DISTRIBUTION OF PSEUDOMONAS AERUGINOSA IN NAVIGATION POOL NO. 8 OF THE UPPER MISSISSIPPI RIVER

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ABSTRACT

The distribution of Pseudomonas aeruginosa in Navigation Pool No. 8 of the Upper Mississippi River has been investigated. Serological and pyocin typing was performed on 152 isolates, and serological type 1 was found to be the most prevalent (34.2%). Pyocin typing was found to be the most efficient method of differentiation, although serological typing was more practical. A comparison of the total number of positive P. aeruginosa samples from each sample type showed that this pseudomonad was most commonly isolated from sediment samples. Comparisons of each sample source indicated that recovery of P. aeruginosa was more frequent from the surface slime than from the feces of 52 fishes, and from plant parts below rather than above the water surface. Predator fishes rather than non-predator fishes were more commonly infected by the bacterium. Current velocities, availability of oxygen and nutrients, surface tension, and possibly negative phototropism, all play a part in the location of the organism in the waters of Navigation Pool No. 8. The organism was found to be abundant in Pool 8, and it was hypothesized that sediment possibly serves as the reservoir for this opportunistic pathogen in water.

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INTRODUCTION

The role played by members of the Genus Pseudomonas in human disease has received increasing attention during recent years. This is, in part, due to the availability of numerous drugs which have simplified the control of several pathogens. Attention is now being focused on recalcitrant organisms, among which Pseudomonas aeruginosa occupies a unique position because of its notorious resistance to antimicrobial agents, its ability to inhabit various environments, and its role as an opportunistic pathogen.

In hospitals, this opportunistic pathogen has been frequently isolated from sinks (6, 49, 163), mechanical respirators (55, 110), endoscopes (68), disinfectants (1, 29, 31, 147, 164), vegetables (90, 105), and hands of personnel (41, 89, 104, 105). Hospital-aquired Pseudomonas infections are therefore common and reach their greatest morbidity and mortality in intensive care and renal transplant patients having associated major trauma, extensive burn, surgical mishap, immunosuppression, and/or prior antibiotic therapy (36, 37, 73). In the natural environment, P. aeruginosa is commonly found in soil, water, and on vegatation, and has been isolated as a contaminant in drinking water (96), surface water (116), and in swimming pools (58, 79, 116). The bacteria have been implicated in infections of the eye, ear, nose, and throat of swimmers (78).

Investigation of various bodies of water have led to the conclusion that P. aeruginosa is common in water. However, the location of the organism in water has not been reported. In the present study, the distribution of Pseudomonas aeruginosa in a natural body of water was

investigated, with the objectives of the study being to:

(i) determine the abundance of P. aeruginosa in Navigation Pool No. 8 of the Upper Mississippi River;

(ii) examine the correlation between incidence of P. aeruginosa isolations and sample source in Navigation Pool No. 8 of the Upper Mississippi River;

(iii) compare the efficacy of two popular typing methods, serotyping and pyocin typing; and

(iv) determine the abundance and distribution of serological types in Navigation Pool No. 8 of the Upper Mississippi River.

LITERATURE REVIEW

Many bacterial species can produce disease in humans, and this is true of certain members of the genus Pseudomonas. Most are free-living bacteria, widely distributed in soil and water, but some are parasites (and pathogens) of plants and insects. Bergey's Manual of Determinative Bacteriology (26) defines this group as gram negative, strict aerobes (except for those species which can use denitrification as a means of anaerobic respiration), single-celled, straight or curved rods, and motile by polar monotrichous or multitrichous flagella. They are either chemoorganotrophs which utilize glucose oxidatively, or facultative chemolithotrophs, utilizing H_2 or CO as their energy source. They are also catalase positive.

A small number of Pseudomonas species are associated with disease in man. One species, P. mallei, appears to be a specialized mammalian parasite that causes the disease glanders. P. pseudomallei, the agent of melioidosis, and P. maltophilia, causative agent of upper respiratory tract ailments and septicemia, are other important members of the group (98).

The most extensively studied of the aerobic pseudomonads is the fluorescent group, which consists mainly of P. aeruginosa, P. fluorescens, and P. putida, characterized by their ability to produce water-soluble, yellow-green, fluorescent pigments. Their nutritional versatility, hallmarked by their ability to utilize a wide range of organic compounds as sole sources of carbon and energy, is well documented (119, 141).

Blazevic et al. (14) in 1973 determined that P. fluorescens and P. putida comprised less than 1% of all pseudomonads isolated in a clinical

laboratory. P. fluorescens has been recovered from wounds, sputum, pleural fluid, urine, and most important, from blood for transfusion in which the organisms multiply, autolyze, and release endotoxins. Although P. fluorescens and P. putida have been isolated from human disease (14), the species most frequently encountered is P. aeruginosa. Because of the increasing importance of this opportunistic pathogen and its implication in numerous diseases, a magnitude of literature dealing with the different aspects of the organism has accumulated over a short period of time. Pseudomonas aeruginosa is the only fluorescent pseudomonad capable of growth at 41-42 C (141). Nutritionally it is extremely versatile (101, 121, 141), and is able to utilize at least 70 to 80 different compounds for growth, and it is one of the few bacteria capable of obtaining energy through the Entner-Doudoroff, β-ketoadipate, and β-oxidative pathways (17, 63, 128), and of respiring anaerobically only in the presence of nitrate or nitrite.

The necessity for rapid, accurate diagnosis of P. aeruginosa infections prompted many researchers to propose various selective and differential media for use in the clinical laboratory. Since the majority of P. aeruginosa isolated from infected sites and from the environment are pyocyanogenic, most of the available media are for enhancement of this phenazine pigment. Pseudomonas Agars F and P (Difco), first developed by King, Ward, and Raney (87), are widely used in diagnostic laboratories for this purpose. A comparison made between two media, cetrimide agar with lemco base (CTA 1) and cetrimide agar with modified King's base (CTA 2), both containing 0.03% cetrimide, showed that pyocyanin production was better on CTA 1, but that stronger fluorescence was obtained on CTA 2 (22). Sands and Rovira (130) found that

the incorporation of novobiocin, penicillin, and cycloheximide into a standard medium used for fluorescence provided greater selectivity of the medium, while Martineau and Forget (107) claimed that strains producing no pigment on ordinary agar slants will demonstrate intense production of the bluish-green pigment on Sabouraud maltose agar. It was noted by Young (116) that pigment production could be inhibited by several substances. Media containing sufficient glucose (> 1%) to establish and maintain an acid reaction did not support pigmentation. Growth of the organism in potato glycerol broth resulted in profuse production of pyocyanin, but enrichment of this medium with veal infusion or blood inhibited chromogenesis. Azuma and Witter (7) observed pyocyanin production after normally apyocyanogenic strains were grown in the presence of carbobenzoxy-amino acids, then on Klinge's medium. In an attempt to develop a rapid method of identifying P. aeruginosa strains, either of the pyocyanogenic or apyocyanogenic type, Gaby and Hadley (62) proposed a test based on the high concentration of cytochrome oxidase present in the cells of P. aeruginosa.

Approximately 4% of the strains isolated from clinical specimens are non-pigmented (61). Necessity therefore exists for acceptable methods to identify these apyocyanogenic strains. Some authors prefer to rely on a variety of characteristics, for instance, odor, growth at different temperatures, virulence for Swiss mice, and the oxidase reaction (28, 61, 64, 134). Ultraviolet light has become a popular aid in rapid detection of P. aeruginosa cultures (18, 74, 94, 137, 144). However, other methods for speciating Pseudomonas have been used. These include absorption and fluorescence spectra of the water-soluble pigments (160), gas-liquid chromatography (115, 157), and radiometric detection (123).

