J Microbiol Immunol Infect
2005;38:194-199

Alcaligenes xylosoxidans bacteremia: clinical features and microbiological characteristics of isolates

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Received: August 23, 2004 Revised: September 29, 2004 Accepted: October 27, 2004

Bacteremia caused by *Alcaligenes xylosoxidans* is rare. Between 1999 and 2002, 12 cases of bacteremia caused by *A. xylosoxidans* were diagnosed at a tertiary referral center in central Taiwan. The clinical features of these patients and the antimicrobial susceptibilities and pulsed-field gel electrophoresis (PFGE) pattern of their blood isolates were studied. All infections were acquired nosocomially. All of the adult patients had underlying diseases, and 10 (83%) had undergone an invasive procedure. The clinical syndrome included primary bacteremia in 7 patients (58%), and catheter-associated bacteremia, surgical wound infection, pneumonia, urinary tract infection, and empyema in 1 each. Polymicrobial bacteremia was found in 1 patient. The case-fatality rate was 17% (2/12). All isolates were susceptible to piperacillin and ceftazidime and resistant to aminoglycoside, ciprofloxacin and cefepime. Susceptibility to imipenem (67%), ampicillin-sulbactam (75%) and trimethoprim-sulfamethoxazole (92%) was variable. Genetic fingerprints obtained by PFGE showed identical pattern in the isolates from 2 neonates, indicating the epidemiologic relatedness of these infections. We conclude that *A. xylosoxidans* isolates are multi-resistant and *A. xylosoxidans* bacteremia should be considered as a possible etiology of infection after invasive procedures in patients with underlying diseases. Strict infection control is needed to prevent this infection.

Key words: *Alcaligenes xylosoxidans,* antibacterial agents, bacteremia, pulsed-field gel electrophoresis

Alcaligenes xylosoxidans is an aerobic, motile, oxidase- and catalase-positive, non-lactose-fermenting, Gram-negative bacillus first described in 1971 by Yabuuchi and Ohyama [1]. *A. xylosoxidans* has been isolated from the normal flora of the ear and gastrointestinal tract in humans and from aquatic surroundings in hospitals [2]. Previously reported cases of infections caused by *A. xylosoxidans* included bacteremia, meningitis, biliary tract infection, urinary tract infection, pneumonia, and osteomyelitis [3-15]. *A. xylosoxidans* bacteremia is rare among Gram-negative bacillus infections and is thought to occur mostly in immunocompromised patients [5,6,16-18].

In this study, we analyzed 12 cases of *A. xylosoxidans* bacteremia diagnosed in our hospital over a 3-year period, including clinical features of patients and antimicrobial susceptibility and molecular types of isolates.

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Materials and Methods

Bacterial isolates

The records of patients with positive blood culture for *A. xylosoxidans* obtained from the microbiological laboratories of Changhua Christian Hospital for the period July 1, 1999 through June 30, 2002 were reviewed. Blood specimens were processed by the BACTEC 9240 non-radiometric blood culture system (Becton Dickinson, Spark, MD, USA) in BACTEC Aerobic/F and Anaerobic/F. *A. xylosoxidans* was identified by conventional methods and the API 20NE system (bioMerieux SA, Marcy l' Etoile, France).

Antimicrobial susceptibility testing

The minimal inhibitory concentration (MIC) values of 10 antimicrobial agents for bacteremic isolates of *A. xylosoxidans* were determined using the PDM Epsilometer test (E-test) [AB Biodisk, Solna, Sweden] following the manufacturer's instructions [19-21]. The following antimicrobial agents were tested: gentamicin, amikacin, piperacillin, ampicillin-sulbactam, ceftriaxone,

ceftazidime, cefepime, ciprofloxacin, trimethoprim-sulfamethoxazole, and imipenem.

A saline suspension of each isolate was adjusted to a McFarland standard of 0.5 and inoculated over the surface of a Mueller-Hinton agar plate, using a sterile swab to produce an even inoculum. The E-test strips were applied on the plates which were then incubated for 18 h at 37°C. The MIC was defined as the point where the elliptical zone of growth inhibition intersected the MIC scale on the E-test strip, and MICs falling between 2 marks on the E-test strip were rounded up to the next highest doubling dilution, as recommended by the manufacturer. The breakpoints of MICs for susceptibility were determined according to the National Committee for Clinical Laboratory Standards (NCCLS) tentative standard for *Pseudomonas aeruginosa* and non-Enterobacteriaceae [22]. *P aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922 were used as controls.

