C2-3857
ICAAC/IDSA 2008
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
rodrigo-mendes@jmilabs.com

Rapid Emergence and Spread of *Acinetobacter* spp. Producing Carbapenem-Hydrolyzing Oxacillinases: Report from the SENTRY Program

RE MENDES, M CASTANHEIRA, L DESHPANDE, RN JONES
JMI Laboratories, North Liberty, IA, USA

SENTRY ANTIMICROBIAL SURVEILLANCE

ABSTRACT

Background: *Acinetobacter* spp. are Gram-negative pathogens associated with carbapenem resistance and occurrences of epidemic outbreaks. We evaluated the prevalence of carbapenem-hydrolyzing class D ß-lactamases (CHCDß) in a collection of *Acinetobacter* spp. from Europe and Americas collected during the 2007 SENTRY Program.

Methods: 766 *Acinetobacter* spp. from 16 countries were centrally processed using CLSI broth microdilution methods. Isolates showing meropenem and imipenem MIC at >8 µg/ml were further screened for CHCDß by PCR using primers targeting bla_{OXA-23}-, $bla_{OXA-24/40}$- and bla_{OXA-58}-clusters, followed by sequencing.

Results: Isolates were from Latin America (37.5%), Europe (33.2%) and USA (29.3%). 244 isolates (31.8%) were carbapenem-resistant, most commonly in Brazil (27.5%), USA (23.4%), Argentina and Turkey (13.1%), and Italy (12.3%). Among them, 207 (84.8%) harbored CHCDß, which were recovered from bacteremia (50.0%), pneumonia (33.6%) and skin and soft tissue infection (13.5%). These isolates were from Brazil (25.6%), USA (18.4%), Argentina and Turkey (15.5%), Italy (13.0%), Israel (7.2%), Chile (2.9%), Spain (1.4%) and Mexico (0.5%; Table). OXA-23 was most prevalent (60.4%), followed by OXA-58 and -24/40 (21.2 and 19.3%, respectively).

Country	Number of Medical centers/Total	CHCDß type OXA-23	OXA-24/40	OXA-58	% of total
Argentina	2/2	30		4	16.4
Brazil	4/4	52	1		25.6
Chile	2/2	1		5	2.9
Mexico	1/2		1		0.5
Israel	1/1		15		7.2
Italy	1/3	22		5	13.0
Spain	1/2			3	1.4
Turkey	2/2	6		26	15.4
USA	9/26	14	23	1	18.3

Conclusions: This SENTRY Program summary (2007) shows high association of carbapenem-resistance with CHCDß. These genes are known to be endemic in Argentina, Israel and Turkey; however, emergence was noted in Chile, Mexico and Italy (bla_{OXA-23}), and rapid dissemination in Brazil and USA. CHCDß-producing *Acinetobacter* spp. may become globally endemic due to their ability for exchanging foreign DNA, presenting a serious contemporary challenge for controlling dissemination of these carbapenem-resistance genes and/or isolates.

INTRODUCTION

Acinetobacter baumannii is recognized as an important hospital-acquired pathogen, mostly associated with patient-to-patient transmission, particularly in intensive care units. Published reports have described several worldwide occurrences of nosocomial outbreaks caused by *A. baumannii*, and more recently, these outbreaks also include those causing bloodstream infections, osteomyelitis and complicated skin and soft tissue infections in United States military and civilian personnel wounded during the war in the Middle East.

In the last decade, this pathogen has displayed escalating rates of resistance to several antimicrobial classes, and carbapenems have become the drug of choice in numerous cases. However, carbapenem-resistance phenotype has also been increasingly reported and mostly associated with carbapenem-hydrolyzing class D ß-lactamase-encoding genes (CHCDß). In this study, we evaluated the prevalence of CHCDß in a collection of *Acinetobacter* spp. isolated from Europe and the Americas collected during the 2007 SENTRY Antimicrobial Surveillance Program.

