# OXA- and GES-type β-lactamases predominate in extensively drug-resistant *Acinetobacter baumannii* isolates from a Turkish University Hospital

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## Abstract

We determined the antibiotic susceptibility and genetic mechanisms of resistance in clinical strains of *Acinetobacter baumannii* from Istanbul, Turkey. A total of 101 clinical strains were collected between November 2011 and July 2012. Antimicrobial susceptibility was performed using the Vitek 2 Compact system and E-test. Multiplex PCR was used for detecting $bla_{\text{OXA-51-like}}$, $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$ and $bla_{\text{OXA-58-like}}$ genes. IS*Aba1*, $bla_{\text{IMP-like}}$, $bla_{\text{VIM-like}}$, $bla_{\text{GES}}$, $bla_{\text{VEB}}$, $bla_{\text{PER-2}}$, *aac-3-Ia* and *aac-6′-Ib* and *NDM-1* genes were detected by PCR and sequencing. By multiplex PCR, all strains were positive for $bla_{\text{OXA-51}}$, 79 strains carried $bla_{\text{OXA-23}}$ and one strain carried $bla_{\text{OXA-40}}$. $bla_{\text{OXA-51}}$ and $bla_{\text{OXA-23}}$ were found together in 79 strains. IS*Aba1* element was detected in 81 strains, and in all cases it was found upstream of $bla_{\text{OXA-51}}$. GES-type carbapenemases were found in 24 strains (GES-11 in 16 strains and GES-22 in 8 strains) while $bla_{\text{PER-2}}$, $bla_{\text{VEB-1}}$, $bla_{\text{NDM-1}}$, $bla_{\text{IMP}}$- and $bla_{\text{VIM}}$-type carbapenemases were not observed. Aminoglycoside modifying enzyme (*aac-3-Ia* and *aac-6′Ib*) genes were detected in 13 and 15 strains, respectively. Ninety-seven (96%) *A. baumannii* strains were defined as MDR and of these, 98% were extensively drug resistant (sensitive only to colistin). Colistin remains the only active compound against all clinical strains. As seen in other regions, OXA-type carbapenemases, with or without an upstream IS*Aba1*, predominate but GES-type carbapenemases also appear to have a significant presence. REP-PCR analysis was performed for molecular typing and all strains were collected into 12 different groups. To our knowledge, this is the first report of GES-11 and OXA-40 in *A. baumannii* from Turkey.

**Keywords:** *Acinetobacter baumannii*, antimicrobial agents, GES-11, GES-22, OXA-40
**Original Submission:** 2 March 2013; **Revised Submission:** 1 July 2013; **Accepted:** 14 July 2013
Editor: R. Cantón
**Article published online:** 19 August 2013
*Clin Microbiol Infect* 2014; **20:** 410–415
10.1111/1469-0691.12338

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## Introduction

Among the *Acinetobacter* species, the most common and clinically significant is *Acinetobacter baumannii* [1]. It is a gram-negative, opportunistic pathogen that causes a range of infections, including bacteraemia, pneumonia, meningitis, urinary tract infection and wound infection. Although carbapenems are the preferred treatment of choice for serious *Acinetobacter* infections, the resistance rates have increased dramatically over the past decade. Carbapenem resistance in *Acinetobacter* species is most commonly caused by the production of OXA-type carbapenemases (class D β-lactamases) and class B metallo-β-lactamases (MBLs) [2,3]. The OXA-type carbapenemases are comprised of four broad groups: $bla_{\text{OXA-23-like}}$, $bla_{\text{OXA-40-like}}$, $bla_{\text{OXA-58-like}}$ and an

intrinsic $bla_{OXA\text{-}51\text{-}like}$ type [4]. The most common mechanism for *A. baumannii* resistance to the carbapenems is the existence of the OXA-23 subtype [5]. The class D OXA-51--like β-lactamases are intrinsic to *A. baumannii* and have been used as a method of identification [6]. In addition, IS*Aba1* insertion sequence elements have been found in *A. baumannii* strains, which can enhance the expression of OXA-type carbapenemases and mobilize them among strains [7]. All *A. baumannii* strains also encode *AmpC* cephalosporinases chromosomally, which can also be overexpressed by an upstream IS*Aba1* element [1]. Other enzymatic mechanisms of resistance have also been identified in *A. baumannii*, including the clavulanic-acid-inhibited extended-spectrum β-lactamases (ESBLs) (PER-1, PER-2 and VEB-1), MBLs (VIM-1/4, VIM-2 and IMP-1, 2, 4, 5, 6) [8], GES-like enzymes (class β-lactamases) [9] and aminoglycoside modifying enzymes [8].

