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Nosocomial Colonization and Infection by *Achromobacter xylosoxidans*

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Achromobacter xylosoxidans, a bacterial species named in 1971, is often isolated from aqueous environments, but little has been reported about its pathogenicity in humans, its epidemiological pattern, and its susceptibility to antibiotics and antiseptics. We were faced with an epidemic caused by this microorganism for 18 months in an intensive care unit. Two patients had fatal infections and 37 others were colonized. The source was the deionized water of the hemodialysis system. The 46 isolates were identified by comparison with the reference strain *A. xylosoxidans* ATCC 27061. The characteristic cellular fatty acids of this species were demonstrated by gas-liquid chromatography. The minimal inhibitory concentrations of 27 antibiotics were determined. The isolates were susceptible to only two: moxalactam at 4 μg/ml and ceftazidime at 8 μg/ml. The minimal bactericidal concentrations of one disinfectant and three antiseptics were: sodium hypochloride, 109 μg/ml; chlorhexidine digluconate in ethanol solution, 15 to 125 μg/ml; polyvinylpyrrolidone iodine, 750 μg/ml; and iodine ethanol, 312 to 625 μg/ml.

Achromobacter xylosoxidans was described and named by Yabuuchi et al. in 1971 (26). Although the recognition of this species is not accepted by all taxonomists (11, 12, 14, 27), Gilardi (10) and Tatum et al. (23) consider it distinct from other nonfermentative gram-negative rods. *A. xylosoxidans* is often encountered in aqueous environments. Little has been reported about its pathogenicity in humans. A few infections have been described in the literature. Generally, these appeared as sporadic cases. This bacterium is resistant to many antibiotics, but the newer drugs have not been evaluated as yet.

Between June 1981 and November 1982 we isolated 46 strains of *A. xylosoxidans* from an intensive care unit (Hôpital Edouard Herriot, Lyon, France). Two strains were associated with a fatal infection in two patients, 42 others colonized patients, and 2 strains were found in the intensive care unit environment.

We report the characteristics of these bacteria and their susceptibilities to antibiotics and antiseptics. A description of the investigations undertaken to find the source of the epidemic is included.

CASE REPORTS

Case 1. A 40-year-old man was hospitalized on 6 June 1981 with severe multiple trauma and in coma. Acute anuric renal failure justified peritoneal dialysis. From the 6 to 25 June, several episodes of *Enterobacteria* bacteremia responded to antibiotic therapy and the peritoneal fluid remained sterile. On 26 June, the patient developed *A. xylosoxidans* peritonitis and *Pseudomonas maltophilia* septicemia. The *A. xylosoxidans* strain isolated in pure culture from several cloudy peritoneal fluid specimens was susceptible only to moxalactam at a minimal inhibitory concentration (MIC) of 2 μg/ml. Despite peritoneal washing with noxytiolin (Innothera, Arcueil, France) and appropriate antibiotic therapy, the patient died on 10 July.

Case 2. A 47-year-old man with acute myeloblastic leukemia presented on 19 June 1982 in respiratory distress with bilateral interstitial pneumonia, requiring mechanical ventilation. The tracheal aspirate produced a pure culture of *Candida albicans*. The white cell count dropped from 4,000 to 40 in spite of leukocyte transfusions; the platelet count was below 40,000, and intestinal hemorrhages appeared. On 25 June, the blood culture was positive for *A. xylosoxidans*, susceptible only to moxalactam (MIC, 4 μg/ml). *A. xylosoxidans* was found with *Pseudomonas aeruginosa* in the tracheal aspirate sample. The patient died. *A. xylosoxidans* and *P. aeruginosa* were isolated from the lung after death.

MATERIALS AND METHODS

Colonization by *A. xylosoxidans*. During the investigation, *A. xylosoxidans* was found in the following 42 specimens from 37 patients: 30 tracheal aspirates, three blood cultures, three catheter cultures, two peritoneal fluid samples, and two urine samples. These patients had no overt symptoms of infection.

Source detection studies. We performed bacteriological cultures of the following samples. Soaps and antiseptic solutions: chlorhexidine digluconate (4%), polyvinylpyrrolidone iodine (9.6%), Dakin solution; and samples from wash basins, plumbing traps, water deionization equipment, humidifiers and respirators, surfaces, wash basins, and faucets.

