ORIGINAL ARTICLE 10.1111/j.1469-0691.2006.01510.x

Epidemiology of multiple *Acinetobacter* outbreaks in The Netherlands during the period 1999–2001

P. J. van den Broek[1], J. Arends[2], A. T. Bernards[3], E. De Brauwer[4], E. M. Mascini[5], T. J. K. van der Reijden[1], L. Spanjaard[6], E. A. P. M. Thewessen[7], A. van der Zee[8], J. H. van Zeijl[9] and L. Dijkshoorn[1]

[1]Department of Infectious Diseases, Leiden University Medical Centre, Leiden, [2]Department of Medical Microbiology, University Hospital Groningen, Groningen, [3]Department of Medical Microbiology, Leiden University Medical Centre, Leiden, [4]Department of Microbiology, Atrium Medical Centre, Heerlen, [5]Department of Medical Microbiology, University Medical Centre, Utrecht, [6]Department of Medical Microbiology, Academic Medical Centre, Amsterdam, [7]Department of Medical Microbiology and Infection Prevention, Groene Hart Hospital, Gouda, [8]Laboratory of Medical Microbiology, St Elisabeth Hospital, Tilburg and [9]Department of Microbiology, Public Health Laboratory Friesland, Leeuwarden, The Netherlands

ABSTRACT

An increase in the number of outbreaks of *Acinetobacter* infection was notified in The Netherlands during 1999–2001. The present study compared the outbreaks at the species and strain levels, and analysed the epidemiology and control measures at the different locations. For each institute, three representative isolates from three patients were identified to the species and strain levels by genotyping methods. A questionnaire investigated the impact of the outbreak, the control measures that were taken, and the possible effects of the measures. Seven outbreaks were associated with *Acinetobacter baumannii* (three outbreaks with a strain designated strain A, two outbreaks with a strain designated strain B, and one outbreak each with strains designated C and D). An additional outbreak was caused by genomic species 13TU, which is related closely to *A. baumannii*. Strains B and D were identified as European clones III and II, respectively. Except for two hospitals with outbreaks caused by strain A, there was no known epidemiological link between the participating hospitals. In all hospitals the outbreak occurred on one or several intensive care units, and spread to other departments was noted in two hospitals. The number of patients affected ranged from six to 66 over a period of 2–22 months. In most outbreaks, patients were the likely reservoir from which spread occurred. In all hospitals, a large panel of measures was required to bring the outbreak to an end. Extensive environmental sampling yielded numerous positive samples in most but not all hospitals.

Keywords *Acinetobacter baumannii*, cross-infection, environmental contamination, genotypes, molecular typing, outbreak management

Original Submission: 29 July 2005; **Revised Submission:** 14 November 2005; **Accepted:** 26 December 2005

Clin Microbiol Infect 2006; 12: 837–843

INTRODUCTION

Acinetobacter is isolated regularly from clinical specimens in hospitals. Different species are found under endemic circumstances, with species belonging to the *Acinetobacter calcoaceticus–Acinetobacter baumannii* (Acb) complex constituting more than half of the isolates [1,2]. Nosocomial outbreaks of *Acinetobacter* occur frequently and are caused almost always by *A. baumannii* [3]. A common source is found in about half of all outbreaks, and elimination of this source leads rapidly to the end of the outbreak. When no common source is identified and cross-contamination seems to be the driving force of the outbreak, bringing the outbreak to an end is much

Corresponding author and reprint requests: P. J. van den Broek, Department of Infectious Diseases, C5-P, Leiden University Medical Centre, PO Box 9600, 2300 RC Leiden, The Netherlands
E-mail: p.j.van_den_broek@lumc.nl

more difficult. It is likely that survival of the microorganism in the environment, as shown by positive environmental cultures, plays an important role in the persistence of outbreaks.

