Molecular findings and antibiotic-resistance in an outbreak of *Acinetobacter baumannii* in an intensive care unit

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Summary. We investigated an outbreak of *Acinetobacter baumannii* in the intensive care unit (ICU) of a hospital in Rome, Italy. The outbreak involved 14 patients whose isolates were most frequently recovered from bronchoalveolar lavage. All isolates were multidrug-resistant and showed diminished susceptibility or resistance to carbapenems. *A. baumannii* strains with a similar antibiotic susceptibility pattern were isolated from the environment. Pulsed-field gel electrophoresis identified a single clone from both the patients' and environmental isolates. Because of the lack of a single source of infection, the eradication of the epidemic required a broad approach, including contact isolation and cohorting, aggressive environmental disinfection, and close monitoring of the ward staff's performance. Infected patients were successfully treated with combined therapy. Although considered of low virulence, *A. baumannii* can be particularly aggressive and difficult to treat in ICU patients.

Key words: Acinetobacter baumannii, outbreak, intensive care unit, multi-drug resistance.

Riassunto *(Indagine molecolare e resistenza agli antibiotici in un'epidemia di* Acinetobacter baumannii *in una unità di terapia intensiva)*. Un'epidemia di *Acinetobacter baumannii* è stata studiata in una unità di terapia intensiva (UTI) presso l'Ospedale S. Giovanni Addolorata di Roma. L'epidemia ha coinvolto 14 pazienti e gli isolamenti provenivano soprattutto dal lavaggio broncoalveolare. Tutti gli isolamenti presentavano resistenza multipla agli antibiotici con ridotta sensibilità o resistenza ai carbapenemici. Ceppi di *A. baumannii* con simile spettro di sensibilità antibiotica sono stati isolati dall'ambiente. L'elettroforesi in campo pulsato su gel ha identificato un solo clone comune a pazienti ed isolamenti ambientali. Data la mancanza di un'unica sorgente di infezione, il controllo dell'epidemia ha richiesto un approccio complesso con isolamento dei contatti, disinfezione aggressiva dell'ambiente e stretto monitoraggio delle procedure del personale. I pazienti infettati sono stati efficacemente trattati con terapia di associazione. Sebbene *A. baumannii* sia considerato un batterio a bassa virulenza, può tuttavia rivelarsi particolarmente aggressivo e difficile da trattare nei pazienti ricoverati in UTI.

Parole chiave: Acinetobacter baumannii, epidemia, unità di terapia intensiva, multifarmaco-resistenza.

INTRODUCTION

Organisms belonging to the genus *Acinetobacter* are important pathogens, often causing nosocomial infections which are difficult to treat and which can be particularly severe in clinically compromised patients [1]. Their ability to survive under dry environmental conditions and to develop resistance to a variety of antimicrobial agents has resulted in their becoming a threat in critical hospital settings [2].

The risk factors for acquiring *Acinetobacter* infection include hospitalization, especially in intensive care units (ICU), poor general health status, the performance of mechanical ventilation, cardiovascular or respiratory failure, previous antimicrobial therapy, and the presence of central venous or urinary catheters [3, 4]. A review of a number of hospital outbreaks has confirmed that *Acinetobacter* infection represents a serious risk for hospital patients, in that it is an important cause of ventilator-associated pneumonia (VAP) and/or other severe clinical manifestations [1, 3]. In hospital settings such as ICUs, several reports have stressed the increasing frequency of the antimicrobial resistance of *Acinetobacter* to many drug classes, including carbapenems.

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We recently described an outbreak of *Acinetobacter baumannii* infection in an ICU in Italy [5]. The risk factors for infection were investigated by performing a case-control study, which revealed tracheostomy as the only risk factor among those patients with respiratory-tract involvement, who constituted nearly all of the cases. We also performed molecular typing of the *Acinetobacter* isolates, which confirmed that the occurrence of these cases did indeed constitute an outbreak. Although the results of this study have in part been previously described, in the present report we focus on antimicrobial susceptibility and the results of molecular typing, which is an important tool for better understanding epidemic dynamics and identifying determinants of transmission, which could be due either to a common source of infection or to cross-transmission from multiple strains.