Special media for the isolation and enumeration of P. aeruginosa have also been developed. Brodsky and Nixon (19) described a membrane filter technique in which black membrane filters, MacConkey agar, and ultraviolet light were used. In another study (77), a modified medium containing asparagine as the sole carbon and nitrogen source gave higher estimated most probable numbers (MPNs) than media listed in Standard Methods. Carson et al. (32) found that the type of filter and nature of the diluents employed, as well as pH of the assay medium, greatly influenced recovery of the organism. Other authors have used inhibitors and elevated incubation temperatures to aid in recovery (99), while Solberg et al. (138) found that incorporation of 200 ppb of 2-hydroxy-2', 4, 4'-trichloro-diphenyloxide and 10 ppm of cetyl-trimethyl-ammoniumbromide (cetrimide) in tryptic soy agar resulted in a medium (CETCH agar) that required a short incubation period prior to plate counting and provided greater recoveries.

There is no evidence of a correlation between strains of P. aeruginosa and types of infections caused in humans. However, for preventative measures, it is often important to trace the pattern of infection of these numerous strains. This has been done mainly through the use of three methods, serological, pyocin, and bacteriophage typing. Combinations of two or all of these methods have been widely used as epidemiologic tools (15, 48, 49, 93, 108). The use of serological typing combined with antibiotic sensitivity patterns (antibiograms) have also been reported (40). The usefulness, effectiveness, and preference of any of these methods seem to depend primarily on the author and purpose of the study.

The most popular of the serological methods is that developed by

Fisher et al. (57) in which seven groups of cross-protective homogeneity were defined. Another method described by Homma et al. (80), consists of type sera 1 to 10. In the antigenic schema of Lányi (95), serogroups 3, 4, 5, 7, and 10 of the 13 O antigen groups defined were further divided into 15 subgroups to increase sensitivity of the method. Difco Laboratories recently proposed a schema representing 17 tentative serotypes (42). In this schema, types 1 through 12 were prepared using Hab's cultures 1 through 12, type 13 using Veron's O13 (Sandvik's type II), type 14 using Verder and Evan's O5, type 15 using Lanyi's O12, and type 16 using Meitert's type X strain.

Perhaps the most tedious but widely used procedure is that of pyocin typing. Pyocin, a strain-specific bacteriocin elaborated by some strains of P. aeruginosa, selectively inhibits the growth of other Pseudomonas strains. Electron micrographs of negatively stained pyocin preparations revealed that pyocin is made up of rod-shaped particles which resemble the contractile tail protein of the T-even phages of Escherichia coli (76). Pyocin precipitates as a homogeneous, jelly-like substance (86), and is stable at pH 7-8 (72). Typing of P. aeruginosa by pyocin production using 8 indicator strains of P. aeruginosa was first done by Gillies and Govan (65). A few years later, pyocin type 1 from the Gillies and Govan strains was subtyped using 5 strains of P. aeruginosa (66). The 27 indicator strains of Farmer and Herman (54), the 18 indicator strains chosen by Jones et al. (85), and the 12 strains used by Zabransky and Day (169), are other sets of pyocin typing strains used. Jones et al. (84) proposed that strains of P. putida and P. fluorescens be used as pyocin indicator strains, since only the fluorescent pseudomonads are sensitive to pyocins produced from P. aeruginosa.

To promote a more sensitive method for pyocin typing, Bergan (11) found that it was necessary to totally remove the strip of agar on which the Pseudomonas grew before indicator strain inoculation. He noted that indicator strains rendered better differentiation when using bacterial suspensions of standard density yielding nearly confluent growth. Jones et al. (83) have proposed the use of TSB (without glucose, BBL) plus 1% potassium nitrate for obtaining good yields of pyocin. It was also found that incorporation of 10^{-5}M iodoacetic acid, 0.1% sodium citrate, and 0.1% dipotassium hydrogen phosphate into the medium used would suppress the action of pyocin-inhibiting substances and result in better pyocin activity (158).

The susceptibility of P. aeruginosa isolates to bacteriophage is another method used in identifying strains. The typing system of Sutter et al. (146) utilizes a selection of 18 phages and a sodium lactate medium. Bradley (16) discovered that Pseudomonas phage show a great variety of forms, some of which resemble two coliphages. Takeya et al. (149) described a bacteriophage which had a rod-shaped head and a long tail. Detailed characterization of Pseudomonas bacteriophage have been made (117).

P. aeruginosa strains synthesize many extracellular substances, some of which are responsible for the pathogenic properties of the organism. Strains may synthesize various combinations of pyocyanin, pyoverdin (fluorescein), pyorubin, and pyomelanin. As noted earlier, environmental conditions greatly influence pyocyanin production. In a dry environment, strains lose their ability to produce pigments (52). Frank and DeMoss (59) showed that synthesis of pyocyanin was accompanied by protein synthesis and was dependent on the presence of magnesium,

phosphate, and a sulfur source in addition to an amino acid substrate. It was also found that certain hydrocarbons were readily oxidized to form pyocyanin (97). This blue-green phenazine derivative which is soluble in water and in chloroform, may be an accessory respiratory enzyme (60) that can increase the respiration of living celly by a great degree. The pigment undergoes a cycle from the oxidized to the reduced and back to the oxidized form and is only effective in the oxidation of certain substances closely associated with the bacterial body. Phenazine pigments are not toxic but are known to be antimicrobials which might suppress other microbial flora and result in replacement of them by colonies of P. aeruginosa (121).

Pyocyanin synthesis may be masked by presence of the other pigments. Pyomelanogenic (brown to black water-soluble pigment) strains of P. aeruginosa comprise less than 3% of clinical Pseudomonas specimens (98). Strains which synthesize pyomelanin or pyorubin are sometimes reluctant to produce acid from carbohydrates which are usually oxidized by the species. Pyoverdin, the greenish-yellow fluorescent pigment, soluble in water but not in chloroform, was found to be the causative agent of fluorescence in the mucus of a land snail (8). At a pH between 6 and 8.5, production of fluorescein by P. aeruginosa was inversely related to the concentration of iron in the media (150). In the presence of excess iron, however, penicillin stimulated production of the pigment, while chloramphenicol, streptomycin, and 8-hydroxy-quinoline in the concentrations tested failed to increase production.

In addition to the fluorescent pigment, the formation of slime is supported by presence of sulfite (118). Purified slime exhibited 2 to 3 times more toxicity to mice than lipopolysaccharide (LPS) from the

same organisms. The response of mice was identical to that observed in lethal infection initiated with viable bacilli (133). Sensakovic found that active and passive immunization against slime prevented these responses and that protection was type-specific. Analysis of slime revealed that it contained mannose and galactose, both absent in LPS; an unidentified orcinol-positive component was in the neutral sugar pattern of LPS but not in slime. Qualitative differences were also observed in the fatty-acid compositions of slime and LPS. In a study on the protein-lipopolysaccharide complex, Rubio and Lopez (129) isolated two low-molecular-weight substances which played no antigenic role.

P. aeruginosa is known to produce several exotoxins, with the concentration of phosphate appearing to be the most critical factor involved in their production. Glucose was apparently used both as an energy source, and for the production of these toxins. Liu (103) determined that P. aeruginosa tend to produce more toxins in abnormal tissues than in healthy ones, enhancing the ability of the organism to be a successful opportunist.