Between 1999 and 2001, eight hospitals in The Netherlands experienced *Acinetobacter* outbreaks. The simultaneous occurrence of so many outbreaks in a relatively short period prompted an investigation with the following questions: (i) were some or all of the outbreaks caused by the same strain of *Acinetobacter*; (ii) what was the consequence for patients, measured as morbidity and mortality; (iii) what control measures were necessary to stop the outbreaks; and (iv) have environmental cultures been helpful in understanding transmission and taking control measures?

MATERIALS AND METHODS

Bacteriological investigations

Isolates were identified presumptively to the species level at each participating hospital using local diagnostic procedures, including use of API 20NE, Vitek 1 or Vitek 2 (bioMérieux, 's-Hertogenbosch, The Netherlands), or identification was limited to the genus level. From each hospital, three representative isolates from three patients (one from the beginning of the outbreak, one mid-way through, and one from the end) were sent to the Department of Infectious Diseases, Leiden University Medical Centre, where species identification was peformed by amplified rDNA restriction analysis (ARDRA) and amplified fragment length polymorphism (AFLP) analysis (see below). AFLP fingerprint analysis was also used to type isolates. Antibiotic susceptibility was determined in the Clinical Microbiology Laboratory of the Leiden University Medical Centre.

ARDRA

ARDRA was carried out as described previously [4]. In brief, separate aliquots of amplified 16S rDNA were digested with five restriction endonucleases (*Cfo*I, *Alu*I, *Mbo*I, *Rsa*I, *Msp*I). Fragments were separated by electrophoresis in agarose 2.5% w/v gels. Species identification was performed by comparing the profiles with those of a library of strains of (genomic) species described previously [4].

AFLP fingerprinting

Selective amplification of genomic restriction fragments using AFLP was performed as described by Nemec *et al*. [5]. Briefly, purified DNA was digested using *Eco*RI and *Mse*I, and this was followed by amplification with a Cy5-labelled *Eco*RI+A primer and an *Mse*I+C primer (A and C are selective bases). The ALFexpress II DNA analysis system (Amersham Biosciences, Roosendaal, The Netherlands) was used for fragment separation. Fingerprints of fragments in the range 50–500 bp were investigated by cluster analysis with Bionumerics v.2.0 (Applied Maths, Sint-Martens-Latem, Belgium), using the Pearson product moment coefficient (r) as similarity measure and the unweighted pair-group method with arithmetic averages (UPGMA) for grouping. For species identification, isolates were compared with a library of strains of all (genomic) species described previously, using a cut-off level of 50%, above which strains are considered to belong to the same species [5]. Isolates were considered to belong to the same strain if they grouped together at ≥90%.

ERIC-PCR fingerprinting

Comparative typing was based on fragments obtained by PCR using enterobacterial repetitive intergenic consensus (ERIC) sequences with two primers: ERIC1R, 5′-ATGTAAGCT-CCTGGGGATTCAC, and ERIC2, 5′-AAGTAAGTGACTGGG-GTGAGCG [6]. Three different PCR amplifications were performed: one with ERIC1R, one with ERIC2, and one with both primers. The results of the three fingerprints were combined to generate a single type, independent of the AFLP fingerprint analysis.

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed with the Vitek 2 system according to the manufacturer's instructions. Allocation of the results to the categories S (susceptible), I (intermediately-susceptible) and R (resistant) was according to the Dutch guidelines [7]. One isolate from each centre was tested in an AST-N020 Gram-negative susceptibility card, except for the Utrecht centre, from which three isolates were included.

Questionnaire

A questionnaire was sent to the eight participating hospitals at the end of 2001 to obtain the following information about the outbreaks: number of patients involved; number of patients who had clinical disease or died because of the outbreak strain; times at which the first and the last patient were detected; number and type of departments involved; whether a common source was identified; total number and number of positive environmental cultures, and, if environmental cultures were positive, at which sites *Acinetobacter* was found; total number and number of positive cultures from medical equipment, and, if positive cultures were found, type of medical equipment; and what control measures were taken.

RESULTS

The eight outbreaks occurred in hospitals scattered throughout The Netherlands (Fig. 1). The outbreaks lasted for 2–22 months and the number of patients involved varied between six and 66 per outbreak, with a total number of 169 patients (Table 1). In all hospitals, one or more intensive care units were affected. In two hospitals, *Acinetobacter* had spread to general wards.

