Acinetobacter Bacteremia in Hong Kong: Prospective Study and Review

Hong Siau, Kwok-Yung Yuen, Pak-Leung Ho, Samson S. Y. Wong, and Patrick C. Y. Woo

From the Department of Microbiology, The University of Hong Kong, Hong Kong

The epidemiological characteristics of 18 patients with acinetobacter bacteremia were analyzed. Patients (mean age, 55.5 years) developed bacteremia after an average of 14.1 days of hospitalization. Fifteen of 16 patients survived bacteremia caused by *Acinetobacter baumannii*. Cultures of blood from the remaining two patients yielded *Acinetobacter lwoffii*. Most patients (78%) resided in the general ward, while four patients (22%) were under intensive care. Genotyping by arbitrarily primed polymerase chain reaction analysis and the temporal sequence of isolation were more useful than phenotyping by antimicrobial susceptibility in the determination of the source of bacteremia, and the intravascular catheter was the leading infection source (39% of cases). The possibility of an association of glucose with the pathogenesis of acinetobacter infection was raised.

In previous studies [1–4], the clinical presentation of acinetobacter bloodstream infection ranged from mild catheter-related bacteremia to sepsis (secondary to respiratory tract infection) requiring intensive care. The incidence of bacteremia secondary to catheter infection may be underestimated in these retrospective studies, as catheter tips may not have been cultured routinely. Because we previously demonstrated that *Acinetobacter* species are endemic at our center in Hong Kong [5], we conducted a prospective study to elucidate the epidemiological and clinical characteristics of patients with bacteremia. In addition, genotyping of isolates by arbitrarily primed PCR (AP-PCR) analysis was employed to determine the source of bacteremia.

See editorial response by Seifert on pages 31–2.

Patients and Methods

Patient selection and evaluation. The prospective study was carried out at the 1,350-bed Queen Mary Hospital in Hong Kong over a 5-month period (May to October 1995); patients whose first pair of blood cultures yielded an *Acinetobacter* species were included in the study. Demographic details, body temperature, and WBC count at the time of positive blood culture; health and underlying disease history; invasive or diagnostic procedures; duration of medication before the onset of bacteremia; presence of prosthetic devices; total parenteral nutrition and use of 5% (w/v) iv glucose at the time of positive blood culture; and outcome were recorded. Clinical specimens from suspected sources of bacteremia (CNS, respiratory or urinary tract, and/or wound) were obtained for culture. When the source of bacteremia was not clinically evident, the site of insertion of intravascular catheters was examined. Central catheters were removed for culture when inflammation and pus were observed at the insertion site, and peripheral catheters were removed for culture irrespective of the local clinical presentation. Antimicrobial therapy was initiated after clinical assessment.

Definitions. Clinically significant bacteremia was defined as fever (temperature, >38.5°C) and/or leukocytosis ($>11 \times 10^9$ WBCs/L) for at least 8 hours. Bacteremia was hospital-acquired if blood for positive culture(s) was obtained from a patient hospitalized for >48 hours. Polymicrobial bacteremia occurred when more than one organism was isolated from blood culture(s). Criteria for a favorable outcome included resolution of fever and subsequent negative blood cultures. Death was attributed to bacteremia if the patient died while receiving therapy within 72 hours of the first positive blood culture.

The source of bacteremia was determined clinically and/or microbiologically by isolation of an identical *Acinetobacter* strain (i.e., one exhibiting complete genetic relatedness as determined by AP-PCR analysis) from a distant site before or during the bacteremic episode. The intravascular catheter was designated as the primary portal of entry if the same organism was isolated from the blood and the catheter tip and not from any other body sites (modification of criteria of Raad and Bodey [6]) and there were >15 cfu in culture. No attempts to recover *Acinetobacter* strains from stool specimens were made. The source of bacteremia was unknown when clinical and microbiological evidence was lacking.