The best studied of the protein exotoxins is Exotoxin A which has a mouse LD_{50} of 0.75 ug (156). In contrast, endotoxin is often not fatal to mice in doses of several milligrams. Renal and pulmonary necrosis, hemorrhage, and fatty degeneration of the liver all result from Exotoxin A, and may indicate that Exotoxin A is the most important factor in producing the lethal effects of P. aeruginosa (121).

Strains of P. aeruginosa can produce four types of enzymes--protease, lecithinase, elastase, and hemolysins. Carney and Jones (30) found that virulent strains produced more of these enzymes, and produced them sooner, than did the avirulent strains. Proteolytic enzymes, the actions of which

are similar to the action of the enzyme elastase (132), are produced by almost all strains of P. aeruginosa and may be responsible for the localized necrosis often associated with Pseudomonas infections of the skin. Elastase-positive strains produce more proteinases than do elastase-negative strains (114). At least two hemolytic factors, the activities of which are lost upon contact with anionic exchange material (12), could be produced. Phospholipase C, one of the factors, is inhibited by phosphate ions and causes wide-spread necrosis. Glycolipid, the other hemolytic factor, is heat-resistant and solubilizes lecithin, greatly enhancing the activity of phospholipase C (121). The only body fluid capable of supporting the rapid production of phospholipase C was bronchial fluid.

The pathogenicity and importance of P. aeruginosa reaches high proportions because of the unusual resistance of this organism to antibiotics (46). Only polymyxin, colistin, gentamicin, carbenicillin, and tobramycin have significant in vitro activity against most isolates. However, use of these antimicrobial agents in the treatment of human infections is limited. Essentially, the amount that can be administered is limited by the toxicities of the drugs, which are predominantly renal but also may impair the vestibular function of the eighth nerve (162). Results from in vitro tests done to determine the minimal inhibitory concentrations (MICs) of these drugs to a particular strain of P. aeruginosa may not parallel their activity in vivo. D'amato et al. (38) found that the MICs of the antibiotics tetracycline, gentamicin, polymyxin B, and carbenicillin, were increased when the cations calcium and magnesium were added to the test media. He proposed that susceptibility tests be done with Mueller-Hinton broth supplemented with

physiological concentrations of calcium and magnesium to better approximate the in vivo activities of these antibiotics. In addition, purulent material decreases the effective concentrations of some antibiotics (25).

Combinations of drugs, e.g., carbenicillin and gentamicin (39, 51, 88, 120, 139), carbenicillin and tobramycin (112), and sulfamethoxazole-trimethoprim and ethylenediaminetetraacetic acid (124), have generally been found to be more effective than individual usage (9), since the chances of development of resistant strains are greatly reduced (75). Some authors, however, found that usage of individual drugs are sometimes more effective (3, 88), and that the drug used is dependent on the amount of phospholipid in wall fractions of the organism (20) and on the sensitivity of the strain to oxygen (21). For expediency and effective drug usage, proposals were made by several authors for correct interpretations of inhibition zones around sensitivity discs (88, 151, 152).

Development of antibiotic resistance in strains of P. aeruginosa is mainly dependent on acquisition of R factors (23, 24, 56, 70, 81, 82, 91, 131). R-factor transfer between P. aeruginosa strains (82, 91), between P. aeruginosa and Escherichia coli (23, 24, 70, 127), and between P. aeruginosa and Proteus mirabilis (70), have been reported. These R factors may specify resistance to one or more antimicrobial agents. Bryan (23) described a study in which a change in pyocin type was noted upon acquisition of R factors. Isolation of R factors revealed that they are extrachromosomal, double-stranded, satellite DNA (56, 131).

Studies have revealed that various mechanisms are involved in the resistance of strains to antibiotics. Tseng (155), in studying streptomycin resistance, found that high-level resistant strains were mediated by R factors or resistant ribosomes, whereas low-level resistance

was dependent on reduced permeability to the antibiotic. In addition, penicillin resistance was dependent on a permeability barrier which prevented the antibiotic from reaching its site of action in the cell wall (143). In the case of actinomycin, this barrier may be formed by the outer layers of the cell wall which include the lipopolysaccharide component of the double-track layer (106). Enzymes may also play a part in conferring resistance by the inactivation of antibiotics (45, 161).

Many strains of P. aeruginosa are susceptible to complement together with specific antibodies. However, strains highly resistant to the action of serum alone make up most of the blood culture isolates of infected patients (167). In uninfected adults, protection is dependent on phagocytosis and intracellular killing of the organism. Patients with serious Pseudomonas diseases develop high levels of type-specific antibodies to the infecting strain, and this might promote phagocytosis and intracellular killing in the absence of complement (162).

P. aeruginosa presents the greatest problem in burn patients, and is the cause of 75% of burn-related deaths (121). Since the various immunotypes of Pseudomonas appear to be distinctly different in studies of immunization and cross-infection, polyvalent Pseudomonas vaccines have been used to treat burn patients. A significant reduction in mortality from Pseudomonas sepsis was noted after administration of a polyvalent vaccine to patients with greater than 20% burns (4). Hyperimmune globulin, when used in conjunction with the vaccine, did not markedly decrease overall septic mortality, but a significant reduction in Pseudomonas related deaths was observed (121). In cystic fibrosis patients, administration of the Parke-Davis heptavalent vaccine resulted in very good antibody responses in the majority of cases. This response

was found to persist for four to six months (121). Mucoid strains of P. aeruginosa have been isolated only in cases of patients with cystic fibrosis (33, 43, 44, 109, 170).

In experiments using rats and mice, completely different patterns of pulmonary bacterial clearance were observed (140). Purified pyocin, administered parenterally to mice infected with sensitive strains of P. aeruginosa, afforded significant protection against two of the three strains studied (111).

One of the major problems posed by P. aeruginosa is its ability to survive and multiply in the least expected environments. Luckily, it has been infrequently recovered from habitats lacking moisture (136). In the hospital environment, P. aeruginosa has been isolated in large numbers from surfaces of sinks (6, 49, 105), sink waste-traps (6, 163), floor-cloths, dishcloths, and mops (105, 163), adhesive tape (13), delivery-room sink aerator and resuscitator units (55), mechanical respirators (110), endoscopes (68), suction apparatus and nailbrushes (105), vegetables (90, 105), and from the hands of personnel (41, 89, 104, 105). Colonization of hospital disinfectants by the organism is a common phenomenon (1, 29, 31, 147, 164). Cross-infection through contaminated equipment or personnel could probably account for many of the reported cases of nosocomial Pseudomonas infections (41, 89, 104, 145, 165), with wounds being the major reservoir (165). Autoinfection of wounds and other sites is also a possibility (142, 165).

A thorough review of the literature revealed that the distribution of P. aeruginosa outside of the hospital environment has not been adequately investigated. High counts of the organism have been obtained from drinking and surface waters (96, 116). In swimming pool waters

(79, 116), it was found that single predominant types prevailed at different times (79), that coliform bacteria occurred usually in smaller numbers than did P. aeruginosa (116), and that concentrations of ammonia and the chlorine stabilizer, cyanuric acid, decreased the rate of kill by chlorine (58). P. aeruginosa can be consistently isolated from sewage (96, 116, 126, 148). Although it is believed that human feces serve as the inoculum for sewage (126) and drinking water (96), it appears that the organism is a resident of the intestinal tract in few individuals but is transient in most others (27, 80, 148). Homma et al. (80) determined that abundance of the organism in the intestines of patients was dependent on chemotherapy treatment. He also found that P. aeruginosa was usually not present in the mouth of healthy individuals.