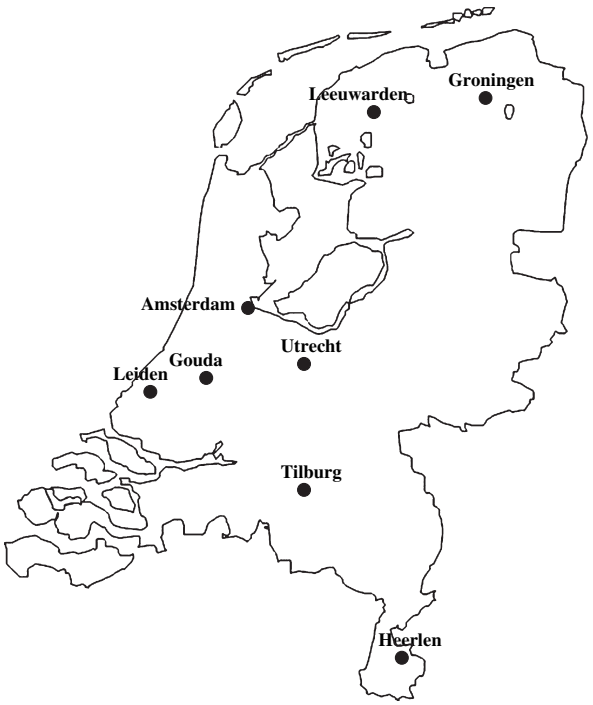

Fig. 1. Location of hospitals that experienced outbreaks of *Acinetobacter* infection.

In 27 (16%) cases, the epidemic strain of *Acinetobacter* was considered to be a definite cause of disease. In another 26 (15%) cases, the epidemic strain was considered to have possibly caused disease. In two cases, death was considered to be the direct consequence of infection with the outbreak strain. In 13 (8%) cases, *Acinetobacter* had possibly played a role in the death of the patients.

Bacteriology

The isolates from seven of the eight hospitals were identified as *A. baumannii* by comparison of ARDRA (*Cfo*I, *Alu*I, *Mbo*I, *Rsa*I, *Msp*I profile 1 1 1 2 1) [4] and AFLP profiles with those of the Leiden University Medical Centre reference libraries. The exception was Utrecht, where the outbreak was caused by *Acinetobacter* 13TU [8]. The AFLP typing results showed that isolates from the same outbreak were linked by >90% similarity, indicating that one strain was involved (Fig. 2). In addition, isolates from some hospitals clustered together at this level, indicating a common type, which was corroborated by results obtained by ERIC-PCR fingerprinting and antibiotic susceptibility profiling (data not shown). The outbreaks in Tilburg and Gouda were caused by the same type (designated strain A) of *A. baumannii*. Likewise, an identical type (strain B) was found in Groningen, Leeuwarden and Heerlen. In Leiden and Amsterdam, unique types were found (designated strains C and D, respectively). The *A. baumannii* strain B was identified with clone III (AFLP linkage level 81%) of multiresistant strains found in Spain, Belgium and France [9], and strain D clustered at 81% with European clone II, which has been found in north-west Europe and the Czech Republic [10,11] (data not shown). All *A. baumannii* strains were resistant to penicillins, cephalosporins and quinolones. *Acinetobacter* genomic species 13TU was sensitive to all tested antibiotics; however, in one patient the outbreak strain acquired

Table 1. Data concerning outbreaks of *Acinetobacter* infection in The Netherlands