METHODS

In April 2005, an increase in the incidence of *A. baumannii* clinical isolates began to be observed at the central laboratory of the "San Giovanni Addolorata" Hospital in Rome Italy, leading to suspicions of an outbreak and a subsequent epidemiological investigation. Given that the majority of the isolates had been obtained from adult ICU patients, both the investigation and the infection-control efforts were focused on this unit, which consists of 16 beds (8 beds in each of the ICU's two sub-units).

To identify the possible source of the outbreak, environmental samples were collected with cotton swabs from the hands of the healthcare workers in the ICU, mechanical ventilator circuits, monitors, and surfaces of tables for carrying medical instruments; microbiologic culture was then performed. We also reviewed the medical records of those patients from whom *A. baumannii* had been isolated so as to obtain information on age, gender, diagnosis upon ICU admission, transfer from other wards or hospitals, duration of ICU stay, performance of invasive procedures, use of antimicrobial agents, date and site of *A. baumannii* isolation, clinical infection, and antimicrobial sensitivity of *A. baumannii*.

Microbiological analysis and genotyping

Isolation of bacteria from clinical samples was obtained by standard procedures. Isolates were identified using the Vitek 2 automatic system (bioMerieux, Marcy l'Etoile, France). Antimicrobial susceptibility tests were performed by the Vitek 2 system and by a reference broth microdilution method (Sensititre panel, Biomedical s.r.l., Scorzè, Venice, Italy), in accordance with the recommendations of the Clinical Laboratory Standard Institute (CLSI) [6]. The CLSI breakpoints were applied for the interpretation of the results. Susceptibility to colistin was determined with a disk-diffusion test using 10 μg disk and the interpretative criteria suggested by Gales *et al.* [7].

The typing of *A. baumannii* was performed by means of pulsed field gel electrophoresis (PFGE). The preparation of genomic DNA was performed as previously described [8].

RESULTS

Outbreak description

From April 21st to June 27th 2005, a total of 57 *A. baumannii* isolates were obtained from 14 patients admitted to the ICU *(Table 1)*. Isolates were most frequently recovered from bronchoalveolar lavage (n. = 37); the other isolates were recovered from nasal/pharyngeal swabs (n. = 7), blood (n. = 5), pressure ulcer/wound (n. = 4), central venous catheter tip (n. = 3), and cerebrospinal fluid (n. = 1). Overall, 10 patients had at least 1 isolate obtained from respiratory secretions, 1 only from blood, 1 only from CVC tip, 1 from cerebrospinal fluid only, and 1 from both nasal and wound swab only. Ten of the 14 patients developed VAP; 1 patient had a diagnosis of meningitis, 1 a bloodstream infection, 1 a wound infection, and 1 had no clinical infection. For 4 of the VAP patients, *Pseudomonas aeruginosa* was also isolated from broncho-alveolar lavage.

The most common diagnoses upon admission to the ICU were trauma, coma, and cerebral hemorrhage. After excluding the 2 patients who were already upon admission to the ICU, the median time that had elapsed between admission and isolation of *A. baumannii* was 14 days (interquartile range, IQR: 7.5-22). The duration of mechanical ventilation at the moment of the first *A. baumannii* isolation ranged from 6 to 29 days.

During the ICU stay, 4 patients died, though none of the deaths was directly attributable to *A. baumannii* infection.

All patients but one had already undergone antibiotic treatment or prophylaxis before *A. baumannii* was isolated, in particular, beta-lactamic (n. = 4 patients), a beta-lactamic plus another antibiotic (*i.e.,* cotrimoxazole, glycopeptide, carbapenemic) (n. = 4); and beta-lactamic plus at least two other classes of antibiotics (n. = 5).

Antimicrobial susceptibility

Thirteen isolates from patients involved in the outbreak (1 isolate each) were available for testing for antimicrobial susceptibility. All isolates were resistant to piperacillin, piperacillin-tazobactam, ceftazidime, ciprofloxacin, chloramphenicol, trimethoprim/sulphametoxazole, and tobramycin *(Table 2)*. All isolates but 2 were susceptible to gentamycin. In 10 of the 13 patients, the first isolate was susceptible to ampicillin-sulbactam, whereas subsequent isolates and isolates from 1 patient were resistant (data not shown).

