Outbreak of multidrug-resistant *Acinetobacter baumannii* in an intensive care unit

Marco Dettori[1], Andrea Piana[1], Maria Grazia Deriu[1], Paola Lo Curto[1], Andrea Cossu[1], Rosario Musumeci[2], Clementina Cocuzza[2], Vito Astone[3], Maria Antonietta Contu[3], Giovanni Sotgiu[4]

[1]Department of Biomedical Sciences, Hygiene and Preventive Medicine, University of Sassari, Sassari, Italy;
[2]Department of Surgery and Interdisciplinary Medicine, University of Milano-Bicocca, Italy;
[3]Service Laboratory Medicine, San Francesco Hospital, A.S.L. 3 Nuoro, Sardinia, Italy;
[4]Epidemiology and Medical Statistics Unit, Department of Biomedical Sciences, University of Sassari-Research, Medical Education and Professional Development Unit, AOU Sassari, Sassari, Italy

SUMMARY

Acinetobacter baumannii is a ubiquitous microrganism often able to colonize and survive in different environments. Currently it is one of the most common pathogens responsible for nosocomial infections, including outbreaks, especially in long-term care facilities. The aim of this study was to show the results of an environmental investigation and genotyping analysis of multidrug-resistant *Acinetobacter baumannii* associated with an outbreak in an intensive care unit of a tertiary hospital located in Northern Sardinia, Italy.

Positive cultures of MDR *Acinetobacter baumannii* were reported during the month of June 2012, after the collection of biological samples from ten patients. *Acinetobacter baumannii* was isolated during the following environmental investigation from the headboard of two beds. All the strains were genotyped by performing multiplex PCR to identify the presence of genes encoding carbapenemases. The results showed specific bands of $bla_{\text{OXA-51-like}}$ gene and of the $bla_{\text{OXA-23-like}}$ gene.

PFGE highlighted minimal differences in genomic fingerprints, while the cluster analysis grouped the isolated microorganisms into two closely related clusters, characterized by Dice's similarity coefficient equal to 95.1%. MLST showed that the strains belonged to ST31.

The results of the study highlight the need, especially in high-risk areas, to adopt strict hygiene practices, particularly hand hygiene, and to ensure an appropriate turnover of personal protective equipment, which could be responsible for the spread of biological agents, such as MDR *Acinetobacter baumannii*.

KEY WORDS: Nosocomial outbreak, Multidrug resistance, Molecular epidemiology.

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INTRODUCTION

Acinetobacter baumannii is one of the reported biological agents responsible for nosocomial infections, particularly in long-term care settings (Peleg, 2008; Popova, 2012; Towner, 2009). It is a gram-negative microorganism, strictly aerobic, non-fermenting, non-mobile, catalase positive, oxidase negative, which is ubiquitous and can colonise medical devices as well as the skin and the airways of patients and hospital staff (Beggs, 2006; Morgan, 2010; Lambiase, 2012).

The biological properties of this bacterial species are associated with intrinsic characteristics, including the expression of the OmpA protein involved in formation of the biofilm (Heritier, 2005; Zarrilli, 2013).

Several reports pointed out the epidemiological impact of *Acinetobacter baumannii* infections in health-care facilities, involving several healthy and ill individuals who could favor the circulation of the strains. Identification and adequate management of epidemic nosocomial

Corresponding author
Prof. Andrea Piana
Department of Biomedical Sciences
Hygiene and Preventive Medicine
University of Sassari, Sassari, Italy
Via Padre Manzella, 4 - 07100 Sassari, Italy
E-mail: piana@uniss.it

events in a timely manner, as a consequence of a specific nosocomial public health strategy, is crucial for successful control of an infection related to health care practices (WHO, 2002).
It was proved that poor infection control measures were responsible for epidemics caused by *Acinetobacter baumannii*; the economic and health consequences were deemed relevant (Lee, 2010; Ayraud-Thévenot S, 2012) .
On this basis, the aim of this study is to show the findings of an environmental investigation and molecular analysis of a multidrug-resistant (MDR) *Acinetobacter baumannii* outbreak in an intensive care unit (ICU) of a tertiary hospital located in Northern Sardinia, Italy, and its successful control after the implementation of basic infection control measures.