Culture, identification, and susceptibility testing of Acinetobacter strains. Ten milliliters of blood was inoculated into two bottles for aerobic and anaerobic incubation in BACTEC NR 660 (Becton Dickinson, Cockeysville, MD). When results were flagged positive, the contents were cultured on solid media. The method for semiquantitative culture of catheter tips of Maki et al. [7] was used. All other clinical specimens were

Received 17 March 1998; revised 23 June 1998.

Financial support: This study was supported in part by the Committee of Research and Conference Grants, The University of Hong Kong.

Reprints or correspondence: Dr. Hong Siau, Department of Microbiology, The University of Hong Kong, University Pathology Building, Queen Mary Hospital Compound, Pokfulam Road, Hong Kong (hongsiau@hkusua.hku.hk).

Clinical Infectious Diseases 1999; 28:26–30

1058–4838/99/2801–0005$03.00

Table 1. Characteristics of 18 patients with clinically significant acinetobacter bacteremia at Queen Mary Hospital in Hong Kong from May to October 1995.

Patient no.	Sex/age (y), type of patient	Underlying condition(s)/ nutritional supplement (duration in d)	Interval (d) between admission and onset of bacteremia/type of bacteremia	Other culture-positive specimen	Antibiotic therapy at the time of blood sample	Antibiotic therapy
1	F/73, surgical	Liver CA/D5 (10)	10/monomicrobial	iv catheter	No	Amoxicillin/clavulanate and gentamicin
2	M/50, surgical	Liver CA/D5 (1)	2/monomicrobial	No further specimens obtained	No	Aztreonam
3	M/54, surgical	Esophagus CA, alcoholic cirrhosis, DM	11/monomicrobial	None	Yes	Amoxicillin/clavulanate
4	M/65, surgical	Stomach CA/TPN (0.5)	9/monomicrobial	iv catheter	Yes	Imipenem
5	M/56, surgical	Pancreas CA, DM/TPN (7)	36/monomicrobial	iv catheter	Yes	Netilmicin
6	M/62, surgical	Pancreas CA/D5 (0.5)	14/monomicrobial	iv catheter	No	Cefoperazone
7	F/21, medical	Lung CA, chemotherapy	0/monomicrobial	None	No	Amoxicillin/clavulanate
8	F/60, ICU	Burkitt's lymphoma, ventilatory support/ TPN (0.5)	2/polymicrobial	None	No	Imipenem and amikacin
9	M/66, surgical	Pancreatitis, ischemic heart disease, DM	13/monomicrobial	None	No	Imipenem
10	M/80, ICU	Acute cholangitis, liver abscess/D5 (27)	27/monomicrobial	Sacral sore	No	Imipenem
11	M/86, surgical	Intestinal obstruction, liver cirrhosis/D5 (4)	4/monomicrobial	Central venous catheter	Yes	Imipenem
12	M/25, medical	AML, chemotherapy/D5 (0.5)	0/monomicrobial	Cuff of Hickman catheter	No	Ceftazidime and amikacin
13	F/35, medical	AML, chemotherapy/D5 (7)	7/monomicrobial	None	Yes	Ceftazidime
14	M/40, medical	After BMT, steroid therapy	59/monomicrobial	None	No	Imipenem
15	F/36, ICU	Meningioma, steroid therapy, ventilatory support/D5 (7)	10/polymicrobial	Endotracheal aspirate	Yes	Imipenem
16	F/60, ICU	CVA, steroid therapy, ventilatory support, DM	9/monomicrobial	Tracheostomy aspirate	No	Trimethoprim-sulfamethoxazole
17	M/73, medical	Ischemic heart disease, ventilatory support	29/monomicrobial	Tracheostomy aspirate	Yes	Imipenem
18	F/57, medical	Myasthenia gravis, steroid therapy, ventilatory support/iv heparin (6)	12/monomicrobial	iv catheter, tracheostomy aspirate	No	Ceftazidime

NOTE. AML = acute myelogenous leukemia; BMT = bone marrow transplantation; CA = carcinoma; CVA = cerebrovascular accident; DM = diabetes mellitus; D5 = 5% glucose; ICU = intensive care unit; TPN = total parenteral nutrition.

processed and cultured accordingly. *Acinetobacter* organisms were identified according to their ability to oxidize glucose in Hugh and Leifson's medium [8]. The genospecies of each blood isolate was determined through PCR analysis of the conserved spacer region between 16S rRNA and 23S rRNA [9]. Susceptibility of *Acinetobacter* species to antimicrobial agents was determined by the disk diffusion method [10].

