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Outbreak Caused by a Clone of OXA-23- or OXA-58-Producing *Acinetobacter baumannii* in a Rome Hospital: First Outbreak of bla_{OXA-23}-carrying *A. baumannii* in Italy

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SENTRY ANTIMICROBIAL SURVEILLANCE

AMENDED ABSTRACT

Background: *Acinetobacter* spp. are an important opportunistic pathogen associated with increasing reports of carbapenem resistance, mainly due to carbapenem-hydrolyzing oxacillinases (CHCDß). We report an outbreak caused by OXA-23- or -58-producing *Acinetobacter* spp.

Methods: 34 *Acinetobacter* spp. were consecutively collected during 2007 at a single hospital in Rome and centrally processed using CLSI broth microdilution methods. Isolates showing MIC of ≥8 µg/ml for carbapenems were screened for CHCDß and MßL by PCR, followed by sequencing. Plasmid analysis and gene location were performed by Southern blot and hybridization. Clonality was evaluated by PFGE.

Results: 30 (88.2%) *Acinetobacter* spp. isolates met the screening criteria and were PCR-positive for bla_{OXA-23} (90.0%) or bla_{OXA-58} (10.0%). All isolates showed multidrug-resistance phenotype, remaining susceptible only to polymyxin B, colistin and tigecycline. Isolates were recovered from bacteremia (86.7%) or pneumonia (13.3%). The majority (66.6%) of the patients were in the ICU and 1 (3.3%) death was attributed to the infection (sepsis). Regardless of the CHCDß, 25 isolates were genetically related (type A) and the remaining isolates, including a carbapenem-susceptible strain, clustered within type B. No record of international travel was documented for the index cases. Among isolates belonging to clone A, bla_{OXA-23} was chromosomally- and plasmid-located, while bla_{OXA-23} or bla_{OXA-58} harbored by isolates belonging to clone B were plasmid-located.

Conclusions: This appears to be the first report of an outbreak due to OXA-23-producing *Acinetobacter* spp. in Italy. These isolates were responsible for a persistent outbreak mostly due to clonal dissemination. Although the attributable mortality rate was low, these findings emphasize the ability of this pathogen to spread, and to acquire and spread carbapenem-resistance genes present in the hospital environment.

INTRODUCTION

During the past two decades, several reports have described the occurrence of nosocomial outbreaks caused by *Acinetobacter* spp. The most clinically relevant species within this genus, *A. baumannii*, possesses the ability to survive for prolonged time periods throughout healthcare environments, colonizing either dry surfaces or human skin; thereby, contributing to the nosocomial spread of this microorganism.

A. baumannii has also been associated with increasing rates of carbapenem resistance, usually due to the acquisition of metallo-ß-lactamase- (MßL), and more usually carbapenem-hydrolyzing class D ß-lactamase-encoding genes (CHCDß). In this study, we investigated the dissemination and mechanisms of carbapenem resistance among *A. baumannii* recovered from patients hospitalized in different units of the Policlinic Agostino Gemelli – Università Cattolica del Sacro Cuore in Rome, Italy.

MATERIALS AND METHODS

Bacterial isolates. During 2007, a total of 34 non-repetitive *Acinetobacter* spp. (one per patient) were recovered from patients hospitalized in the Policlinic Agostino Gemelli (Rome, Italy). This 2,000-bed university hospital is one of the largest and more important medical institutions in Italy, treating more than 70,000 inpatients each year. Isolates were later forwarded to a central monitoring laboratory (JMI Laboratories, North Liberty, Iowa, USA) as part of the SENTRY Antimicrobial Surveillance Program.

Antimicrobial susceptibility testing. Isolates were 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 by the M100-S18 document (CLSI, 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 published ranges.