P. aeruginosa has been constantly labelled as a common inhabitant of the soil, though few studies have been done to confirm this. Green et al. (67), from a study done in California, concluded that soil is a reservoir for the organism which has the capacity to colonize plants during favorable conditions of temperature and moisture. In a lake in Washington, the occurrence of Pseudomonas-like bacteria was found to increase with depth, the greatest number of bacteria being isolated from the sediment samples. In addition to the many sources for P. aeruginosa, the organism was also isolated from ornamental aquarium plants (153) and from hydrophilic contact lenses (113).

P. aeruginosa has been implicated in numerous types of infections. These include sepsis in burn patients (10, 36), urinary tract infections (10, 36, 47, 159), respiratory tract infections (36), eye infections (113), green nail syndrome, toeweb infection, external otitis, ecthyma gangrenosum, and Pseudomonas septicemia (71, 135).

Between 8 and 16% of all hospitalized patients (ca. 2 million individuals) each year develop hospital-associated or nosocomial infections (5), with gram-negative bacilli causing four times as much infection as gram-positives (110). Serious Pseudomonas infections are usually confined to the intensive care and renal transplant units, and are associated with major trauma, burn-cases, surgical mishap or immunosuppression, and prior antibiotic therapy (36, 37, 73).

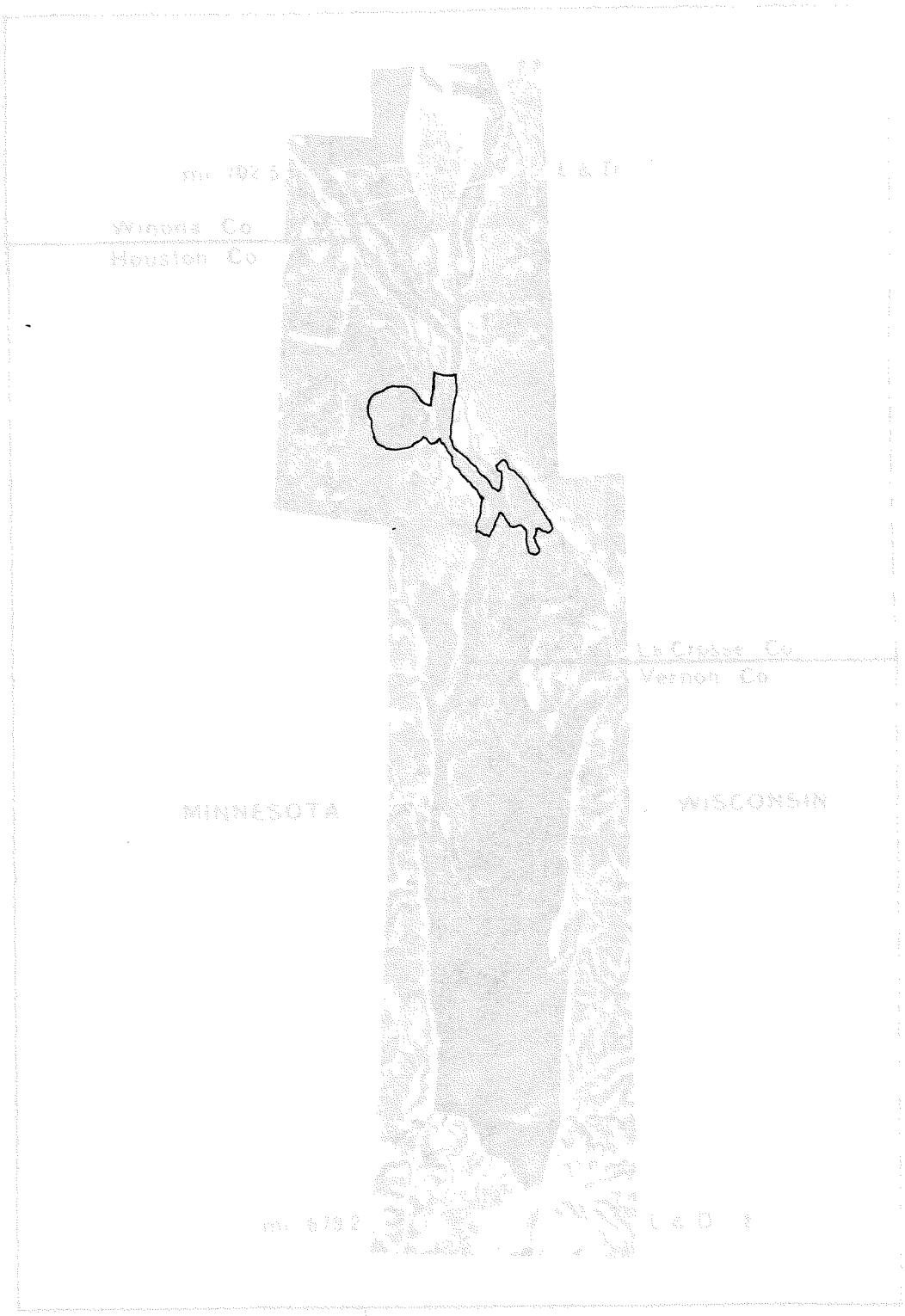
MATERIALS AND METHODS

Study area. The body of water studied was part of Navigation Pool No. 8 of the Upper Mississippi River (Fig. 1). This pool extends from Lock and Dam No. 7 which is 702.5 river miles above the mouth of the Ohio River, to Lock and Dam No. 8. Pool 8, covering 23.3 river miles, is composed of a main channel having a minimum depth of 9 ft and a minimum width of ca. 400 ft, and backwater sloughs. The pool separates Wisconsin from Minnesota, and has two tributaries, the Root River entering at river mile no. 693.8 from the Minnesota side, and the LaCrosse River entering at river mile no. 698.1 from the Wisconsin side. Pool 8 has a humid-continental type climate, with a yearly average temperature of 46 C, and an annual rainfall of 29.9 in. Wide extremes in temperature are experienced.

The study area was divided into 9 sampling areas designated A through I (Fig. 2). Areas A, D, and G, were designated as lotic habitats, because they had a measured current velocity of $\geq$0.1 m/s. The other areas (B, C, E, F, H, and I) were defined as lentic habitats, based on a measured current velocity of $<$0.1 m/s (34).

