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Molecular Characterization of Carbapenem-Resistant *Acinetobacter baumannii* Isolated in Two Turkish Medical Centers in 2006: Report from the SENTRY Antimicrobial Surveillance Program

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AMENDED ABSTRACT

Objectives: To evaluate the mechanisms of resistance (R) to carbapenems (CARB) and the epidemiologic typing of CARB-R *Acinetobacter* spp. (ASP) isolated in Turkey through the SENTRY Program in 2006. We also evaluate the antimicrobial susceptibility (S) of ASP strains collected in Turkey over the last 7 years (2000-2006).

Methods: A total of 303 ASP strains were submitted by two Turkish medical centers (MCs; Ankara and Istanbul) from 2000 to 2006, 57 in 2006. The isolates were mainly from bloodstream (64.1%) and respiratory tract (26.0%) infections and were S tested by reference broth microdilution methods to >30 antimicrobials according to CLSI guidelines (2006). Strains R to imipenem and meropenem (MIC, >16 mg/L) collected in 2006 were screened for production of carbapenemases (IMP, VIM, SPM and OXA groups) by PCR and epidemiologically typed by PFGE.

Results: The most active antimicrobials overall were: polymyxin B (99.2% S), imipenem (51.3% S), meropenem (51.0% S) and tobramycin (42.4% S). S to imipenem decreased from 80.4% in 2000 to only 29.8% in 2006. Thirty nine of 57 (68.4%) ASP strains collected in 2006 were CARB-R with high rates of cross-R to all other antimicrobials tested, except polymyxin B (100.0% S) and tigecycline (100.0% inhibited at <=2 mg/L), and were selected for molecular characterization. All 39 strains were PCR-positive for bla_{OXA-51}, indicating *A. baumannii*. Thirteen strains were from one site and these strains showed identical or similar PFGE patterns and were PCR-positive for bla_{OXA-58}. Twenty five of the remaining 26 CARB-R strains also showed PFGE pattern identical/similar to each other and distinct from that of MC 068. All 25 strains had PCR positive results for bla_{OXA-23}. One isolate with distinct PFGE pattern had positive PCR results for bla_{OXA-58}.

Conclusions: Clonal dissemination of R strains caused a significant increase in the prevalence of CARB-R ASP in the Turkish medical centers evaluated by the SENTRY Program. CARB-R was found to be largely driven by OXA-type carbapenemases, OXA-58 being prevalent in Ankara and OXA-23 in Istanbul. The polymyxins and tigecycline represent the only antimicrobials with reasonable in vitro activity against ASP in the Turkish MCs evaluated.

INTRODUCTION

Acinetobacter spp. represent an important cause of nosocomial infections, including septicemia, ventilator associated pneumonia, and urinary tract infections. Furthermore, this pathogen may colonize hospitalized patients, especially those with severe underlying illnesses. This organism is usually resistant to multiple antimicrobial agents, and the carbapenems, imipenem or meropenem represent the "drug of choice" for the treatment of *Acinetobacter* infections caused by multidrug-resistant (MDR) strains.

Resistance to carbapenems in *Acinetobacter* spp. occurs due to interplay of various resistance mechanisms including over-production of Amp-C ß-lactamases associated with loss of outer membrane porins, and/or overexpression of efflux pump AdeABC. The production of metallo-ß-lactamases (Ambler class B), or oxacillinases (class D) may also confer high-level resistance to carbapenems in this species. Although metallo-ß-lactamases have been identified in a wide variety of gram-negative species, this class of enzymes is not common in *Acinetobacter* spp., where the oxacillinases represent the most common acquired carbapenem-hydrolyzing enzymes. Carbapenem resistance due to OXA-carbapenemases has been reported from diverse geographical origins including Spain, Turkey, Greece, Romania, Austria, Italy and UK, in Europe and also from Kuwait, Iraq and Argentina.

