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Alert surveillance of intensive care unit-acquired *Acinetobacter* infections in a Sicilian hospital

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ABSTRACT

The epidemiological impact of *Acinetobacter baumannii* nosocomial infections in a Sicilian intensive care unit (ICU) was investigated to determine the *Acinetobacter*-specific infection rates, to estimate the preventable proportion of *Acinetobacter* infections, i.e., those resulting from cross-transmission, and to investigate the molecular epidemiology of antimicrobial resistance in *Acinetobacter*. The impact of *Acinetobacter* nosocomial infection in the ICU was determined to be 3.0 new cases per 100 admissions. Site-specific rates confirmed that ICU-acquired pneumonia was the most important infection type. The incidence rate, adjusted by the number of patient-days, was 3.3 infections/1000 patient-days. The estimated preventable proportion of *A. baumannii* nosocomial infections in the ICU was 66.7%. A class 1 integron, characterised by its gene cassette content, was present in all *A. baumannii* isolates of four different pulsed-field gel electrophoresis types, and was associated significantly with clones implicated in cross-transmission episodes. Furthermore, the same integron was detected in two genetically distinct isolates responsible for recurrent infection in the same patient, suggesting the occurrence of horizontal gene transfer *in vivo*. Even in an endemic setting with low infection rates, spread of *A. baumannii* was caused mainly by infection control shortcomings that require appropriate surveillance and control policies.

Keywords *Acinetobacter baumannii*, cross-transmission, integron, nosocomial infection, pulsed-field gel electrophoresis, *recA*

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INTRODUCTION

Epidemiological surveillance is a critical and essential element of any successful infection control programme and is especially recommended in intensive care units (ICUs) in order to increase awareness and to identify areas for improvement. ICU patients are at five- to ten-fold greater risk of acquiring a nosocomial infection, and the ICU is often the epicentre of emerging infection problems in a hospital [1].

Multiresistant *Acinetobacter* spp. have become established as 'alert' nosocomial pathogens, particularly in ICUs, and are associated with outbreaks of nosocomial infection, often attributable to medical equipment and materials that may have had contact with water of uncertain quality, as well as dry environmental surfaces [2]. Although the genus *Acinetobacter* currently comprises at least 32 different species, with many as yet unclassified strains, *Acinetobacter baumannii* is reported most frequently among epidemic isolates [3,4]. Nosocomial infections caused by *A. baumannii* are difficult to treat because of the multiple antibiotic resistance of this species. Studies of antibiotic resistance mechanisms in *A. baumannii* strains have demonstrated the presence of specific genes located on integrons, and molecular typing studies have shown that type 1 and type 2 integrons bearing antimicrobial resistance genes are often associated with epidemic clones [5,6].

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Microbial transmission may occur via the endogenous route (i.e., a patient's own flora) or via the exogenous route (e.g., a healthcare worker's hands or equipment). Therefore, it is important to determine whether infections are attributable to the severity of the patient's illness, or whether they are caused by infection control shortcomings that result in cross-transmission within a unit [7]. Although various risk-factors predisposing to infection with *Acinetobacter* spp. have been identified, including previous antimicrobial therapy that alters the normal flora and results in the selection of resistant *Acinetobacter* strains, cross-transmission and the hospital environment are probably the most likely sources of this organism [3,8]. Ideally, the diagnostic laboratory should be involved in active surveillance, providing systematic observation and measurement of disease, as well as molecular typing of microbial pathogens [9]. Epidemiological typing may enable conclusions to be reached concerning the possible prevention of exogenous infections through infection control measures. However, although this approach has been used frequently in outbreak investigations, it has been used less frequently for surveillance studies under endemic conditions.

