Prevalence of β-lactamase-encoding genes and molecular typing of Acinetobacter baumannii isolates carrying carbapenemase OXA-24 in children

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Abstract

Background: β-Lactam antibiotics have been broadly used for the treatment of Acinetobacter baumannii infections, resulting in development of β-lactam inactivating β-lactamases. Here, we described antibiotic resistance rate, prevalence of β-lactamase-encoding genes, and clonal relationships of A. baumannii strains isolated from children referred to Children's Medical Center in Tehran, Iran, during 2019–2020.

Methods: A total of 60 non-replicate A. baumannii isolates were recovered from clinical specimens of pediatric patients. Antibiotic susceptibility testing was done by the disc diffusion method. Colistin susceptibility of isolates was performed by the broth microdilution method. β-lactamase-encoding genes were characterized by PCR. The presence of ISAb1 element upstream of the several oxacillinase genes was also checked. Genetic relatedness of isolates was determined by using random amplification of polymorphic DNA (RAPD) typing.

Results: The antimicrobial susceptibility tests showed that 83.3% of A. baumannii isolates were MDR, and 40% XDR. Both MDR and XDR A. baumannii isolates were susceptible to colistin. The frequency of blaOXA-51-like, blaOXA-23-like, blaTEM, blaOXA-24-like, blaPER, blaSHV, blaCTX-M, blaOXA-58-like, and blaIMP was 100, 93.33, 60, 36.67, 28.33, 8.33, 5, 3.33, and 1.67%, respectively. Coexistence of ISAb1-blaOXA-23-like and ISAb1-blaOXA-51-like was observed in 65% and 85% of isolates, respectively. RAPD analysis revealed 4 common types and 2 single types of A. baumannii isolates.

Conclusions: The multiple clones harboring blaOXA-23-like, ISAb1-blaOXA-51-like, and ISAb1-blaOXA-23-like were responsible for the spread of A. baumannii isolates in our clinical wards. Dissemination of the well-established clones is worrisome and would become therapeutic challenges due to the possible transferring genetic elements associated with resistance.

Keywords: Antimicrobial susceptibility, Pediatrics, Acinetobacter baumannii, RAPD-PCR, β-Lactamases, blaOXA-24-like
This study was conducted on 60 non-duplicate Clinical specimens, bacterial isolates, and identification
Materials and methods in Tehran, Iran, during 2019–2020. A. baumannii strains isolated from children referred to Children’s Medical Center during investigations.

Antimicrobial susceptibility test
Antimicrobial susceptibility testing was carried out by the Kirby–Bauer disc diffusion method according to Clinical and Laboratory Standards Institute (CLSI) [12] and breakpoint interpretations for including amikacin (30 µg), gentamicin (10 µg), tobramycin (10 µg), trimethoprim–sulfamethoxazole (25 µg), tigecycline (15 µg), doxycycline (30 µg), tetracycline (30 µg), minocycline (30 µg) ceftriaxone (30 µg), cefazidime (30 µg), cefepime (30 µg), cefotaxime (30 µg), pipercillin/tazobactam (100/10 µg), ampicillin-sulbactam (10/10 µg), imipenem (10 µg), meropenem (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), (Mast Group Ltd., Bootle, UK). The minimum inhibitory concentrations (MICs) of colistin (Colistin sulfate salt powder, Sigma-Aldrich, St. Louis, MO, USA) were determined using the broth microdilution method according to CLSI guidelines (CLSI, 2020). Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27,853 strains were used as the standard strains.

Detection of β-lactamase-encoding genes
Genomic DNA was extracted by BioFact™ Genomic DNA Prep Kit GD141-100 (BioFact, Daejeon, Korea) according to the manufacturer’s recommendations. The primer sequences, product sizes, and annealing temperatures for blaTEM, blaSHV, blaCTX-M, PER, VEB, and GES primers for blaOXA-51-like and blaOXA-23-like in A. baumannii [9, 10]. The presence of ISAbal insertion upstream of blaOXA-51-like and blaOXA-23-like genes was explored by using the forward primer for ISAbal and the reverse primers for blaOXA-51-like and blaOXA-23-like. PCR was carried out using the ready-to-use 2X Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) in a 25 µL total volume reaction containing ~30 ng of DNA template and 10 pmol/mL of forward and reverse primers. PCR was performed under conditions of initial denaturation at 94 °C for 5 min, 35 cycles of denaturation at 94 °C for 45 s, annealing ranging from 47 to 58 °C for 45 s, extension at 72 °C for 45 s, and final extension at 72 °C for 5 min. PCR products were separated on agarose gel 1.5% by electrophoresis and visualized under ultraviolet light.

