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In Vitro Activity of Tigecycline Tested against *Acinetobacter* spp. Producing Carbapenem-Hydrolyzing Oxacillinases: a Global Study of 320 Strains

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ABSTRACT

Background: *Acinetobacter* spp. are frequently involved in nosocomial infections and are increasingly recognized as carbapenem-resistant. We evaluated the activity of tigecycline and other broad-spectrum agents against clinical strains of multidrug-resistant, carbapenem-hydrolyzing oxacillinase-producing *Acinetobacter* spp.

Methods: 320 carbapenem-hydrolyzing oxacillinase-producing *Acinetobacter* spp. were collected in 2004-2007 in 19 countries through various surveillance programs and centrally processed using CLSI broth microdilution methods and interpretive criteria. Tigecycline breakpoints established by the USA-FDA for Enterobacteriaceae (≤2 and ≥8 µg/ml for susceptible and resistant) were used for comparison purposes only. The presence of oxacillinase genes was confirmed by PCR using primers targeting bla_{OXA-23}-, $bla_{OXA24/40}$- and bla_{OXA-58}-clusters, followed by sequencing.

Results: Isolates were from the Asia-Pacific region (36.1%), Latin America (30.2%), Europe (28.0%), USA (3.7%) and South Africa (1.9%). The sites of infection were mainly bacteremia (47.8%) and pneumonia (40.8%). bla_{OXA-23} was observed in 233 strains (72.8% of total) from APAC (94), Latin America (82), Europe (51) and South Africa (6); $bla_{OXA-24/40}$ in 14 strains (4.4%) from USA (6), Asia-Pacific (4), Latin America (3) and Europe (1) and bla_{OXA-58} in 56 strains (17.5%) from Europe (38), Latin America (9), USA (6) and Asia-Pacific (3). Thirteen, one and three strains co-produced OXA-23 and OXA-58, OXA-24/40 and OXA-58, and OXA-23, OXA-24/40 and OXA-58, respectively. Tigecycline was the only compound showing consistent in vitro activity against carbapenem-hydrolyzing oxacillinase-producing *Acinetobacter* spp.

Antimicrobial	MIC_{50}	MIC_{90}	% susceptible	% resistant
Tigecycline	1	2	92.0[a]	0.6[a]
Amikacin	>32	>32	6.9	87.8
Tobramycin	>16	>16	20.3	77.8
Levofloxacin	>4	>4	1.6	86.6
Ciprofloxacin	>4	>4	0.6	99.1
Ampicillin/sulbactam	>16	>16	1.2	81.2
Imipenem	>8	>8	0.3	98.8

a. USA-FDA breakpoints for Enterobacteriaceae were applied for comparison purposes only.

Conclusions: Few therapeutic options remain available to treat infections caused by multidrug-resistant carbapenem-hydrolyzing oxacillinase-producing *Acinetobacter* spp. The role of tigecycline in the treatment of infections caused by these microorganisms warrants further evaluation due to sustained in vitro activity against this pathogen.

INTRODUCTION

The genus *Acinetobacter* has undergone significant taxonomic modification over the last years and its most important representative, *Acinetobacter baumannii*, has emerged as one of the most troublesome pathogens for health care institutions globally. *Acinetobacter* spp. has been frequently involved in nosocomial infections probably due to their ability to colonize and survive in human skin and dry surfaces. This opportunistic pathogen commonly targets the most vulnerable hospitalized patients, including immunocompromised patients and those with serious underlying diseases or subjected to invasive procedures.

Acinetobacter spp., in particular *A. baumannii*, has become increasingly resistant to clinically available antimicrobial agents. This susceptibility profile change is attributed to its ability to acquire or upregulate resistance determinants, including ß-lactamases that inactivate carbapenems. The objective of this study was to evaluate the activity of tigecycline and other broad-spectrum agents tested against clinical strains of multidrug-resistant (MDR) *Acinetobacter* spp. that harbored acquired carbapenem-hydrolyzing Ambler class D ß-lactamase-encoding genes (oxacillinases; OXA).

MATERIALS AND METHODS

Bacterial isolates. A total of 320 carbapenem-non-susceptible OXA-producing *Acinetobacter* spp. clinical isolates collected during 2004-2007 from 19 countries through several surveillance programs were evaluated. In addition, randomly selected OXA-negative carbapenem-non-susceptible (69) and carbapenem-susceptible (446) *Acinetobacter* spp. isolated during the same time period and in the same medical centers as the OXA-producing isolates were included in the study.