The aim of this study was to characterize the susceptibility profile and genetic mechanisms of resistance of *Acinetobacter* species collected from a major teaching hospital in Turkey.

## Materials and Methods

### Bacterial strains and antimicrobial susceptibility testing

In this study, consecutive strains of *A. baumannii* ($n$ = 101) isolated from clinical specimens that had been collected at Bezmialem University Hospital in Turkey between November 2011 and July 2012 were included. The isolates were obtained from respiratory specimens (tracheal aspirates 17%, sputum 36%), wounds (21.9%), blood (19%), catheters (3%), an abdominal specimen (0.9%), cerebrospinal fluid (0.9%), pleural fluid (0.9%) and urine (0.9%). The strains were identified by VITEK 2 (bioMerieux, Durham, NC, USA) according to the manufacturer's instructions. Antimicrobial susceptibility testing was carried out using the Vitek 2 Compact system (bioMerieux, Craponne, France), including colistin and tigecycline, and confirmed with the E-test. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines.

### DNA extraction

Genomic DNA was obtained from bacterial suspensions grown overnight in Luria Broth with shaking at 37°C. Bacterial suspensions were centrifuged at 12 470 *g* for 5 min, and pellets were suspended in 500 μL distilled water and subsequently boiled for 10 min. Debris was centrifuged at 12 470 *g* for 5 min and 500 μL of supernatant was obtained. Five microlitres of each supernatant were used as a template for PCR [10].

### REP-PCR analysis

REP-PCR was used in the genotyping of *A. baumannii* strains. The highly conserved REP sequence is approximately 35 nucleotides long, includes an inverted repeat, and can occur in the genome singly or as multiple adjacent copies. The primer pair of REP 1 (5′-IIIGCGCCGICATCAGGC-3′) and REP 2 (5′-ACGTCTTATCAGGCCTAC-3′) was used [11]. A single reaction mixture contained: 5 μL of genomic DNA, 20 pM of each primer, 10 μL reaction buffer (Promega, Madison, WI, USA), 3 μL of 25 mM $MgCl_2$, 200 μM of dNTPs and 1.5 U of Go *Taq* Flexi Polymerase (Promega) in a final volume of 50 μL. The PCR amplification condition was as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 45 s at 94°C, 1 min at 45.8°C and 8 min at 72°C with a final extension of 16 min at 72°C. The amplified DNA fragments were separated by electrophoresis on 1.5% agarose gel with 1-kb DNA ladder (Sigma Chemicals, Ontario, Canada) as the size marker. The amplified DNA bands were visualized following ethidium bromide staining under UV light and the banding patterns of each strain were captured in an UVP bioimaging system (UVP, Upland, CA, USA). The REP-PCR fingerprints were analyzed by the Phoretix gel analysis package (Nonlinear USA, Inc., Durham, NC, USA).

### Multiplex PCR for detection of $bla_{OXA}$ genes

Multiplex PCR was used for detecting $bla_{OXA\text{-}51\text{-}like}$, $bla_{OXA\text{-}23\text{--}like}$, $bla_{OXA\text{-}40\text{-}like}$ and $bla_{OXA\text{-}58\text{-}like}$ genes [12]. Primers used for the detection of $bla_{OXA}$ genes are shown in Table 1. PCRs were performed in a final volume of 50 μL and included 5 μL of genomic DNA, 20 pM of each primer, 10 μL reaction buffer (Promega), 3 μL 25 mM $MgCl_2$, 200 μM of each dNTPs and 1.5 U of *Taq* Polymerase (Promega). PCR amplification conditions were as follows: initial denaturation at 94°C for 3 min followed by 30 cycles of 25 s at 94°C, 40 s at 52°C and 50 s at 72°C with a final extension of 5 min at 72°C.

