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# Airway Colonization by *Flavobacterium* in an Intensive Care Unit

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A total of 195 patients admitted to a respiratory-surgical intensive care unit became colonized with species of *Flavobacterium* during a 70-month prospective study. By biochemical, cultural, and morphological criteria and a comparison of antibiotic susceptibilities, all patient isolates of *Flavobacterium* were apparently related. The origin of these organisms was sought. *Flavobacterium* were recovered from different water-associated areas of the hospital and from the hands of respiratory-surgical intensive care unit staff. The organisms were also found in university dormitory sinks. The isolation of these organisms from tap water led to their recovery from reservoirs supplying drinking water to the city of Boston and surrounding communities. These organisms are resistant to chlorine concentrations found in municipal water. There was no proven case of pneumonia caused by *Flavobacterium* in 2,329 consecutive patients studied in our respiratory-surgical intensive care unit.

The genus *Flavobacterium* was first recognized 90 years ago as ubiquitous in water (15). Only in the last 25 years has this group of ill-defined gram-negative bacilli been associated with the degradation of pesticides (20), deterioration of petroleum products (3), spoilage of food (9), and human infection (2, 5, 6, 8, 13, 17, 21, 24, 25, 27, 29, 31, 34). The appearance of these organisms as the etiological agents in disease, especially in nosocomial and related outbreaks has been associated with the discovery of reservoirs of *Flavobacterium* in the hospital environment (18, 26, 28, 30). Numerous sputum cultures of patients in our respiratory-surgical intensive care unit (R-SICU) showed that *Flavobacterium* species was a common and easily transmitted saprophyte. A 70-month prospective study in the R-SICU was conducted, and the origin, incidence, and importance of *Flavobacterium* as potential pathogens was evaluated. This report describes 195 related incidents of upper airway colonization and suggests that the organisms entered the hospital via the municipal water supply.

(This study was presented at the 78th Annual Meeting of the American Society for Microbiology, Las Vegas, Nev., 1978.)

## MATERIALS AND METHODS

**Investigation of airway colonization.** During the study period, 2,329 consecutive patients were admitted to the R-SICU. Within 24 h of admission tracheal aspirates, throat cultures, and stool cultures were obtained from each patient to ascertain initial colonization by bacteria. Tracheal aspirates were collected daily after morning chest physical therapy and evaluated by Gram stain and culture. Urine cultures were performed at the time of bladder catheterization and when clinically indicated.

Airway colonization was evaluated semi-quantitatively, as described in previous studies (14, 16, 22).

**Epidemiological investigation. (i) Beth Israel Hospital environment.** Environmental surveillance was carried out periodically throughout the study. Areas within or organizationally connected with the R-SICU were cultured, especially those having contact with water. Cultures were taken from ventilator traps, nebulizers, respiratory equipment tubing, sink drains, ice machines, stainless-steel counter tops and splash boards, waterbaths, and the Tissot spirometer. Samples were taken with sterile swabs moistened with dextrose phosphate broth and inoculated onto Trypticase soy agar containing 5% sheep blood. Samples (1 or 0.1 ml) of water from taps and from ice sources were inoculated by spreading on blood agar plates for standard plate counts. Samples of prepared food were cultured by direct inoculation of a food sample onto 5% sheep blood agar plates. Hand cultures of R-SICU personnel were taken by two methods. Personnel were asked to wash their hands with 100 ml of dextrose phosphate broth. The contaminated broth was then collected on sterile 150-mm petri dishes. Portions (1 ml each) of the washings were inoculated by spreading onto 5% sheep blood agar plates. Personnel were then asked to impress each moistened hand firmly onto a Trypticase soy agar plate (diameter, 150 mm) with 5% sheep blood.

Airborne contamination was monitored by placing settling plates containing 5% sheep blood agar at var-

ious locations in the R-SICU during an intense cleaning operation. Plates were exposed for 2 h, recovered, and incubated.