Fluids were filtered through 0.45-μm membranes (Millipore Corp., Bedford, Mass.), cultivated on sheep blood agar (Biomerieux, Lyon, France). For the antiseptics, the membranes were rinsed three times with distilled water and cultivated on tryptic soy agar with 1% Tween 80 (Biomerieux); a membrane filtration method (3) was used to neutralize the antiseptics and disinfectants.

The swabs were plated on 5% sheep blood agar and brain heart broth (Biomerieux). The incubation period was 8 days at 37°C.

Bacteriological studies. The following bacteria were studied: *A. xylosoxidans* reference strain ATCC 27061, 39 clinical isolates (2 infecting, 37 colonizing), and 2 environmental isolates.

The biochemical identifications were performed by the method of Gilardi (10) (Table 1); the flagellar stain was done

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TABLE 1. Characteristics of *A. xylosoxidans* strains[a]

Characteristic	Reference strain ATCC 27061	Environmental strains (n = 2)	Patient strains (n = 39)	Criteria (10)
Growth				
Cetrimide	+	+	+	+
Salmonella-shigella agar	+	+	+	+
MacConkey agar	+	+	+	+
NaCl (6.5%)	−	−	− (1+)	−
TTC (1%)	−	+	+ (3−)	±
42°C	+	+	+ (1−)	+
MBM acetate	+	+	+	+
Acid				
Glucose (1%)	+	+	+	+
Lactose	−	−	−	−
Maltose	−	−	−	−
Xylose	+	+	+	+
Fructose	−	−	−	−
Galactose	+	+	+	±
Mannose	+	+	+	±
Rhamnose	−	−	−	−
Mannitol	−	−	−	−
Saccharose	−	−	−	−
Hydrolysis				
Urease	−	−	−	−
Desoxyribonuclease	−	−	−	−
Esculin	−	−	−	−
Gelatinase	−	−	−	−
Tween 80	−	−	−	−
Acetamide	+	+	+	+
2-Ketogluconate	+	+	+	+
Nitrite production	+	+	+	+
Nitrogen gas production	+	+	+ (1−)	±
o-Nitrophenyl-β-D-galactopyranoside	−	−	−	−
Indole	−	−	−	−
Hydrogen sulfide	−	−	−	−
Pyoverdin	−	−	−	−
Phenylalanine deaminase	−	−	−	−

[a] The number of strains giving an atypical result is shown in parentheses.

as described by Forbes (9). Gas-liquid chromatography of methyl-ester fatty acids was carried out on a capillary silica column (50 m by 0.22 mm inner diameter; no. 427, Packard Instrument Co., Inc., Downers Grove, Ill.) coated with SE 30. The temperature was varied from 160 to 280°C at the rate of 4°C per min. The method used was described by Dees and Moss (5), and the standards were from SUPELCO (Bellefonte, Pa.).

MICs were determined by agar dilution (7). The antibiotics studied, (all were obtained from companies in France) included 16 β-lactams (carbenicillin, ticarcillin [Beecham Sevigne, Paris], azlocillin, mezlocillin [Bayer Pharma, Sens], piperacillin [Lederle, Oullins], cephalothin, cefamandole, moxalactam [Eli Lilly et Cie., St. Cloud], cefoxitin [Merck, Sharp, Dohme, Chibret, Paris], cefotaxime [Roussel, Romainville], cefuroxime, ceftazidime [Glaxo, Paris], cefmenoxime [Cassene Takeda, Paris], cefoperazone [Pfizer, Orsay], cefotetan [I.C.I. Pharma, Bordeaux], and ceftizoxime [Delagrange, Paris]); five quinolones (nalidixic acid, rosoxacin [Winthrop, Clichy], oxolinic acid [Substancia, Courbevoie], pefloxacin [Roger Bellon, Neuilly-sur-Seine], and flumequin [Ricker, Pithiviers]; and others (chloramphenicol [Roussel], minocyclin [Lederle], colistin [Roger Bellon], rifampin [Lepetit, Paris], trimethoprim-sulfamethoxazole [Roche, Neuilly-sur-Seine], and novobiocin [Upjohn, Le Vaudreuil]). β-Lactamases were detected by the nitrocefin method (17).