MATERIALS AND METHODS

Bacterial isolates. A total of 2,999 non-fermentative Gram-negative bacilli were collected during 2007 from 17 countries located in Europe and the Americas as part of the SENTRY Program. Among these isolates, 766 *Acinetobacter* spp. were recovered from hospitalized patients in 16 medical centers, only one isolate per patient was included in the study and species identification was performed by standard biochemical tests and use of the Vitek System (bioMerieux, Hazelwood, MO), when necessary.

Antimicrobial 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 in 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 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 the published ranges.

Screening for CHCDß genes. Isolates showing MIC values for imipenem and meropenem >8 µg/ml were screened using primers able to detect and distinguish alleles encoding three subgroups of acquired CHCDß-encoding genes (bla_{OXA-23}-, $bla_{OXA-24/40}$- and bla_{OXA-58}-like) and the intrinsic subgroup of bla_{OXA-51}-like in a multiplex PCR assay format. Amplicons obtained were sequenced on both strands. The nucleotide sequences and deduced amino acid sequences were analyzed using Lasergene software package (DNASTAR, Madison, WI) and compared with the sequences available through the internet using BLAST (http://www.ncbi.nlm.nih.gov/blast/).

RESULTS

- *Acinetobacter* spp. isolates were collected from hospitalized patients in medical centers located in Latin America (37.5%), Europe (33.2%) and the USA (29.3%).
- Overall, only tigecycline and polymyxin B showed an acceptable spectrum of activity (susceptibility at >90.0%) against *Acinetobacter* spp. collected during the 2007 SENTRY Program (Table 1).

Table 1. In vitro activity of selected antimicrobial agents tested against a collection of *Acinetobacter* spp., carbapenem-resistant and CHCDß-producing isolates collected during the 2007 SENTRY Program.

Antimicrobial agent	*Acinetobacter* spp. (766) MIC50	MIC90	% susceptible[a]	Carbapenem-resistant (244) MIC50	MIC90	% susceptible[a]	CHCDß (207) MIC50	MIC90	% susceptible[a]
Ampicillin/sulbactam	16	>16	39.3	>16	>16	2.4	>16	>16	2.4
Ceftazidime	>16	>16	28.5	>16	>16	2.9	>16	>16	2.9
Cefepime	16	>16	32.5	>16	>16	2.9	>16	>16	2.9
Imipenem	2	>8	59.0	>8	>8	0.0	>8	>8	0.0
Meropenem	2	>8	57.4	>8	>8	0.0	>8	>8	0.0
Amikacin	32	>32	45.4	>32	>32	20.0	>32	>32	16.4
Gentamicin	>8	>8	36.4	>8	>8	12.2	>8	>8	12.6
Tobramycin	4	>16	54.3	>16	>16	30.2	>16	>16	29.5
Levofloxacin	>4	>4	54.3	>4	>4	2.0	>4	>4	1.9
Polymyxin B	≤0.5	≤0.5	99.5	≤0.5	≤0.5	100.0	≤0.5	≤0.5	100.0
Tetracycline	8	>8	40.6	>8	>8	17.6	>8	>8	16.4
Tigecycline	0.5	2	93.3	1	4	84.5	1	4	97.9

a. 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).

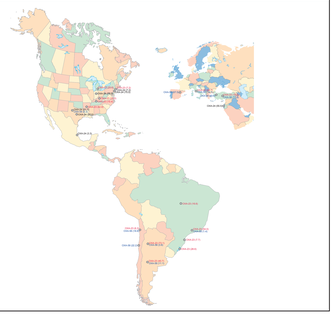
Table 2. Number of non-fermentative Gram-negative bacilli clinical isolates collected from medical centers located in Europe and the Americas, and number of carbapenem-resistant and CHCDß-producing *Acinetobacter* spp., and the respective CHCDß.

