 88.6% and 28.6% of isolates carried the *intI*, *intII* and *intIII* genes, respectively. The prevalence of *aadB*, *dfrA1*, *bla-OXA$_{30}$* and *aadA1* genes were 94.3%, 77.1%, 40% and 5.7%, respectively. The current study showed that a high level of *A. baumannii* isolates harbor integrons in our therapeutic center, which may lead to distribution of multiple antimicrobial resistance. The different types of gene cassette arrays in the present study highlight the important role of geographical features in MDR isolates dissemination which could be credited to different profiles of drug consumption in different areas. The findings emphasized that the need for continuous surveillance to prevent distribution of multidrug resistance among *A. baumannii* strains in Iran.

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is an important opportunistic pathogen responsible for a variety of healthcare-associated infections (HAIs) including, ventilator-associated pneumonia (VAP), bacteremia, secondary meningitis, surgical-site infections, wound infection and urinary tract infections (UTI), especially in intensive care units (ICUs) [1]. Control of multidrug-resistant *A. baumannii* (MDR-AB) infections is a great concern of physicians and clinical bacteriologist [2]. Mobile genetic elements (MGEs) such as plasmids, transposons (TEs) and integrons are most effective MGEs, which play an important role in acquisition, evolution and distribution of resistance elements in *A. baumannii* strains. Integrons, gene-capture systems, are conserved sequences of bacterial genome and capable of obtaining the gene cassettes, which can carry antimicrobial resistance determinants [3]. The most common classes of integrons are the transferable class I followed by class II and III, respectively. These genetic elements constitute of three parts based on conserved sequence (CS): the 5′-CS, the variable segment and the 3′-CS [4,5]. The integron is specified with the presence of an *intI* (encoding integrase), *attI* (a site specific recombination site) and P_C (a promoter) genes [6]. Many resistance genes including, *dfr* genes (dihydroflavonol-4-reductase/trimethoprim), AMEs (disinfectants and aminoglycoside-modifying enzymes), *sul1* gene (sulfonamide) qacEΔ1 (tetravalent ammonium

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compounds) and broad-spectrum β-lactamase present in gene cassettes of class 1 integrons [6,7]. Class II of Integrons and their gene cassette arrays consisting of dfrA1, aadA1 (gene encodes Adenylate transferase), *sat1* and *aadB* (encodes streptothricin acetyltransferase) confer resistance to trimethoprim, streptomycin/spectinomycin, streptothricin, kanamycin and tobramycin, respectively [7]. So, class II integron-mediated resistance cassettes carry a $bla_{PSE\text{-}1}$ β-lactamase gene encode resistance to ampicillin in the bla_{TEM}-negative isolates [8].

This rapid rise of MDR is not only due to the inherent resistance genes carried by MDR-AB but also to its prominent capacity to acquire resistant factors from other bacteria. So, the ability of the MRAB in acquiring resistance mechanisms, resistance to available drugs and lack of new and effective antimicrobial agents is the most important risk factors for MDR-AB infections [9]. Therefore, the aim of the current study was to determine integron types, gene cassettes and antimicrobial resistance profile in *A. baumannii* isolated from bronchoalveolar lavage (BAL) samples from patients admitted in an ICU of a teaching hospital in Babol, north of Iran.

2. Materials and methods

2.1. Clinical samples and laboratory identification

In this cross-sectional study, 35 non-duplicative clinical isolates of *A. baumannii,* were collected over a 15 months period from May 2015 to July 2016 from BAL samples of patients admitted to the ICU at Ayatollah Rohani hospital, Babol, north of Iran. These isolates were confirmed as *A. baumannii* by conventional biochemical, microbiological and 20NE API tests (BioMerieux, Inc). The PCR of the intrinsic *gyrB* gene was performed to confirm *A. baumannii* species. All strains were stored in Luria–Bertani broth (Merck, Germany) containing 20% glycerol at −80 °C for further use.

2.2. Antibiotic susceptibility test

In accordance with Clinical and Laboratory Standards Institute (CLSI document M100-S14) guideline [10], antimicrobial susceptibility was performed on the Mueller-Hinton agar plates (Merck, Germany) using the standard disk agar diffusion method for antimicrobials below: gentamicin (GM, 10 μg), cefepime (FEP, 30 μg), amikacin (AK, 30 μg), Tobramycine (TOB, 10 μg), ciprofloxacin (CP, 5 μg), pipracilin (PIP, 100 μg), Ampicillin/sulbactam (SAM, 10/10 μg), Imipenem (IPM 10 μg), Co-trimoxazole (SXT, 5 μg), cefotaxime (CTX, 30 μg), ceftazidime (CAZ, 30 μg), Colistin (CL, 10 μg), ticarcillin-clavulanic acid (TIM, 75/10 μg), meropenem (MEM, 10 μg) and piperacillin-tazobactam (TZP, 100/10 μg) (MAST Diagnostics, Merseyside, UK). *A. baumannii* ATCC 19606 was used as a positive quality control.