Sample collection. Samples were collected during the months of September and October of 1975 for a duration of two weeks. A modification (Sherman, L. 1977. The bacterial flora in Navigation Pool 8 of the Upper Mississippi River. M.S. thesis, Univ. of Wisconsin--LaCrosse) of Edwards' technique (50) was employed for taking water samples at a depth of 1 m, while surface water samples were collected by means of grab sampling with Whirl-pak bags (NASCO). Water samples were not taken

Fig. 1. Aerial photograph of Navigation Pool No. 8 of the Upper Mississippi River with study area delineated on the cover page.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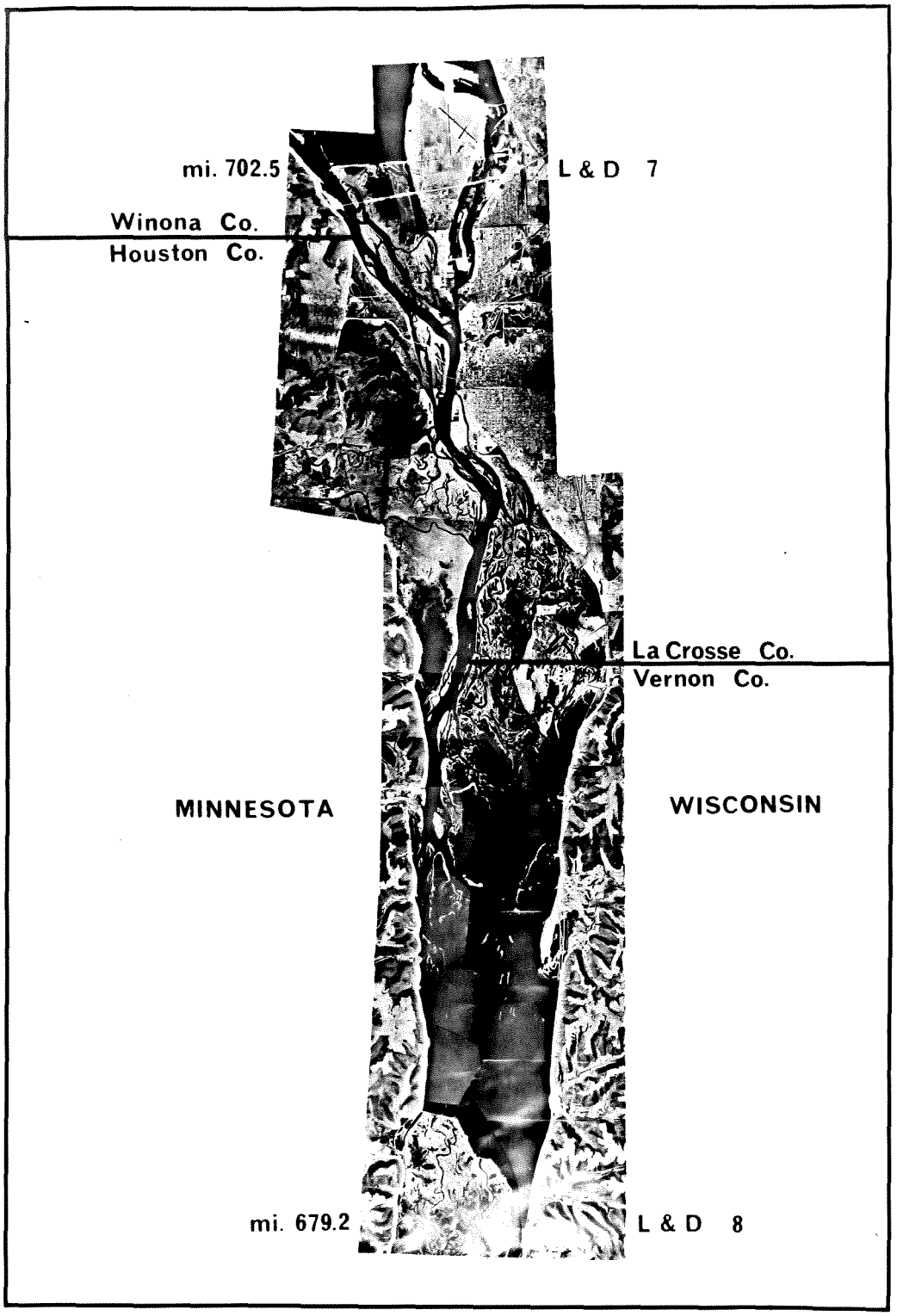
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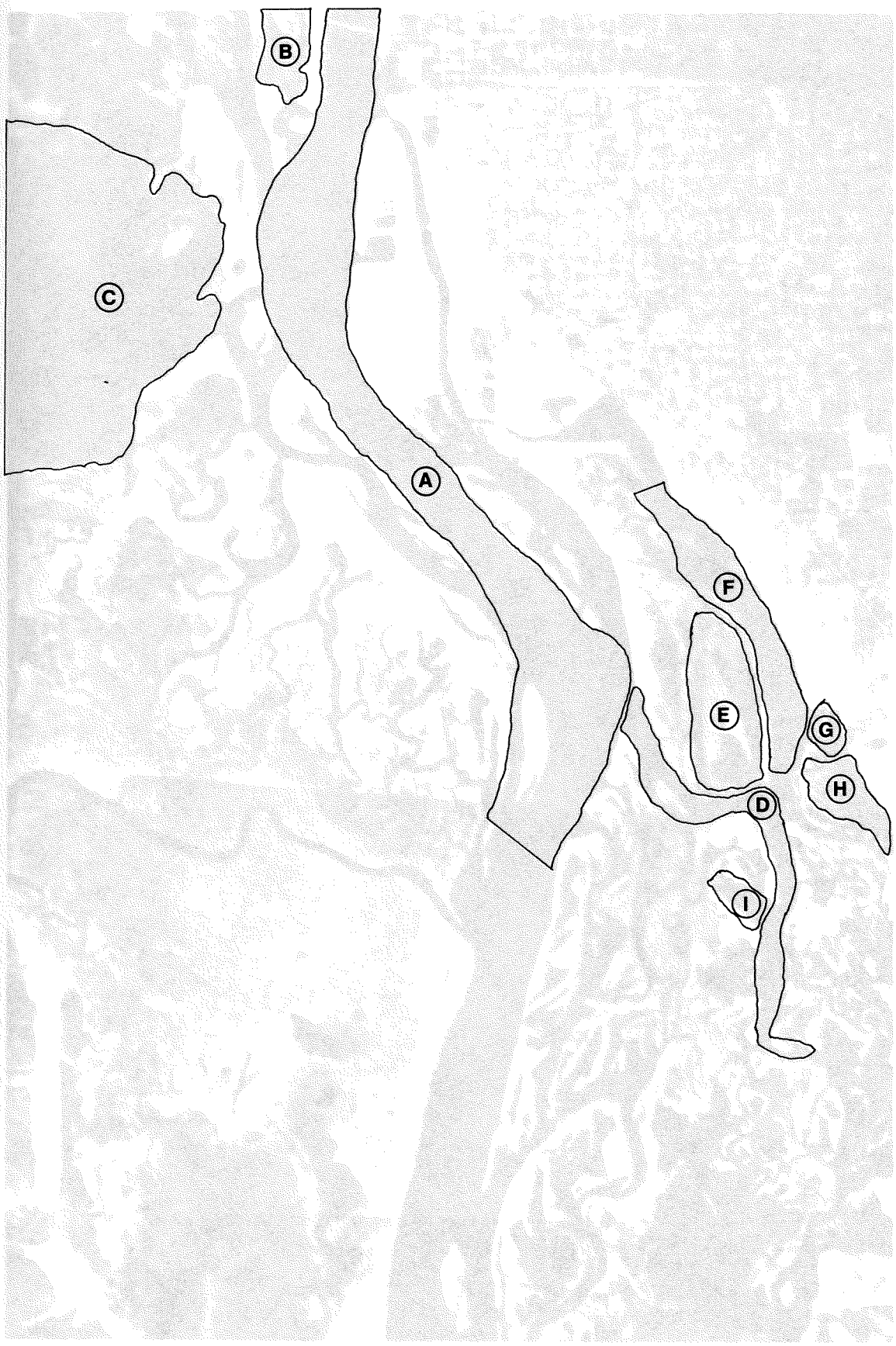
Fig. 2. Aerial photograph of study area indicating 9 sampling areas A through I.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at depths greater than 1 m. A petite Ponar grab dredge (Wildlife Supply Co., Saginaw, MI 48602) was used for acquiring sediment samples. Fecal samples from small fish were collected by dissection and removal of intestines. Large fish were first swabbed for removal of slime from the anal region prior to insertion of a second sterile swab into the anal pore for fecal sampling. Collection of all other specimens was by means of sterile cotton swabs.