Continent/Country/State/City	No. of medical sites	No. of non-fermentative Gram-negative bacilli	No. of *Acinetobacter* spp. (%)	No. of carbapenem-resistant *Acinetobacter* spp. (%)	No. of CHCDß-producing (%)[a]	OXA 23[a]	OXA 24/40[a]	OXA 58[a]
North America								
United States								
002 - Indiana		38	12 (31.6)	9 (75.0)	9 (75.0)		9 (75.0)	
003 - Michigan		36	8 (22.0)	2 (25.0)	2 (25.0)	2 (25.0)		
024 - Texas		60	23 (38.3)	7 (30.4)	6 (26.1)		5 (21.7)	1 (4.3)
025 - Texas		45	4 (8.9)	1 (25.0)	1 (25.0)		1 (25.0)	
027 - Kentucky		38	15 (38.5)	3 (20.0)	3 (20.0)	3 (20.0)		
082 - New York		56	14 (25.0)	7 (50.0)	2 (14.3)	1 (7.2)	1 (7.2)	
107 - Kentucky		48	13 (27.1)	2 (15.4)	2 (15.4)	2 (15.4)		
117 - Arkansas		52	14 (26.9)	6 (42.8)	6 (42.9)	6 (42.8)		
129 - New Jersey		22	10 (45.6)	7 (70.0)	7 (70.0)		7 (70.0)	
Total	26	1053	225 (21.4)	58 (25.8)	38 (16.9)	14 (6.2)	23 (10.2)	1 (0.5)
Europe								
Belgium	1	23	4 (17.4)	0 (0.0)				
France	3	265	15 (5.6)	0 (0.0)				
Germany	3	135	7 (5.2)	0 (0.0)				
Ireland	2	76	11 (14.5)	0 (0.0)				
Israel	1	73	27 (36.9)	15 (55.5)	15 (55.5)		15 (55.5)	
Italy								
086 - Rome		81	34 (42.0)	28 (82.4)	27 (79.4)	22 (64.7)		5 (14.7)
Total	3	175	38 (21.7)	30 (78.9)	27 (71.0)	22 (57.9)		5 (13.2)
Poland	1	71	33 (46.5)	0 (0.0)				
Spain								
064 - Sevilla		43	8 (18.6)	3 (37.5)	3 (37.5)			3 (37.5)
Total	2	80	9 (11.3)	4 (44.5)	3 (30.0)			3 (30.0)
Sweden	2	43	7 (16.3)	0 (0.0)				
Turkey								
068 - Ankara		121	57 (47.1)	16 (28.1)	16 (28.1)	6 (10.5)		10 (17.5)
069 - Altunizade		81	40 (49.4)	16 (40.0)	16 (40.0)			16 (40.0)
Total	2	202	97 (48.0)	32 (33.0)	32 (33.0)	6 (6.2)		26 (26.8)
United Kingdom	2	42	5 (11.9)	0 (0.0)				
Switzerland	1	35	0 (0.0)	0 (0.0)				
South America								
Argentina								
039 - Buenos Aires		84	26 (30.1)	20 (76.9)	20 (76.9)	19 (73.1)		1 (3.8)
040 - Buenos Aires		66	27 (40.9)	12 (44.4)	12 (44.4)	11 (40.7)		3 (11.1)
Total	2	150	53 (35.3)	32 (60.4)	32 (60.4)	30 (56.6)		4 (7.5)
Brazil								
046 - Florianopolis		86	26 (30.2)	2 (7.7)	2 (7.0)	2 (7.7)		
048 - São Paulo		131	74 (56.5)	55 (74.3)	41 (55.4)	40 (54.0)	1 (1.4)	
057 - Porto Alegre		49	14 (28.6)	4 (28.6)	4 (28.6)	4 (28.6)		
101 - Brasilia		91	36 (39.5)	6 (16.6)	6 (16.6)	6 (16.6)		
Total	4	357	150 (42.0)	66 (44.0)	53 (35.3)	52 (34.6)	1 (0.7)	
Chile								
042 - Santiago		37	16 (43.2)	4 (25.0)	4 (25.0)	1 (6.3)		3 (18.8)
043 - Santiago		59	9 (15.2)	2 (22.2)	2 (22.2)			2 (22.2)
Total	2	96	25 (26.0)	6 (24.0)	6 (24.0)	1 (4.0)		5 (20.0)
Mexico								
115 - Guadalajara		69	30 (43.5)	1 (3.3)	1 (3.3)		1 (3.3)	
Total	2	123	60 (48.8)	1 (1.7)	1 (1.7)		1 (1.7)	
Total		2999	766 (25.5)	244 (31.8)	207 (27.0)	125 (16.3)	40 (5.2)	66 (8.6)