After 59 months of patient surveillance, sink traps in 90 locations throughout the hospital were sampled for the presence of *Flavobacterium*. Of the 90 locations sampled, 35 were inpatient areas, including operating room scrub sinks and ward bathrooms, 21 were outpatient areas, which included clinic bathrooms, and 34 were nonpatient areas, including administration offices.

**(ii) University dormitory environment.** To assess whether sink colonization by *Flavobacterium* species was unique to the hospital environment or common to the community environment, university dormitories housing a population of healthy individuals were examined.

The bathroom sinks of four dormitories of a large urban university dependent on the same water supply as the Beth Israel Hospital were studied. Each university dormitory houses an average of 380 students. A total of 86 sinks were cultured in a single day.

**(iii) Greater Boston municipal water system.** The metropolitan water district of the Metropolitan District Commission supplies water to approximately $2 \times 10^6$ people, comprising 44 municipalities in the greater Boston area. The water originates from the Quabbin Watershed, 65 miles (ca. 104.6 km) to the west of metropolitan Boston. Traveling by gravitational flow via aqueducts and tunnels, the water empties into smaller reservoirs that surround the metropolitan area. Because of severe restrictions on recreation and industrial use and public access to these water sources, no extraordinary methods of purification other than the addition of chlorine and ammonia near the points of distribution are considered necessary.

Permission was granted by the director of the Metropolitan District Commission Water Division to examine surface water samples collected from Metropolitan District Commission reservoirs. These samples were in the form of total plate counts, membrane filter (Millipore Corp., Bedford, Mass.) samples, and actual water specimens collected by the investigator. These samples were analyzed for the presence of *Flavobacterium* species. Water specimens were also collected from the Charles River, the main metropolitan waterway not used for drinking water.

**(iv) Bacteriological techniques.** Water samples were processed through an Amicon LP-1 pump fitted with an XM-300 Diaflo membrane filter (Amicon, Inc. Lexington, Mass.). After filtration of 100-ml portions, each membrane was removed and cut into four quarters, using aseptic techniques, and pressed, upside down, onto a plate containing Trypticase soy agar with 5% sheep blood. The exposed side of the membrane was gently rubbed to assure the transfer of organisms to the blood agar plate. The membrane was removed, and the inoculated area was streaked in a standard way. The plates were then incubated overnight at 37°C.

After overnight incubation, all plates were kept at room temperature and examined daily for 6 days for the appearance of organisms. All cytochrome oxidase-positive gram-negative bacilli isolated from the primary plates were identified. Initial identification of *Flavobacterium* was based on colony morphology, pigmentation, production of indole, and the liquefaction of gelatin. Further classification of *Flavobacterium* was based on the traditional biochemical criteria of Weaver et al. (33) and the API enteric identification system (Analytab Products, Inc. Plainview, N.Y.) (32). Antibiotic susceptibility tests were performed on all *Flavobacterium*, using the U.S. Food and Drug Administration modified method of the disk diffusion antibiotic susceptibility test of Bauer et al. (4). Serological analysis was carried out by a rapid slide agglutination test, utilizing absorbed rabbit antisera representing *F. meningosepticum* serotypes A through F (Difco Laboratories, Detroit, Mich.).

Resistance to chlorine was measured by the introduction of a known quantity of organisms into standardized dilutions of a saturated chlorine solution in sterile distilled water. Dilutions ranged from 0.1 to 100 mg/kg. Tubes were sealed with rubber stoppers coated with silicon grease lubricant. A control containing organisms without chlorine was included. A contact time of 24 h was allowed, after which dilutions were filtered through 0.45-μm membrane filters followed by rinsing with 200 ml of sterile distilled water. Membranes were transferred to petri dishes containing Trypticase soy agar containing 5% sheep blood cells and incubated overnight at 37°C. Colonies were enumerated to determine survival in chlorine.

## RESULTS

**Bacteriology of *Flavobacterium* species.** Because no universally accepted taxonomic schema exists to identify *Flavobacterium* to species level, identification of all clinical and environmental strains was not carried past the genus level, although most strains resembled the description of *Flavobacterium* species group IIB given by Weaver et al. (33).