The objective of this study was to evaluate the mechanism of resistance and the epidemiology of carbapenem-resistant *Acinetobacter* spp. isolated in Turkey through the SENTRY Antimicrobial Surveillance Program in 2006. We also evaluated the antimicrobial susceptibility of *Acinetobacter* spp. strains collected in Turkey through the SENTRY Program over the last 7 years (2000-2006).

MATERIALS AND METHODS

Bacterial isolates: A total of 303 *Acinetobacter* spp. strains were submitted by two Turkish medical centers located in Ankara and Istanbul as part of the SENTRY Program from 2000 to 2006. The isolates were mainly from bloodstream (67.4%) and respiratory tract (22.0%) infections. Fifty-seven isolates have been submitted in 2006 at the time we performed this investigation (isolated from January through July 2006).

Susceptibility testing: All *Acinetobacter* spp. strains were susceptibility tested by reference broth microdilution methods to >30 antimicrobials according to CLSI guidelines (2006), using validated dry form panels and cation-adjusted Mueller-Hinton broth. Susceptibility test results were interpreted using CLSI M100-S17 (2007) criteria where available. Tigecycline breakpoints approved by the US-FDA for some Enterobacteriaceae species (susceptible/intermediate/resistant at ≤2/4/≥8 mg/L) were used 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 screens: Strains (n=39) resistant to imipenem, meropenem (MIC, ≥16 mg/L) and ceftazidime (MIC, ≥32 mg/L) collected in 2006 were screened for production of metallo-ß-lactamase using the MßL Etest (AB BIODISK, Solna, Sweden). Isolates with a positive metallo-ß-lactamase test were screened for IMP-, VIM- and SPM-types by PCR using generic primers. Those isolates showing a negative metallo-ß-lactamase test (no inhibition with EDTA) were screened for OXA-type carbapenemases using a multiplex PCR strategy described by Woodford et al. (2006), which included primers specific for bla_{OXA-51} (chromosomal OXA enzyme inherent of *A. baumannii*) as well as bla_{OXA-23}-like, bla_{OXA-24}-like and bla_{OXA-58}-like genes, which confer carbapenem resistance. Representative isolates of each clonal type from both medical centers were also screened for PER-1 ESBL by PCR.

Pulsed-Field Gel Electrophoresis (PFGE): All carbapenem resistant isolates were also epidemiologically typed by PFGE. Bacterial cells grown overnight were embedded in agarose, lysed and deproteinated to isolate near intact genomic DNA. The DNA was digested with restriction endonuclease SmaI. The restriction fragments were separated by electrophoresis on the CHEF DR II (BioRad, Hercules, CA) under following conditions: 1% agarose, 0.5 X TBE, 200V and a switch interval ranging from 5-30 seconds over a 23 hour electrophoresis period. Ethidium bromide stained gels were examined visually. Isolates showing ≤3 bands differences were considered identical or similar and clonally related.

RESULTS

- Antimicrobial susceptibility profiles of all *Acinetobacter* spp. isolates are listed in Table 1. The most active compounds overall were: polymyxin B (99.2% susceptible), tigecycline (98.9% susceptible), imipenem (51.3% susceptible), meropenem (51.0% susceptible) and tobramycin (42.4% susceptible).

- Overall imipenem susceptibility rates decreased from 80.4% in 2000 to only 29.8% in 2006 (Table 2). Susceptibility to meropenem followed a similar trend. The dramatic decrease in carbapenem susceptibility rates in 2006 was attributed to clonal dissemination of OXA-type-producing strains.

- Thirty nine of 57 (68.4%) *Acinetobacter* spp. strains collected in 2006 were resistant to carbapenems with high rates of cross-resistance to all other antimicrobials tested, except polymyxin B (100.0% susceptible) and tigecycline (100.0% inhibited at ≤2 mg/L). This collection (n=39) consisted of isolates from bloodstream infections (53.8%) and from patients hospitalized with pneumonia (46.2%).

- All carbapenem-resistant *Acinetobacter* spp. strains were identified as *Acinetobacter baumannii* based on positive PCR result for bla_{OXA-51}.