a. Number of CHCDß-producing isolates and percentage according to the total number of *Acinetobacter* spp. per medical center or country.

- Among *Acinetobacter* spp., 244 (31.8%) showed resistance phenotype toward carbapenems, which were mostly recovered from Italy (78.9%), Argentina (60.4%), Israel (55.5%), Spain (44.5%), Brazil (44.0%), Turkey (33.0%), USA (25.8%), and Chile (24.0%), see Table 2.
- *Acinetobacter* spp. isolates were not forwarded from Switzerland and strains from Poland, France, Ireland, Sweden, Germany, United Kingdom and Belgium did not exhibit carbapenem-resistance phenotype.
- Overall, CHCDß-producing isolates represented 27.0% of all *Acinetobacter* spp. obtained by the SENTRY Program coordinator laboratory in 2007. These isolates were recovered from bacteremia (50.0%), pneumonia (33.6%) or skin and skin structure infections (13.5%).
- High rates of CHCDß-producing strains were noted among *Acinetobacter* spp. in Italy, Argentina, and Israel (71.0, 60.4 and 55.5%; Table 2).
- Among carbapenem-resistant isolates, 207 (84.8%) harbored acquired CHCDß and these isolates were the majority of the carbapenem-resistant strains (>80.0%) in most countries, except in Spain and USA where these rates were 75.0% and 65.5%, respectively.
- CHCDß-producing *Acinetobacter* spp. were detected among nine USA medical sites located in Indiana, Michigan, Texas (2), Kentucky (2), New York, Arkansas and New Jersey (Figure 1).
- OXA-23 was the most prevalent CHCDß detected in *Acinetobacter* spp. (16.3%), followed by OXA-58 and -24/40 (8.6 and 5.2%, respectively).

Figure 1. Schematic representation of CHCDß-encoding genes distribution detected among *Acinetobacter* spp. recovered from 16 medical centers located in Europe and the Americas during the 2007 SENTRY Antimicrobial Surveillance Program. Values in parenthesis represent the percentage of CHCDß-producing isolates according to the number of *Acinetobacter* spp. recovered from the respective medical center during 2007.



CONCLUSIONS

- High rates of carbapenem resistance were observed in *Acinetobacter* spp. clinical isolates collected during the 2007 SENTRY Program, which were mostly associated with CHCDß genes (84.8% of carbapenem-resistant isolates).
- CHCDß-encoding genes are known to be endemic in Argentina, Israel and Turkey; however, emergence was also noted in Chile, Mexico and Italy (bla_{OXA-23}), and rapid dissemination of these determinants was observed in Brazil and USA.
- CHCDß-producing *Acinetobacter* spp. appears to be becoming globally endemic due to the ability for exchanging foreign DNA, presenting a serious contemporary challenge for controlling dissemination of carbapenem resistance genes and/or isolates.

SELECTED 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. Jones RN (2003). Global epidemiology of antimicrobial resistance among community-acquired and nosocomial pathogens: A five-year summary from the SENTRY Antimicrobial Surveillance Program (1997-2001). *Semin Respir Crit Care Med* 24: 121-134.
4. Peleg AY, Seifert H, Paterson DL (2008). *Acinetobacter baumannii*: Emergence of a successful pathogen. *Clin Microbiol Rev* 21: 538-582.
5. Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al (2006). Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter* spp. *Int J Antimicrob Agents* 27: 351-353.