



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Document heading doi: 10.1016/S2221-1691(13)60149-5 © 2013 by the Asian Pacific Journal of Tropical Biomedicine. All rights reserved.

Detection of class 1 integron in *Acinetobacter baumannii* isolates collected from nine hospitals in Turkey

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PEER REVIEW

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Comments

This is a good study in which authors evaluated the antibiotic resistance and carriage class 1 integron in clinical isolates of *A. baumannii*. The results are very valuable and suggested that class 1 integron is common in *A. baumannii* isolated from different hospitals in Turkey.

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ABSTRACT

Objective: To investigate the antibiotic resistance genes inserted into class 1 and class 2 integrons in *Acinetobacter baumannii* (*A. baumannii*) isolates obtained from nine different cities in Turkey.

Methods: A collection of 281 *A. baumannii* clinical isolates were collected from nine different state hospitals in Turkey and were confirmed as *A. baumannii* by conventional biochemical, API testing and *bla*_{-OXA-51} specific PCR. The isolates were examined by PCR for existence of class 1 and 2 integron gene cassettes.

Results: They were characterized by antimicrobial susceptibility testing and the highest resistance rates were determined for piperacillin (90.03%), ciprofloxacin (87.54%), cefepime and trimethoprim/sulfamethoxazole (81.13%). The lowest resistance rates was for cefotaxime (3.55%). class 1 integrons were detected in 6.4% (18/281) of *A. baumannii* strains and no class 2 integron was detected. The gene cassettes of class 1 integrons *AacC1-AAC(3)-I-aadA1*, *AacC1-aadA1*, *AAC(3)-I*, *AAC(3)-I-AAC(3)-I-aadA1*, *TEM-I*, *AAC(3)-I-aadA1* - *AAC(3)-I-AAC(3)-I*, *AAC(3)-I-AAC(3)-I-AAC(3)-I-aadA1*, *AAC(3)-I-aadA1*, *AAC(3)-I-aadA1*, *AAC(3)-I-AAC(3)-I*, *AAC(3)-I-aadA1* - *AAC(3)-I-aadA1*, *AAC(3)-I-AAC(3)-I-aadA1* were detected in eighteen strains. The *aac* genes family were most frequently found integrated into the class 1 integrons and it was followed by *aadA* genes and *TEM-I* genes.

Conclusions: This is an extensive study on the distribution of class 1 integron among *A. baumannii* in Turkey. In addition to these, two new alleles were observed. Their percentage rates of similarity to other cassettes are 95% *aadA1* (TKA18) and 89% *aadA1* (ANKA3).

KEYWORDS

Acinetobacter baumannii, Class 1 integron, Gene cassette, Resistance

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Foundation Project: Supported by Recep Tayyip Erdogan University (Grant No. BAP-2012.102.03.4. and BAP-2013.102.03.4).

Article history:

Received 26 Jun 2013

Received in revised form 30 Jun, 2nd revised form 3 Jul, 3rd revised form 13 Jul 2013

Accepted 23 Aug 2013

Available online 28 Sep 2013

1. Introduction

Acinetobacter baumannii (*A. baumannii*) is an important opportunistic pathogen responsible for a nosocomial infections, such as pneumonia, urinary tract infections, bacteraemia and meningitis[1–3]. Its clinical significance is due to its rapid acquisition of a wide variety of antibiotic resistance genes so it has caused difficulties in the control[4]. This extremely rapid development of resistance has caused serious therapeutic problems worldwide[5,6]. A major acquisition and dissemination of resistance determinants is mobile elements, including plasmids, transposons and integrons[7–9]. Integrons are one of the main types of mobile elements currently known to be the natural gene capture systems in bacteria. Class 1 integrons are consisted of a 5′-conserved segment (5′CS) carrying a gene named *intI* encoding a site-specific integrase protein, and a variable region containing one or more antibiotic resistance genes[10,11]. Class 2 integrons are related with a mobile genetic element, *Tn7* transposon, and their structures resemble to class 1 integrons[10,12]. The goals of this study were to screen the antibiotic resistance genes inserted into class 1 and class 2 integrons in *A. baumannii* isolates obtained from nine different cities in Turkey. This is also first nationwide investigation of the class 1 and 2 integrons among isolates of *A. baumannii* in Turkey.