### PCR amplifications of the IS*Aba1* gene, ESBLs, MBLs and aminoglycoside-resistance genes

The primers used are shown in Table 1. A single reaction mixture contained: 5 μL of genomic DNA, 20 pM of each primer, 10 μL reaction buffer (Promega), 3 μL 25 mM $MgCl_2$, 200 μL of dNTPs and 1.5 U Go *Taq* Flexi Polymerase (Promega) in a final volume of 50 μL. PCR amplification conditions were as follows: initial denaturation at 95°C for 5 min followed by 35 cycles of 45 s at 95°C, 45 s at 56°C for IS*Aba1* and $bla_{VEB}$, 55°C for $bla_{GES}$, 50°C for $bla_{PER\text{-}2}$, 55°C for *aac-3-Ia*, 54°C for *aac-6′Ib*, 58°C for $bla_{VIM\text{-}like}$, 56°C for $bla_{IMP\text{-}like}$, 57°C for $bla_{NDM\text{-}1}$ and 3 min at 72°C with a final extension of 5 min at 72°C. All PCR results were analyzed on 1% agarose containing 0.5 mg/L ethidium bromide, and subsequently visualized under UV light.

**TABLE 1. Primers used in the amplification of selected genes**

| Primers | 5′→3′ | Amplicon size | $T_m$ | References |
|---|---|---|---|---|
| OXA-51 | F: TAATGCTTTGATCGGCCTTG<br>R: TGGATTGCACTTCATCTTGG | 353 | 52°C | [12] |
| OXA-23 | F: GATCGGATTGGAGAACCAGA<br>R: ATTTCTGACCGCATTTCCAT | 501 | | [12] |
| OXA-24 | F: GGTTAGTTGGCCCCCTTAAA<br>R: AGTTGAGCGAAAAGGGGATT | 246 | | [12] |
| OXA-58 | F: AAGTAT TGGGGCTTGTGCTG<br>R: CCCCTCTGCGCTCTACATAC | 599 | | [12] |
| *ISAba1* | F: CACGAATGCAGAAGT TG<br>R: CGACGAATACTATGACAC | 549 | 56°C | [13] |
| GES | F: ATGCGCTTCATTCACGCAC<br>R: CTATTTGTCCGTGCTCAGGA | 863 | 56°C | [14] |
| VEB | F:ATTTCCCGATGCAAAGCGT<br>R: TTATTCCGGAAGTCCCTGT | 542 | 55°C | [14] |
| PER-2 | F: ATGAATGTCATCACAAAATG<br>R: TCAATCCGGACTCACT | 927 | 50°C | [15] |
| Aac-3-Ia | F: ATGGGCATCATTCGCACA<br>R: TCTCGGCTTGAACGAATTGT | 484 | 55°C | [16] |
| Aac-6-Ib | F: ATGACTGAGCATGACCTT G<br>R: AAGGGT TAGGCAACACTG | 524 | 54°C | [16] |
| IMP | F: CATGGTTTGGTGGTTCTTGT<br>R: ATAATTTGGCGGACTTTGGC | 488 | 56°C | [17] |
| VIM | F: ATTGGTCTATTTGACCGCGTC<br>R: TGCTACTCAACGACTGAGCG | 780 | 58°C | [17] |
| NDM-1 | F GAGATTGCCGAGCGACTTG<br>R CGAATGTCTGGCAGCACACTT | 497 | 57°C | In this study |

PCR products were sent to Macrogen Inc., Seoul, Korea, for sequencing by using the same primers used in PCR reactions. Sequencing results were analysed using the alignment search tool BLAST (http://www.ncbi.nlm.nih.gov/BLAST) and the multiple sequence alignment program CLUSTALW (http://www.ebi.ac.uk/Tools/msa/clustalw2/).

## Results

A total of 101 non-duplicate clinical *A. baumannii* strains were collected from Bezmialem University hospitals in Turkey over a 9-month period. Most of the isolates were obtained from respiratory specimens (tracheal aspirates 17%, sputum 36%), followed by wounds (21.9%), blood (19%), catheters (3%), an abdominal specimen (0.9%), cerebrospinal fluid (0.9%), pleural fluid (0.9%) and urine (0.9%). Eighteen (17.8%) and 83 (82.2%) of all isolates were determined to be originated from the community and hospital, respectively. All patients were hospitalized in several units: 61 patients (60.3%) in an intensive care unit, 12 patients (11.8%) in the internal units (nephrology, pulmonology, etc.), 20 patients (19.8%) in surgical clinics and 7 (6.9%) in paediatric intensive care unit and only one patient (0.9%) was children. The results showed that 31.7% and 68.3% of *A. baumannii* were from colonization and an infectious agent, respectively. The diagnoses of the patients were cirrhosis (1.6%), heart disease (6.4%), acute and chronic renal failure (16.1%), diabetes mellitus (DM) (16.9%), malignancy (17.7%), chronic obstructive pulmonary disease, pneumonia, respiratory failure, pulmonary and thoracic diseases, pneumothorax (28.2%), traffic accidents and gunshot wounds (4.1%), cerebrovascular accident, epilepsy, brain pathologies such as vertebral fracture (8%), and Behcet's disease (0.8%). All strains were identified as *A. baumannii* by the VITEK GNI card and $bla_{OXA-51}$ PCR for specify for the *A. baumannii* species.