Biochemical characteristics of a representative colonizing strain were as follows. The strain reacted positively to tests for: cytochrome oxidase; urease production; indole production; gelatin liquefaction; nitrate reduction; pigment production (very yellow); esculin hydrolysis; catalase; growth at 25°C; growth at 37°C; and growth on MacConkey agar. The strain reacted negatively to tests for: β-D-galactosidase; arginine decarboxylase; lysine decarboxylase; ornithine decarboxylase; citrate utilization; $H_2S$ production; tryptophane deaminase; Voges-Proskauer; motility; polymyxin sensitivity; and growth at 42°C. Results for the assimilation of fructose and of cellobiose (both OF base) were positive. Those for the assimilation of the following carbohydrates (API 20E, unless otherwise indicated) were negative: mannitol; inositol; sorbitol; rhamnose; sucrose; melibiose; amygdalin; arabinose; and lactose (OF base). Glucose (OF base) assimilation by the strain tested negatively at first, and then positively after 7 days.

There was no reaction of the *Flavobacterium* strains with *F. meningosepticum* typing sera A through F. These organisms demonstrated a remarkable resistance to antibiotics (Table 1).

Relative resistance of the colonizing strain to chlorine was also checked, and organisms were found to survive in chlorine concentrations above 1.0 mg/kg.

**Airway colonization.** Of the 2,329 patients studied, 195 (8.4%) had their respiratory tracts colonized during their stay in the R-SICU with species of *Flavobacterium* (Fig. 1). These organisms accounted for the greatest incidence of colonization by any gram-negative organisms. Four of the patients showed pulmonary infiltrates on chest X-ray films during periods when sputum specimens revealed dense concentrations of *Flavobacterium* bacilli. Other organisms were also present in their sputum.

TABLE 1. *Antibiotic susceptibility testing of Flavobacterium species*

| Antibiotic | Disk strength | Zone diam (mm) | Sensitivity[a] |
|---|---|---|---|
| Ampicillin | 10 μg | 7.0 | R |
| Carbenicillin | 100 μg | 14.0 | R |
| Cephalothin | 30 μg | 6.0 | R |
| Chloramphenicol | 30 μg | 17.7 | I |
| Clindamycin | 2 μg | 11.3 | R |
| Erythromycin | 15 μg | 15.0 | I |
| Gentamicin | 10 μg | 13.7 | S |
| Kanamycin | 30 μg | 6.0 | R |
| Penicillin | 10 U | 9.3 | R |
| Polymyxin | 300 U | 6.0 | R |
| Streptomycin | 10 μg | 6.0 | R |
| Tetracycline | 30 μg | 19.0 | I |
| Vancomycin | 30 μg | 18.0 | S |
| Sulfonamide | 300 μg | 15.3 | S |
| Nitrofurantoin | 200 μg | 15.3 | R |

[a] S, Sensitive; I, intermediately sensitive; R, resistant.

**Epidemiological investigations of the Beth Israel Hospital.** During the period of patient surveillance, *Flavobacterium* species were initially isolated, from a sink drain located near the bed of a colonized patient, on three separate occasions. Hand cultures of two R-SICU house officers, two R-SICU nurses, and one research fellow grew *Flavobacterium* species out of a total of 13 staff members examined at a time when one patient was actively exhibiting these organisms. Quantitation of the hand levels of *Flavobacterium* species contamination ranged from 20 to 1,000 organisms/ml. Recovery of *Flavobacterium* species from the hospital environment is summarized in Table 2.

*F. meningosepticum* type B was found in tube feedings being prepared in an area of the main kitchen. These feedings were prepared daily but usually left covered in unsterilized containers under refrigeration for periods of time ranging from 1 h to 20 h before use. A sink trap in an outpatient clinic and a sink in the main kitchen of the hospital yielded a strain similar to the colonized strains. Species of *Flavobacterium* were additionally isolated on two occasions from specimens of tap water drawn from a faucet in an anesthesia research laboratory and from a newly opened patient care facility. There were no organisms resembling *Flavobacterium* recovered from settling plates.

