Diversity of Plasmids in *Achromobacter xylosoxidans* Isolates Responsible for a Seemingly Common-Source Nosocomial Outbreak

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*Achromobacter xylosoxidans*, an uncommon yet highly resistant opportunistic pathogen, was isolated from nine hospitalized patients during an 8-month period. It had been isolated from only seven patients with either nonfatal infection or colonization from 1981 to 1984. From June 1985 to January 1986, *A. xylosoxidans* was isolated 18 times from seven different sites (sputum, 7 times; urine, 4 times; blood, 3 times; and lung, pleural fluid, wound tissue, and tracheal aspirate, 1 time each). Four patients died, including the three with bacteremia. All but two patients had nosocomial infections and either were on the same ward or were cared for by the same staff members. Eleven *A. xylosoxidans* strains yielded eight distinct plasmids (8, 21, 23, 26, 38, 50, 51, and 64 megadaltons). Whole-cell peptide patterns of 10 of these strains were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Isolates from the same patient contained the same plasmids and had identical peptide patterns but differed from other strains in both parameters. Plasmids were absent from the two community-acquired isolates. Although nosocomial strains showed similar antibiotic resistance patterns (only moxalactam and ticarcillin-clavulanic acid were uniformly active) and cross-contamination was strongly suggested epidemiologically, results of plasmid and peptide analyses did not support the possibility of a single-strain outbreak.

*Achromobacter xylosoxidans* (15), also known as *Alcaligenes denitrificans* subsp. *xylosoxidans*, is an aerobic gram-negative rod with peritrichous flagella which produces oxidase and catalase, reduces nitrate, oxidizes glucose and xylose, and grows well on citrime agar (16). *A. xylosoxidans* is an infrequent but potentially serious nosocomial pathogen with a predilection for compromised patients (2, 6-14). Community-acquired infections are mainly confined to the middle ear (12, 15). Resistance to multiple antibiotics is fairly constant among clinical isolates of this organism, perhaps because of the failure of antibiotics to accumulate intracellularly or the presence of β-lactamases (5). *A. xylosoxidans* has been isolated from a number of sites in the environment, including deionized water (13), nonbacteriostatic saline (7), and chlorhexidine solutions (14). It may exist in the colons and the lower ilea of healthy persons (3), and can apparently contaminate clinical samples such as blood, urine, and peritoneal fluid of patients with no overt signs of infections (12, 13).

An unexpected increase in the number of *A. xylosoxidans* identified within a short period of time in specimens from hospitalized patients prompted an epidemiologic evaluation of cases and isolates in an effort to establish the mode of spread of the organism. *A. xylosoxidans* isolates were further characterized through antibiotic susceptibility studies and plasmid and peptide analyses.

**MATERIALS AND METHODS**

**Epidemiologic studies.** A prospective study of patients with *A. xylosoxidans* infection was started in July 1985 after one patient, who had shared a room with another patient infected with *A. xylosoxidans*, died of *A. xylosoxidans* bacteremia. The charts of infected or colonized patients were reviewed to determine age, ward, underlying illnesses, signs of infection, antibiotic therapy, invasive diagnostic and therapeutic procedures (vascular access lines, indwelling urethral catheters, respiratory therapy, dye contrast and radioactive tracer studies, parental alimentation, and chemotherapy), and physician and nursing staff coverage.

Environmental cultures were obtained, by using sterile saline-soaked swabs, from four different patient care areas (the medical intensive care unit [MICU] and three patient rooms) from sinks, shower heads, fan coil units, suction gauges, overhead vents, countertops, irrigation solutions, oxygen delivery systems, and ventilation cascades. Urine, sputum, and any open wounds of all MICU patients were screened for *A. xylosoxidans* in mid-July 1985.

**Bacteriologic studies.** Organisms were identified by using an automated biochemical technique (gram-negative identification cards no. 51-1306, 2503, and 9411; Vitek Systems, Inc., Hazelwood, Mo.). Antibiotic susceptibility studies were done by microdilution (gram-negative susceptibility cards no. 51-9411 and 2503; Vitek) and disk diffusion methods. MBCs were determined by using antimicrobial test panels (MicroScan; American Scientific Products, McGaw Park, Ill.) by streaking 10 μl of broth from each well that contained no visible growth onto a blood agar plate. After overnight incubation, a 99.8% kill was considered bactericidal. *Alcaligenes denitrificans* subsp. *xylosoxidans* ATCC 27061 was used as a control.

A total of 11 *A. xylosoxidans* isolates were analyzed for plasmids by the method described by Kado and Liu (4), and whole-cell peptide patterns of 10 of these isolates were determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (1). Seven isolates were also examined by Bristol Laboratories, Syracuse, N.Y., for the presence of aminoglycoside-inactivating enzymes.

