

Increasing carbapenem resistance due to the clonal dissemination of oxacillinase (OXA-23 and OXA-58)-producing *Acinetobacter baumannii*: report from the Turkish SENTRY Program sites

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A significant increase in carbapenem-resistance rates among *Acinetobacter baumannii* isolates collected in two Turkish medical centres was detected in the 2000–2006 period (20–60 %) by the SENTRY Antimicrobial Surveillance Program. Carbapenem-resistant strains from 2006 were evaluated for the presence of encoding genes and epidemic clonality. OXA-58-like and OXA-23-like carbapenemase-producing strains were detected in both medical institutions. Seventeen out of 18 strains from Ankara were positive for *bla*_{OXA-58} primers and belonged to the same clone, whilst 26 isolates (25 from Istanbul and one from Ankara) harboured *bla*_{OXA-23}-like genes and showed identical or similar PFGE patterns. Isolates producing OXA-23-like carbapenemases were more resistant than OXA-58-like carbapenemase producers to non-carbapenem antimicrobial agents. Carbapenem resistance in these institutions was observed to be largely driven by the dissemination of clones producing OXA-type carbapenemases.

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INTRODUCTION

Acinetobacter baumannii represents an important cause of nosocomial infections, including septicaemia, ventilator-associated pneumonia and urinary tract infections (Perez *et al.*, 2007). Furthermore, this pathogen may colonize hospitalized patients, especially those with severe underlying illnesses. *Acinetobacter* strains are usually resistant to multiple antimicrobial agents (Poirel & Nordmann, 2006), and the carbapenems (imipenem or meropenem) represent an important option for the treatment of *Acinetobacter* infections caused by multidrug-resistant (MDR) isolates (Perez *et al.*, 2007).

Resistance to carbapenems in *Acinetobacter* species occurs due to the accumulation of various resistance mechanisms including β -lactamase production, which can be associated with the loss of outer-membrane porins and/or over-expression of efflux pumps or more rarely with penicillin-binding protein alterations (Poirel & Nordmann, 2006). The production of metallo- β -lactamases (M β Ls; Ambler class B) or carbapenem-hydrolysing oxacillinases (class D) may confer high-level resistance to carbapenems in

Acinetobacter species (Poirel *et al.*, 2007). Although M β Ls have been identified in a wide variety of Gram-negative species, this class of enzyme is not common in *A. baumannii*, in which the oxacillinases represent the most commonly acquired carbapenem-hydrolysing enzymes (Brown & Amyes, 2006). Carbapenem resistance due to OXA-carbapenemases has been reported from diverse geographical origins including European countries, Latin America, Kuwait, Iraq and countries in the Western Pacific area (Marque *et al.*, 2005; Poirel & Nordmann, 2006).

Here, we evaluated the antimicrobial susceptibility of *A. baumannii* strains collected in Turkey through the SENTRY Antimicrobial Surveillance Program over a period of 7 years (2000–2006), and the mechanisms of resistance and epidemiology of carbapenem-resistant *A. baumannii*.

METHODS

Bacterial isolates and susceptibility testing. A total of 321 *A. baumannii* strains collected from 2000 to 2006 in medical centres located in Ankara and Istanbul, Turkey, were submitted to JMI Laboratories (North Liberty, IA, USA). These isolates were susceptibility tested by reference broth microdilution methods against >30 antimicrobials according to Clinical and Laboratory Standards

Abbreviations: FDA, Food and Drug Administration; M β L, metallo- β -lactamase.

Institute guidelines (CLSI, 2006), using validated panels produced by TREK Diagnostics and cation-adjusted Mueller–Hinton broth. Susceptibility test results were interpreted using CLSI M100-S18 (CLSI, 2008) criteria, where available. Tigecycline breakpoints approved by the US Food and Drug Administration (FDA) for indicated *Enterobacteriaceae* species (susceptible/intermediate/resistant at $\leq 2/4/\geq 8 \mu\text{g ml}^{-1}$) were applied for comparison purposes. *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853 were routinely included in the testing for quality assurance.

Carbapenemase screening and PCR experiments. *A. baumannii* strains collected showing resistance to imipenem, meropenem ($\text{MIC} \geq 16 \mu\text{g ml}^{-1}$) and ceftazidime ($\text{MIC} \geq 32 \mu\text{g ml}^{-1}$) were screened for production of M β L using the M β L Etest (AB BIODISK). OXA-type carbapenemases were assessed using a multiplex PCR strategy as described previously (Woodford *et al.*, 2006). The association of *bla*_{OXA} and the insertion sequences IS*Aba*-1, -2 and -3 was also assessed.