To date, there have been few surveillance studies of *Acinetobacter* nosocomial infections in Italy [6,10]. In the ICU of a Sicilian hospital, the Azienda Ospedaliera S. Giovanni di Dio, Agrigento, *Acinetobacter* spp. were found only sporadically before 2001. The subsequent observation in the ICU of *A. baumannii* infections, with neither an evident common source nor a linked chain of transmission, prompted a prospective investigation to determine the rates of *A. baumannii*-associated infections. The present study was designed to evaluate the epidemiological impact of *A. baumannii* nosocomial infections in the ICU through a unit-based surveillance, using the Hospitals in Europe Link for Infection Control through Surveillance (HELICS) protocol [1]. The specific aims of the study were: (1) to determine the *Acinetobacter*-specific infection rates within the ICU; (2) to estimate the preventable proportion of *Acinetobacter* infections; and (3) to characterise the integron contents and sequences of the strains involved in order to investigate the molecular epidemiology of antimicrobial resistance in *Acinetobacter*.

MATERIALS AND METHODS

Nosocomial infection surveillance methods

Between November 2001 and February 2004, all 665 patients admitted to the ten-bed combined surgical and medical ICU of the Azienda Ospedaliera S. Giovanni di Dio, a 339-bed hospital, were enrolled into the laboratory-based alert surveillance, performed by trained personnel using a unit-based protocol for continuous surveillance. Infection data were collected for each episode of *Acinetobacter* infection with onset within the surveillance period. The number of new admissions and the number of patient-days for patients staying >2 days in the ICU were also collected. *Acinetobacter*-associated infections were identified using standard definitions [1]. If the infection criteria were not met, colonisation was defined by the presence of *Acinetobacter* in clinical specimens. Surveillance data were analysed using SPSS v. 11.0 (SPSS Inc., Chicago, IL, USA).

Bacterial isolates

In total, 25 *Acinetobacter* isolates from 22 patients (17 from the ICU, two from orthopaedics, one from the medical ward, one from paediatrics and one from the neonatal ICU) were obtained from clinical specimens and identified presumptively by standard methods (ID 32 GN; bioMèrieux, Marcy l'Etoile, France).

Antimicrobial susceptibilities

Antimicrobial resistance was determined by the NCCLS disk-diffusion method [11]. Isolates showing an intermediate level of susceptibility were classified as resistant. If isolates were resistant to imipenem and meropenem by disk-diffusion, the imipenem MIC and the presence of metallo-β-lactamase (MBL) activity were determined with Etest MBL strips (AB Biodisk, Solna, Sweden), as described previously [12].

Genotypic identification

The genomic species of each *Acinetobacter* isolate was determined by *Tsp*509I restriction analysis of an amplified internal sequence of the *recA* gene as described previously [13]. In brief, crude template DNA was prepared by suspending two to three colonies from an overnight agar plate in 100 μL of ultrapure water, heating to 95°C for 15 min to lyse the cells, cooling on ice and centrifuging for 20 s at 12 000 *g* to remove cell debris [14]. PCR amplification of the *recA* gene was performed using forward primer rA1 and reverse primer rA2 [13]. PCRs contained 200 μM dNTPs, 0.5 μM each primer, 0.2 μg of target DNA and 2.5 U of DNA polymerase in a total volume of 50 μL. PCRs comprised 35 cycles of 94°C for 1 min, 57°C for 1 min and 72°C for 2 min; amplified products were visualised following electrophoresis on agarose 1% w/v gels. Amplified DNA (10 μL) was digested for 2 h at 65°C in a 25-μL volume with *Tsp*509I. Digested samples were electrophoresed in a polyacrylamide 12% w/v gel. *Tsp*509I restriction fragment length polymorphism (RFLP) profiles were compared with representative patterns of known genomic species [13].

Macrorestriction analysis

Molecular typing by pulsed-field gel electrophoresis (PFGE) of *Apa*I-digested genomic DNA was performed as described previously [15]. In brief, isolates were grown overnight on

nutrient agar, suspended in 3 mL of SE buffer (75 mM NaCl, 25 mM EDTA, pH 7.5) and adjusted to a density equivalent to a 2× McFarland standard. Cells were washed twice with 3 mL of SE buffer before being resuspended finally in 1 mL of SE buffer. Cell suspensions were mixed with an equal volume of low-melting-point agarose 2% w/v and pipetted into a plug mould. Plugs were allowed to solidify for 5 min at – 20°C, and were then lysed at 56°C overnight with proteinase K 1 mg/mL. Digestion with *Apa*I (30 U/plug) was performed at 25°C for 5 h. Macrorestriction fragments were separated with a CHEF-DR III apparatus (Bio-Rad Laboratories, Hercules, CA, USA) at 8°C for 20 h, with an initial pulse time of 5 s and an end pulse time of 13 s, at 6 V/cm. A concatamer ladder of lambda phage DNA was used as a size marker. Interpretation of genomic relatedness was performed using well-established criteria [16]. Isolates with patterns that differed by two or three fragments were considered to be subtypes of the same clone; different, although possibly related, clones were defined as those showing at least four DNA fragment differences; patterns that differed by seven or more fragments were considered to be unrelated.