**RESULTS**

**Epidemiologic studies.** From June 1985 through January 1986, *A. xylosoxidans* was isolated 18 times from nine
TABLE 1. Salient clinical and molecular aspects of A. xylosoxidans outbreak

| Patient no. (outcome)* | Admission date | Ward(s) | Date organism isolated | Isolation site | No. of isolates tested | Plasmid size (MDa)* | Peptide pattern
|------------------------|----------------|---------|------------------------|----------------|------------------------|---------------------|-----------------
| 1 (S)                  | 5 November 1984| 5E      | 25 June 1985           | Sputum         | 2                      | 38                  | A                
| 2 (D)                  | 19 June 1985   | 5E, MICU| 1 July 1985            | Urine          | 1                      | (21), 23, 26, (64) | A'               
| 3 (D)                  | 16 June 1985   | MICU    | 7 July 1985            | Sputum, urine  | 2                      | 8                   | B                
| 4 (D)                  | 15 August 1985 | MICU, 5W| 24 August 1985         | Blood, sputum, pleural fluid | 3                      | (50)                | C                
| 5 (D)                  | 3 October 1985 | 5W      | 28 October 1985        | Sputum         | Not tested             |                     |                  
| 6 (S)                  | 19 November 1985| 4W     | 20 November 1985       | Sputum         | 1                      | None                | C                
| 7 (D)                  | 2 November 1985| 4E, MICU| 22 November 1985       | Blood          | 1                      | 51                  | C'               
| 8 (S)                  | 9 December 1985| 4E      | 10 December 1985       | Urine          | 1                      | None                | D                
| 9 (S)                  | 12 December 1985| MICU, 3W| 1 January 1986        | Sputum         | Not tested             |                     |                  

* Four patients survived (S), and five died (D), but patient 1 died shortly after discharge from the hospital. Underlying medical conditions included ruptured cerebral aneurysm, chronic obstructive lung disease, prostatic carcinoma, non-Hodgkin’s lymphoma, bronchogenic carcinoma, cerebrovascular accident, chronic active hepatitis, and amyotrophic lateral sclerosis.

a Plasmids whose sizes are shown in parentheses were present in small and variable amounts; that of patient 4 was not always detectable.

b The different peptide patterns were arbitrarily given letter designations (see Fig. 2 for examples of each pattern). The designations A' and C' indicate variants of patterns A and C, respectively.

patients by our clinical microbiology laboratory. Of the 18 isolates, 7 were recovered from sputum; 4 from urine; 3 from blood; and 1 each from pleural fluid, tracheal aspirate, and wound and lung tissue. Only two isolates were considered community acquired. This organism had been isolated seven times during the preceding 4 years.

All nine patients from whom A. xylosoxidans was recovered were males, with ages ranging from 28 to 79 years. The index patient was a permanently comatose man with long and repeated hospitalizations to the same ward (5E [fifth floor]). This patient had A. xylosoxidans in the sputum and urine. His roommate was transferred to the MICU, where he died with A. xylosoxidans bacteremia. The third patient died in the MICU 16 days later with A. xylosoxidans in his sputum and lungs (postmortem). Patient 4 was admitted to the oncology ward (5W), where he died 1 month later with A. xylosoxidans in his sputum, blood, and pleural fluid. He had been treated in the MICU and transferred back to the oncology ward before his death. Patient 5 was also admitted to the oncology ward and developed A. xylosoxidans colonization of the upper respiratory tract. He died 3 months later, but the cause of death was not ascertained. Patient 6 was transferred from another hospital, and A. xylosoxidans was isolated from sputum obtained 1 day after admission. Patient 7 had end-stage liver disease and was transferred to the MICU, where he died with A. xylosoxidans bacteremia 3 months after the demise of patient 4. Patient 8 had a community-acquired A. xylosoxidans urinary tract infection. Patient 9 had amyotrophic lateral sclerosis, and A. xylosoxidans was isolated from tracheal secretions in January 1986. He had been a patient in the MICU in September 1985. Therefore, seven of the nine patients had nosocomial acquisition of A. xylosoxidans, and five of these seven were treated in the MICU.

There was no diagnostic or therapeutic intervention common to all patients. Eight patients received antibiotics and eight had vascular access lines. Of significance, perhaps, was the sharing of nursing staff from the ward of the index patient and the MICU during June and July 1985.

A. xylosoxidans was not recovered from the environment or from other empirically cultured patients in the MICU. Pseudomonas fluorescens, Pseudomonas species, Aeromonas hydrophila, and Serratia marcescens were isolated from sinks in different locations.

Bacteriologic studies. All A. xylosoxidans strains were identified by the Vitek system with a 91% or greater degree of probability. The results of biochemical reactions conformed with those described previously (16) for this genus. Eight plasmids with molecular masses of 8, 21, 23, 26, 38, 50, 51, and 64 megadaltons (MDa) were identified among the 11 isolates tested (Table 1 and Fig. 1). Whole-cell peptide analysis of 10 of these isolates was done by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 2). A. xylosoxidans isolates from the same patient contained plasmids with the same molecular weights and with the same peptide patterns. Isolates from different patients, however, differed in one or both parameters. No plasmids were recovered from the two community-acquired isolates.