MATERIALS AND METHODS

Positive cultures of MDR *Acinetobacter baumannii* were reported during the month of June 2012, after the collection of biological samples from ten patients admitted in an ICU of a tertiary hospital located in Northern Sardinia, Italy. The hospital has a total number of 390 beds and hosts several wards, including surgical (*i.e.*, 9) and medical (*i.e.*, 14) specialties; eight diagnostic units work for in- and out-patient individuals. The ICU where the infected cases were admitted has ten beds.
Identification of the isolates was performed by the local microbiology laboratory and confirmed by the Sardinian reference microbiology laboratory located in the Hygiene unit of the university hospital of Sassari, Italy.
It was not possible to identify an index patient, the source of secondary cases, because of the delayed involvement of the epidemiological team of the university hospital of Sassari, Italy. During the investigation only five patients out of the ten positive for MDR *Acinetobacter baumannii* were hospitalized.
Rectal and throat swab samples were collected twice, a week apart, from the patients and the health care workers after official notification of the outbreak, following the recommendations of the epidemiological team. All *Acinetobacter baumannii* isolates showed a resistance profile associated with the most prescribed groups of antibiotics in the ICUs (aminoglycosides, carbapenems, fluoroquinolones, tetracyclines, penicillins, cephalosporins), although a sensitivity to colistin was demonstrated *in vitro*.
The epidemiological investigation was followed by the environmental analysis of the ICU, which is spatially divided into three areas called 'intensive care', 'isolation room' and 'semintensive therapy/care'. Environmental sampling was performed in two out of three areas, *i.e.* in the 'intensive care' and in the 'semintensive therapy/care. In the 'isolation room' samples were not collected because during the outbreak a sputum smear-positive tuberculosis patient was admitted.
Air sampling was performed with the Surface Air System (SAS) equipment near the beds of the patients and the air-conditioning system of the ICU. At each sampling point 200 liters of air per minute were collected using specific bacterial and fungal culture media. Average values of air bacterial and fungal counts per m^3 were computed.
Acinetobacter spp. was isolated in plates containing ground Tryptone Soy Agar (Oxoid). The plates were transported in refrigerated containers at 4° C to the reference laboratory and then incubated at 37°C ±1 for 48 hours. Surfaces sampling was carried out using RODAC contact plates containing Tryptone Soy Agar and a neutralizer of any disinfectants (D'Alessandro, 2013). Sterile swabs were used for non-planar surfaces (Dolan, 2011).
Genotyping of the *Acinetobacter baumannii* isolates was performed using multiplex PCR to assess the presence of genes encoding carbapenemases (*i.e.*, bla_{IMP}, bla_{OXA-23}, $bla_{VIM-like}$, $bla_{OXA-24-like}$, $bla_{OXA-51-like}$, $bla_{OXA-58-like}$). The PCR process included an initial denaturation at 94°C for 5 minutes, 33 amplification cycles at 94°C for 25 seconds, at 53°C for 40 seconds, and at 72°C for 50 seconds, followed by an elongation step at 72°C for 6 minutes. The PCR products of 501 bp ($bla_{OXA-23-like}$), 353 bp ($bla_{OXA-51-like}$), 246 bp ($bla_{OXA-24-like}$) and 599 bp ($bla_{OXA-58-like)}$ were visualized after agarose gel electrophoresis and staining with ethidium bromide. The PCR process for metallo-beta-lactamase bla_{VIM} and bla_{IMP} genes included 30 cycles of amplification under the following conditions: denaturation at 95°C for 30 seconds, annealing for 1 min-

ute at specific temperatures (for bla_{VIM} gene 66°C, and for bla_{IMP} gene 45°C), and extension at 72°C for 1 minute. Cycling was followed by a final extension at 72°C for 10 minutes. The PCR products of 500 bp (*i.e.*, bla_{VIM} gene) and 432 bp (*i.e.*, bla_{IMP} gene) were visualized after agarose gel electrophoresis and staining with ethidium bromide (Mostachio, 2009; Amudhan, 2011).