AP-PCR analysis. The genetic relatedness between the first *Acinetobacter* strains isolated from blood and other body site specimens from each patient was determined by AP-PCR analysis with use of the primer $(GTG)_5$ (Molecular Medicine Unit, Rayne Institute, London) [5] as follows: 2 cycles at 94°C for 1 minute, 45°C for 2 minutes, and 72°C for 1 minute; followed by 25 cycles at 94°C for 1 minute, 55°C for 0.75 minute, and 72°C for 1 minute; and a terminal extension at 72°C for 6 minutes.

Results

Study population. Eighteen patients (11 males and seven females) had clinically significant acinetobacter bacteremia

Table 2. Comparison of antibiograms and AP-PCR profiles for *Acinetobacter* strains isolated from blood and other site specimens from 18 patients with bacteremia at Queen Mary Hospital in Hong Kong from May to October 1995.

Patient no.	Antibiogram* (date [d/mo] of first isolation) for *Acinetobacter* strain from indicated specimen		Comparative AP-PCR profile
	Blood	Other body site(s)†	
1	SRSSSSSS (24/5)	Intravascular catheter, SRSSSSSS (26/5)	Identical
2	RSSSSSSS (24/9)	None	ND
3	SRSSSSSS (14/8)	None	ND
4	SRRSSSSS (29/5)	Intravascular catheter, SRRSSSSS (1/6)	Identical
5	SRSRSRRS (2/7)	Intravascular catheter, SRSR**R**RRS (1/7)	Identical
6	SRSSSSSS (31/10)	Intravascular catheter, SRSSSSSS (3/11)	Identical
7	SRSSSSSS (12/8)	None	ND
8	SRSSSSSS (5/8)	None	ND
9	RRRRSSRR (17/7)	None	ND
10	RRRRSRRR (18/10)	Sacral sore, RRRRSRRR (22/10)	Identical
11	SRSRRSSS (19/10)	Central venous catheter, SRSRRSSS (21/10)	Identical
12	SRSSSSSR (5/7)	Cuff of Hickman catheter, **RR**R**R**SS**R**S (24/7)	Identical
13	RRSSSSRS (15/8)	None	ND
14	RRRSSSSS (23/9)	None	ND
15	RRSSSSSS (13/9)	Endotracheal aspirate, **S**RSSSSSS (21/9)	Different
16	SRSSSSSS (18/7)	Tracheostomy aspirate, SRSSSSSS (14/7)	Identical
17	SRSSSSSS (20/9)	Tracheostomy aspirate, SRSSS**RR**S (18/9)	Different
18	SRSRSSRS (12/9)	Intravascular catheter, SRSRSSRS (16/9); tracheostomy aspirate, SRSSSSSS (25/9)	Identical; identical

NOTE. AP-PCR = arbitrarily primed PCR; ND = not determined; R = resistant; S = susceptible.

* Included amoxicillin/clavulanate, cefuroxime, ceftazidime, gentamicin, netilmicin, amikacin, trimethoprim-sulfamethoxazole, and ofloxacin in that order. Strains exhibiting intermediate susceptibility were regarded as being resistant. Differences in susceptibility when compared with that for the blood isolate are shown in bold type.

† Other body site(s) specified before antibiogram(s).

during the 5-month study period (table 1). One-third of the patients were 60 years of age or older (mean age ± SD, 55.5 ± 18.2 years; range, 21–86 years). Fourteen patients (78%) resided in general wards (where two intubated patients were cared for by the intermediate care unit), and four were in the intensive care unit (ICU). The mean interval between hospital admission and the onset of bacteremia was 14.1 days (range, 0–59 days). Two patients (nos. 7 and 12) who became bacteremic during the first day of admission had been hospitalized in the preceding 2 weeks, and the possibility of them acquiring acinetobacter infection during the previous hospitalization could not be excluded. All patients had fever and/or leukocytosis. Two patients (11%) had polybacteremia with concomitant organisms (*Enterobacter cloacae,* patient 8; *Pseudomonas aeruginosa,* patient 15).