Clinical data

Data collected from the medical records of patients whose blood cultures were positive for *A. xylosoxidans* included demographic characteristics, underlying diseases, other associated conditions (e.g., use of an indwelling catheter or ventilator), the clinical syndrome, day of first in-hospital blood culture positive for *A. xylosoxidans*, polymicrobial bacteremia, other sites of isolation of the organism, antibiotic regimen after blood culture results were available, and outcome.

Definitions

Bacteremia was considered to be nosocomial if the first blood culture that yielded positive findings was obtained more than 72 h after admission. Recent surgery was defined as a surgical procedure that had been performed within 1 month of the onset of *A. xylosoxidans* bacteremia. Antibiotic therapy was considered to be appropriate if 1 or more antimicrobial agents were effective in vitro against the corresponding isolates.

Pulsed-field gel electrophoresis analysis

Pulsed-field gel electrophoresis (PFGE) analysis was performed as described by Gautom [23], with the following modifications. The *A. xylosoxidans* genomic DNA embedded in agarose plugs was restricted with 10 units/plug-slice of *Xba*I restriction enzyme (Promega Co., Madison, WI, USA), following the manufacturer's recommendations. Restriction fragments were separated by PFGE through 1% SeaKem Gold agarose gels (Unimed Healthcare Inc., Taipei, Taiwan) in 0.5X Tris-borate-ethylenediaminetetraacetic acid (EDTA) [45 mM Tris-borate, 1 mM EDTA, pH 8.3 (TBE)] buffer at 14°C in a CHEF Mapper (Bio-Rad Laboratories, CA, USA). Electrophoresis conditions were as follows: initial switch time 2.2 s, final switch time 35 s, 6 V/cm, at an angle of 120° for 18 h. DNA fragments of the *Xba*I-digested genomic DNA of *Neisseria meningitidis* M413 were used as the reference standard markers in the electrophoresis. After electrophoresis, the PFGE patterns were imaged using a digital camera system with a resolution of 1792 × 1200 pixels. Chromosomal DNA restriction patterns produced by PFGE were interpreted using the categories of Tenover et al [24].

Results

Bacterial isolates

A total of 13 blood isolates of *A. xylosoxidans* from 12 episodes in 12 patients were collected, accounting for 0.11% of the total number of positive blood cultures in this hospital over the 3-year period of this study. All patients but 3 (all newborns) had at least 2 sets of blood cultures positive for *A. xylosoxidans*. *A. xylosoxidans* was also isolated simultaneously from another site in 5 patients (Table 1). One patient (patient 7) had polymicrobial bacteremia and the concomitant organism was oxacillin-resistant *Staphylococcus aureus*. Another patient (patient 4) had 2 blood isolates drawn 7 days apart during the same episode of bacteremia. All bacteremic episodes were nosocomial.

Antimicrobial susceptibility

The results of susceptibility testing of isolates from 12 episodes to 10 antimicrobial agents are shown in Table 2. All isolates were uniformly susceptible to piperacillin (MIC_{90}, 0.75 μg/mL) and ceftazidime (MIC_{90}, 6 μg/mL) , 11 (92%) were susceptible to trimethoprim-sulfamethoxazole (MIC_{90} [for trimethoprim] 4 μg/mL), 9 (75%) were susceptible to ampicillin-sulbactam (MIC_{90}, 64 μg/mL), and 8 (67%) were susceptible to imipenem (MIC_{90}, 8 μg/mL). All of the isolates were resistant to cefotaxime, cefepime, ciprofloxacin, gentamicin and amikacin.

Clinical characteristics of the patients

The clinical characteristics of the 12 patients with *A. xylosoxidans* bacteremia are summarized in Table 1. Male patients predominated (67%). The mean age of the patients was 47.4 years (range, 1 month to 92 years).