**Epidemiological investigation of the university dormitories.** Species of *Flavobacterium* were found in a total of five sinks in the dormitories (5.8%). Of these five sinks, two strains were *Flavobacterium* species resembling those recovered from the infected patients and the environmental locations in the Beth Israel Hospital. The percentage of *Flavobacterium* species sink colonization was remarkably similar to the colonization by these organisms in the

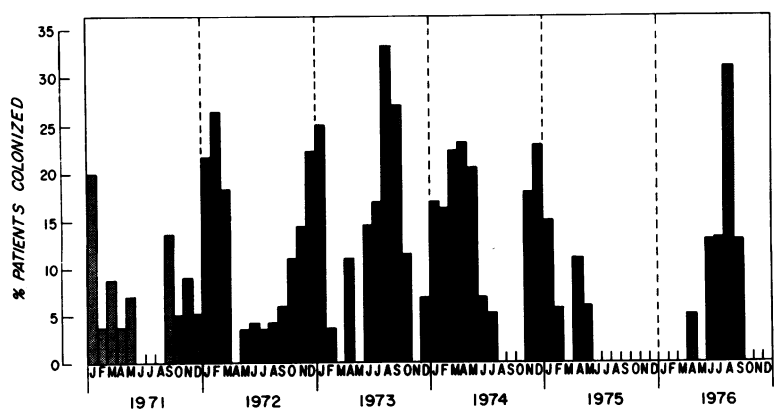


FIG. 1. *Incidence, by month, of respiratory tract colonization in our R-SICU patients with Flavobacterium species.*

nonpatient sinks in the Beth Israel Hospital.

**Bacteriological investigation of municipal water supplies.** During the 5 months of analysis of municipal water supplies, species of *Flavobacterium* were recovered from open-water reservoirs supplying the metropolitan Boston area (Fig. 2). Strains of *Flavobacterium* were recovered from five of the nine reservoirs sampled. In one reservoir, a strain of *Flavobacterium* species was isolated, exhibiting biochemical characteristics and antibiotic susceptibility patterns similar to strains colonizing patients in the R-SICU.

Water sampling of the Charles River, an untreated river carrying significant amounts of raw sewage as well as industrial waste through the metropolitan area, failed to demonstrate any organisms resembling the genus *Flavobacterium*.

## DISCUSSION

*Flavobacterium* species was responsible for airway colonization in 195 seriously ill patients.

TABLE 2. *Recovery of Flavobacterium species from the environment*

| Area sampled | No. of isolations/areas cultured |
|---|---|
| Beth Israel Hospital | |
| Tap water | 2/27 |
| Sink drains | 8/101 |
| Personnel (hand cultures) | 5/13 |
| Ice machine (R-SICU) | 2/7 |
| Water baths | 1/2 |
| Main kitchen areas | 3/9 |
| University dormitories | |
| Sink drains | 5/86 |

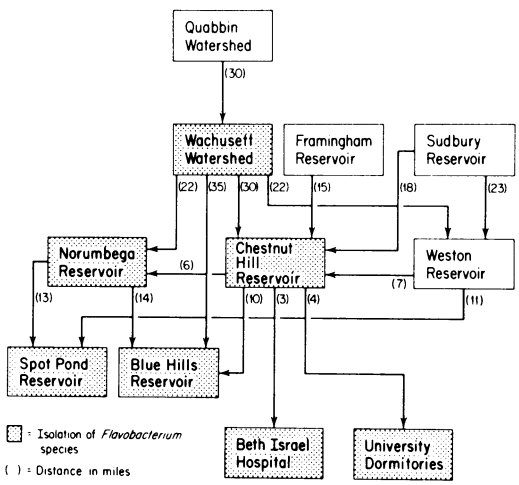


FIG. 2. *Isolation of Flavobacterium species from metropolitan Boston reservoirs.*