All patients had underlying diseases, including malignancy (10 patients), diabetes mellitus (4), conditions requiring steroid therapy (4), intraabdominal infections (3), and/or ischemic heart disease (2). All patients had intravascular catheters; five were intubated, and four had urinary tract catheters. Eleven patients were receiving iv glucose (mean duration, 7.1 days; range, 0.5–27 days) or total parenteral nutrition (mean duration, 2.7 days; range, 0.5–7 days) before the onset of bacteremia. Eleven patients did not receive antibiotic therapy when the first blood sample was obtained for culture. Following the institution of appropriate antibiotic treatment, all patients except one (patient 2) survived.

Microbiological characteristics. *Acinetobacter baumannii* was isolated from 16 patients, and *Acinetobacter lwoffii* was recovered from two (patients 7 and 14). All isolates were resistant to ampicillin and cephalothin and were susceptible to imipenem (these antimicrobial agents were omitted in the antibiogram). The genotypic (AP-PCR) and phenotypic (antibiogram) patterns of isolates from the bloodstream and from other sites are shown in table 2. Patients 1, 4, 6, 10, 11, 16, and 18 had blood and catheter, wound, or tracheostomy aspirate isolates with identical genotypic and phenotypic patterns, while patients 5 and 12 had isolates with identical genotypes but distinct phenotypes (figure 1). However, the blood and respiratory isolates from patients 15 and 17 had different genotypic and phenotypic patterns. Hence, the sources of bacteremia that were identified for the 18 patients were the intravascular catheter (7 patients), wound (1), respiratory tract (1), and unknown (9).

Discussion

The characteristics of acinetobacter bacteremia, especially those cases caused by *A. baumannii* (the most prevalent *Acinet-*

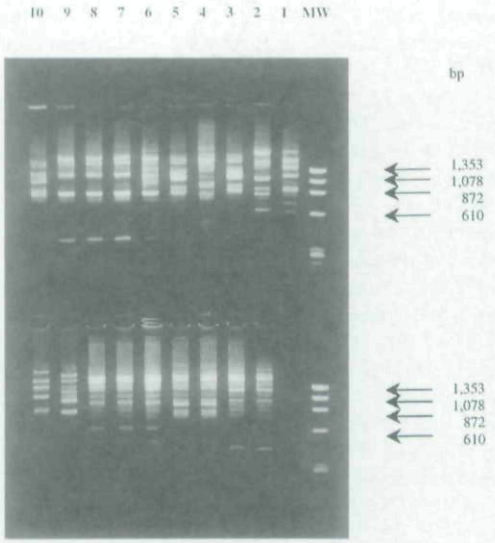

Figure 1. Representative arbitrarily primed PCR profiles for *Acinetobacter* strains isolated from blood and other body site specimens from patients with bacteremia at Queen Mary Hospital in Hong Kong. Upper panel: lane MW, *Hae*III-digested ϕX174 DNA molecular size marker; lanes 1 and 2, endotracheal aspirate isolates from patient 15; lanes 3–5, tracheostomy aspirate isolates from patient 17; lane 6, blood isolate from patient 17; lanes 7–9, tracheostomy aspirate, catheter, and blood isolates from patient 18, respectively; lane 10, positive control *Acinetobacter baumannii* (American Type Culture Collection [ATCC] 19606). Lower panel: lane MW, the same DNA molecular size marker as in the upper panel; lane 1, negative control; lanes 2 and 3, tracheostomy aspirate and blood isolates from patient 16, respectively; lanes 4 and 5, Hickman catheter cuff and blood isolates from patient 12, respectively; lane 6, wound isolate from patient 10; lanes 7 and 8, blood isolates from patient 10; lanes 9 and 10, blood and endotracheal aspirate isolates from patient 15, respectively.

obacter isolate from blood), have been reported recently. In contrast to attributable mortality rates of 19%–34% [1, 2, 4], there was only one death in our study. Fifteen patients (94%) survived bacteremic episodes caused by *A. baumannii*. Smego [11] also reported a recovery rate of 88% (18 patients). The difference in mortality rates may be attributed to the different study populations.