Molecular typing by PFGE. All carbapenem-resistant isolates from 2006 were also evaluated by PFGE. Additionally, these isolates were compared with susceptible *A. baumannii* strains from the same year. Bacterial cells grown overnight were embedded in agarose, lysed and deproteinated, and digested with *Sma*I. The restriction fragments were separated by electrophoresis on a CHEF DR II (Bio-Rad) under the following conditions: 1% agarose, 0.5 $\times$ TBE, 200 V and a switch interval ranging from 5 to 30 s over 23 h. Isolates showing three or fewer band differences were considered to be identical or similar and clonally related.

RESULTS AND DISCUSSION

A total of 321 *A. baumannii* isolates collected in medical centres located in Ankara and Istanbul (Turkey), mainly recovered from bloodstream (66.4%) and respiratory tract (24.3%) infections, were evaluated. The antimicrobial

susceptibility profiles of the *A. baumannii* isolates are listed in Table 1. The most active compounds against these isolates were polymyxin B (99.3% susceptible) and tigecycline (99.0% susceptible). Overall, the isolates showed approximately 52.0% susceptibility to imipenem and meropenem; these antimicrobials were followed in coverage by tobramycin (43.9% susceptible). However, the stratification of the imipenem susceptibility rates in the 7-year period demonstrated a gradual and significant decrease in these rates, from 80.4% in 2000 to only 40.0% in 2006 [$P < 0.001$, OR=6.17 (95% CI 2.42–16.10)], with meropenem following a similar trend, decreasing from 71.7% in 2000 to 40.0% in 2006 [$P < 0.001$, OR=3.18 (95% CI 1.61–9.1)] (Fig. 1).

Among the 75 isolates collected in 2006, 44 strains (58.6%) resistant to imipenem, meropenem and ceftazidime were screened for the presence of carbapenemases. All 44 isolates showed no reduction in the imipenem MIC values in the presence of EDTA, suggesting the absence of M β Ls. Genes encoding oxacillinases showing carbapenemase activity (OXA-23, -24 and -58 clusters) were detected in all carbapenem-resistant *A. baumannii* isolates: 26 (59.1%) strains harboured *bla*_{OXA-23}-like genes, and 18 (40.9%) carried *bla*_{OXA-58}-like genes. All but one *bla*_{OXA-58}-carrying isolate were collected in Ankara, whilst the vast majority (25/26) of the strains carrying *bla*_{OXA-23}-like genes were isolated in Istanbul.

Molecular typing results demonstrated that all strains producing OXA-58-like carbapenemases from Ankara had identical or similar PFGE patterns (Table 2), indicating the clonal dissemination of OXA-58-like carbapenemase-producing isolates in this medical centre. The isolate harbouring a *bla*_{OXA-58}-like gene from Istanbul showed a

Table 1. Antimicrobial susceptibility patterns of *A. baumannii* isolated from medical centres in Turkey (SENTRY Program 2000–2006)

Antimicrobial agent	All sites, all years (<i>n</i> =321)			% Susceptible by medical centre*†	
	MIC ₅₀ ($\mu\text{g ml}^{-1}$)	MIC ₉₀ ($\mu\text{g ml}^{-1}$)	% Susceptible*	Ankara (<i>n</i> =39)	Istanbul (<i>n</i> =36)
Imipenem	4	>8	52.6	51.3	27.8
Meropenem	4	>16	52.3	51.3	27.8
Ampicillin/sulbactam	32	>32	25.5	28.2	8.3
Piperacillin/tazobactam	>64	>64	21.1	20.5	5.5
Aztreonam	>16	>16	6.9	7.7	2.8
Ciprofloxacin	>4	>4	26.8	25.6	5.5
Levofloxacin	>4	>4	32.7	28.2	5.5
Gentamicin	>8	>8	25.6	23.1	2.8
Tobramycin	16	>16	43.9	45.6	25.0
Tetracycline	>8	>8	35.5	35.9	52.8
Trimethoprim/sulfamethoxazole	>2	>2	39.3	23.1	19.4
Polymyxin B	≤ 1	≤ 1	99.3	100.0	100.0
Tigecycline	0.5	2	99.0	100.0‡	100.0‡

*Susceptibility criteria were those of CLSI M100-S18 (CLSI, 2008).

†Isolates collected in 2006 only.

‡US FDA susceptibility breakpoint ($\leq 2 \mu\text{g ml}^{-1}$) approved for indicated *Enterobacteriaceae* species was used for comparison purposes only.

