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Molecular typing of XDR *Acinetobacter baumannii* strains in an Italian ICU

Caratterizzazione molecolare di *Acinetobacter baumannii* isolati da pazienti ricoverati in un reparto di terapia intensiva

COMMUNICATIONS C4
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Abstract

Objective. To investigate the antimicrobial susceptibility and clonal relationship of *Acinetobacter baumannii* strains isolated in an Italian ICU.

Design. Epidemiological, observational, retrospective, longitudinal study.

Setting and participants. The ICU of the University Hospital of Sassari, Italy.

Main outcome measures. Pulsed Field Gel Electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST) were used to evaluate the genomic features of the isolated strains.

Results. Drug susceptibility testing for all isolated strains showed the same resistance pattern, characterized by resistance to the most important antibiotics, with the only exception of colistin. PFGE showed a very poor between-strain variability; three distinct clusters, 11, 4, and 1 isolates in size, were identified (Dice's coefficient: 92.11%). MLST showed that all isolated strains belonged to sequence type 2 (ST2). All isolates collected from the environment and the human samples were positive for the following genes: blaOXA-23, blaOXA-51-like, blaVIM-like, blaIMP-like, andISAba1; however, blaOXA-24-like, blaOXA-58-like, and blaNDM-like were not detected.

Conclusions. The survey identified XDR strains belonging to the same cell clone, confirming the wide circulation and environmental persistence of this microorganism.

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Key words: *Acinetobacter baumannii*, outbreak, PFGE, MLST

Riassunto

Obiettivo. Descrivere le caratteristiche feno- e genotipiche dei microrganismi di più frequente isolamento nei reparti dell'Azienda Ospedaliero-Universitaria di Sassari.

Disegno. Studio epidemiologico osservazionale, retrospettivo.

Setting e partecipanti. Unità di rianimazione e terapia intensiva, Ospedale universitario di Sassari, Italia.

Principali misure di outcome. Profilo fenotipico e genotipico dei ceppi di *Acinetobacter baumannii* isolati.

Risultati. I microrganismi isolati hanno evidenziato un profilo XDR con resistenza ai carbapenemi, ma sensibilità alla colistina. L'analisi di macrorestrizione ha evidenziato minime differenze tra i ceppi (coefficiente di Dice: 97,15%), facendo supporre una possibile discendenza clonale tra essi. I templati allelici ottenuti dall'analisi di sequenza hanno evidenziato l'appartenenza all'allele 2 per tutti geni considerati La combinazione allelica ottenuta corrisponde al ST2. Tutti gli stipiti hanno mostrato positività per i geni blaOXA23-like, blaVIM-like e blaIMP-like, ISAba1, mentre sono risultati negativi i test per la ricerca dei geni blaOXA24-like, blaOXA58-like e blaNDM-like.

Conclusioni. L'indagine condotta sugli stipiti isolati dai pazienti ricoverati nel reparto ha permesso di identificare ceppi MDR appartenenti allo stesso clone cellulare confermando l'ampia circolazione e la particolare persistenza ambientale di tale microrganismo.

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Parole chiave: *Acinetobacter baumannii*, *outbreak*, PFGE, MLST

INTRODUCTION

Acinetobacter baumannii is one of the most frequent opportunistic microorganisms detected in healthcare settings.[1] Clinical care of infections caused by *Acinetobacter baumannii* has been complicated by the emergence and spread of multi-drug resistant strains.[2] Morbidity and mortality can be relevant in some settings, particularly in intensive care units (ICUs), as a consequence of life-threatening infections, including ventilator-related pneumonia, sepsis, urinary tract infections, and skin and soft tissue disorders.[1,3-5]

Complicated drug resistance patterns have been recently described in nosocomial outbreaks where the isolates were resistant to all antibiotics with the only exception of polimixines.[6] Extensively drug-resistant (XDR) *Acinetobacter baumannii* infections, implying resistance to three or more antibiotic classes, can be deemed a serious public health issue worldwide because of the few current therapeutic options, high infection rates, and poor patient outcomes.[7]

Several Authors highlighted the crucial role of the *Acinetobacter baumannii* resistance to carbapenems, mediated by oxacillinases (OXA-class D) and, less frequently, by metallo-β-lactamases (MBL-class B).[8,9] In particular, five OXA subgroups are associated with *Acinetobacter baumannii* resistance: ^{bla}OXA-51-like, ^{bla}OXA-23-like, ^{bla}OXA-40-like, ^{bla}OXA-58-like, and ^{bla}OXA-143-like.[10] It was found that the insertion sequence ISAba1 can activate ^{bla}OXA-23-like and ^{bla}OXA-51-like genes.[11,12]