In the previously reported literature, 40%–91% of those patients who developed bacteremia were in the ICU [1–4, 11], and they were often infected by multidrug-resistant isolates [1–4]. The severity of underlying illnesses in the ICU patients and the inappropriate use of antibiotic therapy for patients infected with multidrug-resistant isolates were independent risk factors associated with mortality [1, 4]. Both characteristics were absent in our study population: most patients were not critically ill (only 22% were in the ICU), and most *A. baumannii* isolates from blood cultures were susceptible to a number of antibiotics (table 2), probably associated with the finding that 61% of the patients had no previous antibiotic exposure (table 1). The finding of fewer cases of *A. baumannii* bacteremia in ICU patients is consistent with the results of a previous 5-year study conducted in this hospital [5], where the proportion of glucose-oxidizing *Acinetobacter* isolates from bacteremic patients in the ICU and general wards was 1:6. In addition, the major source of *A. baumannii* bacteremia in our patients was the intravascular catheter, instead of the lower respiratory tract as reported by other investigators [1, 2, 4, 11]. Better prognoses were reported for patients with *A. baumannii* bacteremia originating from intravascular catheterization [1].

The portal of entry of *Acinetobacter* strains was established by recovery of an isolate with genotypic pattern (as demonstrated by the AP-PCR profile) identical to that of the blood isolate from a different site. We found isolates with identical genotypes that had different susceptibilities to one to four antimicrobial agents (table 2). Scerpella et al. [12] also reported *Acinetobacter* strains with complete genetic relatedness for which different antibiograms were found. Apart from the genotypic relationship, the temporal sequence of positive cultures of blood and other body site specimens was useful in establishing the source of bacteremia (e.g., in the case of patient 18).

The intravascular catheter and the lower respiratory tract are two major portals of entry for *A. baumannii;* the intravascular catheter was the leading infection source in this and other studies [4, 13, 14], and the respiratory tract was the leading infection source in other investigations [1–3, 11]. As the venous access served as the major source of bacteremia in our study (seven patients), there may be common carriage of the nonfermenter in patients and/or staff in this hospital; further study is required for confirmation of this hypothesis. Seifert et al. [4] reported that 57% of all *Acinetobacter* isolates from blood were *A. baumannii* and that the leading source of bacteremia was the indwelling catheter; their study included 79 patients (of whom 79% were in the ICU) in two large and 10 small hospitals during 1990–1991. However, when Seifert and co-workers [15] surveyed the skin surface (forehead, ear, nose, throat, axilla, hand, groin, perineum, and toe web) of 40 cardiology patients in general wards at one of the large hospitals during 1993–1994, *A. baumannii* was found rarely (0.5% of cases). We note that both studies were carried out during different periods and involved different patient populations; the length of hospital stay for those patients from whom surveillance swabs were obtained was not stated, and the insertion site or proximal surface of foreign bodies was not swabbed. There may be attributable differences among the genospecies in their adherence to foreign bodies and their virulence leading to disease manifestations.

The source of bacteremia could not be established in nine patients (50%). Other researchers [1–3] reported that the portal of entry of *Acinetobacter* strains was not known for 17%–43% of bacteremic patients. No further clinical specimens were obtained from a patient who died (patient 2). Intravascular catheters from patients without clinical foci of bacteremia (patients 3, 7, 9, 13, and 14) either were cultured after the institution of antimicrobial therapy (two cases) or were removed without being cultured (three cases). Since oropharyngeal

and/or rectal carriage of multidrug-resistant *A. baumannii* is common in ICU patients [16], the digestive tract may serve as a source (e.g., patient 8 had *E. cloacae* isolated concurrently with *A. baumannii* in the blood culture).

