Adquired Carbapenem Hydrolyzing β-Lactamases among Clinical Strains of *Acinetobacter* spp. from Latin America: Report from the SENTRY Antimicrobial Program

Ana C. Gales, Rodrigo E. Mendes, Fernanda M. Castrucci, Rodrigo Cayô, Ronald N. Jones, Hélio S. Sader

Infectious Diseases Division, Department of Medicine, Universidade Federal de São Paulo, (UNIFESP/E PM), São Paulo, Brazil.
JMI Laboratories, North Liberty, Iowa, USA.

Poster C2-3856
ICAAC/IDSA 2008

Ana Cristina Gales
Laboratório ALERTA
Division of Infectious Disease
Universidade Federal de São Paulo
São Paulo - SP - Brazil
Phone: ++ 55 11 5084-6538
ana.gales@gmail.com

Abstract

Background: *Acinetobacter* spp. is an important cause of hospital infections in Latin America, where high carbapenem resistance rates have been reported. Carbapenem- hydrolyzing Class D β-lactamases (class D carbapenemases) represent the main mechanism of carbapenem resistance in *Acinetobacter*spp.. We evaluated the frequency of class D carbapenemases encoding genes in *Acinetobacter* spp. isolated from Latin America medical centers (MC). **Methods:** 288 *Acinetobacter* spp. were collected from 10 MC in 4 countries in 2007. The isolates were susceptibility tested by CLSI broth microdilution methods and interpr etative criteria. *Acinetobacter* spp. isolates showing MICs > 8 µg/ml for imipenem and meropenem were screened for class D- and class B (MβL)-encoding genes by PCR followed by sequencing. Clonality among class D carbapenemases-producing isolates was accessed by PFGE. **Results:**105 of 288 (36.4%; 9 MC) *Acinetobacter* spp. met the screening criteria and 91 (86.6%; 9 MC) carried class D carbapenemases. The isolates were mainly isolated from blood (52.4%) and respiratory tract (35.3%). bla_{OXA-23} was detected in 82 (78.1%) of carbapenem-resistant *Acinetobacter* spp. collected from Brazilian (51/66), Argentinean (30/32), and Chilean (1/6) MC, while bla_{OXA-58} was identified in 9 (8.6%) strains from Argentinean (4/32) a nd Chilean (5/6) MC. Isolates possessing both $bla_{OXA-23}$and bla_{OXA-58}were observed in Argentinean (2 unrelated isolates) MC and a single clone was noted to carry bla_{OXA-23} or bla_{OXA-58} in one Chilean (2 isolates) MC. MβL genes were not detected. Polymyxin B (MIC90, ≤ 0.5 µg/ml; 100.0% susceptible) showed the highest activity against *Acinetobacter*spp., followed by minocycline (MIC90, 1 µg/ml; 96.2 % susceptible). The spread of a predominant PFGE pattern was observed among the *Acinetobacter* spp. carrying bla_{OXA-23} from one Brazilian MC. **Conclusions:** Class D carbapenemase-producing *Acinetobacter*spp. was identified in 9 of 10 Latin American SENTRY sites. High frequency of bla_{OXA-23} was mainly due to the spread of an epidemic clone in one Brazilian MC. The occurrence of isolat es carrying bla_{OXA-23} and bla_{OXA-58} confirms the ability of *Acinetobacter*spp. to accumulate additional mechanisms of resistance.

Introduction

Acinetobacter spp. is an important cause of nosocomial-acquired infections in Latin America, and carbapenems constitute the main therapeutic option for treatment of serious *Acinetobacter* spp. Infections. However, carbapenem-resistant *Acinetobacter*spp. emerged and rapidly disseminated in Latin American Hospitals, imposing a serious therapeutic challenge. In fact, high rates of pan-resi stant (susceptible only to polymyxins) *Acinetobacter* spp. Have been observed among Latin American hospitals for more than one decade.

Among *Acinetobacter* spp. isolates, resistance t o carbapenems has been mainly associated with acquisition of carbapenem hydrolyzing class B (metallo- β-lactamases; MβL) or class D βlactamases enzymes since naturally occurring AmpC βlactamase and class D OXA-51/69 variants have a little impact on susceptibility to carbapenems. The main objective of this study was to evaluate the frequency of classes B and D carbapenemase- encoding genes among *Acinetobacter*spp. isolates collected from Latin American medical centers.

Material & Methods

Bacterial Strains. A total of 288 *Acinetobacter* spp. isolates were collected from ten Latin American medical centers during the year of 2007. The participant medical centers were located in nine cities of four countries: São Paulo, Brasília, Florianópolis and Porto Alegre in Brazil, Bueno Aires and San Isidro in Argentina, Santiago in Chile (two sites), Guadalajara and Durango in Mexico. The isolates were collected from diverse body sites of infection. Only a single isolate per patient was evaluated. All isolates were identified at the participating institution by routine methodologies in use at each laboratory.

