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**

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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.


**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 blaNMD-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.


**Parole chiave:** *Acinetobacter baumannii*, outbreak, PFGE, MLST
Acinetobacter baumannii is one of the most frequent opportunistic microorganisms detected in healthcare settings. Clinical care of infections caused by Acinetobacter baumannii has been complicated by the emergence and spread of multi-drug resistant strains. 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.

Complicated drug resistance patterns have been recently described in nosocomial outbreaks where the isolates were resistant to all antibiotics with the only exception of polymixines. 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.

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). In particular, five OXA subgroups are associated with Acinetobacter baumannii resistance: OXA-51-like, OXA-23-like, OXA-40-like, OXA-58-like, and OXA-143-like. It was found that the insertion sequence ISAba1 can activate OXA-23-like and OXA-51-like genes.

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). MLST analysis was performed according to the Protocol of the Pasteur Institute. Fragments of seven internal housekeeping genes (i.e., \textit{cpn60}, \textit{fsuA}, \textit{glaE}, \textit{pyrG}, \textit{rplB}, \textit{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 \textit{Acinetobacter baumannii} genes encoding carbapenemases (blaOXA-23, 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. blaOXA51-like and OXA 23-like alleles were simultaneously genotyped together with the insertion sequence ISAba1 in order to evaluate the resistance to carbapenemases.

Statistical analysis

An \textit{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 (StatCorp, College Station, Texas).

RESULTS

Nine patients (median [range] age: 72 [4-88] years; males [\%]: 719 [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 \textit{Acinetobacter baumannii}.

Conventional microbiology

Nasal and broncho-aleolar 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). 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).
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 parental 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 electroforetic 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 gthA 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.

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**Figure 1.** Dendrogram and electrophoretic bands of the isolated *Acinetobacter baumannii* strains.

**Figura 1.** Dendrogramma e profilo elettroforetico degli isolati di *Acinetobacter baumannii*.
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

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**Table 1.** Epidemiological, phenotypic and genotypic data of the *Acinetobacter baumannii* isolates and of the patients enrolled in the study.

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

**Tabella 1.** Dati epidemiologici, fenotipici e genotipici degli isolati di *Acinetobacter baumannii* e dei pazienti inclusi nello studio.