FIG. 1. Plasmids (molecular masses) found in the A. xylosoxidans strains from the indicated patients. Lane 1. Patient 3 (8 MDa); lane 2. patient 1 (38 MDa); lane 3. patient 2 (21, 23, 26, and 64 MDa); lane 4. patient 6 (no plasmid); lane 5. patient 4 (no plasmid was seen in this preparation, although a 50-MDa plasmid was recovered on two other occasions); lane 6. control 80-MDa plasmid of Legionella pneumophila. The 51-MDa plasmid of patient 7 is not shown.
Twelve *A. xylosoxidans* isolates were tested against a battery of antibiotics. All were resistant to amikacin, gentamicin, tobramycin, colistin, cefazolin, ceftriaxone, cefotaxime, ampicillin, cefotaxime, and chloramphenicol. Another nine antibiotics showed activity, but only moxalactam and ticarcillin-clavulanic acid inhibited all 12 isolates. Six of nine isolates were inhibited by trimethoprim-sulfamethoxazole, but none of the isolates was killed by this drug (MBC >2/38 μg/ml). Piperacillin, mezlocillin, cefoperazone, and ticarcillin also exhibited wide differences between bactericidal and bacteriostatic levels against one or more isolates. Only two isolates produced β-lactamase when tested by an acidimetric method (βLAC test kit; Analytab Products, Plainview, N.Y.), and they were both from patient 1. Seven isolates, each from a different patient, were tested for aminoglycoside-inactivating enzymes by Bristol Laboratories, but none were found.

**DISCUSSION**

Judging from the few published reports, nosocomial outbreaks of *A. xylosoxidans* are distinctly rare, yet a number of common features are apparent. Infected patients usually have serious underlying illnesses, the outbreaks are short-lived, and an environmental source is common but not convincingly implicated. Mortality rates of systemically infected patients tend to be elevated, probably because of the patient population affected and because *A. xylosoxidans* is resistant to most of the antibiotics available. The outbreak in our hospital was similar to those in others in that seven serious or critically ill patients were involved and the responsible organism was resistant to many antibiotics. In addition, if not the primary cause of death, *A. xylosoxidans* was a significant contributing factor in the deaths of four of the nine patients (44%) that harbored the organism. A fifth patient died after discharge from the hospital. Although an environmental source of infection was not identified, it appeared that the organism was being transmitted from person to person by cross-contamination since the cases were clustered in the MICU and the fifth floor of the hospital. We reasoned that no more than one or two strains of such an infrequently isolated pathogen could have been involved in this limited outbreak. To our surprise, however, plasmid and peptide analyses failed to corroborate this impression since no molecular identity could be established between isolates from different patients. These analyses were methodologically sound since they have been validated previously (1), and all isolates from the same patients were identical in plasmid content and peptide pattern. In addition, the plasmid and peptide profiles were stable on passage of the isolates. Perhaps the organisms were readily able to exchange genetic material with other clinical or environmental organisms, or the variability may have resulted from the effect of some mobile genetic element. The two community-acquired isolates were just as resistant to antibiotics as were the nosocomial ones, even though they contained no plasmids. *A. xylosoxidans* was isolated once during the 12 months after the outbreak subsided.

A finding which deserves emphasis was the inability of trimethoprim-sulfamethoxazole to kill any *A. xylosoxidans* isolates tested during this outbreak, even though growth inhibition was demonstrated, suggesting that this drug should not be used in neutropenic patients infected with this organism. This phenomenon has been described previously in a single isolate of *A. xylosoxidans* (11). Tolerance was also observed with ticarcillin, mezlocillin, piperacillin, and cefoperazone. Only an agent which is shown to be bactericidal in vitro should be relied on for treating life-threatening *A. xylosoxidans* infections in the patients that are typically infected with this organism. The peculiar results of the molecular studies are difficult to reconcile with the epidemiologic evidence. Further scrutiny of these strains and those involved in future outbreaks might provide an explanation.

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**LITERATURE CITED**


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**FIG. 2.** Peptide patterns of *A. xylosoxidans* strains obtained from the various patients. Lane 1, Patient 3, isolate 1 (pattern B); lane 2, patient 1, isolate 1 (pattern A); lane 3, patient 2 (variant of pattern A); lane 4, patient 3, isolate 2 (pattern identical to that of the isolate in lane 1); lane 5, patient 1, isolate 2 (pattern identical to that of the isolate in lane 2); lanes 6 to 8, patient 4, isolates 1 to 3, respectively (pattern C); lane 9, patient 7 (variant of pattern C); lane 10, patient 8 (pattern D).
MOLECULAR DIVERSITY IN A. XYLOSOXIDANS OUTBREAK