Characterisation of class 1 integrons and metallo-β-lactamase genes

PCR amplification of class 1 integrons and mapping of resistance genes were performed with 0.5 μg of genomic DNA as described previously [17]. Primers for the detection of class 1 integrons were located in the 5′-conserved segment (CS) and in the 3′-CS [5]. Detection of class 1 and class 2 integrons by integrase gene PCR was performed as described by Koeleman *et al.* [5]. PCR products were purified by low-melting-point agarose gel electrophoresis, phenol–chloroform extraction and ethanol precipitation, and were then sequenced (see below). PCR analysis of bla_{IMP}-like or bla_{VIM}-like genes was performed with degenerate primers as described by Nordmann and Poirel [18].

DNA sequencing

Cycle sequencing of purified PCR products was performed with the ABI PRISM BigDye Terminator v. 3.0 Ready Reaction Cycle Sequencing Kit as recommended by the manufacturer (Applied Biosystems, Foster City, CA, USA). DNA products were analysed with an AB 3100 Genetic Analyser (Applied Biosystems). Similarity searches of the DNA sequences obtained were performed with the BLAST program against nucleic acid sequence databases.

Identification of episodes of cross-transmission

The presence of two indistinguishable strains in two patients was considered to represent one episode of cross-transmission. In general, the number of cross-transmissions that occurred in a ward was calculated by subtracting 1 from the number of indistinguishable strains in different patients [7].

RESULTS

Nosocomial infection surveillance

Of the 25 *Acinetobacter* spp. isolates collected, 20 were associated with infections of ICU patients: 13 with ICU-acquired pneumonia, five with urinary tract infections (UTIs) and two with bloodstream infections. Three isolates were associated with infections acquired outside the ICU: one surgical site infection in an orthopaedic patient, and two UTIs (one each in a medical and an orthopaedic patient). Finally, two isolates, one from a neonatal ICU patient and one from a paediatric patient, were classified as colonising isolates. All 25 clinical isolates were identified presumptively as *A. baumannii* by phenotypic characterisation.

There were 3.0 new *Acinetobacter* infections/100 admissions to the ICU (20 infections and 665 admissions). Of these, 65% were ICU-acquired pneumonia, 25% were UTIs, and 10% were bloodstream infections. The incidence rate, adjusted by the number of patient-days, was 3.5 infections/1000 patient-days (20 infections and 5675 patient-days). Site-specific rates were 2.3% for ICU-acquired pneumonia, 0.9% for UTIs, and 0.3% for bloodstream infections.

Genotypic identification

Genomic species identification was performed by *Tsp*509I RFLP analysis of the amplified internal sequence of the *recA* gene. This method confirmed the biochemical identification of 21 (84%) isolates, including 19 isolates from ICU patients and two isolates from orthopaedic patients, as *Acinetobacter* genomic sp. 2, i.e., *A. baumannii*. Four (16%) isolates were misidentified with standard identification methods: one (4%) isolate failed to give any amplification product, leaving this strain unidentified by the *recA*-based assay, while the other three (12%) isolates, one each from the neonatal ICU, the paediatric ward and the medical ward, gave identical patterns that did not match with any known reference profile [13]. The amplicons obtained from these three isolates were sequenced and shown to have homology with the *recA* gene of *Acinetobacter* genomic sp. 3 (Table 1).

Thus, following genotypic identification, the final *A. baumannii* infection rate in the ICU was 3.3% (19 infections and 5675 patient-days).