2. Materials and methods

2.1. Bacterial strain and antibiotics susceptibilities

A total of 281 isolates of *A. baumannii* were investigated in this study. All strains were isolated from different hospitals in Turkey between 2011 and 2012. All clinical isolates were identified using the API 32GN system (bioMerieux, Marcy-l’Etoile, France). The isolates were also identified using *bla*_{OXA-51} specific PCR[13]. VITEK cards for antibiotic susceptibility testing (GNS-424 cards) were inoculated and incubated according to their commendations of the manufacturer (BioMerieux, Hazelwood, Mo.). The following antibiotics were tested: ampicillin-sulbactam, piperacillin, ceftazidime, cefepime, amikacin, gentamicin, tobramycin, ciprofloxacin, cefotaxime, levofloxacin, tetracycline, trimethoprim/sulfamethoxazole, imipenem meropenem according to Clinical and Laboratory Standards Institute[14].

2.2. DNA extraction

Genomic DNA used as a template for PCR assays was obtained from bacterial suspension grown overnight in Luria Broth with shaking incubator at 37 °C. Bacterial suspension

was centrifuged at 13 000 r/min for 5 min. Pellet was suspended in 500 µL distilled water and subsequently boiled in a water bath for 10 min. Debris was centrifuged at 13 000 r/min for 5 min. Five microliters of supernatant was used as a template for PCR assays.

2.3. PCR assays for integrons

All isolates were tested for the presence of class 1 and class 2 integrases conserved region. Primers used for detection for *intI1*: *intI1F* (5′-ACATGTGATGGCGACGCACGA-3′) and *intI1R* (5′-ATTTCTGTCTGGCTGGCGA-3′); for *intI2*: *intI2F* (5′-CACGGATATGCGACAAAAAGGT-3′) and *intI2R* (5′-GTAGCAAACGACTGACGAAATG-3′); for class 1 integron gene cassette: 5′CS (5′-GGCATCCAAGCAGCAAG-3′) and for 3′CS (5′-AAGCAGACTTACCTGA-3′). PCRs were performed in a final volume of 50 µL. PCR mix component was as follows; 5 µL of 10X PCR buffer [0.1 mol/L Tris-HCl (pH 8.8), 0.5 mol/L KCl, 1% Triton X-100], 3 µL of 0.025 mol/L MgCl₂, 5 µL of 10X dNTP (0.002 mol/L dATP, dCTP, dGTP and dTTP), 2 µL each of primer (25 pmol/µL), 34 µL deionised sterile water, 1 U of *Taq* DNA polymerase and 5 µL of template DNA. PCR amplification condition was as follows: initial denaturation at 94 °C for 3 min for initial denaturation, 94 °C for 45 seconds, 55 °C for 1 min, 72 °C for 3 min followed by 34 cycles and 5 min at 72 °C with a final extension[15,16].

2.4. DNA sequencing and analysis

Electrophoresis of PCR products was performed with 1% agarose containing 0.5 µg/mL ethidium bromide, and subsequently visualized under UV light. The PCR products were ligated into pGEM-T easy vector (Promega) at 16 °C for 16 h by T4 DNA ligase. Ligation mixture were then transformed into *Escherichia coli* JM101 strain prepared according to Sambrook *et al.* and spread in ampicilline plates (50 µg/mL) containing X-gal (40 µg/mL)[17]. We collected the white colonies and plasmids were purified from these colonies by using Promega Plasmid Purification Kits. Recombinant plasmids carrying amplicons of class 1 integrons were sent to Macrogen Inc., Seoul, Korea for sequencing by using the universal oligonucleotide primers, T7 and SP6. Sequencing results were analysed using alignment search tool, BLAST (<http://www.ncbi.nlm.nih.gov/BLAST>)[18] and the multiple sequence alignment program, CLUSTAL W(<http://www.ebi.ac.uk/clustalw>).

3. Results

All isolates were tested for susceptibility to 14 antimicrobials. The frequency of resistance of the two

hundred and eighty-one clinical isolates to each of the antibiotics tested can be observed in Figure 1. Resistance to tobramycin (40.56%), to piperacillin (90.03%), to ceftazidime (80.78%), to amikacin (70.01%), to gentamicin (34.16%), to imipenem (80.07%), to meropenem (78.29%), to ciprofloxacin (87.54%), to levofloxacin (69.75%), to cefepime (81.13%), to ampicillin–sulbactam (79.35%), to cefotaxime (3.55%), to tetracycline (70.46%) and to trimethoprim/sulfamethoxazole (81.13%) were observed. The lowest resistance rates was for cefotaxime (3.55%). The highest resistance rates were for piperacillin (90.03%), ciprofloxacin (87.54%), cefepime and trimethoprim/sulfamethoxazole (81.13%).

