

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

A. C. Cicek¹, A. Saral², M. Iraz³, A. Ceylan³, A. O. Duzgun⁴, A. Y. Peleg^{5,6} and C. Sandalli⁷

1) Department of Medical Microbiology, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, 2) Department of Biology, Faculty of Arts and Sciences, Artvin Coruh University, Artvin, 3) Department of Medical Microbiology, Faculty of Medicine, Bezmialem Foundation University, Istanbul, 4) Department of Biology, Faculty of Arts and Sciences, Giresun University, Giresun, Turkey, 5) Department of Microbiology, Monash University, 6) Department of Infectious Diseases, The Alfred Hospital, Melbourne, VIC, Australia and 7) Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, Rize, Turkey

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*_{OXA-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-40-like} and *bla*_{OXA-58-like} genes. *ISAbal*, *bla*_{IMP-like}, *bla*_{VIM-like}, *bla*_{GES}, *bla*_{VEB}, *bla*_{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*_{OXA-51}, 79 strains carried *bla*_{OXA-23} and one strain carried *bla*_{OXA-40}. *bla*_{OXA-51} and *bla*_{OXA-23} were found together in 79 strains. *ISAbal* element was detected in 81 strains, and in all cases it was found upstream of *bla*_{OXA-51}. GES-type carbapenemases were found in 24 strains (GES-11 in 16 strains and GES-22 in 8 strains) while *bla*_{PER-2}, *bla*_{VEB-1}, *bla*_{NDM-1}, *bla*_{IMP}- and *bla*_{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 *ISAbal*, 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

Corresponding author: C. Sandalli, Microbiology and Molecular Biology Research Laboratory, Department of Biology, Faculty of Arts and Sciences, Recep Tayyip Erdogan University, 53100 Rize, Turkey
E-mail: cemal.sandalli@erdogan.edu.tr

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*_{OXA-23-like}, *bla*_{OXA-40-like}, *bla*_{OXA-58-like} and an

intrinsic *bla*_{OXA-51-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*AbaI* 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*AbaI* 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 (bioMérieux, Durham, NC, USA) according to the manufacturer's instructions. Antimicrobial susceptibility testing was carried out using the Vitek 2 Compact system (bioMérieux, Craaponne, 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 μ L distilled water and subsequently boiled for 10 min. Debris was centrifuged at 12 470 g for 5 min and 500 μ L 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 μ L of genomic DNA, 20 pM of each primer, 10 μ L reaction buffer (Promega, Madison, WI, USA), 3 μ L of 25 mM MgCl₂, 200 μ M of dNTPs and 1.5 U of Go Taq Flexi Polymerase (Promega) in a final volume of 50 μ L. 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-51-like}, *bla*_{OXA-23-like}, *bla*_{OXA-40-like} and *bla*_{OXA-58-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 μ L and included 5 μ L of genomic DNA, 20 pM of each primer, 10 μ L reaction buffer (Promega), 3 μ L 25 mM MgCl₂, 200 μ M 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*AbaI* gene, ESBLs, MBLs and aminoglycoside-resistance genes

The primers used are shown in Table 1. A single reaction mixture contained: 5 μ L of genomic DNA, 20 pM of each primer, 10 μ L reaction buffer (Promega), 3 μ L 25 mM MgCl₂, 200 μ L of dNTPs and 1.5 U Go Taq Flexi Polymerase (Promega) in a final volume of 50 μ L. 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*AbaI* and *bla*_{VEB}, 55°C for *bla*_{GES}, 50°C for *bla*_{PER-2}, 55°C for *aac-3-Ia*, 54°C for *aac-6'Ib*, 58°C for *bla*_{VIM-like}, 56°C for *bla*_{IMP-like}, 57°C for *bla*_{NDM-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.

Primers	5'→3'	Amplicon size	T _m	References
OXA-51	F: TAATGCTTTGATCGGCCTTG R: TGGATTGCACCTTCATCTTGG	353	52°C	[12]
OXA-23	F: GATCGGATTGGAGAACCAGA R: ATTTCTGACCCGATTTCCAT	501		[12]
OXA-24	F: GGTTAGTTGGCCCCCTTAAA R: AGTTGAGCGAAAAGGGGATT	246		[12]
OXA-58	F: AAGTAT TGGGGCTTGTGCTG R: CCCCTCTGCGCTCTACATAC	599		[12]
ISAbal	F: CACGAATGCAGAAGT TG R: CGACGAATACTATGACAC	549	56°C	[13]
GES	F: ATGCGCTTCATTACGCAC R: CTATTTGTCCGTGCTCAGGA	863	56°C	[14]
VEB	F: ATTTCCCGATGCAAAGCGT R: TTATTCCGGAAGTCCCTGT	542	55°C	[14]
PER-2	F: ATGAATGTCATCACAATAATG R: TCAATCCGGACTCACT	927	50°C	[15]
Aac-3-la	F: ATGGGCATCATTGCGACA R: TCTCGGCTTGAACGAATTGT	484	55°C	[16]
Aac-6-lb	F: ATGACTGAGCATGACCTT G R: AAGGGT TAGGCAACTG	524	54°C	[16]
IMP	F: CATGGTTTGGTGGTCTTGT R: ATAATTTGGCGGACTTTGGC	488	56°C	[17]
VIM	F: ATGGTCTATTTGACCCGCTC R: TGCTACTCAACGACTGAGCG	780	58°C	[17]
NDM-1	F GAGATTGCCGAGCGACTTG R CGAATGTCTGGCAGCACACTT	497	57°C	In this study