Macrorestriction analysis

Macrorestriction analysis of the *Acinetobacter* isolates identified eight unrelated PFGE types,

Table 1. Molecular epidemiology of *Acinetobacter* isolates included in the study

Number of isolates and ward of isolation	Associated infections (*n*)	*recA*-based identification	PFGE type	Class 1 integron
1 (ICU)	UTI (1)	*A. baumannii*	A	Absent
4 (ICU)	P (4)	*A. baumannii*	B	2.2 kb
12 (ICU)	P (8), UTI (4)	*A. baumannii*	C	2.2 kb
2 (OW)	UTI (1), SSI (1)	*A. baumannii*	C	2.2 kb
1 (ICU)	P (1)	*A. baumannii*	D	2.2 kb
1 (MW)	UTI (1)	*Acinetobacter* sp. 3	E	Absent
1 (ICU)	BSI (1)	*A. baumannii*	F	2.2 kb
1 (NICU)	Col (1)	*Acinetobacter* sp. 3	G	Absent
1 (PW)	Col (1)	*Acinetobacter* sp. 3	H	Absent
1 (ICU)	BSI (1)	Unidentified	ND	Absent

ICU, intensive care unit; OW, orthopaedics ward; MW, medical ward; NICU, neonatal intensive care unit; PW, paediatric ward; UTI, urinary tract infection; P, ICU-acquired pneumonia; SSI, surgical site infection; BSI, bloodstream infection; Col, colonisation; ND, not determined.

named A–H. Six PFGE types were single patterns associated with sporadic isolates; the remaining two *A. baumannii* PFGE types were associated with cross-transmission of infection. Type B was common to four isolates from four patients in the ICU, and type C was common to 12 isolates from ten patients in the ICU, and by two isolates from two patients in the orthopaedic ward (Table 1). No PFGE profile was obtained for the isolate that failed to give a PCR amplification product. The occurrence of two infections (ICU-acquired pneumonia and UTI) was associated with PFGE type C in two patients. Two serial isolates from the same patient showed two distinct macrorestriction patterns; the first isolate belonged to PFGE type B, while an isolate with the unrelated sporadic type D profile was identified 1 month later.

Antimicrobial susceptibility patterns

Analysis of antimicrobial susceptibility patterns showed that *Acinetobacter* isolates with different PFGE profiles all had a multiresistant antibiotype characterised by resistance to monobactams and ceftriaxone, and resistance or intermediate susceptibility to piperacillin–tazobactam, broad-spectrum cephalosporins, aminoglycosides, ciprofloxacin and trimethoprim–sulphamethoxazole (Table 2). Seven of eight *Acinetobacter* PFGE types were susceptible to ampicillin–sulbactam and carbapenems, and all *Acinetobacter* PFGE types were susceptible to colistin. *A. baumannii* isolates of PFGE type C were resistant to most antimicrobial agents, but were sensitive to ampicillin–sulbactam, carbapenems and colistin. *A. baumannii* isolates of PFGE type B were characterised by resistance to most of the antimicrobial agents tested, including ampicillin–sulbactam, but all type B isolates were susceptible to colistin, and three of four isolates were susceptible to carbapenems (Table 2). With this single exception, all *Acinetobacter* isolates with the same PFGE profile had the same antibiotype.

Table 2. Antimicrobial susceptibility patterns[a] of *Acinetobacter* PFGE types

	PFGE types							
Antimicrobial agent	A	B	C	D	E	F	G	H
Ampicillin–sulbactam	s	r	s	s	s	s	s	s
Piperacillin	r	r	r	r	s	r	s	s
Piperacillin–tazobactam	r	r	r	s	s	s	s	s
Cefepime	r	r	r	r	s	s	s	s
Ceftazidime	r	r	r	r	s	s	s	s
Ceftriaxone	r	r	r	r	r	r	r	r
Aztreonam	r	r	r	r	r	r	r	r
Imipenem	s	s/r	s	s	s	s	s	s
Meropenem	s	s/r	s	s	s	s	s	s
Amikacin	r	r	r	r	s	s	s	s
Gentamicin	s	r	r	r	s	s	s	s
Netilmicin	r	r	r	r	s	r	s	s
Tobramycin	s	r	r	s	s	s	s	s
Ciprofloxacin	r	r	r	r	s	s	s	s
SXT	s	r	r	s	s	s	s	s
Colistin	s	s	s	s	s	s	s	s

PFGE, pulsed-field gel electrophoresis; SXT, trimethoprim–sulphamethoxazole; s, susceptible; r, resistant.
[a]Antimicrobial resistance was determined by the disk-diffusion method. Isolates showing an intermediate level of susceptibility were classified as resistant.