For all isolates, MICs to carbapenems ranged from 4 to 16 μg/ml, clustering around the breakpoints for susceptibility and resistance [6]. Based on this clustering, the isolates were categorized as exhibiting

Table 1 | *Characteristics of patients with* A. baumannii *infection/colonization*

Case	Date of Admission	Age/sex	Duration of stay in ICU at AB isolation (days)	Site of isolation (n.)	Admission diagnosis	Infection/ colonization	Other isolation from the same site	Outcome
H25	27/3/05	31/M	25	BAL (4) Blood (1) Pharyngeal swab (1) Nasal swab (1)	Trauma	VAP		Died
H23	19/4/05	53/F	10	BAL (4)	Cerebral haemorrage	VAP	*P. aeruginosa* from BAL	Discharged to ward
H34	30/4/05	46/M	7	CSF (1)	Trauma	Meningitis		Discharged to ward
H24	19/4/05	61/F	28	BAL (2) CVC tip (1)	Cerebral haemorrage	VAP	*P. aeruginosa* from BAL	Discharged to ward
H4	6/5/05	100/M	8	BAL (4) Wound swab (1)	Vertebral collapse	VAP		Died
H35	6/5/05	38/M	13	CVC tip (1)	Trauma	Colonization		Discharged to ward
H33	5/5/05	68/M	19	BAL (2) Nasal swab (1)	Cardiovascular	VAP	*P. aeruginosa* from BAL	Discharged to ward
H3	19/5/05	19/M	6	BAL (4) Wound swab (2) Nasal swab (1) Pharyngeal swab (1)	Trauma	VAP		Discharged to ward
H21	21/5/05	23/M	7	BAL (7)	Trauma	VAP		Discharged to ward
H20	15/5/05	53/F	15	Wound swab (1) Nasal swab (1)	Pancreatitis	Wound infection	*P. aeruginosa* and *E.coli* from wound swab	Discharged to ward
H8	14/6/05	74/M	-7*	BAL (4) Nasal swab (1)	Coma	VAP		Died
H6	20/4/05	65/F	51	CVC tip (1) Blood (4)	Coma	Blood stream infection	*E. faecalis* from blood	Died
H5	13/6/05	84/M	-2**	BAL (4)	Coma	VAP		Discharged to ward
H22	23/5/05	58/M	24	BAL (2)	Trauma	VAP	*S. marcescens* and *P. aeruginosa* from BAL	Discharged to ward

**While the patient was in Post-surgical intensive care.*
***While the patient was in Sub-intensive care.*
AB: A. baumannii; BAL: broncho-alveolar lavage; CSF: cerebrospinal fluid; CVC: central venous catheter; VAP: ventilator-associated pneumonia.

diminished susceptibility (MIC = 4 μg/ml), intermediate susceptibility (MIC = 8 μg/ml), or low resistance (MIC = 16 μg/ml). MICs to meropenem were in general 1 dilution lower than MICs to imipenem. MICs to carbapenem tended to be higher for isolates obtained towards the end of the outbreak. The disk-diffusion test for colistin was performed on 7 isolates, all of which were susceptible.

Two *A. baumannii* isolates were also obtained in the same period from patients admitted to other wards. One of these isolates, which was obtained in May 2005 from a patient in the neurosurgery ward, had a multiresistant profile, though it was susceptible to carbapenems (imipenem MIC = 4 μg/ml; meropenem MIC = 2 μg/ml), to bramycin (MIC = 2 μg/ml), and trimethoprim/sulphametoxazole (MIC ≤2 μg/ml). The other