Glucose-oxidizing *Acinetobacter* strains cause browning of blood agar incorporated with glucose [9]. The proportion of diabetic patients with acinetobacter bacteremia in Hong Kong (19%–22%; this study and [2]) was significant compared with that (4%–6%) in Vancouver, British Columbia, Canada, and Cologne, Germany [3, 4] ($P < .005$, χ^2 test). Ng et al. [2] showed that diabetes mellitus may be a risk factor associated with mortality in patients with acinetobacter bacteremia, and Lye et al. [17] suggested that diabetes mellitus could be a predisposing factor for the development of acinetobacter peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. Whether this association reflects a difference among the patient populations studied or that diabetes mellitus may be a risk factor for acinetobacter infections in regions of endemicity requires further evaluation.

References

1. Cisneros JM, Reyes MJ, Pachón J, et al. Bacteremia due to *Acinetobacter baumannii:* epidemiology, clinical findings, and prognostic features. Clin Infect Dis **1996**;22:1026–32.
2. Ng TKC, Ling JM, Cheng AFB, Norrby SR. A retrospective study of clinical characteristics of acinetobacter bacteremia. Scand J Infect Dis Suppl **1996**;101:26–32.
3. Tilley PAG, Roberts FJ. Bacteremia with *Acinetobacter* species: risk factors and prognosis in different clinical settings. Clin Infect Dis **1994**; 18:896–900.
4. Seifert H, Strate A, Pulverer G. Nosocomial bacteremia due to *Acinetobacter baumannii.* Clinical features, epidemiology, and predictors of mortality. Medicine (Baltimore) **1995**;74:340–9.
5. Siau H, Yuen KY, Wong SSY, Ho PL, Luk WK. The epidemiology of acinetobacter infections in Hong Kong. J Med Microbiol **1996**;44: 340–7.
6. Raad II, Bodey GP. Infectious complications of indwelling vascular catheters. Clin Infect Dis **1992**;15:197–210.
7. Maki DG, Weise CE, Sarafin HW. A semiquantitative culture method for identifying intravenous-catheter-related infection. N Engl J Med **1977**; 296:1305–9.
8. Weaver RE, Actis LA. Identification of *Acinetobacter* species. J Clin Microbiol **1994**;32:1833.
9. Siau H, Yuen KY, Ho PL, et al. Identification of acinetobacters on blood agar in the presence of D-glucose by a unique browning effect. J Clin Microbiol **1998**;36:1404–7.
10. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. NCCLS document M2-A4. Villanova, Pennsylvania: National Committee for Clinical Laboratory Standards, **1997**.
11. Smego RA. Endemic nosocomial *Acinetobacter calcoaceticus* bacteremia. Arch Intern Med **1985**;145:2174–9.
12. Scerpella EG, Wanger AR, Armitage L, Anderlini P, Ericsson CD. Nosocomial outbreak caused by a multiresistant clone of *Acinetobacter baumannii:* results of the case-control and molecular epidemiologic investigations. Infect Control Hosp Epidemiol **1995**;16:92–7.
13. Glew RH, Moellering RC, Kunz LJ. Infections with *Acinetobacter calcoaceticus* (*Herellea vaginicola*): clinical and laboratory studies. Medicine (Baltimore) **1977**;56:79–97.
14. Rolston K, Guan Z, Bodey GP, Elting L. *Acinetobacter calcoaceticus* septicemia in patients with cancer. South Med J **1985**;78:647–51.
15. Seifert H, Dijkshoorn L, Gerner-Smidt P, Pelzer N, Tjernberg I, Vaneechoutte M. Distribution of *Acinetobacter* species on human skin: comparison of phenotypic and genotypic identification methods. J Clin Microbiol **1997**;35:2819–25.
16. Corbella X, Pujol M, Ayats J, et al. Relevance of digestive tract colonization in the epidemiology of nosocomial infections due to multiresistant *Acinetobacter baumannii.* Clin Infect Dis **1996**;23:329–34.
17. Lye WC, Lee EJC, Ang KK. Acinetobacter peritonitis in patients on CAPD: characteristics and outcome. Adv Perit Dial **1991**;7:176–9.