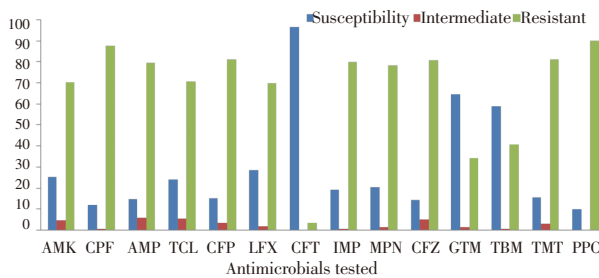


Figure 1. Antibiotics susceptibility of *A. baumannii* strains.

AMK: Amikacin; CPF: Ciprofloxacin; AMP: Ampicillin–sulbactam; TCL: Tetracycline; CFP: Cefepime; LFX: Levofloxacin; CFT: Cefotaxime; IMP: Imipenem; MPN: Meropenem; CFZ: Cefazidime; GTM: Gentamicin; TBM: Tobramycin; TMT: Trimethoprim/sulfamethoxazole; PPC: Piperacillin.

Among the 281 isolates analysed by PCR, eighteen *A. baumannii* isolates were found to have the class 1 integron and no class 2 integron was detected. Integrase gene (*IntI*) were detected in 38.7% (109/281) of the total isolates. Of the 109 *IntI* positive isolates which 18 of them were integron positive. A total of 12 different gene cassettes were observed in 18 isolates. The integrons were observed to contain 1–4 gene cassettes and location of the cassettes differ (Figure 2).

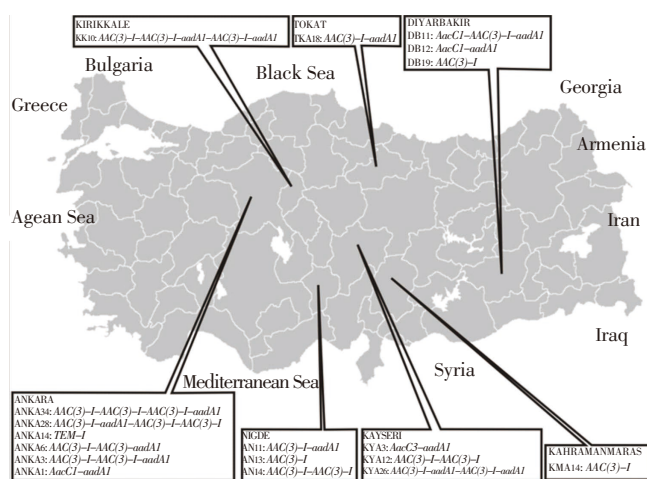


Figure 2. The geographical distribution of class 1 integrons found in *A. baumannii* strains in Turkey.

The gene cassettes of class 1 integrons *AacC1*–*AAC(3)*–*I*–*aadA1*, *AacC1*–*aadA1*, *AAC(3)*–*I*, *AAC(3)*–*I*–*AAC(3)*–*I*–*aadA1*, *TEM*–*I*, *AAC(3)*–*I*–*aadA1*–*AAC(3)*–*I*–*AAC(3)*–*I*, *AAC(3)*–*I*–*AAC(3)*–*I*–*AAC(3)*–*I*–*aadA1*, *AAC(3)*–*I*–*aadA1*, *AAC(3)*–*I*–*AAC(3)*–*I*,

AAC(3)–*I*–*aadA1*–*AAC(3)*–*I*–*aadA1*, *AAC(3)*–*I*–*AAC(3)*–*I*–*aadA1*–*AAC(3)*–*I*–*aadA1*. Among these profiles, *aac* genes family were found most frequently in class 1 integrons gene cassettes.

In addition to these, two new gene cassettes array were observed. Their percentage rates of similarity to other cassettes are 95% *aadA1* (TKA18) and 89% *aadA1* (ANKA3).

4. Discussion

A. baumannii is resistant to various antimicrobial agents such as penicillins, cephalosporines, aminoglycosides, tetracyclines[19,20]. Because of the multidrug resistance quickly spread in hospital population, *A. baumannii* has a clinical significance, requiring epidemiologic monitoring as a measure for control of nosocomial infection[20].

Integrons play a significant role in the dissemination of antimicrobial resistance through horizontal transmission among Gram–negative bacteria[21,22]. In the present study, we screened and characterised integrons in *A. baumannii* isolates from nine center in Turkey. 109 of 281 isolates contained integrase gene and 18 of *IntI* positive strains included gene cassettes. According to these results, 18 isolates contain gene cassettes and 91 strains do not include. The first reasons for this may be mutations or deformation at the 3'CS. The second reasons may be gene cassette array in novel, complex or unusual class 1 integrons[23,24]. And finally perhaps variable region was too long to be amplified. The gene cassettes included those encoding resistance to aminoglycosides and the β –lactamase. Among 18 gene cassettes, there were 12 different gene arrays in the integron gene cassette systems and two new alleles were observed (95% *aadA1* and 89% *aadA1*) in *A. baumannii*.