Table 2 | *Antimicrobial susceptibility patterns* of* A. baumannii *isolates*

	MIC (µg/ml)																	
Isolate	**AMP**	**SAM**	**PIP**	**TZP**	**TIM**	**ATM**	**TIC**	**FEP**	**CAZ**	**CIP**	**SXT**	**CHL**	**AMK**	**GEN**	**TOB**	**IPM**	**MEM**	**COL**
H3	**>16**	**16**	**>256**	**>256**	**>128**	**>32**	**>128**	**32**	**>64**	**>16**	**>4**	**>64**	**>64**	4	**8**	**16**	**16**	NT[a]
H4	**>16**	**>16**	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**64**	4	**8**	**>16**	**16**	NT
H5	**>16**	8	**>256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**>64**	4	**8**	**8**	**16**	S
H6	**>16**	8	**256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**>64**	4	**8**	**16**	4	S
H8	**>16**	8	**>256**	**>256**	**>128**	**>32**	**>128**	**64**	**>64**	**>16**	**>4**	**>64**	**>64**	4	**8**	**16**	**8**	NT
H20	**>16**	8	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**>64**	4	**8**	**16**	**8**	S
H21	**>16**	**16**	**256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**16**	**>4**	**>64**	**32**	4	**8**	4	4	S
H22	**>16**	**16**	**>256**	**>256**	**>128**	**>32**	**>128**	**32**	**>64**	**>16**	**>4**	**>64**	**64**	**8**	**32**	**8**	**8**	NT
H23	**>16**	**>16**	**256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**16**	**>4**	**>64**	**32**	**8**	**8**	4	4	S
H24	**>16**	8	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**64**	4	**8**	**16**	**8**	NT
H25	**>16**	**>16**	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**>64**	**64**	4	**8**	**16**	**8**	S
H33	**>16**	**>16**	**256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**64**	**64**	4	**8**	**8**	4	S
H34	**>16**	4	**>256**	**>256**	**>128**	**>32**	**>128**	**32**	**>64**	**>16**	**>4**	**>64**	**32**	4	**8**	**16**	**8**	NT
[b]H1	**>16**	4	**256**	**256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	≤2	**>64**	**64**	**8**	2	4	2	S
[c]H30	**16**	≤2	8	≤1	≤4	8	≤8	1	2	≤0.5	≤2	≤8	1	≤1	0.5	≤0.5	≤1	NT
[d]E15	NT	NT	**>256**	**>256**	**>128**	**>32**	**>128**	**>64**	**>64**	**>16**	**>4**	**>64**	**>64**	NT	**16**	**16**	**8**	NT
[d]E16	NT	NT	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**64**	**64**	NT	**8**	**8**	4	NT
[d]E17	NT	NT	**256**	**256**	**>128**	**>32**	**>128**	**32**	**>64**	**16**	**>4**	**64**	**32**	NT	**8**	4	4	NT
[d]E18	NT	NT	**>256**	**>256**	**>128**	**>32**	**>128**	**>64**	**>64**	**>16**	**>4**	**>64**	**>64**	NT	**16**	**16**	**16**	NT
[d]E31	NT	NT	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**64**	**>64**	NT	**16**	**8**	**8**	NT
[d]E32	NT	NT	**>256**	**>256**	**>128**	**>32**	**>128**	**16**	**>64**	**>16**	**>4**	**64**	**>64**	NT	**16**	**8**	**8**	NT

Antibiotic abbreviations are as follows: AMP, ampicillin; SAM, ampicillin-sulbactam; PIP, piperacillin;TZP, piperacillin-tazobactam; TIM, ticarcillin/clavulanic acid; ATM, aztreonam; TIC, ticarcillin; FEP, cefepime; CAZ, ceftazidime; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; CHL, chloramphenicol; AMK, amikacin; GEN, gentamicin; TOB, tobramycin; IPM, imipenem; MEM, meropenem ; COL, colistin.
Non susceptibility is indicated in boldface.
**Antimicrobial susceptibility was determined by the sensititre panel, except for ampicillin, ampicillin-sulbactam, gentamycin.*
[a]NT, not tested; [b]strain from neurosurgery ward; [c]strain from pneumology ward; [d]strain from environment.

isolate, collected from a patient in the respiratory disease ward in July 2005, was fully susceptible to all of the antibiotics mentioned above, except for imipenem and meropenem *(Table 2)*.