TABLE 1. Primers used in the amplification of selected genes

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

Antibiotics	Antibiotic susceptibility ($n = 101$)		
	% 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]. MIC₅₀ and MIC₉₀ 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-23-like} gene and one strain carried a *bla*_{OXA-40-like} gene. All strains had a *bla*_{OXA-51-like} gene and importantly, 80% of these had an upstream *ISAbal* element. Sixty-six strains had *bla*_{OXA-51-like} with an upstream *ISAbal* and *bla*_{OXA-23-like} together. The one strain with *bla*_{OXA-40-like} also had *ISAbal* upstream from *bla*_{OXA-51-like}. *bla*_{GES-like} genes were detected in 24 strains (GES-11 in 16 strains, GES-22 in eight strains). The aminoglycoside modifying enzyme gene *aac-3-la* 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-51-like} with an upstream *ISAbal*, *bla*_{OXA-23-like}, *bla*_{GES-11} and *aac-6'-Ib*, whereas four strains had the same combination of resistance mechanisms but with *aac-3-la* instead of *aac-6'-Ib*, and another eight strains had similar combinations but with *bla*_{GES-22}, *bla*_{OXA-58-like}, *bla*_{VEB}, *bla*_{PER-2}, *bla*_{IMP-like}, *bla*_{VIM-like} genes and *bla*_{NDM-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.

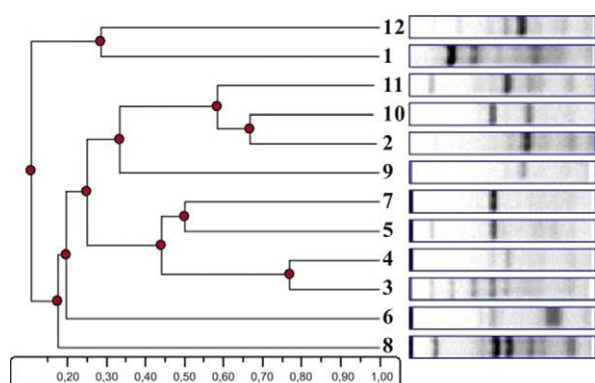


FIG. 1. REP-PCR profiles of 101 *Acinetobacter baumannii* isolates. Twelve different genotypes were determined and most of the isolates were clustered into genotypes 6 and 9.

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 ceftepime), 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 classes, 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-51-like} gene, supporting the identification of the strains as *A. baumannii* [22]. Importantly, an *ISAbal* element was upstream of *bla*_{OXA-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-51} with an upstream *ISAbal* 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*AbaI* upstream of *bla*_{OXA-51}. IS*AbaI*-*bla*_{OXA-51}, IS*AbaI*-*bla*_{OXA-51}-*bla*_{OXA-23}, IS*AbaI*-*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 β -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) β -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 imipe-

nem-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*AbaI* 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 (APPI047916 and APPI010114).

Transparency Declaration

The authors have no conflicts of interest to declare.

References

1. Peleg AY, Seifert H, Paterson DL. *Acinetobacter baumannii*; emergence of a successful pathogen. *Clin Microbiol Rev* 2008; 21: 538–582.
2. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. *Clin Infect Dis* 2006; 43: 49–56.
3. Thomson JM, Bonomo RA. The threat of antibiotic resistance in gram-negative pathogenic bacteria: beta-lactams in peril!. *Curr Opin Microbiol* 2005; 8: 518–524.
4. Merkier AK, Centron D. Bla(OXA-51)-type beta-lactamase genes are ubiquitous and vary within a strain in *Acinetobacter baumannii*. *Int J Antimicrob Agents* 2006; 28: 110–113.
5. Adams-Haduch JM, Paterson DL, Sidjabat HE et al. Genetic basis of multidrug resistance in *Acinetobacter baumannii* clinical strains at a tertiary medical center in Pennsylvania. *Antimicrob Agents Chemother* 2008; 52: 3837–3843.
6. Lee YT, Kuo SC, Chiang MC et al. Emergence of carbapenem-resistant non-*baumannii* species of *Acinetobacter* harboring a *bla*_{OXA-51}-like gene that is intrinsic to *A. baumannii*. *Antimicrob Agents Chemother* 2011; 56: 1124–1127.
7. Zhou H, Pi BR, Yang Q et al. Dissemination of imipenem-resistant *Acinetobacter baumannii* strains carrying the IS*AbaI* *bla*_{OXA-23} genes in a Chinese hospital. *J Med Microbiol* 2007; 56: 1076–1080.
8. Nordmann P, Poirel L. *Acinetobacter baumannii* –basic and emerging mechanisms of resistance. *Eur Infect Dis* 2008; 2: 94–97.