The production of aminoglycoside modifying enzymes (AMEs) confer to bacteria the ability to modify antibiotic's amino or hydroxyl functions and it's the main mechanism for the bacteria that provide resistance to aminoglycosides. Aminoglycoside modifying enzymes include the aminoglycoside acetyltransferase (AAC), aminoglycoside adenytransferase (AAD), aminoglycoside nucleoside transferase (ANT), aminoglycoside phosphotransferase (APH) and their isozyme[25]. *AAC(3)*–*I* and *AAC(6')*–*I* were found frequently in integron gene cassettes[26]. In our study, in *A. baumannii*, aminoglycoside resistance determinants, including *aac(3)*–*I*, *aadA1* were predominantly. The high prevalence of aminoglycoside resistance genes in the *A. baumannii* integrons described here was comparable to that observed in other studies[27–30]. However our study is the

first to report the presence of *AacCI-AAC(3) I-aadAI*, *AacCI-aadAI*, *AAC(3)-I*, *AAC(3)-I-AAC(3)-I-aadAI*, *TEM-1*, *AAC(3)-I-aadAI-AAC(3)-I-AAC(3)-I*, *AAC(3)-I-AAC(3)-I-AAC(3)-I-aadAI*, *AAC(3)-I-aadAI*, *AAC(3)-I-AAC(3)-I*, *AAC(3)-I-aadAI-AAC(3)-I-aadAI*, *AAC(3)-I-AAC(3)-I-aadAI-AAC(3)-I-aadAI* in *A. baumannii* from clinical isolates in Turkey. Aminoglycoside resistance genes containing isolates were found in seven of nine different cities in Turkey. This finding indicates that the aminoglycoside resistance genes are predominant in *A. baumannii* in Turkey, to our knowledge.

The main mechanism of resistance to β -lactams in *A. baumannii* is enzymatic degradation by β -lactamases^[31]. AmpC cephalosporinase is chromosomally encoded by *A. baumannii*, other β -lactamases TEM-92, TEM-116, SHV-12, CTX-M-2, TEM-1 and TEM-2 have been described, and we have identified TEM-1 type beta-lactamases in one strain (ANKA14). In conclusion, class 1 integron appear to be widely disseminated amongst clinical isolate of *A. baumannii* from Turkey. These data show that, 18 resistance gene cassettes were detected and 12 different gene cassette arrays were identified from *A. baumannii*. The wide distribution of integron and *A. baumannii* may pose a serious threat to the development of future effective antimicrobial therapies. This is the first extensive study on the distribution of class 1 integron among *A. baumannii* in Turkey.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

This work was supported by Recep Tayyip Erdogan University, Research Fund Grants BAP-2012.102.03.4. and BAP-2013.102.03.4.

Comments

Background

A. baumannii is an opportunistic pathogen that is mostly cause of septicaemia, pneumonia and urinary tract infection. Recently, antibiotic resistance has increased in *A. baumannii*. The reason for this is gene acquisition. The gene acquisition is mediated by mobile genetic elements such as plasmids, transposons and integrons.

Research frontiers

A. baumannii is the important nosocomial infection agent in Turkey. It has acquired quickly wide variety of antibiotic resistance genes. Therefore, this is important to investigate the antibiotic resistance genes in these bacteria in Turkey.

Related reports

Mirnejad *et al.* (2013) have studied prevalence of integrons class 1 and class 2 in *A. baumannii* in Tehran, Iran. They showed that class 1 and class 2 integrons are prevalent in clinical isolates of *A. baumannii* in Iran.

Innovations and breakthroughs

This is first nationwide study of class 1 integron among clinical isolates of *A. baumannii* in Turkey. This study has found two novel alleles and 12 different gene cassettes have determined. Data showed that aminoglycoside modifying enzymes are widely found in Turkey.

Applications

It may be important to know the existence of class 1 integron in *A. baumannii* and distribution of *A. baumannii* in hospitalized patients. The results of this study suggest that class 1 integron is an important role in the spread of antibiotic resistance among clinical isolates in Turkey. Therefore, it is significant to monitor antibiotic resistance gene by PCR and sequence analysis.

Peer review

This is a good study in which authors evaluated the antibiotic resistance and carriage class 1 integron in clinical isolates of *A. baumannii*. The results are very valuable and suggested that class 1 integron is common in *A. baumannii* isolated from different hospitals in Turkey.

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