Materials and methods
Clinical specimens, bacterial isolates, and identification
This study was conducted on 60 non-duplicate A. baumannii isolates obtained from pediatric patients, aged from 4 days–14 years old, and hospitalized in different wards of Children’s Medical Center in Tehran, Iran, during 2019–2020. The isolates recovered from various clinical specimens were identified by the routine microbiological tests as A. baumannii. Then isolates were confirmed by PCR amplification of partial RNA polymerase β-subunit (rpoB) gene using a set of forward and reverse primers of 5’-TAYGYYAAAGAYTTGAAAGG-3’ and 5’-CMACACCTTGTGCMCCR TGA-3’, as described previously [11]. All identified A. baumannii strains were stored at −70 °C until further investigations.

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Random amplification of polymorphic DNA (RAPD)-PCR fingerprinting
The genetics relatedness of \( \text{bla}_{\text{OXA-24-like}} \)-positive strains was assessed using RAPD-PCR analysis with a random oligonucleotide 5'–CCGCAGCCAA-3' (TAG Copenhagen, Denmark). Amplification of random sequences was done by a conventional PCR as follows: initial denaturation at 94 °C for 10 min, followed by 30 cycles consisting of denaturation at 94 °C for 1 min, annealing at 43 °C for 1 min, extension at 72 °C for 3 min, and final extension at 72 °C for 10 min. The RAPD patterns were compared by GelCompar® II v.4.1 software (Applied Maths BVBA, Sint–Martens–Latem, Belgium) and clustered with unweighted pair group method (UPGMA).

Statistical analysis
Data analysis was performed using the Minitab® statistical software package 17 (MINITAB, Inc., State College, Pennsylvania, USA). The \( P \) value ≤ 0.05 was considered statistically significant for the analysis.

Results
Clinical isolates and demographic characteristics of pediatric patients
A total of 60 non-replicate \( A. \) baumannii clinical isolates were collected from the Children's Medical Center in a period of April 2019 to December 2020. 43 out of 60 (71.6%) and 17 out of 60 (28.3%) isolates of \( A. \) baumannii were belonged to male and female pediatric patients, respectively with an age range of 4 days to 14 years. The majority of isolates were obtained from patients admitted to the neonatal and pediatric intensive care units (NICU, PICU) (65%), followed by the other wards (35%). Distribution of collected isolates from the specimen sources was follows: CSF (6.66%), BAL (23.33%), tracheal tube