Susceptibility testing. Isolates were centrally tested for susceptibility using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (CLSI; M7-A7, 2006). Cation-adjusted Mueller-Hinton broth was used n validated panels manufactured by TREK Diagnostics (Cleveland, OH). MIC values were interpreted according to the M100-S18 document (2008) for *Acinetobacter* spp., except for tigecycline MIC results that were interpreted according to the *Enterobacteriaceae* breakpoints approved by the United States Food and Drug Administration (USA-FDA; ≤2 and ≥8 g/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 the published ranges.

Detection of class D and class B carbapenemase-encoding genes. *Acinetobacter* spp. isolates showing MIC values >8 g/ml for imipenem and meropenem were screened for acquired carbapenem-hydrolyzing class D- and class B-encoding genes . Multiplex PCR assay was used to detect four groups of class D carbapenemase genes, including bla_{OXA-25}like, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$, and bla_{OXA-58}like genes as described before (Woodford, 2006). MβL screening was performed using generic primers able to detectVIM- and IMP-like, SPM-1-, GIM-1- and SIM-1-encoding genes in a multiplex real-time platform. (Mendes, 2007). DNA sequencing was performed by direct sequencing with an ABI Prism 3100 genetic analyzer (Applied Byosistems, Foster City, CA, USA). Similarity searches and alignments for both nucleotide and predicted protein sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAS T).

Material & Methods

Genetic relatedness. Clonality among class D carbapenemase-producing *Acinetobacter*spp. isolates was accessed by pulsed field gel electrophoresis (PFGE). Apal-digested genomic DNA were separated on a CHEF-DRIII system (BioRad, California, USA) for 23 h at 14◦C with 5 to 20 s of linear ramping at 6V/cm. The PFGE pattern was designated ba sed on the number of the medical center followed by a capital letter (A, B, C). Isolates were assigned with the same PFGE pa ttern when all bands matched. When 2-6 band differences were observed, isolates were assigned as a sub-type or variant of the major type, which was designated with the same capital letter followed by an Arabic number (Example: C1, C2, C3), as reco mmended by Tenover *et. al.* In addition, one representative isolate belonging to the previously described epidemic clone dissem inated in the Southern region of Brazil (Curitiba, Paraná) was utilized for comparison purposes.

Results

● Polymyxin B (MIC_{90} ≤ 0.5 µg/ml; 100.0% susceptible) was the most active compound tested against *Acinetobacter* spp. Followed by minocycline (MIC_{90} 2 µg/ml; 93.1% susceptible; Table 1).

Table 1. In vitro activity of selected antimicrobial agents tested against a collection of *Acinetobacter* spp. isolated from Latin America during the 2007 SENTRY Program.

Antimicrobial agent	*Acinetobacter* spp. (288)		
	MIC_{50}	MIC_{90}	% susceptible[a]
Ampicillin/sulbactam	16	> 16	29.9
Ceftazidime	16	> 16	20.1
Cefepime	16	> 16	24.7
Imipenem	2	> 8	58.0
Meropenem	2	> 8	56.3
Amikacin	> 32	> 32	27.8
Gentamicin	> 8	> 8	26.4
Tobramycin	8	>16	47.8
Levofloxacin	> 4	>4	19.1
Polymyxin B	≤ 0.5	≤ 0.5	100.0
Tetracycline	8	> 8	42.7
Minocycline	0.5	1	96.2
Tigecycline	0.5	2	93.1

a. Tigecycline MIC results were interpreted according to the Enterobacteriaceae breakpoints approvedby the USA-FDA (≤2 and ≥8 g/ml for susceptibility and resistance, respectively).

●Overall, among the 288 *Acinetobacter* spp. collected, 105 (36.4%) strains exhibited MIC values >8 g/ml for imipenem and meropenem and met the screening criteria for carbapenemase production. Carbapenem resistance was highest in Argentina (60.4%), followed by Brazil (44.0%), Chile (24.0%) and Mexico (3.3%). These isolates were mainly collected from blood (52.4%) and respiratory tract (35.3%).

●No MβL-encoding genes were detected among the carbapenem-resistant*Acinetobacter* spp. isolates studied. However, 91 of 105 (86.6%) carbapenem-resistant *Acinetobacter* spp. isolates carried genes encoding for class D carbapenemases (Table 2).

●bla_{OXA-51} was identified in all 105 carbapenem-resistant Acinetobacter spp. isolates, indicating that these isolates are presumably*A. baumannii* .

●bla_{OXA-23} was the most frequent class D carbapenemase-encoding gene being detected in 82 of 105 (78.1%) carbapenem-resistant *Acinetobacter* spp. isolates. *Acinetobacter* spp. carryingbla_{OXA-23} were collected from Brazilian (51 isolates), Argentinean (30 isolates), and Chilean (1 isolate) medical centers (Table 2).

●Two *Acinetobacter* spp. strains isolated from an Argentinean medical center had bothbla_{OXA-23} and bla_{OXA-58}. These strains were genetically unrelated.