Screening for CHCDß- and MßL-encoding genes. Isolates showing MIC values for imipenem and meropenem at ≥8 µg/ml were screened using primers able to detect and distinguish three subgroups of acquired CHCDß-encoding genes (bla_{OXA-23}-, bla_{OXA-24}- and bla_{OXA-58}-like) and the intrinsic subgroup of bla_{OXA-51}-like in a multiplex PCR assay format. MßL screening was performed using generic primers able to detect VIM-, IMP-, SPM-1-, GIM-1-, SIM-1-like-encoding genes in a multiplex real-time platform. 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/).

Plasmid analysis and CHCDß-encoding gene location. Plasmid DNA was extracted using the Plasmid DNA Midi Kit (Qiagen GmbH, Hilden, Germany), separated on 1% agarose gel in TAE buffer on a Criterion Sub-cell GT system (Bio-Rad, Hercules, CA). Plasmid sizes were determined with GelCompar II software using plasmid bands from *E. coli* NCTC 50192 as standard references. Band position tolerance and optimization were set at 1.0% and 0.5%, respectively. Total DNA from clinical isolates was digested with I-Ceu-I and DNA fragments and plasmids were transferred onto a nylon membrane by Southern blot. Specific labeled probes for bla_{OXA-23}, bla_{OXA-58} and 16S rRNA were used for hybridization.

Molecular typing. Pulsed-field Gel electrophoresis (PFGE) of bacterial genomic DNA digested with ApaI followed by pattern analysis using the GelCompar II software (Applied Math, Kortrijk, Belgium) were performed to investigate clonality. Percent similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.5% and 0.5%, respectively, and isolates showing similarity coefficient ≥87% were considered as genetically related. One carbapenem-susceptible isolate recovered from the same institution during the study period and representative isolates of European clone I and II were also included in the pattern analysis for comparison purposes (see Figure 1).

RESULTS

- Thirty isolates showed resistance phenotype to imipenem, meropenem, ampicillin/sulbactam and tetracycline. The majority of isolates (80%) were also resistant to aminoglycosides. Only polymyxin B, colistin and tigecycline exhibited acceptable in vitro activity results (susceptibility, >90%; Table 1).
- These isolates were recovered from blood (87.1%) or skin and soft tissue (12.9%), mostly from patients hospitalized in intensive care units (60%; Table 2). Only one death was attributed to the infection and the majority of these patients were treated with colistin or tigecycline.
- PCR followed by sequencing detected bla_{OXA-23} and bla_{OXA-58}. MßL were not detected among the resistant isolates and both types of carbapenamases (CHCDß and MßL) were not detected in the susceptible isolate.
- PFGE results demonstrated the presence of two clones (A and B) harboring either bla_{OXA-23} or bla_{OXA-58}, and the imipenem-susceptible *A. baumannii* clustered within clone B (Figure 1). Representative isolates of European clones I and II did not match with either clone A or B.
- OXA-23-producing *A. baumannii* belonging to cluster A displayed four plasmid bands and bla_{OXA-23} probe hybridised with the 32-kb band (Table 2). This same cluster of isolates showed hybridization signals from the 487-kb chromosomal DNA bands. One isolate belonging to cluster A harboured bla_{OXA-58}, which was located in the 20-kb plasmid band.
- Among those *A. baumannii* belonging to cluster B, two isolates harboured bla_{OXA-23} and showed two plasmid bands (44- and 27-kb) and hybridization signals were obtained from the 44-kb band. Five isolates, also belonging to clone B harbouring bla_{OXA-58}, showed two plasmid bands (44- and 27-kb) and the CHCDß gene was located in the 44-kb plasmid band (Table 2). One OXA-58-producing strain belonging to clone B (subtype B1) exhibited multiple plasmid bands and bla_{OXA-58} hybridization signals were observed in the 49- and 22-kb bands.

Table 1. Activity of antimicrobial agents tested against CHCDß-producing *Acinetobacter* spp. recovered among units at the Polyclinic Agostino Gemelli (Rome, Italy) in 2007.