Most of the *A. baumannii* strains ($n$ = 95) were resistant to imipenem (95%) and meropenem (94%) (Table 2). We observed high rates of resistance to ampicillin-sulbactam (97%), piperacillin (97%), piperacillin-tazobactam (97%), ceftazidime (97%), cefoparozone-sulbactam (87%), cefepime (97%), amikacin (63%), ciprofloxacin (94%), levofloxacin (89%), tetracycline (70%), trimethoprim-sulphamethoxazole (69%) and gentamicin (48%). Tigecycline and netilmicin showed some activity against these isolates (8% and 11% susceptible, respectively); however, colistin was the most active compound

**TABLE 2. Antibiotic susceptibility rounded off to nearest whole per cent**

| | Antibiotic susceptibility ($n$ = 101) | | |
|---|---|---|---|
| Antibiotics | % *R* (no.) | % *I* (no.) | % *S* (no.) |
| Ampicillin-sulbactam | 97 | 0 | 3 |
| Piperacillin | 97 | 0 | 3 |
| Piperacillin-tazobactam | 97 | 0 | 3 |
| Ceftazidime | 97 | 0 | 3 |
| Cefoparozone-sulbactam | 87 | 6 | 7 |
| Cefepime | 97 | 0 | 3 |
| Amikacin | 63 | 2 | 35 |
| Gentamicin | 48 | 10 | 42 |
| Netilmicin | 11 | 14 | 75 |
| Ciprofloxacin | 94 | 3 | 3 |
| Levofloxacin | 89 | 8 | 3 |
| Tetracycline | 70 | 11 | 19 |
| Tigecycline | 8 | 27 | 65 |
| Colistin | 0 | 0 | 100 |
| Trimethoprim-sulphamethoxazole | 69 | 0 | 31 |
| Imipenem | 95 | 2 | 3 |
| Meropenem | 94 | 3 | 3 |

(100% susceptible). Ninety-seven strains (96%) were defined as MDR based on resistance to more than two antibiotic groups (Table 2), and of these, 98% were extensively drug-resistant as defined by resistance to all drugs except ≤2 [18]. $MIC_{50}$ and $MIC_{90}$ values of tigecycline of isolates were also found to be 2 and ≥8, respectively.

The molecular analysis revealed that 79 strains (78%) carried the $bla_{OXA\text{-}23\text{-}like}$ gene and one strain carried a $bla_{OXA\text{-}40\text{-}like}$ gene. All strains had a $bla_{OXA\text{-}51\text{-}like}$ gene and importantly, 80% of these had an upstream IS*Aba1* element. Sixty-six strains had $bla_{OXA\text{-}51\text{-}like}$ with an upstream IS*Aba1* and $bla_{OXA\text{-}23\text{-}like}$ together. The one strain with $bla_{OXA\text{-}40\text{-}like}$ also had IS*Aba1* upstream from $bla_{OXA\text{-}51\text{-}like}$. $bla_{GES\text{-}like}$ genes were detected in 24 strains (GES-11 in 16 strains, GES-22 in eight strains). The aminoglycoside modifying enzyme gene *aac-3-Ia* was identified in 13 strains, and the *aac-6′-Ib* gene in 15 strains. Many of the strains (95%) carried multiple resistance determinants. For example, eight strains were positive for $bla_{OXA\text{-}51\text{-}like}$ with an upstream IS*Aba1*, $bla_{OXA\text{-}23\text{-}like}$, $bla_{GES\text{-}11}$ and *aac-6′-Ib*, whereas four strains had the same combination of resistance mechanisms but with *aac-3-Ia* instead of *aac-6′-Ib*, and another eight strains had similar combinations but with $bla_{GES\text{-}22}$. $bla_{OXA\text{-}58\text{-}like}$, $bla_{VEB}$, $bla_{PER\text{-}2}$, $bla_{IMP\text{-}like}$, $bla_{VIM\text{-}like}$ genes and $bla_{NDM\text{-}1}$ were not observed.