MICs of imipenem and MBL activity were determined for all *A. baumannii* isolates belonging to PFGE type B. Three isolates were susceptible to imipenem (MIC < 4 mg/L) and negative for MBL production (imipenem + EDTA MIC < 1 mg/L), while one isolate was resistant to imipenem (MIC 16 mg/L) and negative for MBL production (imipenem + EDTA MIC 4 mg/L). No amplification products were obtained from these four isolates with PCRs specific for bla_{IMP}-type or bla_{VIM}-type carbapenemase genes (data not shown).

Identification of class 1 integrons in *Acinetobacter* isolates

Single amplification products of *c.* 2.2 kb were obtained from the chromosomal DNA of all the *A. baumannii* isolates belonging to PFGE types B, C, D and F, using primers corresponding to the conserved regions of type 1 integrons [17]. No amplification products were obtained from the sporadic isolates belonging to PFGE types A, E, G and H, and no amplicon was obtained from the unidentified isolate (Table 1). Sequence analysis of the integrons from PFGE types B, C, D and F demonstrated a 2230-bp amplicon containing

three gene cassettes: an *aacA4* allele encoding an AAC(6′)-Ib aminoglycoside acetyltransferase conferring resistance to amikacin, netilmicin and tobramycin; an open reading frame coding for an unknown product; and bla_{OXA-20}, a gene encoding a class D β-lactamase conferring resistance to amoxycillin, ticarcillin, oxacillin and cloxacillin.

The presence of integrons in the *Acinetobacter* isolates was also investigated by integrase gene PCRs specific for the *intI1* and *intI2* genes [5]. Class 1 integrons were detected in all *A. baumannii* isolates belonging to PFGE types B, C, D and F, but not in PFGE types A, E, G and H. Class 2 integrons were not detected in any of the *Acinetobacter* isolates (data not shown).

Identification of cross-transmission episodes and association with class 1 integron sequences

There were 14 episodes of cross-transmission, accounting for 66.7% of all *A. baumannii* nosocomial infections (14 of 21 infections). The presence of class 1 integron sequences ($n = 20$) was shown to be statistically significant ($p < 0.001$) in isolates ($n = 18$) involved in episodes of cross-transmission.

DISCUSSION

The proportion of potentially preventable nosocomial infections has been addressed recently by a systematic review of the published literature describing multi-modal intervention studies and transmission studies performed during the last decade [19]. It was concluded that ≥20% of all nosocomial infections are probably avoidable, but that further research into the feasibility and cost-effectiveness of prevention is required. Few studies have addressed the issue of estimating the preventable proportion of nosocomial infections under routine conditions [19,20], and there are even fewer reports concerning ICU-acquired infections caused by *Acinetobacter* spp. in Italy [6,10]. The present study was designed to assess the preventable proportion of infections caused by *Acinetobacter* in a Sicilian hospital by means of a unit-based surveillance protocol. Although indicators generated by such a 'first-level' surveillance are not suitable for ICU benchmarking of quality of care in terms of infection control, they are appropriate for the follow-up of infections within the same unit and for regional, national and international investigation of trends for pathogen-specific infection rates [1].

In total, 23 of 25 isolates were associated with infection, which was a rather high proportion, since *Acinetobacter* isolates have been reported to be associated most frequently with colonisation [21]. Although the relevance of patient colonisation in the epidemiology of *A. baumannii* is well-known [3,22], the incidence of infection vs. colonisation may vary among wards, or even within the same ward, at different times [6,23]. Possible explanations for the high infection-to-colonisation ratio in this ICU include different criteria for submission of clinical specimens for microbiological culture, and utilisation of the HELICS definitions for infections, particularly those for nosocomial pneumonia; the latter are based on draft CDC criteria that have recently been reported to be in better agreement with clinical assessment than the current CDC definitions [24].