Table 1. Clinical characteristics, treatment and outcome of 12 patients with bacteremia caused by *Alcaligenes xylosoxidans*

Patient no. /age/gender	Underlying disease	Precipitating factor(s)	Clinical syndromes	Other site of *A. xylosoxidans* isolation	Antibiotic treatment	Outcome
1/1 month/F	None	None	Primary bacteremia	None	None	Survived
2/1 month/F	None	None	Primary bacteremia	None	None	Survived
3/1 month/F	None	PIVC	Primary bacteremia	None	Piperacillin, amikacin	Survived
4/40 years/M	Hypoxic encephalopathy, bedridden	CVC, ventilator, indwelling urine catheter	Primary bacteremia	None	Ceftazidime, ampicillin-sulbactam, ceftazime	Survived
5/46 years/M	DM	Decortication of empyema, CVC, ventilator, indwelling urinary catheter	Wound infection	Wound	Cefepime	Survived
6/65 years/M	COPD, DM	Chelecystectomy, ventilator, CVC, indwelling urinary catheter	Catheter-associated bacteremia	CVC tip	Piperacillin-tazobactam, imipenem	Survived
7/63 years/M	DM	Liver abscess after hepatectomy, ventilator, CVC	Primary bacteremia	None	Imipenem, vancomycin	Died
8/56 years/M	DM	Craniotomy, ventilator, PIVC, indwelling urine catheter	Urinary tract infection	Urine	Ampicillin-sulbactam	Survived
9/38 years/M	Alcoholism, acute pancreatitis	Ventilator, CVC, indwelling urine catheter	Primary bacteremia	None	Imipenem	Survived
10/85 years/M	Thyroid carcinoma	Ventilator, PIVC	Empyema	Pleural fluid	Ceftazidime	Survived
11/92 years/M	COPD, obstructive nephropathy	Ventilator, PIVC, indwelling urinary catheter, steroid therapy	Nosocomial pneumonia	Sputum	Ciprofloxacin	Died
12/81 years/F	DM, old CVA	Ventilator, PIVC, indwelling urinary catheter, embolectomy of common iliac artery	Primary bacteremia	None	Imipenem	Survived

Abbreviations: F = female; M = male; DM = diabetes mellitus; COPD = chronic obstructive pulmonary disease; CVA = cerebrovascular accident; PIVC = peripheral intravenous catheter; CVC = central venous catheter

Three patients were newborns. Underlying diseases were present in 9 patients, and included diabetes mellitus in 5, chronic obstructive pulmonary disease in 1, hypoxic encephalopathy in 1, alcoholism and acute pancreatitis in 1, and thyroid cancer in 1. An indwelling intravascular catheter was implanted before the bacteremic episodes in all but 2 patients (patients 1 and 2). Nine patients had used at least 1 antibiotic and had received ventilator support at least 1 week before developing bacteremia. Five patients had undergone recent surgery and only 1 had used long-term steroid for more than 2 weeks prior to developing bacteremia. *A. xylosoxidans* bacteremia developed at least 2 weeks after admission in 8 adult patients. The underlying clinical syndromes included primary bacteremia in 7 patients, catheter-related bacteremia in 1, pneumonia in 1, surgical wound infection in 1, urinary tract infection in 1, and empyema in 1. Five patients had positive *A. xylosoxidans* isolates from another site, including wound in 1 patient, central venous catheter tip in 1, urine in 1, pleural fluid in 1 and sputum in 1.

Appropriate antibiotics were given on the basis of the results of disk susceptibility testing after blood cultures were found to be positive for *A. xylosoxidans* in 8 of 12 patients. Two patients (patients 1 and 2) did not receive appropriate antibiotics because they became afebrile and improved clinically after a positive blood culture result for *A. xylosoxidans* was obtained. One patient (patient 4) initially responded to ceftazidime treatment but bacteremia developed one week later after the antibiotic was switched to ampicillin-sulbactam. The patient then responded to ceftazidime treatment again. Intravascular catheters were removed from all patients. Two of the patients died (17%), 1 on the second and 1 on the seventh day after the onset of bacteremic episodes.