The minimal bactericidal concentrations (MBCs) of antiseptics and disinfectants were established by the micromethod previously described by Surgot et al. (22). The products studied were chlorhexidine digluconate in 0.2% ethanol, 9.6% polyvinylpyrrolidone iodine, 1% iodine ethanol, and 10.9% sodium hypochlorite.

RESULTS

Identification of the strains. The bacteria were gram-negative rods that produced oxidase and catalase. They were motile, with peritrichous flagella. The biochemical characteristics of the strains isolated from patients and the two environmental strains are reported in Table 1. These characteristics correlated well with those of the reference strain studied under the same conditions and also with the criteria described by Gilardi (10).

Gas-liquid chromatography of the reference strain and the

TABLE 2. MICs of 11 antibiotics for *A. xylosoxidans* strains

Antibiotic	MIC (μg/ml) for indicated strains						
	Isolates from colonized patients (n = 37)		Isolates from deceased patients		Environmental strains		Reference strain ATCC 27061
	Modal	Range	Case 1	Case 2	No. 1	No. 2	
Azlocillin	64	≤0.125–128	128	128	0.5	16	4
Piperacillin	64	≤0.125–128	64	128	1	64	4
Moxalactam	4	0.5–32	2	4	4	16	4
Ceftazidime	8	2–32	8	32	8	4	2
Cefoperazone	64	1–64	64	64	8	8	2
Cefamandole	16	8–64	16	32	16	16	64
Rifampin	32	8–128	32	32	16	8	32
Novobiocin	16	4–64	4	32	8	8	1
Minocyclin	16	≤0.125–16	16	16	≤0.125	0.25	≤0.125
Rosoxacin	32	2–64	32	64	16	16	32
Colistin	8	4–128	4	8	4	4	8

isolates from the two deceased patients showed major peaks of hexadecanoic acid and 17-cyclopropanoic acid.

Antibiotic susceptibility. Of 27 antibiotics tested, only 11 had some inhibitory activity: azlocillin, piperacillin, cefoperazone, (modal MIC, 64 μg/ml), rifampin, rosoxacin (modal MIC, 32 μg/ml), cefamandole, novobiocin, minocyclin (modal MIC, 16 μg/ml), ceftazidime, colistin (modal MIC, 8 μg/ml), and moxalactam (modal MIC, 4 μg/ml). The MICs of other antibiotics were all greater than 128 μg/ml. Some strains, including the two from the deceased patients and the environmental strains, produced β-lactamase (Table 2).

The MIC study allowed the strains to be separated into two groups by resistance to β-lactams, excluding moxalactam and ceftazidime (Table 2). The more resistant group included the isolates from the two deceased patients and one environmental isolate (no. 2).

The results obtained with antiseptics and disinfectants are shown in Table 3. The reference strain and our isolates all had the same susceptibility to these agents.

The epidemiological investigation found no *A. xylosoxidans* isolates in antiseptics or soaps, on solid surfaces, or in water obtained from different sources. The bacterium was discovered in deionized water from the faucets of the hemodialysis system in 2 of 14 rooms, but not in the deionization resins (Lewatit Resins, Bayer, Cologne, Federal Republic of Germany). To disinfect the deionized water system, we filled it with sodium hypochlorite solution (10.9%) for 15 min and then rinsed it with running water. Immediately after disinfection, *A. xylosoxidans* was not found in the deionized water. The chlorination process was repeated every 2 months. Since this procedure was initiated, no *A. xyloxosidans* infections have occurred.

DISCUSSION

Several publications (1, 4, 6, 8, 13–15, 18–21, 24, 26) reported the isolation of *A. xylosoxidans* from humans (Table 4). Only one epidemic was reported, by Foley et al. (8).