9. Bogaerts P, Naas T, Garch FE et al. GES extended-spectrum β -lactamases in *Acinetobacter baumannii* isolates in Belgium. *Antimicrob Agents Chemother* 2010; 54: 4872–4878.
10. Ausubel FM, Brient R, Kingston RE et al. *Short protocols in molecular biology*, 2nd edn. New York: John Wiley and Sons, 1995.
11. Andriamanantena TS, Ratsima E, Rakotonirina HC et al. Dissemination of multidrug resistant *Acinetobacter baumannii* in various hospitals of Antananarivo Madagascar. *Ann Clin Microbiol Antimicrob* 2010; 30: 9–17.
12. Woodford N, Ellington MJ, Coelho JM et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 2006; 27: 351–353.
13. Segal H, Garny S, Elisha BG. Is IS(ABA-1) customized for *Acinetobacter*? *FEMS Microbiol Lett* 2005; 243: 425–429.
14. Moubareck C, Bre'mont S, Conroy MC et al. GES-11, a novel integron-associated GES variant in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009; 58: 3579–3581.
15. Celenza G, Pellegrini C, Caccamo M et al. Spread of blaCTX-M-type and blaPER-2 β -lactamase genes in clinical isolates from Bolivian hospitals. *J Antimicrob Chemother* 2006; 57: 975–978.
16. Ning D, De-zhi L, Ji-chao C et al. Drug-resistant genes carried by *Acinetobacter baumannii* isolated from patients with lower respiratory tract infection. *Chin Med J* 2010; 123: 2571–2575.
17. Jeon BC, Jeong SH, Bae IK et al. Investigation of a nosocomial outbreak of imipenem-resistant *Acinetobacter baumannii* producing the OXA-23 β -lactamase in Korea. *J Clin Microbiol* 2005; 43: 2241–2245.
18. Magiorakos AP, Srinivasan A, Carey RB et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; 18: 268–281.
19. Jones RN, Ferraro MJ, Reller LB et al. Multicenter studies of tigecycline disk diffusion susceptibility results for *Acinetobacter* spp. *J Clin Microbiol* 2007; 45: 227–230.
20. Lolans K, Rice TW, Munoz-Price LS et al. Multicity outbreak of carbapenem-resistant *Acinetobacter baumannii* isolates producing the carbapenemase OXA-40. *Antimicrob Agents Chemother* 2006; 50: 2941–2945.
21. Nowak P, Paluchowska P, Budak A. Distribution of blaOXA genes among carbapenem-resistant *Acinetobacter baumannii* nosocomial strains in Poland. *New Microbiol* 2012; 35: 317–325.
22. Turton JF, Woodford N, Glover J et al. Identification of *Acinetobacter baumannii* by detection of the bla_{oxa-51-like} carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006; 44: 2974–2976.
23. Turton JF, Ward ME, Woodford N et al. The role of ISAbal in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006; 258: 72–77.
24. Bonnin RA, Rotimi VO, Al Hubail M et al. Wide dissemination of GES-Type carbapenemases in *Acinetobacter baumannii* isolates in Kuwait. *Antimicrob Agents Chemother* 2013; 57: 183–188.
25. Delbrück H, Bogaerts P, Kupper MB et al. Kinetic and crystallographic studies of extended-spectrum GES-11, GES-12, and GES-14 β -lactamases. *Antimicrob Agents Chemother* 2012; 56: 5618–5625.
26. Higgins PG, Janßen K, Fresen MM et al. Molecular epidemiology of *Acinetobacter baumannii* bloodstream isolates obtained in the United States from 1995 to 2004 using rep-PCR and multilocus sequence typing. *J Clin Microbiol* 2012; 50: 3493–3500.
27. Somily AM, Absar MM, Arshad MZ et al. Antimicrobial susceptibility patterns of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* against carbapenems, colistin, and tigecycline. *Saudi Med J* 2012; 33: 750–755.