	Heerlen	Groningen	Leeuwarden	Utrecht	Tilburg	Gouda	Amsterdam	Leiden
Number of patients	66	14	10	23	11	6	8	31
First patient detected	August 1999	September 2000	October 2000	October 1999	September 2000	July 2001	October 2001	November 2000
Last patient detected	May 2001	November 2000	January 2001	May 2000	November 2000	August 2001	November 2001	October 2001
Morbidity, possible/certain	3/0	5/6	0/1	11/6	1/2	1/2	0/5	5/5
Mortality, possible/certain	0/0	3/1	0/0	0/0	1/0	1/0	5/0	3/1
Departments involved (number)	ICU (3)	ICU (4) General ward (1)	ICU (1) Coronary care unit (1) General ward (3)	ICU (1)	ICU (1)	ICU (1)	ICU (3)	ICU (2)
Common source	Not identified	Not identified	Not identified	Not identified	Not identified	Not identified	Not identified	Ventilator Bear Hugger (likely)
Control measures	A, B, C, D	A, D, E, F	A, C, D, G, H	A, B, C, D, G, I	A, B, D, G, H, J	C, D	A, B, C	A, B, C, D, H

A, patient isolation; B, patient screening; C, intensified standard precautions; D, intensified cleaning and disinfection; E, intensified hand hygiene; F, department closure; G, cohort nursing; H, admission stop; I, limitation of cephalosporin use; J, screening of personnel; ICU, intensive care unit.

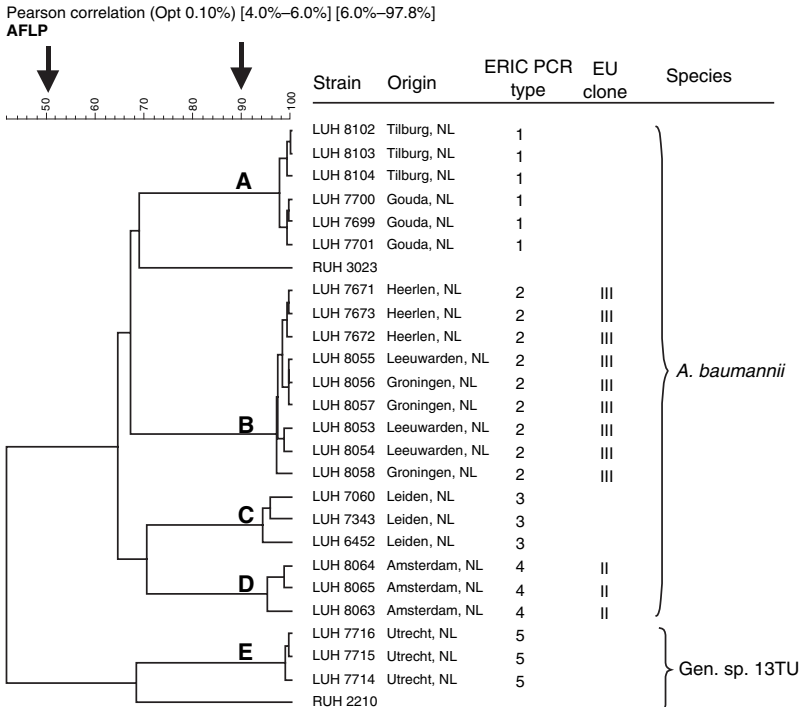

Fig. 2. Dendrogram of cluster analysis of amplified fragment length polymorphism fingerprints of 24 isolates from eight outbreaks of *Acinetobacter* spp. infection in The Netherlands, and the type reference strain of *Acinetobacter baumannii* (RUH 3023 = ATCC 19606^T) and unnamed genomic species 13TU (RUH 2210 = ATCC 17903). The grouping was determined using the unweighted pair-group method with arithmetic averages (UPGMA) and Pearson's product moment correlation coefficient. Arrows denote the strain and species delineation levels (90% and 50%, respectively). EU clones II and III [9–11] are indicated. ERIC, enterobacterial repetitive intergenic consensus.

resistance to aminoglycosides, cephalosporins and quinolones, as described in detail elsewhere [8].

Epidemiological investigations and control measures

In seven of the eight hospitals, samples were taken from the environment, furniture, medical and non-medical equipment, the tap water system and the air-conditioning system. In six hospitals, multi-site contamination with the outbreak strain was observed (Table 2). This was not the case in Leiden, where the outbreak strain was recovered only from the electronic compartment of ventilators and filters from a Bear Hugger, an apparatus from which cold or warm air is passed to the patient's mattress for cooling or warming [12].