^{bla}OXA-58-like, ^{bla}OXA-23-like, and ^{bla}OXA-24-like are the most frequent genes detected in European isolates.[13]

The aim of this study was to investigate the antimicrobial susceptibility and clonal relationship of *Acinetobacter baumannii* strains isolated from human and environmental samples collected during a potential nosocomial outbreak in 2012-2013 in an Italian ICU, located in the University Hospital of Sassari, Italy.

MATERIAL AND METHODS

Setting

The study was carried out in an ICU located in a 500-bed tertiary university hospital in Italy. An epidemiological investigation was performed by the public health specialists of the same hospital after the collection of four multi-drug resistant *Acinetobacter baumannii* strains in a time-period of 10 days (November-December, 2012).

The total duration of the epidemiological surveillance was 10 months (November, 2012-August, 2013).

Biological samples were collected from the environment and several anatomical sites of the patients admitted during the above-mentioned period. The ICU included 10 beds, divided into two different types of room (i.e., single and triple bed). Environmental sampling was performed on circulating air, surfaces, and devices located in the rooms.

We carried out an epidemiological, observational, retrospective, longitudinal study in order to assess the epidemiological characteristics of a potential nosocomial outbreak.

Pulsed Field Gel Electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST) were used to evaluate the genomic features of the isolated strains.

Conventional microbiology

Nasal and broncho-alveolar lavage (BAL) samples were collected from the patients after notification of a potential outbreak. Drug susceptibility testing was carried out on the isolates obtained from solid culture media using the following antibiotics: aminoglycosides, carbapenems, fluoroquinolones, tetracyclines, penicillins, cephalosporins, and colistin (Vitek II, BioMerieux).

Pulsed field gel electrophoresis

PFGE was carried out according to the method recommended by ARPAC (Antibiotic Resistance Prevention and Control).[14] Bacteria were suspended in low melting point agarose disks; then, their DNA was extracted and purified. The agarose disks were cut using the ApaI restriction enzyme. DNA fragments were separated by agarose PFGE using a Clamped Homogeneous Electric Fields DRII SYSTEM (BIORAD). Gel images were analyzed by means of Image Master Program (Pharmacia). The identification of the position of the electrophoretic bands, as well as the definition of the phylogenetic dendrogram, was obtained with the GelCompar II software (Applied Maths).

Multi-locus sequence typing

MLST analysis was performed according to the Protocol of the Pasteur Institute.[15] Fragments of seven internal housekeeping genes (i.e., *cpn60, fusA, gltA, pyrG, recA, rplB, rpoB*) were amplified and sequenced. Sequence analysis was performed with the Bioedit software. Each sequence was compared with sequences deposited in the website of the Pasteur Institute to evaluate the «percentage of identity» and compatibility.

The presence of *Acinetobacter baumannii* genes encoding carbapenemases (blaOXA-23, blaOXA-24-like, blaOXA-51-like, blaOXA-58-like) and metallo-beta-lactamases (blaVIM-like, blaIMP-like, blaNDM-like) was assessed for all the collected isolates using Multiplex and single PCR, respectively.[16,17]

blaOXA51-like and OXA 23-like alleles were simultaneously genotyped together with the insertion sequence ISAba1 in order to evaluate the resistance to carbapenemases.[18-20]

Statistical analysis

An *ad hoc* electronic form was prepared to collect demographic, epidemiological, clinical, and microbiological variables. Frequencies (percentages) and medians and ranges were used to summarize qualitative and quantitative variables, respectively. Data were analyzed using STATA version 13 (StataCorp, College Station, Texas).

RESULTS

Nine patients (median [range] age: 72 [4-88] years; males [%]: 7/9 [77.8]) were admitted to the ICU of the university hospital of Sassari, Italy, between November 2012 and March 2013, and were found positive for *Acinetobacter baumannii*.