Furthermore, the genomic profile of the isolates was investigated using Pulsed Field Gel Electrophoresis (PFGE) and Multi Locus Sequence Typing (MLST).

The PFGE was performed following the methodology recommended by ARPAC (Antibiotic Resistance Prevention And Control) (http://www.hpa.org.uk/webc/HPAwebFile/HPAweb_C/1194947313339). It consisted in 'casting' bacterial suspensions in low melting point agarose disks from which bacterial DNA was subsequently extracted and DNA purified. The agarose disks were then incubated for 8 hours in the presence of 40U of the restriction enzyme ApaI, an infrequent cutter endonuclease. The DNA fragments were then separated by agarose PFGE using a Clamped Homogeneous Electric Fields DRII SYSTEM. The gel was stained with ethidium bromide and viewed under UV. Gel images were analyzed by means of Image Master Program (Pharmacia).

The MLST analysis was performed according to the Protocol of the Pasteur Institute (http://www.pasteur.fr/recherche/genopole/PF8/mlst/references_Abaumannii.html), following amplification and sequence analysis of fragments of seven internal housekeeping genes (*i.e.*, *cpn60*, *fusA*, *gltA*, *pyrG*, *recA*, *rplB*, *rpoB*). Sequence analysis was performed using the Bioedit software. Each sequence was included in the website of the Pasteur Institute under Single locus and Multiple locus query in order to evaluate the 'percentage of identity' and the compatibil-

TABLE 1 - *Qualitative and quantitative air sampling findings.*

Intensive Care Unit Area				
Sampling area	*Mean value (CFU/m³)*	*Bacteria*	*Mean value (CFU/m³)*	*Fungi*
Zone 1	114.6	Environmental flora	10	*Mucor* spp
Zone 2	107.9	Environmental flora	20	*Mucor* spp *Penicillium* spp *Cladosporium* spp
Zone 3	125	*Pseudomonadaceae* and environmental flora	20	*Alternaria* spp *Penicillium* spp *Cladosporium* spp
Semi-intensive therapy area				
Sampling area	*Mean value (CFU/m³)*	*Bacteria*	*Mean value (CFU/m³)*	*Fungi*
Room 1_bed	146.7	Methicillin-sensitive *Staphylococcus aureus*		
		Pseudomonadaceae and environmental flora	146.7	*Mucor* spp
Room 1_air conditioning exhaust	87.5	Environmental flora	87.5	*Mucor* spp *Penicillium* spp *Cladosporium* spp
Room 2_bed	51.7	Environmental flora	10	*Penicillium* spp
Room 2_air conditioning exhaust	67.5	Environmental flora	<1	-

TABLE 2 - *Surface sampling: quantitative method.*

Sampling Points	*UFC*	*Isolated microrganisms*
Drug cart 1	3	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Drug cart 2	4	*Pseudomonadaceae, Aspergillus niger* and environmental flora
Drug cart 3	2	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Drug cart 4	2	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Drug cart 5	2	Environmental flora
Crash cart	6	Environmental flora
Ventilator monitor bed 1	<1	-
Ventilator monitor keyboard_ bed 1	1	Environmental flora
Floor (point n. 1) near to the bed 1	10	*Pseudomonadaceae* and environmental flora
Floor (point n. 2) near to the bed 1	17	Methicillin-resistant *Staphylococcus* non *aureus*, *Pseudomonadaceae* and environmental flora
Ventilator monitor bed 4	2	Environmental flora
Ventilator monitor keyboard_ bed 4	1	Environmental flora
Floor (point n. 1) near to the bed 4	>50	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Floor (point n. 2) near to the bed 4	28	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Ventilator monitor bed 7	8	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Ventilator monitor keyboard_ bed 7	<1	-
Floor (point n. 1) near to the bed 7	17	*Pseudomonas fluorescens* and environmental flora
Floor (point n. 2) near to the bed 7	22	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Manifolds for medical gas-bed 8 (point 1)	<1	-
Manifolds for medical gas-bed 8 (point 2)	<1	-
Monitor infusion pump bed 8	2	*Enterobacter* spp and environmental flora
Monitor infusion pump bed 10	<1	-
Manifolds for medical gas-bed 10	<1	-
Floor near to the bed 10	18	Environmental flora
Ventilator monitor bed 11	<1	-
Floor near to the bed 11	6	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora

ity. Sequences were then compared with those stored in the Pasteur Institute's database. The MLST scheme including amplification and sequencing primers, allele sequences and sequence types (STs) are available at Pasteur's Institute MLSTWeb site (http://www.pasteur.fr/recherche/genopole/PF8/mlst/references_Abaumannii.html).