The isolates were collected from hospitalized patients and only one isolate per patient was selected. Species identification was performed by standard biochemical tests and use of the Vitek System (bioMerieux, Hazelwood, MO), when necessary. Additionally, *A. baumannii* species identification was confirmed by the presence of $bla_{OXA-51/69}$-like. Those species other than *A. baumannii* showing negative PCR results for $bla_{OXA-51/69}$-like were identified by 16S ribosomal RNA gene sequencing.

Molecular characterization of OXA genes. *Acinetobacter* spp. strains resistant to imipenem or meropenem (MIC, > 8 µg/ml) were screened for OXA genes. Results were obtained by PCR using primers able to amplify alleles encoding for three subgroups of acquired oxacillinase genes (bla_{OXA-23}-, $bla_{OXA-24/40}$- and bla_{OXA-58}-like) and the intrinsic subgroup of $bla_{OXA-51/69}$-like in a multiplex PCR assay format. Amplicons obtained were sequenced on both strands and nucleotide sequences analyzed with Lasergene software package (DNASTAR, Madison, WI) and compared with the sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast).

Antimicrobial susceptibility testing. Isolates were tested for susceptibility by the broth microdilution method following Clinical and Laboratory Standards Institute recommendations (CLSI; M7-A7, 2006). Cation-adjusted Mueller-Hinton broth was used in validated panels manufactured by TREK Diagnostics (Cleveland, OH). MIC values were interpreted according to the M100-S18 document (CLSI, 2008) for *Acinetobacter* spp., except for tigecycline MIC results, which were interpreted according to the Enterobacteriaceae breakpoints approved by the United States Food and Drug Administration (USA-FDA; ≤2 and ≥8 µg/ml for susceptibility and resistance, respectively).

Quality control (QC) was performed using *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *Pseudomonas aeruginosa* ATCC 27853. All QC results were within ranges as published in CLSI documents.

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RESULTS

- The OXA-producing isolates were recovered from hospitalized patients in medical sites located in the Asia-Pacific region (36.1%), Latin America (30.2%), Europe (28.0%), USA (3.7%) and South Africa (1.9%). The sites of infection were mainly blood stream (47.8%) and lower respiratory tract (40.8%).

Table 1. Oxacillinase-producing *Acinetobacter* spp. isolates included in this study according to geographic region.

	No. of strains					
Oxacillinase	Asia Pacific	Europe	North America	Latin America	South Africa	Total
OXA-23	94	51		82	6	233
OXA-24/40	4	1	6	3		14
OXA-58	3	38	6	9		56
OXA-23 and OXA-58	11			2		13
OXA-24/40 and OXA-58	1					1
OXA-23, OXA-24/40 and OXA-58	3					3
Total	116	90	12	96	6	320

Table 2. In vitro activity of selected antimicrobial agents tested against a worldwide collection of OXA-producing *Acinetobacter* spp. Carbapenem-susceptible and carbapenem-non-susceptible OXA-negative isolates were included for comparison purposes.

	Carbapenem-susceptible (446)[a]			Carbapenem-non-susceptible					
				OXA-negative (69)[a]			OXA-positive (320)		
Antimicrobial agent	MIC_{50}	MIC_{90}	% susceptible[b]	MIC_{50}	MIC_{90}	% susceptible[b]	MIC_{50}	MIC_{90}	% susceptible[b]
Tigecycline	0.25	1	99.6	1	1	98.6	1	2	92.0
Tetracycline	4	>8	53.4	>8	>8	4.3	>8	>8	16.9
Ceftazidime	>16	>16	39.5	>16	>16	0.0	>16	>16	2.2
Ampicillin/sulbactam	8	>16	54.6	>16	>16	11.6	>16	>16	1.3
Gentamicin	>8	>8	40.1	>8	>8	1.4	>8	>8	8.1
Tobramycin	1	>16	67.0	>16	>16	14.5	>16	>16	20.3
Amikacin	8	>32	53.6	>32	>32	13.0	>32	>32	6.9
Levofloxacin	4	>4	41.7	>4	>4	0.0	>4	>4	1.6
Polymyxin B	≤0.5	≤0.5	100.0	≤0.5	1	100.0	≤0.5	≤0.5	100.0

a. Carbapenem-susceptible and carbapenem-non-susceptible OXA-negative *Acinetobacter* spp. strains were isolated during the same time period and in the same medical centers as the OXA-producing isolates.
b. Tigecycline MIC results were interpreted according to the Enterobacteriaceae breakpoints approved by the USA-FDA (≤2 and ≥8 µg/ml for susceptibility and resistance, respectively).