Isolation medium and culture maintenance. The isolation medium for all samples was acetamide broth (74, 137). All incubations were at 35 C for 24 to 48 hr unless otherwise stated. Portions approximating 1 ml of water or 1 g of sediment were inoculated into tubes of acetamide broth; swabs were placed directly into the medium. Inoculated acetamide broths were incubated for up to 7 days, and checked daily with ultraviolet light (peak intensity at 366 nm) for fluorescence. Cultures that fluoresced were streaked for isolation onto MacConkey agar (Difco) plates. After incubation, typical MacConkey agar colonies that fluoresced under ultraviolet light (18) were considered presumptive P. aeruginosa, and were picked and transferred to slants of Plate Count agar (Difco) for use as stock cultures. Stock cultures were transferred monthly to fresh Plate Count agar slants, and were stored in a refrigerator (5 C) after incubation.

Culture identification. Cultures exhibiting the following properties were considered confirmed P. aeruginosa: negative gram reaction, characteristic cellular morphology, positive oxidase test (92), growth at 41 C in both Brain Heart Infusion (Difco) and acetamide broths, and motility in SIM medium followed by lack of indole and hydrogen sulfide

production (98, 141). Cultures showing atypical reactions were considered confirmed P. aeruginosa based on fluorescence under ultraviolet light, negative gram reaction, characteristic cellular morphology, positive oxidase test, and growth at 41 C.

Serological typing. Rabbit antiserum to each of the seven Fisher immunotypes (57) were supplied by H. B. Devlin, Parke, Davis & Co., Detroit, Michigan. Each biochemically confirmed P. aeruginosa was grown on freshly poured Mueller-Hinton agar (Difco) plates for 18 hr at 30 C. Growth was removed from each plate with a sterile, cotton-tipped applicator and suspended in 0.5 ml physiological saline to form a dense, homogenous suspension. With a micro-hematocrit capillary tube, nine drops of suspension were placed in 9 different areas on a ruled slide. Micro-hematocrit capillary tubes were next used to add a drop of each antiserum to 7 of the cell suspension drops, a drop of saline was added to the 8th drop, and a drop of normal human serum (serum containing no anti-Pseudomonas antibody--student volunteer, University of Wisconsin-LaCrosse) was added to the remaining drop of cell suspension. Thorough mixing for one minute was followed by observation for agglutination. Cultures that agglutinated in saline control were reported as rough, those that agglutinated in both saline and serum control were reported as auto-agglutinating, and those that showed no agglutination were reported as non-typable.

Pyocin typing. The qualitative pyocin typing method of Zabransky and Day (169), as modified by Edmonds (48) was used, and the 27 pyocin indicator strains of Farmer and Herman (54) were supplied by P. Edmonds, University of Wisconsin-Oshkosh, Oshkosh, Wisconsin. Biochemically

confirmed P. aeruginosa cultures were grown in Tryptic Soy broth (BBL) at 32 C for 4 hr. Then, 3 plates of Tryptic Soy agar (glass Petri plates) containing 0.1% sodium citrate, 0.1% dipotassium hydrogen phosphate, and 10^{-5}M iodoacetic acid (158) were each inoculated with a 4-hr Tryptic Soy broth culture by swabbing a single, wide streak across the center of the medium. Plates were then incubated, right side up, for 12 to 14 hr at 32 C at which time they were inverted, and 1 to 2 ml of chloroform was added to the lid of each plate to kill the test cultures. Cultures were exposed to chloroform vapors for 30 min and residual growth was then removed from the agar surface by using a sterile dry swab. The 3 plates representing each isolate to be tested were next cross-streaked with the 27 indicator strains (9 strains per plate) and incubated at 32 C for 12 to 14 hr. Zones of growth inhibition of the indicator strains by the test organism were recorded as positive (+). Indicator strains showing no inhibition of growth in the vicinity of the test organism were recorded as negative (-).

RESULTS

Biochemical reactions. One hundred sixty three of 316 samples collected exhibited growth in acetamide broth and produced fluorescent colonies on MacConkey agar (Appendix 1). However, 11 (6.8%) of these samples were not capable of growing at 41 C in acetamide and Brain Heart Infusion broths (Appendix 2), and were therefore not confirmed as P. aeruginosa. The remaining 152 P. aeruginosa cultures were all oxidase positive. Atypical reactions were given by a small percentage of the 152 isolates: 8 (5.3%) were non-motile, 13 (8.6%) demonstrated hydrogen sulfide production, and 8 (5.3%) were indole positive. However, all of these 152 isolates (48.1% of 316 samples collected) were confirmed as P. aeruginosa as evidenced by biochemical reactions (Appendix 2).

Serological typing. The distribution of P. aeruginosa according to serological types (Appendix 3) is shown in Table 1. Type 1 accounted for 34.2% of the total isolates, followed by types 4, 2, 7, 3, 5, and 6, in decreasing order of incidence. A total of 15 (9.9%) isolates were non-typable by this method.

Pyocin typing. Figs. 3 and 4 demonstrate inhibition of pyocin indicator strains by a pyocinogenic isolate. In Fig. 3, inhibition of indicator strains 4 and 9 by isolate 96 (Appendix 3) is shown, while Fig. 4 depicts inhibition of indicator strains 3 and 4 by isolate 110 (Appendix 3). The 27 reactions obtained for each culture (Appendix 3) were converted into a 9-digit number (53) according to a mnemonic for type designation (Table 2).

Careful examination of the data presented in Appendix 3 revealed

TABLE 1. Distribution of Pseudomonas aeruginosa according to serological types.

Serological type	No. of samples	% of total isolates
1	52	34.2
2	17	11.2
3	15	9.9
4	25	16.5
5	11	7.2
6	1	0.7
7	16	10.5
non-typable	12	7.9
auto-agglutinating	2	1.3
rough	1	0.7
Total	152	100.0