- All 13 carbapenem resistant strains from Ankara were PCR-positive for bla_{OXA-58} and showed identical/similar PFGE patterns (Table 3).

- Twenty five of 26 carbapenem resistant strains from Istanbul had PCR positive results for bla_{OXA-23}, while one isolate yielded PCR product with bla_{OXA-58} primers (Table 3).

- All bla_{OXA-23}-positive strains exhibited identical/similar PFGE patterns, while the one with bla_{OXA-58}-positive PCR result showed different PFGE pattern. PFGE pattern of this isolate was also distinct from the bla_{OXA-58}-positive isolates from Ankara (Table 3).

- Metallo-ß-lactamases and the ESBL PER-1 were not detected in any of the strains tested.

Table 1. Antibiotic susceptibility patterns of *Acinetobacter* spp. isolates from medical centers in Turkey submitted to the SENTRY Program (2000-2006).

	All sites, all years (n=303) MIC (mg/L)			% susceptible[b] by medical center in 2006 (no. tested)[a]	
Antimicrobial agent	50%	90%	% susceptible[b]	Ankara (n=25)	Istanbul (n=32)
Imipenem	4	>8	51.3	48.0	15.6
Meropenem	4	>16	51.0	48.0	15.6
Ampicillin/sulbactam	32	>32	24.3	20.0	6.3
Piperacillin/tazobactam	>64	>64	21.0	16.0	6.3
Aztreonam	>16	>16	6.9	8.0	3.1
Ciprofloxacin	4	>4	29.7	16.0	6.3
Levofloxacin	4	>4	32.6	20.0	6.3
Gentamicin	>8	>8	25.1	12.0	3.1
Tobramycin	16	>16	42.4	36.0	15.6
Tetracycline	>8	>8	34.5	24.0	56.3
Trimethoprim/sulfamethoxazole	>2	>2	39.5	12.0	21.9
PolymyxinB	≤1	≤1	99.2	100.0	100.0
Tigecycline[c]	0.5	2	98.9	100.0	100.0

a. Isolates collected in 2006 only.
b. Susceptibility criteria were those of CLSI (M100-S17; 2007).
c. US-FDA susceptible breakpoint (≤2 mg/L) approved for some Enterobacteriaceae species was used for comparison purposes only.

Table 2. Carbapenem susceptibility of *Acinetobacter* spp. isolated from medical centers in Turkey (SENTRY Program, 2000-2006).

	% susceptibility by year (no tested):						
Antimicrobial agent	2000 (46)	2001 (19)	2002 (9)	2003 (41)	2004 (68)	2005 (63)	2006 (57)
Imipenem	80.4	68.4	66.7	48.8	41.2	55.6	29.8
Meropenem	71.7	73.7	66.7	48.8	42.6	57.1	29.8

Table 3. Summary of molecular and epidemiological characterization of carbapenem resistant *Acinetobacter baumannii* isolates from Turkey.

Medical center location (no. isolates)	Carbapenemase detected (no. isolates)	PFGE patterns[a] (no. isolates)
Ankara (13)	OXA-58 (13)	A1 (6), A2 (7)
Istanbul (26)	OXA-23 (25)	B (7), B1 (5), B2 (13)
	OXA-58 (1)	C (1)

a. Capital letter designation defines the PFGE patterns; subtypes are identified by the number following the capital letter.

CONCLUSIONS

- Polymyxin B and tigecycline were the only antimicrobials with reasonable in vitro activity against *Acinetobacter* spp. isolated in the Turkish medical centers evaluated.

- Clonal dissemination of OXA-carbapenemase producing strains caused a significant increase in the prevalence of carbapenem-resistant *Acinetobacter* spp. in the Turkish medical centers evaluated by the SENTRY Program in 2006.

- Carbapenem resistance was found to be largely driven by OXA-type carbapenemases, OXA-58 being prevalent in Ankara and OXA-23 in Istanbul, both of which have become prevalent in Europe.

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