| Target gene | Primer sequence (5′-3′) | Product size (bp) | Annealing Temperature (°C) | References |
|-------------|--------------------------|------------------|-----------------------------|------------|
| \( \text{bla}_{\text{TEM}} \) | F: GAG TAT TCA ACA TT TTT CCG TG R: TAA TCA GTG AGG CAC CTA TC | 848 | 47 | [34] |
| \( \text{bla}_{\text{SHV}} \) | F: TTA TCT CCC TGT TAG CCA CC R: GAT TTG CTG ATT TCG CTC GG | 797 | 52 | [35] |
| \( \text{bla}_{\text{CTX-M}} \) | F: ACG CTG TTG TTA GTA AGT G R: TTG AGG CTT GGT GAA GT | 759 | 52 | [34] |
| \( \text{bla}_{\text{VET}} \) | F: CGA CTT CCA TTT CCC GAT GC R: GGA CTC TGC AAC AAA TAC GC | 643 | 54 | [36] |
| \( \text{bla}_{\text{GES}} \) | F: TGGCAA TGT GCT CAA CGT TC R: TAT CAC ACA CAA TAT TGT CG | 340 | 58 | [37] |
| \( \text{bla}_{\text{AMP}} \) | F: TGG ACA CTC CAT TTA CTG GTA R: TCA TTT GTT AAT TCA GAT GCA TA | 630 | 48 | [38] |
| \( \text{bla}_{\text{VBI}} \) | F: GAG TGG CTT TTT AGT ATT TCA GGA TA R: TGG ATT GCA CTT CTA CAT AG | 247 | 51 | [39] |
| \( \text{bla}_{\text{NDM}} \) | F: AAC ACA GCC TGA CTT TCG R: TGA TAT TAC CTA TGA CAC | 111 | 53 | [39] |
| IS\( \text{Aba1} \) | F: CAC GAA TGC AGA AGT TG R: CTA GCA ATC CTA TGA CAC | 549 | 48 | [25] |
| \( \text{bla}_{\text{OXA-51-like}} \) | F: TAA TGC TTT GAT CGG CCT TG R: TGG ATT GCA CTT CTA TGG CTC | 353 | 53 | [40] |
| \( \text{bla}_{\text{OXA-24-like}} \) | F: GGT TAG TTG GCC CCC TTA AA R: AGT TGA GCA AAA AGG GGA T | 246 | 54 | [40] |
| \( \text{bla}_{\text{OXA-23-like}} \) | F: GAT CGG ATT AGA GAA GCA GA R: ATT TCT GAC CGC ATT TCC AT | 501 | 52 | [40] |
| \( \text{bla}_{\text{OXA-58-like}} \) | F: AAG TAT TGG GGC TTG TGC TG R: CCC CTC TGC GCT CTA CAT AC | 599 | 56 | [40] |
| IS\( \text{Aba1}/\text{bla}_{\text{OXA-23-like}} \) | F: CAC GAA TGC AGA AGT TG R: ATT TCT GAC CGC ATT TCC AT | 1404 | 50 | [40] |
| IS\( \text{Aba1}/\text{bla}_{\text{OXA-51-like}} \) | F: CAC GAA TGC AGA AGT TG R: TGG ATT GCA CTT CTA TGG CTC | 1223 | 52 | [40] |
(18.33%), blood (30%), urine (3.33%), central venous line (CVL) (3.33%), respiratory secretions (10%), drain discharge (3.33%), and dialysis fluid (1.7%). All isolates were molecularly confirmed as \textit{A. baumannii} using both inherent \textit{rpoB} and \textit{bla}\textsubscript{OXA-51-like} genes. Demographics and clinical features of pediatric patients are accessible in detail in a descriptive Additional file 1: Table S1.

**Antimicrobial susceptibility profile**

Antimicrobial susceptibility testing was displayed that the highest resistance rate was for imipenem and meropenem (53, 88.3%), followed by cefotaxime, cefepime, ceftazidime, amikacin, cotrimoxazole, ciprofloxacin (51, 85%), piperacillin-tazobactam (50, 83.3%), gentamicin (49, 81.67%), tetracycline (44, 73.3%), minocycline (34, 56.6%), doxycycline (32, 53.3%), ampicillin-sulbactam (29, 48.3%), and tigecycline (3, 5%). Fifty-two out of 60 isolates were resistant to both imipenem and meropenem, whereas 6 out of 60 isolates were sensitive to them. However, only one isolate was found to be sensitive to imipenem, and one was susceptible to meropenem.

All isolates were susceptible to colistin with MICs ranging from 0.125 to 2 µg/mL. The MIC\textsubscript{50} and MIC\textsubscript{90} of colistin were 0.5 and 2 µg/mL, respectively. Fifty-one out of 60 (83.3%) \textit{A. baumannii} isolates were categorized as the MDR, and 24 out of 60 (40%) were extensively drug-resistant (XDR).

Antimicrobial susceptibility testing for the isolates is summarized in Fig. 1.

**Characterization of \(\beta\)-lactamase resistance genes profile**

Molecular analysis of \(\beta\)-lactamase resistance genes in \textit{A. baumannii} isolates showed that 93.33% of the clinical isolates (\(n = 56\)) were positive for the \textit{bla}\textsubscript{OXA-23-like} gene, followed by \textit{bla}\textsubscript{TEM} 60% (\(n = 36\)), \textit{bla}\textsubscript{OXA-24-like} 36.67% (\(n = 22\)), \textit{bla}\textsubscript{PER} 28.33% (\(n = 17\)), \textit{bla}\textsubscript{SHV} 8.33% (\(n = 5\)), \textit{bla}\textsubscript{CTX-M} 5% (\(n = 3\)), \textit{bla}\textsubscript{OXA-58-like} 3.33% (\(n = 2\)), and \textit{bla}\textsubscript{IMP} 1.67% (\(n = 1\)). In contrast, we could not detect any \textit{bla}\textsubscript{VIM}, \textit{bla}\textsubscript{GES}, \textit{bla}\textsubscript{VEB}, and \textit{bla}\textsubscript{NDM} genes. Although, the \textit{bla}\textsubscript{OXA-51-like} gene was identified in all \textit{A. baumannii} isolates, we could not conclude the antibiotic resistance associated with its presence alone (\(P\) value > 0.05). The \textit{ISAba1} element was found in 95% of the isolates. However, \textit{ISAba1} was detected in the upstream of \textit{bla}\textsubscript{OXA-23-like} and \textit{bla}\textsubscript{OXA-51-like} genes in 65% and 85% of isolates, respectively. Distribution of \(\beta\)-lactamase-encoding genes and \textit{ISAba1} upstream of \textit{bla}\textsubscript{OXA} genes in pediatric isolates of \textit{A. baumannii} is presented in Fig. 2 and Additional file 1: Table S1.