In seven hospitals, measures to control the outbreak included isolation of patients, and intensification of standard cross-infection precautions, including cleaning and disinfection of the environment, furniture and equipment (Table 1). In four hospitals, an admission stop or closure of wards was necessary. Screening of patients to detect carriers of *Acinetobacter* was undertaken in five hospitals.

DISCUSSION

The *Acinetobacter* outbreaks that occurred between August 1999 and November 2001 in The Netherlands were caused by *A. baumannii* in seven hospitals and by *Acinetobacter* genomic species 13TU in one hospital. Four *A. baumannii* strains were involved. One strain was found in three hospitals (Groningen, Leeuwarden and Heerlen), and a second strain in two hospitals (Tilburg and Gouda), while Leiden and Amsterdam each had a unique strain. The occurrence of indistinguishable strains in more than one hospital implied inter-hospital spread. For the two hospitals sharing AFLP type B (Groningen and Leeuwarden), spread was explained by the transfer of a colonised patient from one hospital to the other. There was no direct link between the other hospitals, and the implied inter-hospital spread came as a surprise. The finding that strains B and D belonged to EU clones III and II, respectively, emphasises the epidemic potential of these strains.

Several reports have documented episodes of extensive spread of multidrug-resistant *A. baumannii* among hospitals, including a multicentre outbreak in the UK [13] and another in the USA [14]. The fact that highly similar multidrug-

Table 2. Surveillance culture results during outbreaks of *Acinetobacter* infection in The Netherlands

	Heerlen	Groningen	Leeuwarden	Utrecht	Tilburg	Gouda	Amsterdam	Leiden
Number of cultures (positive)	6603 (194)		275 (33)	75–90 (9)	80 (10)	Not done	121 (15)	145 (3)
Environment[a]	Light rail Floors in and outside wards		Light rails Floor	Bed curtains Door-handle Cup holder Dialysis water outlet	Dressing rooms		Light rail	Sedimentation plate in patient room
Furniture[a]	Beds Anti-decubitus mattress Pillow Bedside cabinet Desk surface Dustbin	Anti-decubitus mattress	Beds		Desk surface Laundry bag holder Chairs			
Medical equipment[a]	Ventilator Laryngoscope CT scan	Infusion pump Ventilation equipment	Wound dressing trolley Ventilators Dialysis machines Perfusors Stethoscopes	Moisture catcher of ventilator tubing Expiration part of ventilator	Resuscitation car			Ventilator Bear Hugger apparatus
Other equipment[a]	Refrigerator Cleaning basket Computer keyboard		Telephone Computer keyboard		Computer keyboard			
Tap water system[a]	Kitchen sink		Sinks	Wash-stand	Sink		Sinks	
Air-conditioning and ventilation system[a]	Ventilation grate						Ventilation grate	

[a]Sites from which positive samples were obtained.
CT, computed tomography.

resistant *A. baumannii* strains (clones) occur in hospitals of different cities and countries of Europe, and beyond, is a matter for concern. Early recognition and careful monitoring of these strains should be part of a strategy to halt their spread.

The overall impact of *Acinetobacter* on morbidity and mortality is not known, and crude mortalities have been approximated to fluctuate between 17% and 52% [15]. In the present study, *Acinetobacter* caused disease in about one-third of patients, while disease was definitely attributed to the organism in about half of these patients. Typically, *Acinetobacter* is an opportunistic pathogen, causing disease in severely-ill patients. All the outbreaks occurred primarily in intensive care units. In *c.* 9% of the patients, *Acinetobacter* could have contributed to mortality. Death was considered to be directly caused by the organism in two patients.