RESULTS

In the 'intensive care' area of the ICU total bacterial counts found in air samples, including environmental flora and *Pseudomonas* spp., ranged from 125 to 107.9 CFU/m^3 (Tab. 1). Values were below the suggested cut-off of 180 CFU/m^3 (National Health Service, 1994; UNI EN ISO, 2007). Furthermore, a few colonies of *Mucor* spp, *Alternaria* spp., *Penicillium* spp and *Cladosporium* spp. were isolated. Strains of methicillin-sensitive *Staphylococcus aureus* (MSSA), *Pseudomonas* spp. and environmental bacterial flora were collected in the semi-intensive area of the ICU. Quantitative analysis showed values within the recommended limits of detection (Tab. 2 and 3).
Methicillin-resistant coagulase-negative *Staphylococcus* spp., *Pseudomonas* spp., environmental microbial flora, *Aspergillus niger,* and *Pantoea* spp. were isolated from surfaces. The headboards of two beds were contaminated by MDR *Acinetobacter baumannii* strains.
The molecular analysis to identify genes encoding carbapenemases, showed specific bands of $bla_{\text{OXA-51-like}}$ gene and of the $bla_{\text{OXA-23-like}}$ gene. The macrorestriction analysis, performed on the 12 isolated microorganisms (*i.e.*, 10 from patients and 2 from the beds of the patients) did not identify major differences between strains, characterized by a similar electrophoretic band pattern. Cluster analysis, performed using the GelCompare II software, obtained by applying the Dice coefficient on electrophoretic profiles using ApaI, showed two distinct clusters of the isolates, characterized by a Dice similarity coefficient of 95.1% (Fig. 1).
In order to verify a possible clonal lineage with the strain of *Acinetobacter baumannii* RUH875, one of the most frequently described in Europe, we performed a macrorestriction analysis profiling of the 12 'local' strains together with the RUH875 collection strain (ATCC 17978). The strains were grouped into three clusters, with an overall similarity coefficient of 91.87%.
MLST showed that strains belonged to ST31 (*i.e., cpn60*: 1 allele, *fusA*: 2 allele, *gltA*: 2 allele, *pyrG*: 2 allele, *recA*: 11 allele, *rplB*: 1 allele, *rpoB*: 1 allele).

TABLE 3 - *Surface sampling: qualitative method.*

Sampling points	*Isolated microrganisms*
Headboard of bed 1	*Acinetobacter baumannii* MDR, *Pantoea* spp and environmental flora
Right patient call button - bed 1	-
Left patient call button - bed 1	-
IV rod near to the bed 1	*Pantoea* spp
Headboard of bed 4	Environmental flora
Right patient call button - bed 4	-
Left patient call button - bed 4	-
IV rod near to the bed 4	Environmental flora
Headboard of bed 7	*Acinetobacter baumannii* MDR and environmental flora
Right patient call button - bed 7	Environmental flora
Left patient call button - bed 7	-
IV rod near to the bed 7	Methicillin-resistant *Staphylococcus* non *aureus* and environmental flora
Headboard of bed 1	*Enterobacter cloacae*
Right patient call button - bed 11	-
Left patient call button - bed 11	-
IV rod near to the bed 11	-
Telephone	Environmental flora