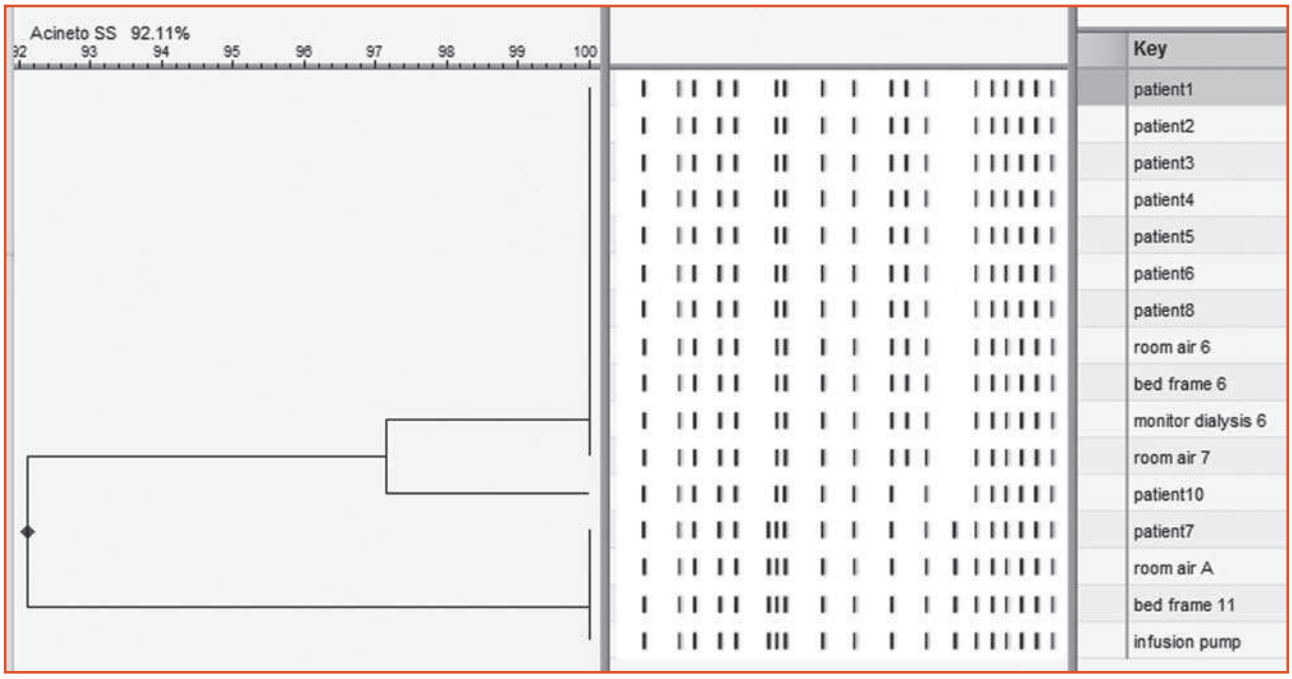

Figure 1. Dendrogram and electrophoretic bands of the isolated *Acinetobacter baumannii* strains.
Figura 1. Dendrogramma e profilo elettroforetico degli isolati di *Acinetobacter baumannii*.

In particular, drug susceptibility testing for all isolated strains showed the same resistance pattern, characterized by resistance to the most important antibiotics, with the only exception of colistin.

The median hospital stay was 58 days (range 20-163). Unfortunately, 4/9 (44.5%) died; 60-day mortality was 3/9 (33.3%). The majority of them (6/9, 66.7%) were admitted in a medical ward before their transfer to the ICU.

Furthermore, 7 XDR *Acinetobacter baumannii* were isolated from surfaces located in the patients' rooms (i.e., dialysis machine and parenteral administration monitors, and headboard of the beds), together with *Pseudomonadaceae*, *Enterobacteriaceae*, and fungi.

In total, 16 *Acinetobacter baumannii* strains sharing the same phenotypic profile were collected in a short time period (November 2012 – March 2013), suggesting a potential outbreak supported by environmental sources. PFGE showed a very poor between-strain variability, with a number of electrophoretic bands ranging from 18 to 22 and a molecular weight ranging from 100 Kb to >1,000 Kb.

Three distinct clusters (11, 4, and 1 isolates in size) were identified using the GelCompar II software, with a Dice's coefficient of 92.11%.

MLST showed that all isolated strains belonged to allele 2 following the sequence typing of the fusA, pyrG, recA, rplB, cpn60, rpoB, and gltA genes, corresponding to sequence type 2 (ST2).

All isolates collected from the environment and the human samples, were positive for the following genes: blaOXA-23, blaOXA-51-like, blaVIM-like, blaIMP-like, and ISAba1; however, blaOXA-24-like, blaOXA-58-like, and blaNDM-like were not detected.