2.3. Polymerase chain reaction method

In line with genomic DNA purification kit (Fermentas, Co., Germany) protocol, genomic DNA was extracted from *A. baumannii* colonies. The primer sequences used in this study are listed in Table 1. The total volume of PCR reaction mixture was 25 μl, contained of 1.0 μl of extracted template DNA, 2.0 μl of 10 × PCR buffer, 0.6 μl $MgCl_2$ (50 mM), 0.6 μl dNTPs (10 mM), 0.5 μl of each primer, 0.7 μl of Taq DNA polymerase (5 U/μl) (Amplicon Co., Denmark) and 19.6 μl ddH_2O. Amplification was performed in a Techne TC-512 thermocycler (Eppendorf, Hamburg, Germany) as follows: initial denaturation at 95 °C for 60s, 30 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 60 °C and extension for 60 s at 72 °C, and a final extension for 6 min at 72 °C. PCR products were subjected to electrophoresis in a 1.0% agarose gel, stained with 0.5 mgL^{-1} ethidium bromide (EtBr) and photographed with ultraviolet illumination (Bio-rad, Hercules, USA). The PCR products were purified from the agarose gel using a QIAquick Gel Extraction kit (Qiagen, Hilden, Germany). DNA strands of the PCR product were sequenced using an ABI automatic DNA sequencer (model 3730xl; Perkin-Elmer). Nucleotide sequences data were analyzed at the National Center for Biotechnology Information (NCBI), available at the (http://www.ncbi.nlm.nih.gov/BLAST/) website.

3. Results

3.1. Bacterial isolation

During 15- month period (May 2015 till July 2016), 35 non-duplicative clinical isolates of *A. baumannii* were collected from BAL samples of patients admitted to the medical ICU of Ayatollah Rohani Hospital, (Babol, north of Iran). The mean age was 68.22 ± 10.15 years of 19 (54.28%) male and 16 (45.71%) female.

3.2. Antibiotic resistance profile

All strains were screened for resistance to 15 antimicrobials. The resistance rates to GM, FEP, AK, TOB, CP, PIP, SAM, IPM, SXT, CTX, CAZ, CL, TIM, MEM, and TZP were 85.7%, 100%, 91.4%, 68.5%, 94.3%, 88.5%, 97.1%, 94.3%, 100%, 100%, 100%, 0.0%, 91.4%, 94.3% and 91.4%, respectively (Table 2). 91.4% of isolates were MDR phenotype. All isolates were susceptible to CL. So, CL is the best effective therapeutic option. The highest resistance rate was related to FEP, SXT, CTX and CAZ.

3.3. Integron types and gene cassette

Of 35 *A. baumannii* isolates, 94.3% (n; 33) carried *int* gene. The distribution analysis of *int* genes showed that 25.7% (n; 9), 88.6% (n; 31) and 28.6% (n; 10) of isolates carried the *intI*, *intII* and *intIII* genes, respectively. The prevalence of *aadB*, *dfrA1*, $bla\text{-}OXA_{30}$ and *aadA1* gees were 33 (94.3%), 27 (77.1%), 14 (40%) and 2 (5.7%), respectively. Of all *aadB*-positive strains, 66.6% (n; 22) were resistant to TOB. All *aadA1*-positive strains (n; 2; 100%), were resistance to STR. Twenty-five (92.6%) of *dfrA1* and 12 (85.7%) of $bla\text{-}OXA_{30}$ positive strains were resistance to SXT and FEP, respectively.

3.4. Nucleotide sequence accession number

The nucleotide sequences data described in the current study have been deposited in the Pubmed/NCBI/GenBank nucleotide sequence database under accession numbers for *intIII* (KX094891.1 and KX094890.1) and $bla\text{-}OXA_{30}$ (KX602151, KX602152, and KX602153) genes.

4. Discussion

In agreement with our findings, several studies showed that antibiotic resistance in *A. baumannii* is increasing due to misuse, overuse and uncontrolled use of antimicrobials, so that 91.4% of tested *A. baumannii* indicated MDR phenotype. Bayugo et al. [15], Joshi et al. [16], Mirnejad et al. and Zhu et al. [3], showed that 45%, 75%, 82% and 97% of strains were MRAB, respectively. Except CL, resistance ratios to each tested antimicrobial agents were all over than 80% in our study. The susceptibility of clinical isolates to TOB and CL were 31.4% and 100%, respectively. These data are similar to Huang et al. [17]. These findings highlighted that the severe challenge of MRAB nosocomial infection.

Of 35 *A. baumannii* isolates, 33 strains carried the *int* gene. Molecular analysis of class I, II and III integrons showed that, 25.7%