The main measures taken to stop the outbreaks were barrier nursing of patients, intensification of standard cross-infection precautions, including cleaning and disinfection of patient rooms and medical equipment, etc., stopping admissions or temporary closure of departments, and screening of patients for colonisation with *Acinetobacter*. No common source was identified with certainty for any of the outbreaks, with Leiden being the possible exception. In Leiden, the outbreak strain was found in the electronic compartments of ventilators and a Bear Hugger. After removing dust from the electronic compartments of the ventilators and changing the filters of the Bear Hugger apparatus, the outbreak came to an end, suggesting that this apparatus was indeed the source of the outbreak [12]. In the other hospitals, cross-contamination was considered to be the main mechanism sustaining the outbreak.

Contamination of the environment is described in about half of the outbreaks for which information is given concerning environmental sampling. In 16 single-strain outbreaks with multi-site contamination of the environment, no common source was identified and cross-contamination was considered to be the method of transmission [8,16–30]. Of 11 single-strain outbreaks with negative environmental cultures, seven were identified as common source outbreaks; in four the transmission route remained unknown [31–

41]. The Dutch outbreaks fit this general pattern of *Acinetobacter* outbreaks, in that multi-site environmental contamination suggests cross-transmission, while negative environmental cultures raise high suspicion of a common source.

Based on these results, it seems reasonable to advocate identification of isolates to the species and strain levels and sampling of the environment as the first step in the analysis of an outbreak caused by *Acinetobacter*. When a single strain is found in conjunction with negative environmental cultures, the next step should be to look for a common source. When multiple strains or a contaminated environment are found, measures should be taken to stop cross-transmission. Often, this will also involve closure of the ward.

In the present study, the rates of positive environmental samples varied considerably among the participating hospitals (Table 2). The differences may reflect true variations in environmental prevalence, or may be caused by differences in sampling and cultivation procedures. Control measures are frequently based on results obtained by these procedures, and it is therefore recommended to establish guidelines for environmental tracing and monitoring of epidemic strains of *Acinetobacter*.

ACKNOWLEDGEMENTS

The authors thank J. H. Wagenvoort for his contribution to the work.