This bacterium causes disease more readily in persons with compromised host defenses, including immunosuppressed patients with underlying disease (2, 6, 13, 14, 20) and premature babies (8, 15, 21). Generalized infection is usually severe and very often lethal (13–15, 21). The distribution of *A. xylosoxidans* in nature is not well known; an aqueous environment seems to be preferred (2, 16, 18, 23). This species has been found by Holmes et al. (13) in a swimming pool, by Siegman et al. (14) in dialysis fluids, by Moffet and Williams (16) in distilled, deionized, and tap water, by Foley et al. (8) in distilled water, respirators, and humidifiers, by Shigeta et al. (20) and Holmes et al. (13) in chlorhexidine solutions, and by several groups (8, 15, 16, 21) in incubators.

TABLE 3. MBCs of antiseptics and disinfectants for *A. xylosoxidans* isolates

Antiseptic or disinfectant	MBC (μg of active compound per ml)	
	Modal	Range
Chlorhexidine digluconate in ethanol solution	62.5	15.62–125
Polyvinylpyrrolidone iodine in aqueous solution	750	750–1500
Iodine ethanol	312.5	156.25–625
Sodium hypochloride	109	54.5–109

TABLE 4. Strain sources

Authors (reference no.)	Date	No. of cases	Source of isolate (no.)
Foley et al. (8)	1961	6	Septicemia in prematures
Sindhu (21)	1971	3	Meningitis in newborn
Yabuuchi and Ohyama (25)	1971	7	Ear discharge
Lee and Tan (15)	1972	3	Meningitis in newborn
Shigeta et al. (19)	1974	1	Meningitis
Yabuuchi et al. (26)	1974	55	Ear discharge (16) Spinal fluid (3) Skin, burns, ulcers (7) Throat, sputum, respiratory tract (6) Peritoneal fluid (5) Brain (1) Pus (2) Stool (2) Urine (1) Undetermined (12)
Holmes et al. (13)	1977	11	Blood or blood cultures (4) Urine (1) Sputum (1) Orbit swab (1) Wound swab (2) Chlorhexidine (1) Swimming pool (1)
Shigeta et al. (20)	1978	6	Cerebral ventriculitis
Dworzack et al. (6)	1978	1	Bacteremic pneumonia
Pien and Higa (18)	1978	9	Otitis (5) Surgical wound (2) Tracheal aspirate (1) Herpes zoster vesicle (1)
Chester and Cooper (4)	1979	23	Unknown (3) Blood (4) Wounds (4), abscess (1) Urine (7) Throat (1) Stool (1), vaginal (1) Environmental (1)
Toki and Kitaura (24)	1980	1	Suppurative skin disease
Appelbaum et al. (2)	1980	1	Pancreatic abscess
Igra-Siegman et al. (14)	1980	6	Otitis (2) Urinary tract infection (1) Lung abscess (1) Pharyngitis (1) Peritonitis (1)

Tatum et al. (23) pointed out that *A. xylosoxidans* can be found in normal human stools.

In this epidemic, the common source was deionized water; the route of transmission was probably contamination during nursing care.

A. xylosoxidans grows on many selective and nonselective media. Often confused with *Pseudomonas* spp. and *Alcaligenes* spp. (12, 13, 23, 26), *A. xylosoxidans* can be identified if attention is paid to flagellar morphology and xylose oxidation. Gas-liquid chromatography enabled us to confirm the diagnosis in accordance with the results of Dees and Moss (5).

Most studies of *A. xylosoxidans* susceptibility to antibiotics have been carried out by standard disk diffusion methods (2, 4, 6, 8, 13–15, 18, 20, 21, 24 25); MICs were seldom determined (6, 25).

Carbenicillin, trimethoprim-sulfamethoxazole, nalidixic acid, and polymyxin B and E were most often active against these isolates; penicillin G, methicillin, and ampicillin were always inactive; other antibiotics, such as aminoglycosides,

chloramphenicol, cephalosporins, and macrolides, had intermediate activity. Our strains were resistant to all 27 antibiotics tested except moxalactam and ceftazidime.

Antiseptic and disinfectant susceptibility determinations have rarely been carried out. *A. xylosoxidans* is resistant to quaternary ammonium compounds, and cetrimide is used as a selective agent in culture media (0.3 g/liter). Our strains had the same response to chlorehexidine as that reported by Shigeta et al. (20) and Holmes et al. (13).

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