Antimicrobial agent	MIC_{50}	MIC_{90}	Range	% susceptible/resistant[a]
Ampicillin/sulbactam	>16	>16	16 - >16	0.0 / 90.0
Ceftazidime	>16	>16	4 - >16	3.3 / 93.3
Cefepime	>16	>16	16 - >16	0.0 / 96.7
Imipenem	>8	>8	>8	0.0 / 100.0
Meropenem	>8	>8	8 - >8	0.0 / 93.3
Levofloxacin	>4	>4	>4	0.0 / 100.0
Amikacin	>32	>32	2 - >32	20.0 / 80.0
Gentamicin	>8	>8	≤2 - >8	20.0 / 80.0
Tobramycin	>16	>16	0.5 - >16	20.0 / 80.0
Tetracycline	>8	>8	>8	0.0 / 96.7
Tigecycline	0.5	2	0.25 - 4	93.3 / 6.7
Colistin	≤0.5	≤0.5	≤0.5	100.0
Polymyxin B	≤0.5	≤0.5	≤0.5 - 1	100.0

a. According to CLSI M100-S18, except for tigecycline, which was determined according to the Enterobacteriaceae breakpoints approved by the USA-FDA (breakpoints for susceptibility, ≤2 µg/ml; breakpoints for resistance, ≥8 µg/ml).

Figure 1. PFGE profile of ApaI-digested genomic DNA from representative isolates belonging to the European clones I and II and those found in this study (A and B). Dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.5% and 0.5%, respectively. Isolates showing similarity coefficient ≥87% were considered as genetically related. Similarity coefficient, isolates, PFGE pattern and susceptibility phenotype are shown, as well as the *A. baumannii* COL 20820 reference strain and the Lambda ladder profiles for comparison purposes. NA reads "Not applicable".

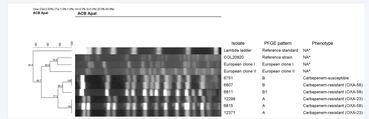

Table 2. Epidemiologic and molecular data from bla_{OXA}-carrying *A. baumannii* isolates evaluated in this study.