All 101 *A. baumannii* strains were typed by REP-PCR. REP-PCR results show that our isolates harboured 12 predominant genotypes (Fig. 1). Genotypes 6 and 9 (19.8% of isolates; $n = 20$) were the most prevalent; 9.9, 3.96, 5.94, 9.9, 13.86, 0.99, 6.93, 1.98, 1.98 and 4.95% of strains were clustered into genotypes 1, 2, 3, 4, 5, 7, 8, 10, 11 and 12, respectively. Two major clusters were obtained, one corresponding to groups 1 and 12 and the second to the other groups. The highest similarity was detected between groups 3 and 4.

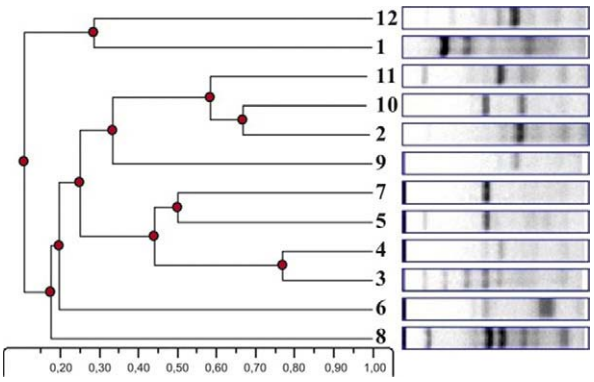


**FIG. 1.** REP-PCR profiles of 101 *Acinetobacter baumannii* isolates. Twelve different genotypes were determined and most of the isolates were clustered into genotypes 6 and 9.

## Discussion

*Acinetobacter baumannii* is an important bacterial pathogen that causes serious infections in immunocompromised patients. It has reemerged among the important infectious agents, especially in healthcare institutions, primarily due to the increasing number of outbreaks with MDR strains. MDR *A. baumannii* is generally defined as resistance to more than two of the following five drug classes: antipseudomonal cephalosporins (ceftazidime or cefepime), antipseudomonal carbapenems (imipenem or meropenem), ampicillin-sulbactam, fluoroquinolones (ciprofloxacin or levofloxacin) and aminoglycosides (gentamicin, tobramycin or amikacin) [1]. In our strains, it was not uncommon for resistance to be observed in all five drug classess, with susceptibility only to colistin. This is termed extensively drug-resistant [18]. We observed no pan-drug-resistant strains, where the bacteria are resistant to all available antibiotics, including colistin and tigecycline. Lately, the use of broad-spectrum cephalosporins such as ceftazidime and cefotaxime, carbapenems and fluoroquinolones has been associated with an increase in multidrug resistance in *Acinetobacter* species [1]. For many years, carbapenems have been considered the drug of choice to treat serious *A. baumannii* infections; however, resistance has become all too frequent around the world, including in Turkey [19].

Our work shows high rates of resistance to the carbapenems (imipenem 95% and meropenem 94%). Carbapenem-resistant strains of *A. baumannii* have been reported worldwide and carbapenemases, MBLs and some oxacillinases are recognized as important contributors to carbapenem resistance in *A. baumannii* [20]. OXA-type carbapenemases have predominated in *A. baumannii*, especially in worldwide outbreaks of OXA-23 [1]. The OXA-40 group has been reported in Portugal, Spain, Iran and the United States. Naturally occurring OXA carbapenemases such as OXA-51-like enzymes (e.g. OXA 64-66, OXA 68-71, OXA 78-80, OXA-82, OXA-86, OXA-92 and OXA104-112) have been identified in *A. baumannii* isolates worldwide. In addition, strains producing OXA-58 derivatives were found in isolates recovered from Italy, Belgium, France, Greece, Iran, the United States and Argentina [21]. OXA-23, OXA-58 and OXA-51 have been reported in Turkey [1]; however, to our knowledge, this is the first report of OXA-40 in a clinical *A. baumannii* strain from Turkey. In our study, all strains had an intrinsic $bla_{OXA\text{-}51\text{-}like}$ gene, supporting the identification of the strains as *A. baumannii* [22]. Importantly, an IS*Aba1* element was upstream of $bla_{OXA\text{-}51}$ in 80% of strains, and this combination has been shown to confer high levels of carbapenem resistance [23]. In fact, in 66 strains, $bla_{OXA\text{-}51}$ with an upstream IS*Aba1* was also