After the last *Acinetobacter baumannii* collection, in August 2013 a new ST2 strain, showing the same phenotypic resistance profile, was isolated from a patient admitted to the same hospital, but the molecular profile showed differences (Dice's coefficient: 79.47%), highlighting a different clonal origin because of >3 different stripes, according to the classification obtained using the between-groups linkage method.[21]

DISCUSSION

The epidemiological investigation and molecular analysis carried out in an ICU of a tertiary university hospital showed that nine patients were infected by XDR *Acinetobacter baumannii* strains sharing the same clonal characteristics.

This report confirmed previous findings, which pointed out the important role played by the environment.[22] It was not possible to assess the potential contamination/infection of the health-care providers who could have favoured the transmission of bacterial strains from the environment to the patients.[23] However, the environmental sampling of several devices and surfaces allowed the identification of the same genotypes in the ICU rooms.

On this basis, it is necessary to underline the importance of epidemiological investigation and implementation of immediate preventive measures to interrupt nosocomial transmission (i.e., isolation of infected patients, hand hygiene, turnover of personal protective equipment, and cleaning/disinfection of contaminated environmental areas). The effectiveness of the infection control interventions implemented in the Italian ICU was proved by the missing identification of *Acinetobacter baumannii* strains after March; the identification of a different strain in August showed the interruption of the epidemiological transmission.

Patient	Sex	Age (years)	PFGE type	ST	Clinical sample	Clinical wards	Date of admission	Date of discharge	Mode of dismission
1	M	68	A	2	BAL	surgical	12/10/2012	21/11/2012	death
2	M	88	A	2	BAL	medical	01/10/2012	28/11/2012	death
3	F	79	A	2	BAL	medical	02/11/2012	05/03/2013	ordinary
4	M	67	A	2	BAL	medical	07/12/2012	16/01/2013	death
5	M	70	A	2	wound swab	surgical	03/09/2012	13/02/2013	death
6	F	4	A	2	BAL	medical	24/12/2012	01/03/2013	ordinary
7	M	72	A1	2	nasal swab	medical	10/02/2013	02/03/2013	ordinary
8	M	74	A	2	nasal swab	surgical	16/02/2013	13/04/2013	ordinary
10	M	83	A2	2	BAL	medical	23/02/2013	26/04/2013	ordinary

Table 1. Epidemiological, phenotypic and genotypic data of the *Acinetobacter baumannii* isolates and of the patients enrolled in the study.
Tabella 1. Dati epidemiologici, fenotipici e genotipici degli isolati di *Acinetobacter baumannii* e dei pazienti inclusi nello studio.

The epidemiological study we conducted raised two important clinical and public health issues: the isolation of XDR strains which did not allow the administration of important antibiotics, particularly in vulnerable patients affected by several co-morbidities (44.4% of the infected individuals died during the initial period of hospitalization) and the rapid transmission of *Acinetobacter baumannii* strains in a high-risk ward. The potential outbreaks caused by those XDR strains should be immediately detected, particularly with the support of advanced molecular techniques, which can assess the clonality of the isolated strains and the genotypic drug-resistance pattern.

PFGE and cluster analysis showed two different clusters with a Dice's coefficient of 97.2%; furthermore, the implementation of MLST confirmed the PFGE results.

Limitations of the present study are related to its retrospective nature and to the missing active surveillance in the hospital, which may have provided a partial evaluation of the epidemiology of the potential outbreak. Furthermore, health-care workers were poorly involved in the bacteriological screening, hindering the possibility of better assessing the transmission chain. However, the use of several molecular techniques and the simultaneous evaluation of both the patients and the environment allowed targeted preventive interventions which stopped further contamination.

It was demonstrated that the knowledge and attitudes of the health-care workers towards this important clinical issue are poor in terms of diagnosis, treatment, and prevention.

Training sessions and microbiological surveillance focused on the admitted patients and the environment, as well as the evaluation of the effectiveness of the preventive measures, represent a priority in high-risk medical and surgical wards in order to reduce the clinical, public health, and economic burden associated with *Acinetobacter baumannii* infections.