Table 3. MIC values distribution for tigecycline against selected carbapenem-susceptible, carbapenem-non-susceptible OXA-negative and OXA-positive *Acinetobacter* spp. clinical isolates.

	No. of isolates (cumulative %) inhibited at tigecycline MIC (µg/ml) of						
Group of isolates	0.06	0.12	0.25	0.5	1	2	4
Carbapenem-susceptible[a] (446)	22 (5.1)	75 (21.7)	87 (41.3)	99 (63.5)	128 (92.2)	33 (99.6)	1 (99.8)
Carbapenem-non-susceptible							
OXA-negative[a] (69)			2 (2.9)	25 (39.1)	36 (91.3)	5 (98.6)	0 (98.6)
OXA-23[b] (233)	1 (0.4)	7 (3.4)	23 (13.3)	85 (49.8)	72 (80.7)	24 (91.0)	20 (99.6)
OXA-58[b] (56)		1 (1.8)	0 (1.8)	13 (25.0)	27 (73.2)	14 (98.2)	0 (98.2)
OXA-24/40[b] (14)			2 (14.3)	3 (35.7)	5 (71.4)	3 (92.9)	1 (100.0)

a. Carbapenem-susceptible and carbapenem-non-susceptible OXA-negative *Acinetobacter* spp. strains were isolated during the same period and in the same medical centers as the OXA-producing isolates.
b. Isolates harboring more than one bla_{OXA} gene were excluded from this analysis.

- The vast majority of isolates (99.0%) were identified as *A. baumannii* based on positive PCR results for $bla_{OXA-51/69}$-like. Two isolates were identified as *A. lwoffii* (Spain and India) and one isolate as *A. junii* (Japan).
- bla_{OXA-23} was the most common acquired OXA gene observed in the study (233 strains; 72.8% of total). OXA-23-producing strains were isolated in the Asia-Pacific region (94 strains; 40.3%), Latin America (82; 35.2%) and Europe (51; 21.9%; Table 1).
- bla_{OXA-58} was the second most common acquired OXA gene (56 strains; 17.5% of total) and OXA-58 producing strains predominated in Europe (38 strains; 67.9%) and Latin America (9 strains; 16.1%), while $bla_{OXA-24/40}$ was present in 14 strains (4.4%). Thirteen, one and three strains co-produced OXA-23 and OXA-58, OXA-24/40 and OXA-58, and OXA-23, OXA-24/40 and OXA-58, respectively (Table 1).
- OXA-producing strains showed high rates of resistance to all antimicrobial agents tested, except tigecycline (MIC_{50}, 1 µg/ml; MIC_{90}, 2 µg/ml; 92.0% inhibited at ≤2 µg/ml) and polymyxin B (MIC_{50} and MIC_{90}, ≤0.5 µg/ml; 100.0% susceptible). Besides these two compounds, the most active agent was tobramycin with only 20.3% susceptibility; Table 2).
- Tigecycline inhibited 99.6% of carbapenem-susceptible strains, 98.6% of carbapenem-non-susceptible OXA-negative strains and 92.0% of OXA-positive strains, at MIC of ≤2 µg/ml (Table 2).
- Although tigecycline was highly active against all OXA producer groups (>90.0% inhibited at ≤2 µg/ml), OXA-24/40 and OXA-23 producers showed tigecycline MIC results slightly lower than OXA-58 producers.

CONCLUSIONS

- Tigecycline was very active against a large collection of OXA-producing *Acinetobacter* spp. strains isolated worldwide.
- Few therapeutic options remain available to treat infections caused by multidrug-resistant carbapenem-hydrolyzing oxacillinase-producing *Acinetobacter* spp.
- OXA-producing isolates were mostly *A. baumannii*, which confirms this species as the main pathogen within the *Acinetobacter* genus, and emphasizes its remarkable ability to develop antimicrobial resistance.
- The role of tigecycline in the treatment of infections caused by these microorganisms warrants further evaluation due to sustained in vitro activity against this pathogen.