The impact of *Acinetobacter* nosocomial infections in the ICU was 3.0 new cases/100 admissions, which is a relatively low rate when compared with epidemic rates reported in the literature, e.g., 14% in Spain [25] and 12.4% in Italy [10]. Site-specific rates showed that ICU-acquired pneumonia is the most important type of *Acinetobacter* infection, as reported previously [6,10]. The incidence rate of 3.3 *A. baumannii* infections/1000 patient-days was lower than the pooled rate of 4.5 *A. baumannii* colonisations or infections/1000 patient-days reported in a multicentre study of Spanish ICUs [23]. The present alert surveillance study estimated the preventable proportion of *A. baumannii* nosocomial infections in the ICU as 66.7%. Thus, exogenous transmission may be considered as a major infection control problem, even in a ward with low baseline rates, indicating that there is still scope for improvement and that the preventive potential is even higher than the figure of 20% determined previously [19].

Commercial identification systems have a limited capability to differentiate *Acinetobacter* genomic species [26]. A variety of genotypic methods have been explored for species identification, including a rapid method based on *recA* PCR *Tsp*509I RFLP analysis that has been shown to be suitable for continuous epidemiological surveillance surveys [13]. Nevertheless, some

limitations were observed in the present study: three isolates, identified presumptively as *A. baumannii*, were excluded as such, but further sequencing was necessary to assign them definitively to *Acinetobacter* genomic sp. 3. Furthermore, *recA* amplification failed for one isolate, identified consistently as *A. baumannii* in repeated biochemical assays. Therefore, the *recA*-based method was not as useful as amplified rDNA restriction analysis, a thoroughly validated genotypic identification assay, for which a comprehensive database has been established [27,28].

Acinetobacter spp. have become resistant to almost all antimicrobial agents that are currently available, including the aminoglycosides, quinolones and broad-spectrum β-lactams [29], and all the *Acinetobacter* PFGE types isolated in this study had multiresistant antibiotypes. In particular, *A. baumannii* isolates of PFGE type B were resistant to most of the antimicrobial agents tested, with the exception of colistin for all isolates and carbapenems for three isolates. *A. baumannii* isolates of PFGE type C were also resistant to most of the antimicrobial agents tested, but were sensitive to ampicillin–sulbactam, carbapenems and colistin. *A. baumannii* nosocomial acquisition has been associated previously with elevated rates of resistance to broad-spectrum cephalosporins, carbapenems and aminoglycosides in ICUs [6].

Additional epidemiological data were provided by molecular typing of the *A. baumannii* isolates. A class 1 integron, characterised by the same set of gene cassettes, was present in all *A. baumannii* isolates belonging to PFGE types B, C, D and F, and was associated significantly with isolates involved in episodes of cross-transmission. This is in agreement with previous studies demonstrating that integrons bearing antimicrobial resistance genes are found in the genome of epidemic *A. baumannii* strains [5,6]. The presence of integrons with the same molecular organisation in *A. baumannii* isolates belonging to different PFGE types suggests that horizontal gene transfer of integrons may have occurred [5,30,31]. In the present study, the same integron was detected in two unrelated isolates responsible for sequential infection in the same patient. Thus, in-vivo horizontal gene transfer may have been selected by the antimicrobial therapy prescribed for a patient in an ICU where epidemic and sporadic clones co-exist.

Even in an endemic setting with low infection rates, *A. baumannii* spread occurs mainly as a result of cross-infection problems that require appropriate surveillance and infection control policies [20]. Although the present study was not designed to identify risk-factors, there was no adequate infection control programme at the time of the study. Cross-transmission may have occurred via inadequate decontamination of patient-care equipment [3,8], poor hand hygiene and insufficient routine cleaning of clinical areas [32]. Feedback of surveillance data to healthcare personnel, including molecular typing data of bacterial isolates and their associated antibiotic resistance genes, is essential to prevent horizontal transmission [9,33]. A molecular-based approach to the control of *Acinetobacter* nosocomial infections would provide an insight into the population dynamics of bacterial circulation in the hospital setting, and would allow the design of evidence-based strategies to decrease specific infection rates to the irreducible minimum.

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