Antibiotic resistance and carriage class I integron in clinical isolates of *Acinetobacter baumannii* from Al Muthanna, Iraq

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Abstract

The goal of this work was to systematically characterize and detect class 1, 2, and 3 integrons with many antibiotic resistance *A. baumannii* strains collected from a clinical environment in Iraq’s Al-Muthanna hospitals. In this investigation, 24 non-replicated clinical strains of *A. baumannii* were evaluated using Chrome agar as a selective medium and PCR of the *rplB* gene. The clonal relatedness of the isolates to class 1 integron was evaluated using a PCR technique. The prevalence of class 1 integron was detected by PCR in only 12 clones of *A. baumannii* followed by *HinfI* digestion analysis showing three identical bands at 160 bp, 1350 bp, and 870 bp. In addition, PCR sequencing confirmed the presence of gene cassette arrays consisting of *aacA4-catB8-aadA1* (100%) in class 1 integron. The sequence analysis of the integron shows 97.87 identity with *A. baumannii* isolates from Australia (GenBank accession number CP054302) among *A. baumannii* isolates. The blast analysis of this class I integron showed that the presence of the *intI1, aacA4-catB8-aadA1* genes can considerably boost the acquisition of MDR phenotypes in *A. baumannii* isolates. We concluded that antibiotics of many types are widely used. The presence of integrons in *A. baumannii* is concerning for public health. In the clinical setting, it appears that the class 1 integron can be used as a predictive biomarker for the presence of MDR phenotypes. In these bacteria, however, the integron does not possess carbapenemases genes.

Introduction

Antibiotic resistance has been linked to high rates of morbidity and mortality in recent years as a result of prolonged hospitalization. With the increasing incidence on healthcare systems around the world, antimicrobial resistance has emerged as a major issue [1].

Antibiotic resistance is a normal occurrence in bacteria, but it is amplified when antibiotics are overused or misused in humans and animals [2]. Highly mobile genetic elements are a crucial factor in the rapid spread of antibiotic resistance. These elements have the ability to multiply and spread amongst bacterial species [3]. *A. baumannii* strains have demonstrated a remarkable ability to rapidly evolve multi-drug resistance in recent decades (MDR). This rapid rise in MDR is attributed not just to these strains’ inherent resistance genes, but also to their exceptional ability to acquire resistant components from other bacteria [4]. *Acinetobacter* bacteria are members of the Gammaproteobacteria class, which includes a wide range of bacteria [5]. Some *Acinetobacter* species invade mammals’ skin and mucous membranes, and they have been found as opportunistic pathogens in humans, especially in hospital patients [6]. *Acinetobacter* species are adaptable to a wide range of situations, including the natural world and hospitals [7]. The Gram-negative opportunistic pathogen *Acinetobacter baumannii* causes a variety of nosocomial diseases. Due to increased levels of antibiotic resistance, treating infections caused by these bacteria has become problematic [8].

Carbapenem-resistant *A. baumannii* strains that are also extensively drug resistant have become a major concern globally [9]. Most *A. baumannii* strains were antibiotic-susceptible prior to the 1970s [10]; however, as antibiotic use increased, multidrug-resistant (MDR) strains quickly evolved, with up to 57% of strains demonstrating MDR in recent research [11]. MDR is caused by the superbugs’
ability to acquire resistance elements from other bacteria through horizontal gene transfers. Integrons are unusual among these mobile elements in that they can carry and express resistance genes [12].

In this study, 12 clones of A. baumannii clinical isolates were tested. These isolates’ antibiotic resistance phenotype and population structure were studied, as well as the genetic properties of class 1 integron in MDR isolates. The class I integron of a sample strain was sequenced and examined to acquire insight into the genetic environment of a common class I integron, as well as additional resistance gene content and context.