The results of this study suggest that the ultimate source of these organisms was the municipal water supply. Airway colonization of seriously ill patients with gram-negative bacilli, including environmental species, has been found to be a common event and is seen as a precursor to bacteriological complications, including pneumonia (23); however, the role of *Flavobacterium* species as a pulmonary pathogen is unclear. *Flavobacterium* species are commonly found in the contamination of heated newborn incubators and mist tents (11). *Flavobacterium* can often be recovered from humidifiers and in the tap water used to fill the humidifier reservoirs (1) and are commonly found in air samples taken in the vicinity of vapor and aerosol humidifiers (10). Jackson et al. showed that *Flavobacterium* species are rapidly cleared from the lungs of mice after experimental aerosolization (19). Teres reported a case of *F. meningosepticum* type D pneumonia from this hospital (31). Since 1959, organisms of the genus *Flavobacterium* have been implicated in human infections including meningitis, septicemia, and endocarditis (2, 5, 6, 8, 12, 17, 18, 21, 24–31, 34). In most cases, the *Flavobacterium* were waterborne. *Flavobacterium* originate as the normal flora of watersheds, open reservoirs, and their surrounding environments (7). Since no extraordinary purification treatment is given these waters, the *Flavobacterium* are carried unimpeded to the metropolitan reservoirs where the first chlorination processes are initiated. The Boston area relies solely upon chlorine and ammonia for water disinfection (G. C. du Moulin, G. Friedland, K. Indorato, and K. D. Stottmeier, Abstr. Annu. Meet. Am. Soc. Microbiol. 1978, Q70, p. 206). Total residual chlorine levels of 0.45 to 0.80 mg/kg are considered adequate to neutralize waterborne pathogens without imparting a characteristic odor or taste to potable water. *Flavobacterium* have been shown to resist chlorination and survive concentrations as high as 100 mg/kg (12, 18). In our situation, *Flavobacterium* were found resistant to concentrations up to 1.0 mg/kg. Maximal concentration of chlorine in municipal waters rarely exceed 0.8 mg/kg, except briefly at pumping stations where disinfectant is added. Therefore, it appears that these organisms encounter no difficulty surviving in municipal waters.

The water supplying the Beth Israel Hospital and the university dormitories described in this study originates in the Quabbin and Wachusett watersheds and, during periods of high water demand, the Chestnut Hill Reservoir in Brookline. Reservoirs in which *Flavobacterium* species were isolated are directly fed by the Wachusett watershed (Fig. 2). These organisms appar-

ently contaminate water taps, sink drains, ice-making machines, and water fountains during the normal use of water.

In the R-SICU, 80% of the patients are post-surgical, and the remainder are medical patients with chronic obstructive lung disease, heart failure, poisoning, or neuromuscular disease. Therapy with broad-spectrum antibiotics for preoperative and perioperative prophylaxis or for the management of specific infections in this patient population is common. The unusual resistance of the *Flavobacterium* to antibiotics directed at gram-negative bacteria allows for favorable competition and subsequent colonization. Transmission of *Flavobacterium* from locations in the hospital to susceptible patients occurred predominantly via contaminated water or ice. The hands of hospital personnel were probably also contaminated by splash back occurring in contaminated sinks during hand washing or by direct contact with colonized patients (23).

Once a patient became colonized, secondary reservoirs were established and organisms were again able to be transmitted to noncolonized patients primarily by hand carriage. It was not infrequent to have more than one patient colonized simultaneously with *Flavobacterium*.

Recovery of *Flavobacterium* species from sink drains were generally infrequent, suggesting that these organisms are poor competitors with other waterborne bacterial species. Sink drains in which *Flavobacterium* species were the predominant organisms isolated generally showed a lesser degree of contamination by other gram-negative organisms. The recovery of these organisms from the oligotrophic reservoirs contrasted markedly with the lack of recovery in the Charles River, which is still eutrophic and which demonstrated a high bacterial presence.

In this investigation, although this organism showed a remarkably high incidence of colonization, infections due to this organism were not documented. Chlorinated tap water can support populations of environmental gram-negative organisms sufficient enough to exert a strong microbiological influence in a critical care environment.

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