associated with $bla_{OXA-23}$. We also observed for the first time in Turkey, $bla_{OXA-40}$ in one strain, and this was associated with IS*Aba1* upstream of $bla_{OXA-51}$. IS*Aba1*- $bla_{OXA-51}$, IS*Aba1*- $bla_{OXA-51}$- $bla_{OXA-23}$, IS*Aba1*- $bla_{OXA-51}$- $bla_{OXA-40}$ combinations in *A. baumannii* were found in a previous study [20].

As mentioned above, carbapenem resistance in *A. baumannii* is most often associated with class D $\beta$-lactamases (OXA-23-like, OXA-40-like and OXA-58-like) and MBLs. Recently, the Ambler class A of the GES and KPC (carbapenemase) types has also been reported in *A. baumannii*. GES (Guiana-Extended-Spectrum) $\beta$-lactamases are Class A carbapenemases and can confer high level resistance to carbapenems. $bla_{GES}$ genes have been reported in several countries in Europe, Asia, South America and South Africa [9]. DNA sequencing of the $bla_{GES}$ genes identified in this study showed two types; GES-11 ($n = 16$ strains) and GES-22 ($n = 8$ strains). GES-11 (AFU25739.1) was previously identified in Belgium from a Turkish patient, suggesting its possible presence in Turkey [9]. Our study confirms its presence in Turkey and, at least in patients from our institution, GES-11 is present in a considerable number of our carbapenem-resistant *A. baumannii* strains.

$bla_{OXA-40}$ and $bla_{GES-11}$ were found together in the same strain in this study. Similar to our results, in one study from Kuwait, $bla_{OXA-23}$ and $bla_{GES-11}$ genes were found together in 33 isolates [24]. The most important characteristic of the GES family of enzymes is their ability to evolve into carbapenemases [25]. GES and OXA-type enzymes are together responsible for high carbapenem resistance in our strains.The OXA-40 harbouring strain was identified from an 88-year-old patient. The patient presented at the emergency department with discharge and redness around the PEG. *A. baumannii* was isolated in pus from this case. The isolate was only susceptible to colistin and tigecycline, moderately susceptible to meropenem and tetracycline, and resistant to all other antibiotics. Tigecycline treatment of patient is admitted to intensive care and after 17 days of hospitalization, the patient had been ex.

REP-PCR was shown to be the discriminatory power almost as much as PFGE. REP-PCR requires less labour compared with PFGE and analysis makes it possible to isolate a large number. REP-PCR was conducted to determine the genomic diversity of *A. baumannii* isolates [26]. Twelve genotypes were observed. The same antibiotic susceptibility pattern and resistance genes were found in various REP genotypes, suggesting horizontal transfer of resistance determinants.

Treatment options for infections caused by members of the genus *Acinetobacter* are limited due to increasing levels of drug resistance and reliance on polymyxins in combinations with other antibiotics. Colistin has become one of the most commonly used antibiotics for the treatment of imipenem-resistant gram-negative rods. The recently approved antibiotic tigecycline has been demonstrated to have activity against *A. baumannii* [20]. In another study, colistin and tigecycline proved to be effective alternative antimicrobial agents for treatment of carbapenem-resistant *A. baumannii* [27]. The emergence of GES-type ESBLs in *A. baumannii* in Kuwait suggests that the Middle East region might be a reservoir for carbapenemase-producing *A. baumannii* [24]. Similarly, according to our results, Anatolia can be a reservoir for GES-type beta lactamase-producing *A. baumannii*. These results support our understanding of carbapenem resistance in *A. baumannii* strains from Turkey, showing that OXA-type carbapenemases, with or without an IS*Aba1* element, and GES-type carbapenemases predominate.

## Acknowledgements

This study was supported by grants from Recep Tayyip Erdogan University (BAP-2012.106.01.11 and BAP-2011.102.03.3). AYP was supported by the Australian National Health and Medical Research Council (APP1047916 and APP1010114).

## Transparency Declaration

The authors have no conflicts of interest to declare.

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