Results

Table 2. Distribution of class D carbapenemase-enconding genes among 105 carbapenem-resistant *Acinetobacter* spp. isolates collected from Latin American medical centers (SENTRY Antimicrobial Surveillance Program, 2007).

OXA genes (N)	Medical Center (N)	Nation	Body Site of Infection (N)	PFGE Pattern
bla_{OXA-23}/bla_{OXA-51} (80)	39 (19), 40 (9), 42 (1), 46 (2)*, 48 (39), 57 (4)*, 101 (6)*	Argentina (28), Chile (1), Brazil (51)	Blood (41), Respiratory tract (29), Wound (10)	39C (3), 39D (1), 39E (4), 39F (5), 39G (1), 39H (1), 39I (1), 39J (3), 40B (4) 40E (2), 40C (1), 40F (2), 42A (1) 48A (28), 48B (6), 48B2 (1), 48B3 (1), 48B4 (1), 48C (3), 48D (1), 48E (1), 57B (1), 57C (1) 101C (1)
bla_{OXA-23}/ bla_{OXA-58}/bla_{OXA-51}(2)	40 (2)	Argentina (2)	Blood (2)	40A (1), 40B (1)
bla_{OXA24}/bla_{OXA-51}(2)	48 (1)*, 115 (1)	Brazil (1), Mexico (1)	Blood (2)	115A (1)
bla_{OXA58}/bla_{OXA-51}(7)	39 (1), 40 (1), 42 (3), 43 (2)	Argentina (2), Chile (5)	Blood (3), Respiratory tract (3), Wound (1)	39C (1), 40D (1), 42A (1) 42B (2), 43A(1), 43B (1)

* Strains not available for typing. Only a single carbapenem-resistant *Acinetobacter*spp. isolated from medical centers 57 and 101were typed by PFGE. Colored PFGE patterns represent local clones harboring different class D carbapenemase-encoding genes.

● Seven carbapenem-resistant *Acinetobacter* spp. carried bla_{OXA-58}. These strains were from Argentinean (4 isolates) and Chilean (five isolates) medical centers. Clonal dissemination of carbapenem-resistant *Acinetobacter*spp. strains was observed in a Chilean medical center (Table 2).

●The PFGE patterns 39C and 42A, were noted to carry bla_{OXA-23}or bla_{OXA-58}in Argentinean and Chilean (2 isolates) medical centers, respectively. In addition, the clone 40B collected from an Argentinean m edical center harbored both bla_{OXA-23} and bla_{OXA-58} .

●Twenty nine of 39 (74.4%) *Acinetobacter*spp. carrying bla_{OXA-23}isolated from a single Brazilian medical center exhibited an unique PFGE pattern (48A), which was related to that displayed by the epidemic OXA-23-producing *Acinetobacter* spp. strain previously reported by Dalla-Costa *et al* (2003) in two distinct hospitals lo cated in Curitiba, Paraná, Brazil (data not shown).

Conclusions

●Class D carbapenemase genes were highly frequent (86.6%) among carbapenem-resistant *Acinetobacter* spp. isolates collected from Latin American medical centers participating in the SENTRY Program.

●The high frequency of bla_{OXA-23} was mainly attributed to the spread of local clones within Brazilian and Argentinean medical centers. Furthermore, a great genetic diversity was observed among bla_{OXA-23} carrying *A. baumannii*, indicating horizontal dissemination of resistance determinants (plasmid DNA).

●The occurrence of isolates concomitantly carrying bla_{OXA-23} and bla_{OXA-58} emphasizes the ability of *Acinetobacter* spp. to accumulate additional mechanisms of resistance.

References

1. Clinical and Laboratory Standards Institute (2006). M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition. Wayne, PA: CLSI.

2. Clinical and Laboratory Standards Institute (2008). M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement. Wayne, PA: CLSI.

3. Dalla-Costa LM, Coelho JM, Souza HA, Castro ME, Stier CJ, Bragagnolo KL, Rea-Neto A, Penteado-Filho SR, Livermore DM, Woodford N. Outbreak of carbapenem-resistant *Acinetobacter baumannii* producing the OXA-23 enzyme in Curitiba, Brazil. J Clin Microbiol. 2003;41(7):3403-6.

4. Mendes RE, Kiyota KA, Monteiro J, Castanheira M, Andrade SS, Gales AC, Pignatari AC, Tufik S, Rapid detection and identification of metallo-beta-lactamase-encoding genes by multiplex real-time PCR assay and melt curve analysis. J C lin Microbiol. 2007;45(2):544-7.

5. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii* mechanisms and epidemiology. Clin Microbiol Infect . 2006;12(9):826-36.

6. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol. 1995 Sep;33(9):2233-9.

7. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii*by detection of th e *bla*OXA-51-like carbapenemase gene intrinsic to this species. J Clin Microbiol. 2006;44(8):2974-6.

8. Woodford N, Ellington MJ, Coel ho JM, Turton JF, Ward ME, Brown S, Amyes SG,Livermore DM. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. Int J Antimicrob Agents. 2006;27(4):351-3.