Table 2. In vitro antimicrobial susceptibilities of the 12 blood isolates of *Alcaligenes xylosoxidans*

Antimicrobial agent	MIC range (μg/mL)	MIC_{50} (μg/mL)	MIC_{90} (μg/mL)	Susceptibility breakpoint (μg/mL)	No. (%) of isolates susceptible
Piperacillin	0.5-2	0.5	0.75	≤16	12 (100)
Cefotaxime	>128	>128	>128	≤8	0
Ceftazidime	4-8	6	6	≤8	12 (100)
Cefepime	>128	>128	>128	≤8	0
Imipenem	2-16	2	8	≤4	8 (67)
Ciprofloxacin	2-128	>128	>128	≤1	0
Trimethoprim-sulmethoxazole	0.016-4	0.032	0.032	≤2	11 (92)
Ampicillin-sulbactam	4-64	6	64	≤8	9 (75)
Gentamicin	>128	>128	>128	≤4	0
Amikacin	>128	>128	>128	≤8	0

Abbreviations: MIC = minimal inhibitory concentration; MIC_{50} = MIC for 50% of isolates; MIC_{90} = MIC for 90% of isolates

Pulsed-field gel electrophoresis

The PFGE fingerprints of 13 isolates are shown in Fig. 1. The PFGE fingerprints of isolate 4 and isolate 5, from 2 newborn patients treated in the intensive care unit (ICU) [patients 2 and 3, respectively], and isolates 7 and 8 (both from patient 4) demonstrated an identical pattern. The PFGE fingerprints of isolate 3 from patient 1, a newborn treated in the ICU, was closely related to isolates 4 and 5. The PFGE fingerprints of isolates other than isolates 3, 4 and 5 were different, indicating that 9 of the 13 isolates were epidemiologically unrelated.

Discussion

A. xylosoxidans is a Gram-negative bacillus that can survive and multiply in environmental water sources. In the laboratory, this organism can be misidentified as *P. aeruginosa*, *Burkholderia cepacia* and *Stenotrophomonas maltophilia* [25]. *A. xylosoxidans* bacteremia is very uncommon [6,26,27]. Records from our hospital on *A. xylosoxidans* bacteremia revealed 12 cases over a 3-year period, accounting for 0.11% of the total number of positive blood cultures. This low number of cases of bacteremia suggests that this organism is either not a common part of the normal human flora or is of low virulence.

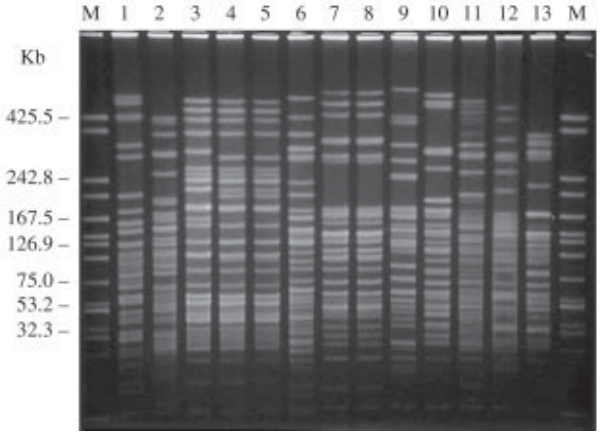

Fig. 1. Pulsed-field gel electrophoresis fingerprints of *Xba*I restriction digestion of chromosomal DNA of 13 bacteremic *Alcaligenes xylosoxidans* isolates. Lane M serves as a molecular size marker; lanes 4-5 exhibit the same pulsotypes from patients 2 and 3, respectively; lanes 7-8 show the same strain isolated from the same patient.

All of the adult patients infected with *A. xylosoxidans* bacteremia had underlying disease. There was a higher percentage of diabetes mellitus in this series (5/12 [42%] vs 3/77 [4%]) and a lower percentage of patients with malignancy (1/12 [8%] vs 23/77 [30%]) compared to a review of the literature by Duggan et al [6]. All patients except 2 neonates had undergone various invasive procedures, such as urinary catheterization, central or peripheral catheterization, respiratory assistance or recent surgery, which suggests that these procedures may have been responsible for the bacteremia. This finding also suggests that a breakdown of infection control techniques is a likely cause of the infection.

All cases of *A. xylosoxidans* bacteremia in this study were hospital-acquired and most (8/12) developed bacteremia at least 2 weeks after admission. In this series, primary bacteremia and catheter-associated bacteremia were the most common clinical syndromes, accounting for 75% of cases (8/12). This finding is in contrast to the review of Duggan et al, who found that these conditions accounted for 39% of cases (30/77) [6].