Outbreak investigation

Environmental investigation

Following the isolation of the first strains of *A. baumannii*, sampling of the ICU environment and of the hands and coats of the staff was carried out. Of the 32 environmental samples, 6 yielded growth of *A. baumannii*. These included 3 samples from gloves worn by the attending nursing staff, 1 from a physician's disposable coat, 1 from a washbasin, and 1 from the cover of the folder containing the patient's clinical chart. The antibiotic susceptibility profiles of the environmental isolates were consistent with those of the clinical isolates: all of the isolates were resistant to all of the antibiotics tested, including carbapenems, except for one isolate which was susceptible to both imipenem and meropenem.

PFGE typing

Twenty-one isolates were available for PFGE typing: 13 isolates from patients involved in the outbreak (1 isolate each); 6 from environmental samples; and 2 from patients in the neurosurgery and respiratory-disease wards. Typing indicated the presence of a single strain, given that the isolates from the ICU (from both patients and the environment) showed > 95% genetic relatedness *(Figure 1)*. Interestingly, the 2 clinical isolates from the other wards showed a genetic relatedness with the outbreak isolates of < 65%.

Intervention

Several measures were adopted to control the outbreak. First, a multidisciplinary task force was created, which consisted of the Hospital health direction, the ICU's head physician, a microbiologist, the ICU's infectious-disease consultant, and epidemiologists and microbiologists from the *Istituto Superiore di Sanità* (Italian National Health Institute). Second, rigorous infection control measures, such as enhanced cleaning of the environment with 4% sodium hypochlorite and strict hand-washing with chlorhexidine gluconate after patient contact, were implemented. All staff members were advised to use gloves when coming into direct contact with a patient and to change gloves between patients. The adoption of all of these measures was stressed during daily briefings.

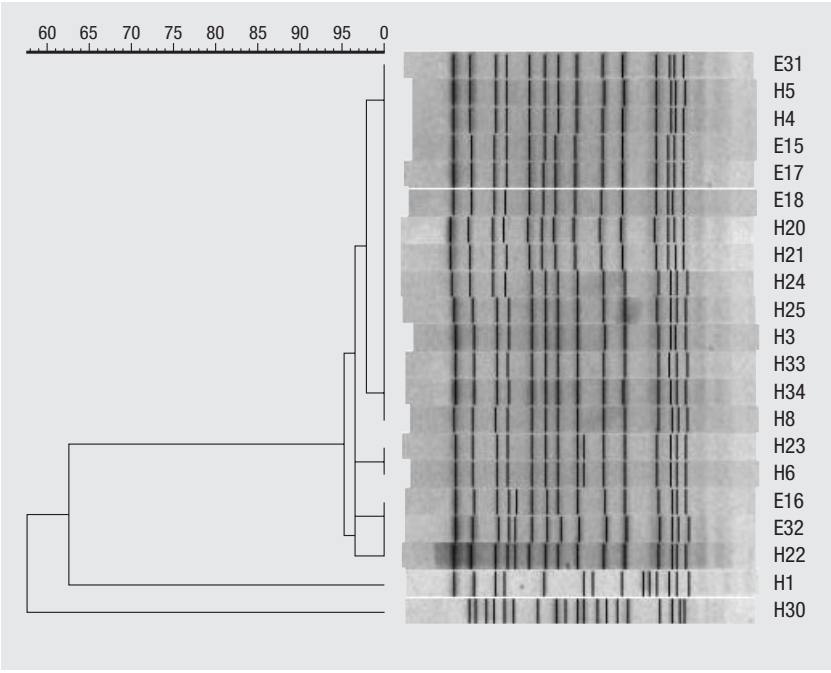

Fig. 1 | *PFGE dendogram illustrating genetic relationships among human (H) and environmental (E) isolates of* A. baumannii. *Among the human isolates, 13 were from patients involved in the ICU outbreak, whilst H1 and H30 isolates were obtained from 2 patients admitted in other wards. All the environmental isolates were obtained in the same ICU.*

Third, cohorting isolation and contact isolation were instituted. Moreover, the ICU was closed for about 21 days for decontamination. Following these interventions, no other *A. baumannii* isolates were reported in the ward. Infected patients were successfully treated with combined therapy consisting of ampicillin-sulbactam, colistin and rifampin.