**RAPD genotyping of \textit{A. baumannii}**

The \textit{A. baumannii} isolates harboring \textit{bla}\textsubscript{OXA-24-like} were designated for further epidemiological analysis by RAPD-PCR. Dendrogram analysis by GelCompar software and UPGMA clustering method by the arithmetic mean calculation and the Dice coefficient (Dice...
similarity index) with an optimization of 0.5% and a tolerance of 1.5% were used for the similarity with a cut-off value of > 85% and considered the same RAPD type. According to the similarity index, we found 4 common-type (A–D) and 2 single-type (E and F). The major RAPD type A has included 12 strains, followed by RAPD type B with 3, RAPD type C with 2, and RAPD type D with 2 strains. Dendrogram of pediatric

**A. baumannii** isolates based on RAPD-PCR analysis is shown in Fig. 3.

**Discussion**

The incidence of MDR **A. baumannii** in pediatric patients is increasing worldwide as far as it has led to therapeutic challenges and has become an ongoing serious public health concern [13]. In this study, we described a high antibiotic resistance rate, rising MDR and XDR strains, and widespread prevalence of β-lactamase-encoding genes in **A. baumannii** isolated from pediatric patients.

Carbapenems, as the last-line group of β-lactam antibiotics, have been used to treat infections associated with MDR **A. baumannii** for a long time; however, carbapenem resistant **A. baumannii** (CRAB) has recently emerged and limited therapeutic options [14, 15]. We found that most isolates were resistant to carbapenem (imipenem and meropenem 88.3% for each, and both imipenem and meropenem 86.6%). A high rate of CRAB in pediatric has been explored in several similar studies [16, 17]. It has been reported that the drug resistance in CRAB is developed by several mechanisms, including production of hydrolyzing enzymes, horizontally transferring resistance determinants, changes in outer membrane permeability, and upregulation of the efflux systems. The major mechanism of carbapenem resistance in **A. baumannii** is related to the production of
carbapenem-hydrolyzing β-lactamases, i.e., carbapenem-hydrolyzing class D β-lactamases (CHDLs), class B metallo-β-lactamases (MBLs), and class A carbapenemases [18]. Distribution of the CHDLs, MBLs, and ESBLs genes has been reported with different prevalence in recent published papers [19–21]. We could not find A. baumannii isolates harboring bla\textsubscript{VIM}, bla\textsubscript{GES}, bla\textsubscript{VEB}, and bla\textsubscript{NDM} genes. In contrast, a high prevalence rate of bla\textsubscript{NDM} gene has been observed in CRAB isolates from Egyptian patients [22]. The bla\textsubscript{OXA-23-like} was more prevalent in our study, which is in agreement with previous studies [17, 23]. Moreover, we also detected the bla\textsubscript{OXA-24-like} and bla\textsubscript{OXA-58-like} genes, but in lower frequencies, corresponding with similar reports [20, 24]. Although the catalytic activity of OXA-type carbapenemases is far less than MBLs, their expression can be influenced by upstream existence of insertion sequences (ISs) elements such as IS\textit{Ab}a1 resulting in increased resistance to carbapenems [25]. Most isolates carried IS\textit{Ab}a1; however, 85% of isolates harbored IS\textit{Ab}a1-bla\textsubscript{OXA-51-like} and 65% were also positive for IS\textit{Ab}a1-bla\textsubscript{OXA-23-like}. We found a statistically significant relationship between the presence of IS\textit{Ab}a1 upstream of bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-51-like} and carbapenem resistance (P value < 0.05). Over 90% of isolates (54 out of 60) were resistant to carbapenems (imipenem and meropenem) with the presence of IS\textit{Ab}a1 upstream of bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-51-like}; though, four isolates despite the existence of IS\textit{Ab}a1 upstream of bla\textsubscript{OXA-23-like} and bla\textsubscript{OXA-51-like} were susceptible to carbapenems; while it might be expected that the co-existence of the determinants should confer resistance to the carbapenems, as described by Turton et al. [25]. Another study has suggested that IS\textit{Ab}a1-bla\textsubscript{OXA-51-like} alone is insufficient to confer resistance to carbapenems [26]. Nevertheless, it has not been completely clarified that the cause of the contradiction in carbapenem susceptibility levels in isolates harboring the IS\textit{Ab}a1-bla\textsubscript{OXA-51-like} gene [27].