Materials and methods

A. baumannii isolates

During the months of September 2021 and February 2022, 100 clinical samples were collected from Al-Muthanna hospitals in Iraq (Hussein Teaching Hospital and Maternity & Children Teaching Hospital). The Ethics Committee of Al-Muthanna University’s Ministry of Higher Education and Scientific Research approved this work.

Bacterial isolation and laboratory diagnosis

Bacterial isolates were obtained from 50 skin burn swabs and 50 urine samples. These samples were grown overnight on MacConkey agar media to isolate a single non-fermenting bacterial colony, then grown overnight on Chrome agar as a selective medium to isolate pure A. baumannii. These isolates were confirmed through the use of standard microbiological and biochemical testing, including the Vitek system (bioMérieux Vitek Systems Inc., Hazelwood, MO), which was used to identify isolates according to [13], the isolates were evaluated based on their physical traits and routine biochemical assays. Then, utilizing the previously published method of amplification of the rpiB gene, a one-tube multiplex PCR assay was performed for the fast identification of A. baumannii [14].

Antimicrobial susceptibility test

The susceptibility of A. baumannii to aminoglycoside drugs such as Amikacin, Gentamicin, Tobramycin, Streptomycin and Kanamycin; cephalosporins such as Cefazidime and Ceftriaxone and Cefepime; penicillins such as Ticarcillin; monobactam such as Aztreonam; carbapenem such as Imipenem and Meropenem; fluoroquinolone antibiotics such as Ciprofloxacin and Levofoxacin; Ampicillin/sulbactam, Piperacillin/tazobactam, Imipenem/EDTA; Sulfamethoxazole/trimethoprim combination (TM Media/India) was tested using the Clinical Laboratory Standard Institute (CLSI) recommended agar dilution method [15], and was evaluated using the CLSI interpretive criteria [15, 16].

DNA extraction

All twenty-four isolates had their genomic DNA extracted using a specialized kit (wizard® genomic DNA purification kit, Promega business, USA) according to the manufacturer’s instructions. The gDNA was eluted in 50 µl of nuclease-free water in the final stage of the extraction processes to produce a good DNA concentration and kept at −20 °C until used in the PCR reaction.

PCR amplification

PCR amplifications were carried out in 20 µl volumes using GoTaq® Green Master Mix, 2X containing GoTaq® DNA Polymerase is supplied in 2X Green GoTaq® reaction Buffer (pH 8.5), 400 µM dATP, 400 µM dGTP, 400 µM dCTP, 400 µM dTTP and 3 mM MgCl2 and including 5 ml of template DNA and 1.25 mM each primer. PCR amplification was performed with the Labnet MultiGene OptiMax Thermal Cycler. Amplification products were resolved by electrophoresis at 100 V for 1 h on 1.5% agarose gels with 0.53 Tris-borate-EDTA buffer containing ethidium bromide and were visualized under UV light. PCR amplification for the detection of class 1 integron cassettes (integron PCR) was performed with primers 5′CS and 3′CS, as described previously [17]. For PCR detection of the IntI1 and IntI2 integrase genes (integrase gene PCR), oligonucleotide primers based on the intI1 and intI2 genes were designed (Table 1). Primers IntIF and IntIR were used to amplify a 160 bp fragment of the intI1 gene. The combination of primers Int2F and Int2R amplified a fragment of 288 bp, specific for the intI2 gene. The combination of primers Int3F and Int3R amplified a fragment of 1041 bp, specific for the intI3 gene was described previously [17]. The positions of the primers relative to the integron are indicated in (Fig. 1). PCR amplification of integrase gene type 1, type 2 and type 3 was performed simultaneously for 35 cycles: 5 min of denaturation at 95 °C, 30 s of annealing at 95 °C, 30 s of annealing at 55 °C, and 1 min of extension at 72 °C while the viable region of the integron, the PCR was performed simultaneously for 35 cycles: 5 min of denaturation at 95 °C, 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 2.5 min of extension at 72 °C. The class 1 integron structure was determined by means of overlapping PCR fragments using the 5 ′CS/aacA6′R or aacA6-F/3′CS that covered from conserve of integron (intI1) to AAC(6′) gene using the primers listed in (Table 1). PCR was performed simultaneously for 35 cycles: 30 s of denaturation at 95 °C, 30 s of annealing at 55 °C, and 1 min of extension at 72 °C [17].
Fragment length polymorphism analysis