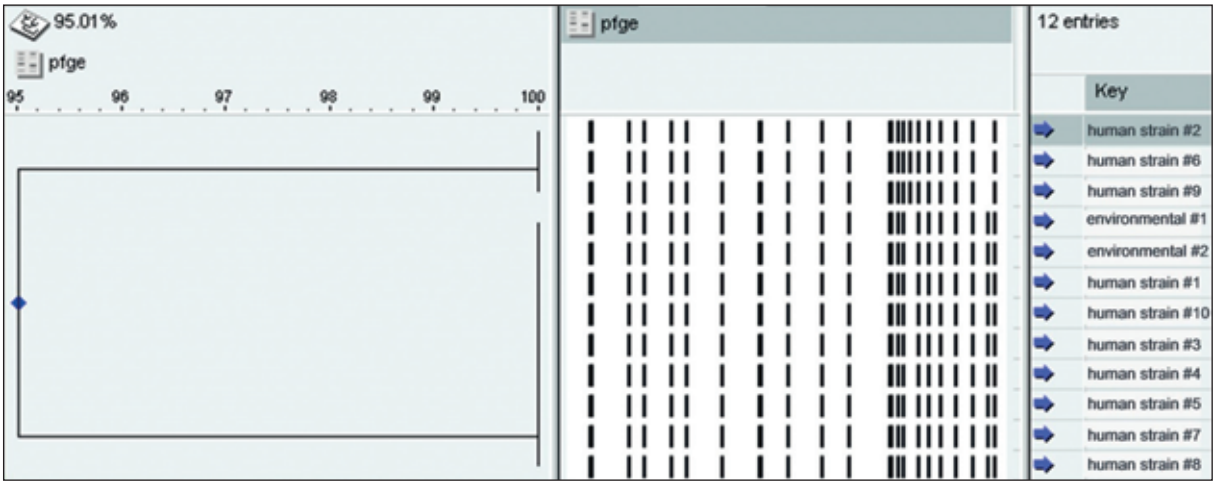

FIGURE 1 - *Cluster analysis of Acinetobacter baumannii patterns obtained by pulsed field gel electrophoresis (ApaI).*

DISCUSSION

Phenotypic and molecular characterization of *Acinetobacter baumannii* strains isolated from the environment and patients admitted to an ICU together with information from the classical epidemiological investigation identified a nosocomial outbreak. PFGE highlighted minimal differences in genomic fingerprints, while the cluster analysis grouped the isolated microorganisms into two closely related clusters, characterized by Dice's similarity coefficient equal to 95.1%. The restriction profile was similar to the European variant of *Acinetobacter baumannii*, RUH875: its antibiotic resistance pattern is similar to those detected in the strains isolated during the investigation (Dijkshoorn, 1996). The MLST method identified the presence of ST31.

It was not possible to detect the source of the infection. The environmental collection of MDR *Acinetobacter baumannii* cannot exclude a human source of infection. However, the environmental contamination, along with ten infected patients, reported during a 30-day period, points to a wide circulation of the microorganism. The combination of the clinical and environmental investigations, including the use of molecular techniques, has been adopted by other authors (Munoz, 2013). Its efficacy in terms of containment of the outbreak, along with infection control measures focusing on the targets infected or contaminated by the bacterial strains was also proved in the Sardinian hospital. Epidemiological and microbiological data were crucial for the implementation and scale-up of accurate and repeated practices of sanitation/disinfection/sterilization of the instruments used in the ICU, the surfaces (at least twice a day and after each care practice), and the environment. Detection of the same strain of *Acinetobacter baumannii* in some of the patients admitted and in the environment did not change the infection control policy, planned before the outbreak. However, stricter recommendations were issued, including microbiological screening of all health care workers and training on the administrative and environmental measures to control the spread of the microorganism. In particular, the importance of handwashing with alcohol-based solutions during the management of patients and the cleaning procedures to be implemented in the environment were highlighted, as well as a change of gloves, gowns and other personal protective equipment used during the care of patients. Furthermore, in our opinion, it was crucial to isolate the infected subjects in specific areas of the ICU using mechanical barriers to avoid the spread of the bacterial strains in the unit, *i.e.* to other individuals and in the environment.

The results of the study highlight the need to adopt strict and proper hygiene practices, particularly hand hygiene (Amudhan, 2011) espe-

cially in high-risk areas, as well as to ensure an appropriate turnover of personal protective equipment, which could be responsible for the spread of biological agents such as MDR *Acinetobacter baumannii* (Agodi, 2013). Training of caregivers on health care-associated infections and environmental microbiological surveillance represent essential measures to prevent outbreaks and their recurrence.

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