Numerous reports have indicated that colistin (polymyxin E) and tigecycline, the only active antibiotics against A. baumannii, have become the last resort of treatment for MDR A. baumannii [28]. Now, resistance to colistin is relatively low; however, colistin-resistant strains have been introduced from different regions of the world [29]. A recent study has reported inhibitory potential effects of methylene blue for improving colistin susceptibility in the treatment of colistin-resistant strains of A. baumannii [30]. We investigated the in vitro activity of colistin, indicating that all A. baumannii isolates were susceptible to colistin according to the MIC\textsubscript{50} and MIC\textsubscript{90} values of <2 µg/ml. Significantly, both MDR and XDR A. baumannii were susceptible to colistin which are in concordance with previous studies [13, 31].

RAPD is a simple and rapid technique and possesses high discriminative power for source tracking of bacteria, and it has been used in epidemiological studies of Acinetobacter species to identify circulating clones in clinical settings [32, 33]. The 21 bla\textsubscript{OXA-24-like}-positive isolates were distributed into 4 RAPD types belonging to four clusters (A, B, C, D) and two additional RAPD types E and F. Twelve strains were categorized in cluster A. All strains belonging to cluster A were MDR. All strains of cluster A were positive for IS\textit{Ab}a1 and bla\textsubscript{OXA-23-like} and the genetic profile of the strains was also varied. However, antibiotic resistance profiles were different between the isolates within the same group. Several strains with the same genetic profile and common origin were observed in clusters B and D. All strains of clusters E and F were susceptible to all antibiotic groups. Significantly, strains were collected from different infection sites were genetically categorized in the same cluster, suggesting the same origin and upsurging threat of multiple infection abilities for these genotypes. We successfully applied RAPD for distinguishing the genetic diversity of A. baumannii clones circulating in clinical setting.

Epidemiological studies on the dissemination of drug-resistant clones and their resistance gene profile are prerequisites to control infection and prevent policies in clinical settings.

There were a number of limitations to the current study; (a) the present literature was limited to single-center experiences, (b) our population size was small, (c) there was not enough data about pediatric patients’ records. Further researches are required to detect the expression level of resistance genes by real-time PCR assay. Other methods such as next-generation sequencing (NGS) can also be considered for well understanding the molecular mechanisms of resistance.

**Conclusions**

The distribution of resistance determinants in A. baumannii isolates is a great concern for our pediatric center and would become therapeutic challenges soon.

The high prevalence of the MDR clones circulating in different wards is a problematic issue for infection control, surveillance programs, and health care strategies, especially in the pediatric population. It is recommended that the development of novel therapeutic strategies or reassessment of old drugs is scheduled to make insight for pediatricians to address and find alternative antibiotics with high efficiency. In addition, longitudinal studies are needed for continuous monitoring and tracking dispersion dynamics of successful clones associated with the persistence of drug-resistant phenotypes.
Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12941-021-00480-5.

Additional file 1: Table S1. Clinical and demographics data of pediatric-patients, drug resistance profile, and distribution pattern of β-lactamase genes in A. baumannii isolates.

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Authors’ contributions

ZG: conception of the research idea, study design, and the drafting of the manuscript; NY, MS, and RD: laboratory works; GE and MV: analysis and data interpretation; BN: collection of the strains SY: write up and language edition; All authors read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during the study were included in this article.

Declarations

Ethics approval and consent to participate

This study was authorized by the ethical committee of Shahid Beheshti University of Medical Sciences with reference number IR.SBMU.MSP.REC.1399.262. Consent for publication Not applicable.

Competing interests

The authors declare that they have no competing interests.

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