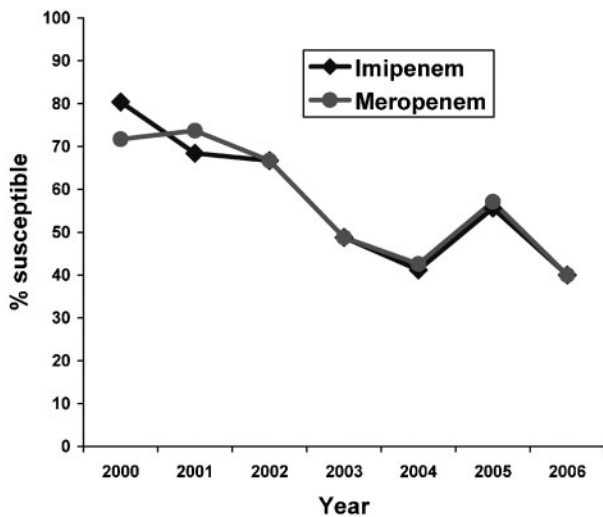


Fig. 1. Susceptibility to imipenem and meropenem of *A. baumannii* isolated in the period 2000–2006 from Turkish medical centres participating in the SENTRY Program.

different PFGE pattern from the OXA-58-like-producing isolates from Ankara. The 26 OXA-23-like-producing isolates (25 from Istanbul and one from Ankara) were found to be genetically related (Table 2), suggesting that this clone disseminated in these two Turkish cities. Oxacillinase-producing strains were distinct from susceptible *A. baumannii* isolates from the same medical sites. Susceptible isolates showed a great genetic diversity when compared with the oxacillinase-producing isolates, showing 11 different PFGE patterns among 18 isolates selected for evaluation. All 44 isolates were confirmed as *A. baumannii* based on a positive PCR result using primers for *bla*_{OXA-51}, a cluster of constitutive oxacillinase genes found in the chromosome of this species.

The insertion sequences *ISAb*₁, -2 and -3 have been associated with carbapenem-hydrolysing oxacillinases (Corvec *et al.*, 2007; Poirel & Nordmann, 2006; Segal *et*

Table 2. Summary of molecular and epidemiological characterization of carbapenem-resistant *A. baumannii* isolates from Turkey

The number of isolates is shown in parentheses.

Medical centre location	Carbapenemase detected	PFGE patterns*
Ankara (18)	OXA-58 (17)	A1 (8), A2 (8), A3 (1)
	OXA-23(1)	B2 (1)
Istanbul (26)	OXA-23 (25)	B (7), B1 (5), B2 (13)
	OXA-58 (1)	C (1)

*The capital letter defines the PFGE pattern, with subtypes identified by a number.

al., 2007). When located upstream of the *bla*_{OXA} genes, these genetic elements can supply a transcriptional promoter that upregulates the expression of these β -lactamases. The *ISAb*₃ element was detected upstream of the *bla*_{OXA-58}-like gene in all isolates; however, none of the *ISAb* elements described were found to be related to *bla*_{OXA-23}-like genes in the isolates evaluated in this study.

In general, the carbapenem-resistant *A. baumannii* isolates from 2006 showed high rates of co-resistance to all other antimicrobials tested, except polymyxin B (100.0 % susceptible) and tigecycline (100.0 % inhibited at $\leq 2 \mu\text{g ml}^{-1}$). Of note, the isolates from Istanbul showed higher rates of resistance to other antimicrobial classes (Table 1). Among 36 *A. baumannii* isolates from Istanbul, 26 produced OXA enzymes corresponding to 72.2 % of the isolates, whilst in Ankara, the proportion of OXA-producing strains was lower (46.2 %). The high occurrence of OXA-producing strains may reflect increasing rates of resistance to non-carbapenem antimicrobial agents, as well as to the carbapenems.

Molecular epidemiology studies in recent years have documented several outbreaks of multidrug-resistant clones of *A. baumannii* in Turkish hospitals (Alp *et al.*, 2006; Vahaboglu *et al.*, 2006). In addition, various carbapenemases have been described in Turkey, including oxacillinase-producing *A. baumannii*. However, isolates producing these enzymes have been observed in small numbers and with restricted geographical diversity (Marque *et al.*, 2005). In this study, we demonstrated that the dramatic decrease in carbapenem susceptibility rates among *A. baumannii* in 2006 was attributed to the clonal dissemination of OXA-producing strains and that carbapenem resistance was found to be driven largely by OXA-58 in Ankara and OXA-23 in Istanbul, both of which have become more prevalent in Europe in recent years (Brown & Amyes, 2006; Poirel & Nordmann, 2006). The dissemination of *A. baumannii* clones carrying oxacillinase genes that were largely recovered from intensive care unit patients (51.4 %) highlights the importance of hospital infection control measures and the need for a more extensive local molecular epidemiology surveillance programme throughout Europe.

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