Resistance of *A. xylosoxidans* to several antibiotics has been widely reported [4,16,28,29]. In the present study, susceptibility testing confirmed uniform susceptibility of isolates to ceftazidime and piperacillin; resistance to all aminoglycosides, cefotaxime,

ciprofloxacin and cefepime; and varying degrees of resistance to imipenem, trimethoprim-sulfamethoxazole and ampicillin-sulbactam. Our data for the resistance pattern of *A. xylosoxidans* to antibiotics are in agreement with findings of other investigators [4,5,29]. In this study, antibiotic susceptibility testing was performed by the E-test according to NCCLS interpretative criteria for *P. aeruginosa* because the NCCLS still has no formal recommendation for the optimal antimicrobial susceptibility testing method for *Alcaligenes* spp. or for the antimicrobial agents that should be tested. In addition, antimicrobial susceptibility data obtained by the disk diffusion method (data not shown) were comparable to that obtained by the E-test using susceptibility breakpoints established for *P. aeruginosa* according to NCCLS criteria. Further studies are needed to standardize microbiologic methodologies for susceptibility testing of *Alcaligenes* spp.

Of the 12 patients, 8 were treated with appropriate antibiotics. Two neonates (patients 1 and 2) did not receive appropriate antibiotics due to the transient nature of their bacteremia. One patient (patient 5) who did not receive appropriate antibiotic treatment may have responded to adequate debridement of a wound. One patient (patient 4) failed to respond to ampicillin-sulbactam and removal of intravascular catheter but responded to ceftazidime treatment. The effectiveness of clinical treatment of *A. xylosoxidans* bacteremia with ampicillin-sulbactam as the sole agent needs further evaluation. Two of the 12 patients with *A. xylosoxidans* bacteremia in this series died (2/12, 17%). One of these patients (patient 7) had polymicrobial bacteremia which did not respond to appropriate antibiotic treatment and removal of intravascular catheter, and the other (patient 11) received inappropriate antibiotic treatment with ciprofloxacin (MIC, 12 μg/mL). The optimal therapeutic regimen for treating *A. xylosoxidans* bacteremia remains unclear because of the small number of patients in this series, and the limited data from previous reports. Some authors have suggested that combination therapy may be necessary for serious infection, and that in vitro study showed synergy or additive effects for the combination of piperacillin-gentamicin, chloramphenicol-minocycline, and ciprofloxacin-imipenem [4,8,24,30].

All cases of *A. xylosoxidans* bacteremia in this study were hospital-acquired. The finding that 12 patients had nosocomial infections caused by this unusual organism at the same hospital may suggest a common source. We used a PFGE method that had been previously used for epidemiologic typing of *A. xylosoxidans* because of its good discriminatory power [17,31]. The PFGE fingerprints of 2 *A. xylosoxidans* isolates (isolates 4 and 5 from patients 2 and 3, respectively) from newborns treated in the ICU had identical patterns, suggesting an outbreak. In addition, the PFGE fingerprints of isolate 3 (patient 1) from a newborn treated in the ICU was closely related to isolate 4 and isolate 5. However, patient 2 and patient 3 developed bacteremia during the same week while patient 1 developed bacteremia 1 year earlier. Thus, the outbreak strain of *A. xylosoxidans* might have colonized in our hospital environment for a long period and been relatively non-pathogenic. In previous studies, *Alcaligenes* species have been recovered from ventilators, humidifiers, disinfectant solutions, intravenous fluid, irrigation and dialysis solutions, well water, tap water, and infant formulas [3, 27,32]. Because of the retrospective nature of this study, it was difficult to determine the sources of infection and the mode of transmission. The PFGE fingerprints of isolates 7 and 8 from patient 4 had an identical pattern due to persistent bacteremia despite treatment with ampicillin-sulbactam for 7 days.

In conclusion, *A. xylosoxidans* bacteremia is a rare nosocomial infection which occurs in patients with underlying diseases and is often associated with invasive procedures. Strict infection control is needed to prevent outbreak of *A. xylosoxidans* infection. The unique resistance of this organism to multiple antibiotics makes it difficult to determine the optimal therapeutic option. Further studies are needed to determine the optimal the treatment and pathogenesis of *A. xylosoxidans* infection.

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