REFERENCES

1. Struelens MJ, Carlier E, Maes N, Serruys E, Quint WGV, van Belkum A. Nosocomial colonization and infection with multiresistant *Acinetobacter baumannii*: outbreak delineation using DNA macrorestriction analysis and PCR-fingerprinting. *J Hosp Infect* 1993; **25**: 15–32.
2. Seifert H, Baginsky R, Schulze A, Pulverer G. The distribution of *Acinetobacter* species in clinical culture materials. *Zentralbl Bakteriol* 1993; **279**: 544–552.
3. Villegas MV, Hartstein AI. *Acinetobacter* outbreaks, 1977–2000. *Infect Control Hosp Epidemiol* 2003; **24**: 284–295.
4. Dijkshoorn L, Van Harsselaar B, Tjernberg I, Bouvet PJ, Vaneechoutte M. Evaluation of amplified ribosomal DNA restriction analysis for identification of *Acinetobacter* genomic species. *Syst Appl Microbiol* 1998; **21**: 33–39.
5. Nemec A, De Baere T, Tjernberg I, Vaneechoutte M, van der Reijden TJ, Dijkshoorn L. *Acinetobacter ursingii* sp. nov. and *Acinetobacter schindleri* sp. nov., isolated from human clinical specimens. *Int J Syst Evol Microbiol* 2001; **51**: 1891–1899.
6. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res* 1991; **19**: 6823–6831.
7. Commissie Richtlijnen Gevoeligheidsbepalingen. Interpretatie van gevoeligheidsonderzoek en gevoeligheidscriteria voor antimicrobiële middelen in Nederland. *Ned Tijdschr Med Microb* 2000; **8**: 79–81.
8. Dessel H, Kamp-Homans TEM, Fluyt AC *et al.* Outbreak of a susceptible strain of *Acinetobacter* species 13 (*sensu* Tjernberg and Ursing) in an adult neurosurgical intensive care unit. *J Hosp Infect* 2002; **51**: 89–95.
9. Van Dessel H, Dijkshoorn L, van der Reijden T *et al.* Identification of a new geographically widespread multiresistant *Acinetobacter baumannii* clone from European hospitals. *Res Microbiol* 2004; **155**: 105–112.
10. Dijkshoorn L, Aucken H, Gerner-Smidt P *et al.* Comparison of outbreak and nonoutbreak *Acinetobacter baumannii* strains by genotypic and phenotypic methods. *J Clin Microbiol* 1996; **34**: 1519–1525.
11. Nemec A, Dijkshoorn L, van der Reijden TJ. Long-term predominance of two pan-European clones among multiresistant *Acinetobacter baumannii* strains in the Czech Republic. *J Med Microbiol* 2004; **53**: 147–153.
12. Bernards AT, van der Reijden TJK, Dijkshoorn L, Harinck HIJ, van den Broek PJ. Persistent *Acinetobacter baumannii*? Look *inside* your medical equipment. *Infect Control Hosp Epidemiol* 2004; **25**: 1002–1004.
13. Turton JF, Kaufmann ME, Warner M, Dijkshoorn L, van der Reijden T, Pitt TL. A prevalent, multiresistant clone of *Acinetobacter baumannii* in South East England. *J Hosp Infect* 2004; **58**: 170–179.
14. Landman D, Quale JM, Mayorga D *et al.* Citywide clonal outbreak of multiresistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in Brooklyn, NY: the preantibiotic era has returned. *Arch Intern Med* 2002; **162**: 1515–1520.
15. Cisneros JM, Rodríguez-Baño J. Nosocomial bacteremia due to *Acinetobacter baumannii*: epidemiology, clinical features and treatment. *Clin Microbiol Infect* 2002; **8**: 687–693.
16. El Shafie SS, Alishaq M, Garcia ML. Investigation of an outbreak of multidrug-resistant *Acinetobacter baumannii* in trauma intensive care unit. *J Hosp Infect* 2004; **56**: 101–105.
17. Allen KD, Green HT. Hospital outbreak of multi-resistant Acinetobacter anitratus: an airborne mode of spread? *J Hosp Infect* 1987; **9**: 110–119.
18. Denton M, Wilcox MH, Parnell P *et al.* Role of environmental cleaning in controlling an outbreak of *Acinetobacter baumannii* on a neurosurgical intensive care unit. *J Hosp Infect* 2004; **56**: 106–110.
19. Roberts SA, Findlay R, Lang SD. Investigation of an outbreak of multi-drug resistant *Acinetobacter baumannii* in an intensive care burns unit. *J Hosp Infect* 2001; **48**: 228–232.
20. Wang SH, Sheng WH, Chang YY *et al.* Healthcare-associated outbreak due to pan-drug resistant *Acinetobacter baumannii* in a surgical intensive care unit. *J Hosp Infect* 2003; **53**: 97–102.
21. Aygun G, Demirkiran O, Utku T *et al.* Environmental contamination during a carbapenem-resistant *Acinetobacter baumannii* outbreak in an intensive care unit. *J Hosp Infect* 2002; **52**: 259–262.
22. Crombach WH, Dijkshoorn L, van Noort-Klaassen M, Niessen J, van Knippenberg-Gordebeke G. Control of an