PCR positive products of using 5'CS and 3'CS primer were digested using HinfI. The digestion reactions were carried out at 37 °C for 2 h. The resulting restriction patterns were analyzed through electrophoresis on 1.5% agarose gels. Size and number of generated fragments are given in (Table 2).

Sequence analysis

The class I integron of using both 5'CS and 3'CS was purified and sent to Macrogen Corporation-Korea using ABI3730XL, automated DNA sequences. The strain A1 and A3 were chosen and sequenced as the digestion of HinfI was shown the same profile for 12 strains of class I integron. The sequences of these products were 100% identical to previously published sequences of the intI1 gene.

Ethical statement

All experiments were carried out in accordance with Al Muthanna University's humane treatment policies in Samawah, Iraq.

Statistical analysis

The $p$ value of Antimicrobial resistance prevalence in integron-positive and integron-negative was analysis by was analyzed using one-way analysis of variance, student $t$ test, using GraphPad Prism 8 software.
Bioinformatics

Geneious 7.2.2 (Biomatters) was used for visualization and alignment of gDNA, primers annotation and determination of restriction enzyme.

Results and discussion

Identification of in A. baumannii

Isolates from 100 samples were assigned to 24 distinct Acinetobacter baumannii based on the results of rplB gene analysis and VITEK-2 Compact system. The PCR amplification of rplB gene showed the expected product of 440 bp (Fig. 2). The rplB gene in A. baumannii is a housekeeping gene that encodes 50S ribosomal protein. It is essential for ribosome activity and is a major component of the peptidyl transferase activity and binding to functionally essential regions of 23S rRNA, one of the rRNA binding proteins that connects the 16S rRNA in the 70S ribosome [18]. Moreover, it has been used in multilocus sequence typing in order to identify phylogenetic relationships for the genus Acinetobacter with local clinical isolates of A. baumannii [19]. The samples were collected from 50 swabs of burned skin and 50 samples of urine. Acinetobacter baumannii’s own class I integron was detected in 10 isolates of A. baumannii, while in the burn sample it was detected in 2 isolates. In a recent study, it was shown that of 103 collected samples from different sources such as tracheal aspirate, catheter, sputum, CSF, and wounds, only 65 non-repetitive isolates were collected and confirmed as MDR A. baumannii [17].