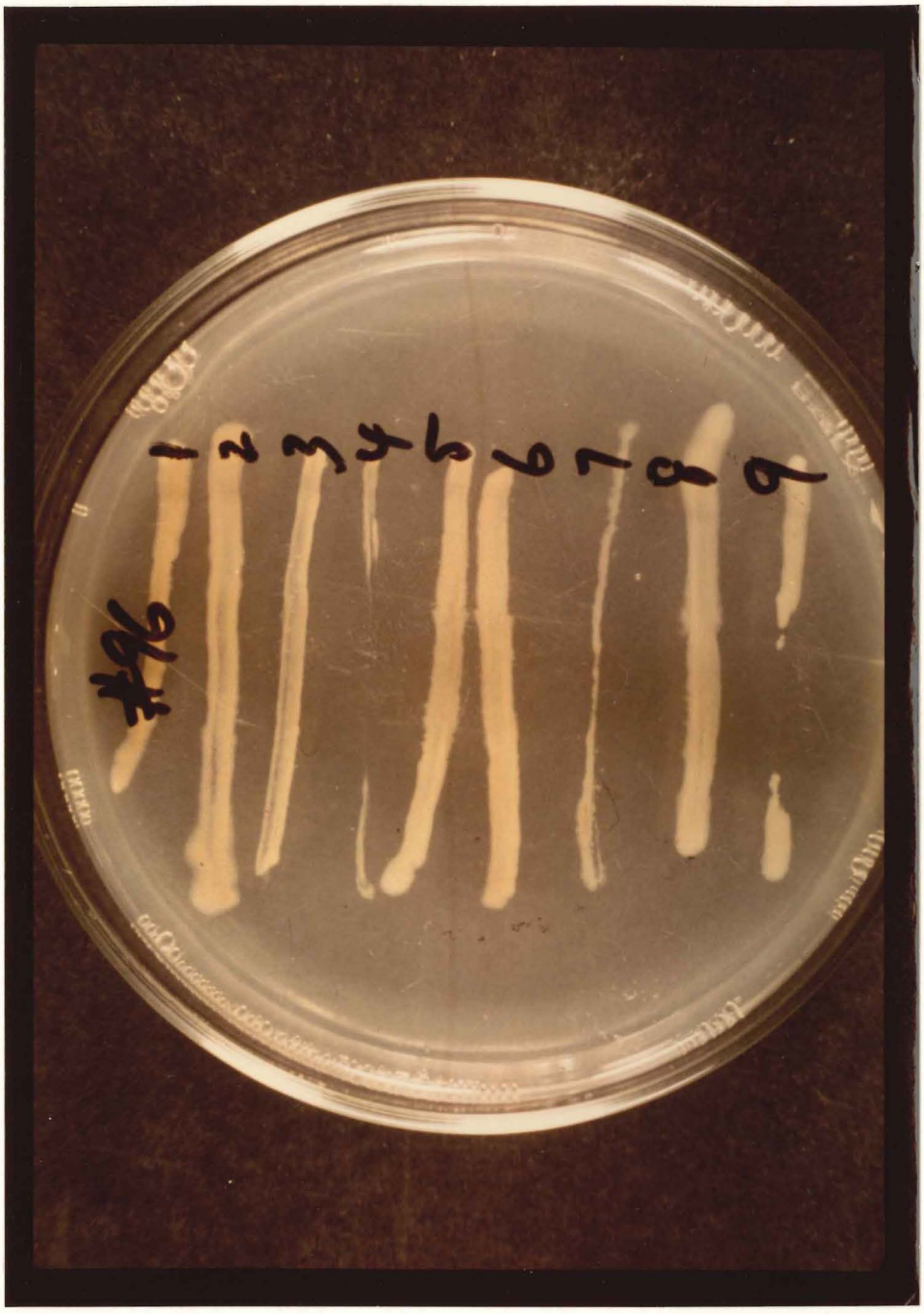

Fig. 3. Inhibition of indicator strains 4 and 9 by isolate 96.

TABLE 2. Mnemonic coding system (53) for reporting pyocin production patterns[a].

Pyocin reaction triplicate	Representation
+++	1
++-	2
+-+	3
-++	4
+--	5
-+-	6
--+	7
---	8

[a] A pyocinogenic test strain with a pyocin production pattern +-- -+- -++ +-+ --- -+- --- --- --- would be coded 564 386 888.

that certain susceptible indicator strains tended to be associated with certain serological types. Specifically, 50.0% of serological type 1 isolates exhibited pyocinogenicity towards indicator strain 2, 65.4% towards indicator strain 20, 42.3% towards both indicator strains 2 and 20, 61.5% towards indicator strain 15, 28.9% towards both indicator strains 15 and 20, and 1.9% towards indicator strain 6. The percentages of serological type 2 that were toxic to indicator strains numbered 7, 26, and 16, were 94.1%, 70.6%, and 5.9%, respectively. Strains 26 (80.0%) and 6 (60.0%) were most susceptible to cultures belonging to serological type 3, while strains 15 (6.7%) and 25 (6.7%) were the least susceptible in this group. Cultures in serological type 4 displayed nearly equal percentages (ca. 24.0%) of inhibition towards all but indicator strains 4 and 6 which were inhibited by 44.0% and 4.0% of type 4 serotypes, respectively. The same phenomenon was noted in serological type 5 cultures, with indicator strains 24, 25, and 27 each showing a 54.6% susceptibility. With serological type 7, indicator strains 2, 4, 2 and 4, 16, 20, 21, and 22 were each inhibited by 37.5% of the serotype, while strain 6 was susceptible to 43.8% of the serotype. In the non-serotypable group, pyocin indicator strain 5 (40.0%) was the most commonly inhibited, with a variety of other strains, including strain 1, having a susceptibility of 6.7%. It should be specifically noted that indicator strain 1 was only inhibited by cultures belonging to the non-serotypable group, and by the only culture belonging to serological type 6.

Distribution. Table 3 shows the distribution of P. aeruginosa according to the type of sample. Sediment samples contained the largest percentage (67.7%) of samples positive for P. aeruginosa. This was

TABLE 3. Sample sources and relative incidence of Pseudomonas aeruginosa.

Source	No. of samples	No. positive for P. aeruginosa	% positive
Water	77	26	33.8
Sediment	31	21	67.7
Tripton	14	8	57.1
Fish	112	52	46.4
Plants	47	22	46.8
Synthetics	10	6	60.0
Metals	9	5	55.6
Misc.[a]	16	12	75.0

[a] This group encompasses samples of oil, rocks, clams, snails, shrimp, water scorpion, water bug, and turtles.

followed, in decreasing order, by synthetic, tripton (dead organisms), metal, plant, fish, and water samples.

The distribution of P. aeruginosa between still and running water is tabulated in Table 4. In lotic waters, the bulk of the organisms (46.7%) originated from water samples taken 1 m below the water surface, while in lentic waters, most (45.0%) were from the surface. Although a higher incidence was noted in sand rather than in mud from both areas (Table 4), a large percentage of mud samples were positive (66.7% and 61.1% in lotic and lentic areas, respectively). Tripton from both areas showed approximately the same percentage of positive samples, while positive samples from synthetics and metals varied greatly. In lotic areas, 80.0% of samples taken from submerged plant parts were positive, with 75.0% positives in lentic areas. In comparison, a less percentage of isolates (35.7% and 41.7% in lotic and lentic areas, respectively) were from plant parts located above the water surface in both areas.

The distribution of fish-borne P. aeruginosa is shown in Table 5. Predator fish demonstrated an 83.9% incidence of the pseudomonad which originated either from the slime layer and/or fecal material, while non-predator fish had a 48.0% incidence of occurrence of P. aeruginosa. A higher percentage of the isolates (51.8%) from both predator and non-predator fish originated from the surface slime, whereas 41.1% was recovered from the fecal material of these fish (Appendix 1). The families Amiidae (bowfin), Centrarchidae (bass, crappie, and bluegill), Esocidae (northern pike), Percichthyidae (white bass), and Clupeidae (gizzard shad), representing both predator and non-predator groups, demonstrated 100% positive samples which originated from the slime and/or fecal material.

TABLE 4. Distribution of Pseudomonas aeruginosa according to current velocity of the sampling area.

SOURCE	[a] LOTIC			[b] LENTIC		
	No. of samples	No. positive	% positive	No. of samples	No. positive	% positive
WATER						
surface	14	3	21.4	20	9	45.0
middle	15	7	46.7	28	7	25.0
SEDIMENT						
mud	9	6	66.7	18	11	61.1
sand	2	2	100.0	2	2	100.0
TRIPTON	5	3	60.0	9	5	55.6
PLANTS						
emergent	14	5	35.7	24	10	41.7
submergent	5	4	80.0	4	3	75.0
SYNTHETICS	1	0	0.0	9	6	66.7
METALS	3	3	100.0	6	2	33.3
MISC.	2	1	50.0	14	11	78.6

[a] Lotic habitats defined as those areas having a current velocity of ≥ 0.1 m/s.