epidemic spread of a multi-resistant strain of *Acinetobacter calcoaceticus* in a hospital. *Intens Care Med* 1989; **15**: 166–170.
23. Simor AE, Lee M, Vearncombe M *et al*. An outbreak due to multiresistant *Acinetobacter baumannii* in a burn unit: risk factors for acquisition and management. *Infect Control Hosp Epidemiol* 2002; **23**: 261–267.
24. Das I, Lambert P, Hill D, Noy M, Bion J, Elliott TJ. Carbapenem-resistant *Acinetobacter* and role of curtains in an outbreak in intensive care units. *J Hosp Infect* 2002; **50**: 110–114.
25. Debast SB, Meis JF, Melchers WJ, Hoogkamp-Korstanje JA, Voss A. Use of interrepeat PCR fingerprinting to investigate an *Acinetobacter baumannii* outbreak in an intensive care unit. *Scand J Infect Dis* 1996; **28**: 577–581.
26. Pina P, Guezenec P, Grosbuis S, Guyot L, Ghnassia JC, Allouch PY. Investigation d'une épidémie à *Acinetobacter baumannii* au centre hospitalier de Versailles. *Pathol Biol (Paris)* 1998; **46**: 385–394.
27. Hsueh PR, Teng LJ, Chen CY *et al*. Pandrug-resistant *Acinetobacter baumannii* causing nosocomial infections in a university hospital, Taiwan. *Emerg Infect Dis* 2002; **8**: 827–832.
28. Podnos YD, Cinat ME, Wilson SE *et al*. Eradication of multi-drug resistant *Acinetobacter* from an intensive care unit. *Surg Infect* 2001; **2**: 297–301.
29. Go ES, Urban C, Burns J *et al*. Clinical and molecular epidemiology of acinetobacter infections sensitive only to polymyxin B and sulbactam. *Lancet* 1994; **344**: 1329–1332.
30. Tankovic J, Legrand P, De Gatines G, Chemineau V, Brun-Buisson C, Duval J. Characterization of a hospital outbreak of imipenem-resistant *Acinetobacter baumannii* by phenotypic and genotypic typing methods. *J Clin Microbiol* 1994; **32**: 2677–2681.
31. D'Agata EM, Thayer V, Schaffner W. An outbreak of *Acinetobacter baumannii*: the importance of cross-transmission. *Infect Control Hosp Epidemiol* 2000; **21**: 588–591.
32. Yoo JH, Choi JH, Shin WS *et al*. Application of infrequent-restriction-site PCR to clinical isolates of *Acinetobacter baumannii* and *Serratia marcescens*. *J Clin Microbiol* 1999; **37**: 3108–3112.
33. Huang YC, Su LH, Wu TL *et al*. Outbreak of *Acinetobacter baumannii* bacteremia in a neonatal intensive care unit: clinical implications and genotyping analysis. *Pediatr Infect Dis J* 2002; **21**: 1105–1109.
34. Cox TR, Roland WE, Dolan ME. Ventilator-related *Acinetobacter* outbreak in an intensive care unit. *Mil Med* 1998; **163**: 389–391.
35. Kappstein I, Grundmann H, Hauer T, Niemeyer CJ. Aerators as a reservoir of *Acinetobacter junii*: an outbreak of bacteraemia in paediatric oncology patients. *J Hosp Infect* 2000; **44**: 27–30.
36. Snelling AM, Gerner-Smidt P, Hawkey PM *et al*. Validation of use of whole-cell repetitive extragenic palindromic sequence-based PCR (REP-PCR) for typing strains belonging to the *Acinetobacter calcoaceticus–Acinetobacter baumannii* complex and application of the method to the investigation of a hospital outbreak. *J Clin Microbiol* 1996; **34**: 1193–1202.
37. Hartstein AI, Rashad AL, Liebler JM *et al*. Multiple intensive care unit outbreak of *Acinetobacter calcoaceticus* subspecies *anitratus* respiratory infection and colonization associated with contaminated, reusable ventilator circuits and resuscitation bags. *Am J Med* 1988; **85**: 624–631.
38. Stone JW, Das BC. Investigation of an outbreak of infection with *Acinetobacter calcoaceticus* in a special care baby unit. *J Hosp Infect* 1986; **7**: 42–48.
39. Sherertz RJ, Sullivan ML. An outbreak of infections with *Acinetobacter calcoaceticus* in burn patients: contamination of patients' mattresses. *J Infect Dis* 1985; **151**: 252–258.
40. Melamed R, Greenberg D, Porat N *et al*. Successful control of an *Acinetobacter baumannii* outbreak in a neonatal intensive care unit. *J Hosp Infect* 2003; **53**: 31–38.
41. Levidiotou S, Galanakis E, Vrioni G *et al*. A multi-resistant *Acinetobacter baumannii* outbreak in a general intensive care unit. *In Vivo* 2002; **16**: 117–122.