DISCUSSION

This outbreak was clinically characterized by a predominance of respiratory-tract involvement, which in most cases had serious consequences, such as VAP. This finding is consistent with reports of other outbreaks that predominantly involved ICU patients requiring intubation and mechanical ventilation.

As previously highlighted in the case-control study, tracheostomy was the only factor associated with *A. baumannii* positivity among patients with respiratory-tract involvement [5]. Several environmental samples collected in the ICU were found to be contaminated. However, the way in which *A. baumannii* was introduced in the ICU remains undefined. Of importance is the finding that the environmental isolates had the same pattern of antimicrobial susceptibility as the isolates from patients. Whereas this confirms that the outbreak was caused by a single clone, it was not possible to establish whether the source was an infected patient or the environment.

Transmission of *Acinetobacter* to multiple patients is enhanced by a combination of multiple-site patient colonization, widespread environmental contamination, prolonged survival on dry surfaces and on hands, and the capacity to develop or acquire resistance to virtually all classes of antimicrobial agents. This occurs when patients who are severely ill and aggressively supported and who had been previously treated with antibiotics are kept in close proximity to one another. Moreover, at the time of the outbreak, there was a shortage of ICU staff, which may have contributed, along with the lack of strict hand hygiene, to less stringent infection-control practices.

Antibiotic resistance is a major problem in treating infection with *A. baumannii*, which has become resistant to almost all currently available antimicrobial agents. Of note is our finding that the strains involved in this outbreak were fully susceptible to gentamycin and colistin only, that they were partially susceptible to ampicillin-sulbactam, and that they showed diminished susceptibility or low resistance to carbapenems. *A. baumannii* isolates with a similar profile of antibiotic resistance have already caused outbreaks in Italy [9]. The activity of ampicillin/sulbactam towards some of the isolates can be attributed to the intrinsic activity of sulbactam alone against *A. baumannii* and not necessarily to its inhibition of *A. baumannii* beta-lactamase [10]. Until very recently, carbapenems were considered to be the only antibiotics with reliable activity against *A. baumannii* [11, 12]. However, outbreaks of carbapenem-resistant *A. baumannii* have occurred in several countries, especially in Spain, France, and Italy [13]. In the present outbreak, the range of MICs indicated diminished susceptibility or low resistance. This range appears to be characteristic of *A. baumannii* isolates carrying the OXA-58 carbapenemase [14], which have been identified in recent outbreaks occurring in other hospitals in Rome [15]. In our isolates, meropenem exhibited a slightly greater activity than imipenem, although the

latter is generally considered more potent than meropenem against this species [11]. It can be hypothesized that the resistance mechanism operating in the isolates, which can involve diminished permeability or efflux factors besides the carbapenemase, was slightly more efficient against imipenem. Colistin showed full activity against the strains tested using a disk-diffusion method. Although this method has been demonstrated to be error-prone [7], the reference MIC testing requires in-house preparation of antibiotic dilutions and is beyond the routine of a hospital clinical laboratory. In any case, the association of antibiotics used was apparently effective. Despite the fact that isolates of *A. baumannii* were not tested for susceptibility against rifampin, this drug has been used by clinicians, in combination with colistin and ampicillin-sulbactam, based on successful clinical evidence [16, 17].

Because of the inability to precisely identify the source of infection, controlling the epidemic required a broad approach, such as contact isolation and cohorting applied to all patients with *A. baumannii*, aggressive environmental disinfection, and close monitoring of the performance of the personnel.

In conclusion, our study confirms that, although considered of low virulence, *A. baumannii* can cause significant morbidity in ICUs. Structural factors, such as nurse-to-patient ratio and the need to strictly adhere to infection-control policies (especially hand and environmental hygiene) are essential conditions for the prevention and control of *A. baumannii* outbreaks.

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