Isolate	Specimen[a]	Admission (2007)	Age	Sex	Culture date (2007)	Mortality[b]	ICU[c]	Primary diagnosis[d]	Service[e]	Code Service	PCR	CHCDß location[f]	PFGE pattern	Plasmid Profile (kb)[g]
12298	B	28-Apr	75	F	3-May	N	N	NEU	NS	144	OXA-23	P/C	A	**32**, 27, 13 and 10
12299	B	30-Apr	74	M	4-May	Y	Y	CAR	ICU	138	OXA-23	P/C	A	**32**, 27, 13 and 10
12302	B	2-May	23	M	7-May	N	Y	TRM	OTH	704	OXA-23	P/C	A	**32**, 27, 13 and 10
12319	B	30-May	73	F	4-Jun	N	Y	NEU	ICU	138	OXA-23	P/C	A	**32**, 27, 13 and 10
12335	B	16-Jun	67	M	20-Jun	N	Y	GI	ICU	138	OXA-23	P/C	A	**32**, 27, 13 and 10
12354	B	10-Jul	17	M	18-Jul	N	Y	NEU	NS	150	OXA-23	P/C	A	**32**, 27, 13 and 10
12371	B	12-Aug	65	M	18-Aug	N	Y	CAR	MED	113	OXA-23	P/C	A	**32**, 27, 13 and 10
12373	B	14-Aug	66	M	19-Aug	N	N	CAR	ICU	139	OXA-23	P/C	A	**32**, 27, 13 and 10
12383	B	31-Aug	70	M	7-Sep	N	N	PULM	MED	148	OXA-23	P/C	A	**32**, 27, 13 and 10
12402	B	26-Sep	58	F	2-Oct	N	N	TRM	ICU	182	OXA-23	P/C	A	**32**, 27, 13 and 10
12403	B	28-Sep	73	M	4-Oct	N	Y	NEU	ICU	138	OXA-23	P/C	A	**32**, 27, 13 and 10
12413	B	6-Oct	55	M	16-Oct	N	Y	TRM	OTH	163	OXA-23	P/C	A	**32**, 27, 13 and 10
12422	B	16-Oct	93	M	21-Oct	N	N	CAR	MED	101	OXA-23	P/C	A	**32**, 27, 13 and 10
12424	B	18-Oct	55	M	23-Oct	N	Y	TRM	ICU	139	OXA-23	P/C	A	**32**, 27, 13 and 10
12432	B	27-Oct	66	F	6-Nov	N	N	CAR	ICU	139	OXA-23	P/C	A	**32**, 27, 13 and 10
12441	B	10-Nov	80	M	16-Nov	N	N	CAR	MED	101	OXA-23	P/C	A	**32**, 27, 13 and 10
12456	B	18-Nov	68	M	26-Nov	N	Y	TRM	OTH	701	OXA-23	P/C	A	**32**, 27, 13 and 10
2851	S	5-Jan	46	M	24-Jan	N	N	CANCER	HEM	174	OXA-23	P/C	A	**32**, 27, 13 and 10
2871	S	5-Nov	50	F	29-Jan	N	Y	TRM	ICU	138	OXA-23	P/C	A	**32**, 27, 13 and 10
12384	B	29-Aug	80	F	8-Sep	N	N	CAR	ICU	139	OXA-23	P/C	A	**32**, 27, 13 and 10
12428	B	26-Oct	80	F	3-Nov	N	Y	NEU	OTH	106	OXA-23	P/C	A	**32**, 27, 13 and 10
6815	B	7-Apr	77	M	15-Apr	N	Y	PULM	ICU	138	OXA-58	P	A	**20**
2850	S	5-Nov	82	F	24-Jan	N	Y	NEU	ICU	138	OXA-23	P	B	**44** and 27
12439	B	5-Nov	80	F	15-Nov	N	Y	NEU	ICU	138	OXA-23	P	B	**44** and 27
2863	S	15-Jan	29	M	26-Jan	N	Y	TRM	ICU	138	OXA-58	P	B	**44** and 27
6807	B	3-Apr	70	F	10-Apr	N	Y	PULM	ICU	138	OXA-58	P	B	**44** and 27
12379	B	22-Aug	70	M	2-Sep	N	Y	PULM	OTH	702	OXA-58	P	B	**44** and 27
12397	B	10-Sep	50	M	15-Sep	N	Y	TRM	ICU	138	OXA-58	P	B	**44** and 27
12359	B	30-Jul	78	F	4-Aug	N	Y	NEU	ICU	138	OXA-58	P	B	**44** and 27
6811	B	5-Apr	75	F	11-Apr	N	Y	PULM	ICU	138	OXA-58	P	B1	**49**, 39, **22** and 14

a. Specimen. B – Blood; S – Skin and soft tissue.
b. Mortality related to infection.
c. Intensive care unit.
d. Primary diagnosis. NEU – Neurologic; CAR – Cardiovascular; TRM – Trauma; GI – Gastrointestinal; PULM – Pulmonary.
e. Service. NS – Neurosurgery; OTH – Other; CAR – Cardiothoracic/Pulmonary; MED - Internal Medicine; HEM - Hematology/Oncology; ICU - Intensive care unit.
f. CHCDß location. P – Plasmid; C – Chromosomal.
g. Plasmid bands showing hybridization signal with bla_{OXA}-specific probes are in bold.

CONCLUSIONS

- This study describes the dissemination of two complex carbapenem-resistant *A. baumannii* clusters among units in a large Italian hospital.
- Only polymyxins and tigecycline remained effective in vitro (Table 1).
- Several studies have reported the detection of OXA-58-producing *A. baumannii* in Italy, suggesting bla_{OXA-58}-carrying *A. baumannii* has become endemic; however, reports about bla_{OXA-23}- or bla_{OXA-24}-like genes are still limited in this country.
- The presence of multiple copies of CHCDß-encoding genes provides increased level of resistance among these clinical isolates.
- Although death related to infection rates were very low in this study (3.2%), these findings emphasize the ability of *A. baumannii* isolates to acquire and also spread different resistance determinants circulating in the hospital environment.

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