Mapping of class 1 integron

The cassette assortments in all the strains were characterized by PCR with the 5′CS and 3′CS primers and by sequencing of the amplification products (Table 1) (Fig. 1 and Fig. 2). The sequences were found to be identical to those identified in integron of other A. baumannii isolates from Australia [20], (GenBank accession number CP054302) (Table 2) when compared using BLAST. In this study, 24 non-duplicated isolates were identified as A. baumannii isolates. The gDNA was extracted, followed by PCR screening for the intI, intII and intIII. The result showed a positive PCR product for only intI at the expected product of 160 bp (Fig. 2) in only 12 strains. Then the variable region for the integron was amplified using 5′CS and 3′CS primers, giving the expected product of 2380 bp (Fig. 1 and Fig. 2). Subsequently, the primers aacA6-F and aacA6R were used in order to identify the integron cassette arrays. This PCR amplification showed the expected product of 508 bp, followed by using an overlap PCR fragment using the aacA6-F/3′CS, giving the expected product of 2204 bp, confirming that AAC(6) is part of the class I integron. In order to differentiate between the classes, the integron class I cassette of 12 clones was PCR product using IntI-F and 3′CS primers and was digested with HinfI restriction enzyme, showing three different bands of 160 bp, 1350 bp, and 870 bp, confirming that the 12 strains contain the same cassette arrays of three different genes (Table 1 and Table 2). These results are different from those that were published, which showed different cassette arrays [21, 22]. Out of twelve, two identical strains were sequenced using 5′CS and 3′CS primers, showing alignment at 97.87 with CP054302 (Table 2) having three encoding genes: AAC(6)-catB8-AadA. Similarly, these A. baumannii strains have aadA6 cassettes, which have been previously described for P. aeruginosa, indicating that integrons can be transferred between these two species via plasmids and/or transposons [21]. In a recent study, the class 1 integron and complex gene cassettes of distinct species of clinical isolates from northern China were described. 383 clinical isolates were collected from northern China, and class 1 integron with gene cassettes were found in gram-negative clinical isolates in large numbers. A. baumannii was the most common isolate, with 78.5% carrying the aacA4-catB8-aadA1 gene cassettes [23]. Twelve strains had an integron containing a single cassette which encoded an AAC(6)-catB8-AadA aminoglycoside-modifying enzyme. Previous studies reported integrons containing genes that confer resistance to aminoglycoside antibiotics such as gentamicin, spectinomycin, streptomycin, amikacin, netilmicin, and tobramycin [21]. Furthermore, the sequencing results show that the integron class I contain catB8, which encodes a chloramphenicol acetyltransferase, and AadA, which have previously been identified as resistance genes for chloramphenicol, spectinomycin, and streptomycin, respectively [17, 21].

| Sources | Identity | Gene cassette array | GenBank accession No. | Amplicon size (bp) | Restriction enzyme | Restriction fragment size (s) (bp) |
|---------|----------|---------------------|-----------------------|-------------------|--------------------|----------------------------------|
| A. baumannii strain MS14413 | 97.87 | aacA4-catB8-AadA | CP054302 | 2380 | HinfI | 160/1350/870 |
Antimicrobial resistance pattern of positive class I integron in A. baumannii isolates

Representatives of the 24 clones of A. baumannii revealed that 12 clones did not contain class I integrons while the other 12 possessed the 2.3 kb class I integron (Table 3). This class I integron cassette array associated with contained the aacA4 gene, the chloramphenicol acetyltransferase gene catB8, and the aadA1 gene were highly resistant to aminoglycoside. Only 6 out of 12 clones were resistant to Imipenem/EDTA at 50% and 66% resistance level against ampicillin/sulbactam and piperacillin/tazobactam. According to a recent study, 75.2% of the 125 examined isolates were imipenem resistant, which was linked to having the blaADC and blaOXA-51 like genes [24].

The most effective antibiotics are imipenem, meropenem, ampicillin/sulbactam, and piperacillin/tazobactam. Class 1 integron have cassettes that don’t normally have a promoter. As a result, they are frequently transcribed from PC, a promoter upstream of the cassette arrays. This results in significantly lower expression levels of downstream gene cassettes [25]. As a result, the class 1 integron usually only comprises 6 gene cassettes [26]. In addition, the integron discovered in this study had 3–5 gene cassettes, which is typical of class 1 integron. The most common type of resistance in these production of resistant bacteria is beta-lactamases [27]. MDR isolates of A. baumannii clones had the highest prevalence of antibiotic resistance, while class I integron contains only three genes with no genes encoding for β-lactamase enzymes, a study has found.
Conclusions

The significant incidence of antibiotic resistance among A. baumannii isolates in clinical samples necessitates a comprehensive antimicrobial stewardship and infection control approach. In clinical strains, repertoires of aminoglycoside-modifying enzymes make up the Class 1 integron. More research is needed to clarify the link between gene pools and antibiotic resistance patterns.

Data availability

All data from this study are accessible upon request from the corresponding author.

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Author contributions

All the work, the analysis, and the writing were done by YAJA.

Compliance with ethical standards

Conflict of interest

The author declares no competing interests.

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