Conflicts of interest: none declared

References/Bibliografia

1. Kelesidis T, Karageorgopoulos DE, Kelesidis I, Falagas ME. Tigecycline for the treatment of multidrug-resistant *Enterobacteriaceae*: a systematic review of the evidence from microbiological and clinical studies. *J Antimicrob Chemother* 2008;62:895-904.
2. Perez F, Endimiani A, Ray AJ, et al. Carbapenem-resistant *Acinetobacter baumannii* and *Klebsiella pneumoniae* across a hospital system: impact of post-acute care facilities on dissemination. *J Antimicrob Chemother* 2010;65: 1807-18.
3. Cisneros JM, Reyes MJ, Pachón J, et al. Bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical findings and prognostic feature. *Clin Infect Dis* 1996;22:1026-1032.
4. Kuo SC, Chang SC, Wang HY, et al. Emergence of extensively drug-resistant *Acinetobacter baumannii* complex over 10 years: Nationwide data from the Taiwan Surveillance of Antimicrobial Resistance (TSAR) program. *BMC Infect Dis* 2012;12:200-209.
5. Towner KJ. *Acinetobacter*: an old friend, but a new enemy. *J Hosp Infect* 2009;73:355-63.
6. Kalin G, Alp E, Akin A, Coskun R, Doganay M. Comparison of colisti and colistin/sulbactam for the treatment of multidrug resistant *Acinetobacter baumannii* verntilator associated pneumonia. *Infection* 2014;42:37-42.
7. Lee JY, Kwan SK. Antimicrobial resistance and clones of *Acinetobacter* species and *Pseudomonas aeruginosa*. *J Bact Virol* 2012; 42:1-8.
8. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 2006;12: 826-36.
9. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem resistant Acinetobacter baumannii. *J Antimicrob Chemother* 2010;65:233-38.
10. Higgins PG, Poirel L, Lehmann M, Nordmann P, Seifert H. OXA-143, a novel carbapenem-hydrolyzing class D beta-lactamase in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* 2009;53:5035-38.
11. Turton JF, Woodford N, Glover J, Yarde S, Kaufmann ME, Pitt TL. Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. *J Clin Microbiol* 2006;44:2974-76.
12. Cicek AC, Saral A, Iraz M, et al. OXA-end GES-type beta-lactamases predominate in extensively drug-resistant *Acinetobacter baumannii* isolates from a Turkish University Hospital. *Clin Microbiol Infect* 2014;20: 410-415.
13. Zarrilli R, Giannouli M, Tomasone F, Triassi M, Tsakris A. A Carbapenem resistance in *Acinetobacter baumannii*: the molecular epidemic features of an emerging problem in health care facilities. *J Infect Dev Ctries* 2009;3:481-89.
14. http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947313339
15. http://www.pasteur.fr/recherche/genopole/PF8/mlst/references_Abaumannii.html
16. Mostachio AK, van der Heidjen I, Rossi F, Levin AS, Costa SF. Multiplex PCR for rapid detection of genes encoding oxacillinases and metallo-beta-lactamases in carbapenem-resistant *Acinetobacter spp*. *J Med Microbiol* 2009;58: 1522-24.
17. Amudhan SM, Sekar U, Arunagiri K, Sekar B. OXA beta-lactamase-mediated carbapenem resistance in *Acinetobacter baumannii*. *Indian J Med Microbiol* 2011;29:269-74.
18. Segal H, Garny S, Elisha BG. Is IS(ABA-1) customized for *Acinetobacter*? *FEMS Microbiol Lett* 2005;243:425-29.
19. Turton JF, Ward ME, Woodford N, et al. The role of ISAbal in expression of OXA carbapenemase genes in *Acinetobacter baumannii*. *FEMS Microbiol Lett* 2006;258:72-77.
20. Mugnier PD, Poirel L, Naas T, Nordmann P. Worldwide dissemination of the blaOXA-23 carbapenemase gene of *Acinetobacter baumannii*. *Emerg Infect Dis* 2010;16:35-40.
21. Zhao SY, Jiang DY, Xu PC, et al. An investigation of drug-resistant *Acinetobacter baumannii* infections in a comprehensive hospital of East China. *Ann Clin Microbiol Antimicrob* 2015;14:7. doi:10.1186/s12941-015-0066-4.
22. Otter JA, Yezli S, French GL. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect Control Hosp Epidemiol* 2011;32:687-99.
23. Cristina ML, Spagnolo AM, Cenderello N, et al. Multidrug-resistant *Acinetobacter baumannii* outbreak: an investigation of the possible routes of transmission. *Public Health* 2013;127:386-91.