[b] Lentic habitats defined as those areas having a current velocity of < 0.1 m/s.

TABLE 5. Distribution of fish-borne Pseudomonas aeruginosa according to families of fishes.

Type of fish	Family	No. of samples	No. positive for P. aeruginosa	% positive
Predator	Amiidae	2	2	100.0
	Centrarchidae	13	13	100.0
	Esocidae	3	3	100.0
	Hiodontidae	1	0	0.0
	Ictaluridae	2	1	50.0
	Percichthyidae	2	2	100.0
	Percidae	8	5	62.5
	Total	31	26	83.9
Non-predator	Atherinidae	1	0	0.0
	Catostomidae	6	3	50.0
	Clupeidae	3	3	100.0
	Cyprinidae	15	6	40.0
	Total	25	12	48.0

The distribution of P. aeruginosa according to sampling areas A through I (Fig. 2) is shown in Table 6. Many of the areas had an almost equal incidence of occurrence of P. aeruginosa (ca. 51.1%), except areas A (30.2%) and C (28.6%). A smaller percentage of positive samples (40.7%) was obtained from areas with current (A, D, and G) than from those with no current (51.1%). A further breakdown of the data (Table 7) shows the relative incidence of P. aeruginosa in areas A through I in relation to the sample source. Although sample sources in these areas differed in their abundance of P. aeruginosa, each source nevertheless demonstrated a high incidence of the organism in many of the sample areas.

TABLE 6. Distribution of Pseudomonas aeruginosa according to sampling area.

Area	No. of samples	No. positive for P. aeruginosa	% positive
A	43	13	30.2
B	45	25	55.6
C	21	6	28.6
D	31	17	54.8
E	96	51	53.1
F	49	26	53.1
G	17	7	41.2
H	8	4	50.0
I	6	3	50.0

TABLE 7. Sample sources and relative incidence of Pseudomonas aeruginosa according to sampling area.

Area	Source	No. of samples	No. positive for P. aeruginosa	% positive
A	water	14	5	35.7
	fish	21	3	14.3
	plants	2	1	50.0
	synthetics	1	0	0.0
	metals	3	3	100.0
	misc.	2	1	50.0
B	water	8	4	50.0
	sediment	4	3	75.0
	fish	21	9	42.9
	synthetics	5	3	60.0
	metals	1	1	100.0
	misc.	6	5	83.3
C	water	8	3	37.5
	sediment	3	0	0.0
	tripton	1	0	0.0
	plants	9	3	33.3
D	water	12	5	41.7
	sediment	9	6	66.7
	tripton	5	3	60.0
	plants	5	3	60.0

TABLE 7.--(Continued)

Area	Source	No. of samples	No. positive for *P. aeruginosa*	% positive
E	water	14	3	21.4
	sediment	3	2	66.7
	tripton	2	0	0.0
	fish	70	40	57.1
	plants	3	2	66.7
	synthetics	1	1	100.0
	misc.	3	3	100.0
F	water	12	4	25.0
	sediment	6	5	83.3
	tripton	6	5	83.3
	plants	13	6	46.2
	synthetics	3	2	66.7
	metals	4	1	25.0
	misc.	5	3	60.0
G	water	3	0	0.0
	sediment	2	2	100.0
	plants	12	5	41.7
H	water	4	2	50.0
	sediment	2	2	100.0
	plants	1	0	0.0
	metals	1	0	0.0

TABLE 7.--(Continued)

Area	Source	No. of samples	No. positive for *P. aeruginosa*	% positive
I	water	2	0	0.0
	sediment	2	1	50.0
	plants	2	2	100.0

DISCUSSION

The purpose of this study was to investigate the distribution of Pseudomonas aeruginosa in Navigation Pool No. 8 of the Upper Mississippi River. Specifically, the objectives of the study were to determine the abundance of P. aeruginosa in Navigation Pool No. 8, to examine the correlation between incidence of P. aeruginosa isolations and sample source in the Pool, to compare the efficacy of two typing methods, serotyping and pyocin typing, and to determine the abundance and distribution of serological types within the Pool.

The results of this study support those obtained by Brodsky and Nixon (18) and by Smith and Dayton (137), that ultraviolet light is an efficient accessory tool for fast recognition of P. aeruginosa cultures grown in acetamide broth or on MacConkey agar. Only 6.8% of all cultures isolated using this method were not confirmed as P. aeruginosa. However, this method does not recognize non-fluorescent strains of P. aeruginosa. It could therefore be used as an initial means of sorting large numbers of samples into a presumptive P. aeruginosa group and a non-fluorescent group. The non-fluorescent group could then be typed by other available methods. Acetamide broth instead of Brain Heart Infusion broth could be used for growth of the organism at 41 C since identical results were obtained in both media (Appendix 2). This would be advantageous because acetamide broth selects for P. aeruginosa cultures. In SIM medium, 5.3% of the isolates were non-motile. However, non-motile varieties of P. aeruginosa have previously been identified (102). Also in SIM medium, 8 (5.3%) of the isolates were indole positive. Most Pseudomonas species are known for their ability to attack both the aromatic rings and side

chains of the amino acid tryptophan, resulting in a negative indole test. Thus, the data obtained in this study cannot be explained:

P. aeruginosa was isolated from 48.0% of the samples taken, indicating that the organism is abundant in this part of the River. In a study by Stanier et al. (141), it was found that 27.6% of the 29 strains characterized demonstrated no fluorescent pigments. Gaby (61) found that 4% of the strains isolated were non-fluorescent. The possibility therefore exists that P. aeruginosa may be more abundant in the pool than was found in this study, since only the fluorescent strains were selected for and used. Isolates belonging to serological type 1 amounted to 34.2% (Table 1). Predominance of type 1 was followed, in decreasing order, by types 4, 2, 7, 3, 5, and 6. A study in a burns hospital revealed that serotypes 1 and 2 were the most prevalent (49). In other hospitals, Young and Moody (168), Dayton et al. (40), and Kurup and Sheth (93) also found that serotype 1 was the most abundant. The findings of this study cannot be compared to studies that have used classification systems other than the seven Fisher immunotypes (95, 116) used in this study, since the serotypes of other systems would represent different sets of P. aeruginosa. However, a comparison of data from this study and others that have used the same classification system tend to support the hypothesis that the relative incidence of P. aeruginosa serotypes in clinical infections parallels their incidence in the natural environment.

The analysis of results on pyocin typing showed that certain susceptible indicator strains were more commonly found in relation to specific serological types. The significance of this finding is not known at the present time. For comparative purposes, the same difficulty is encountered as with serological typing, i.e., the availability of too

many typing methods, the results of which cannot be compared. Standard procedures for serological and pyocin typing should be implemented, and used by investigators, to facilitate maximum benefits from such results.

Serological and pyocin typing are very efficient tools for epidemiological tracing. In this study, 90.1% of the isolates (Table 1) were differentiated by serological typing (57), whereas 100% were typed by pyocin production against indicator strains (54). Other authors have found one or both of these methods to be reliable tools (15, 48, 93), and feel that a combination of methods should be used to promote reliability of results. Serological typing is a very simple, rapid method of defining Pseudomonas isolates, whereas pyocin typing is a very laborious, time-consuming procedure. For expediency purposes, serological typing could initially be used to differentiate isolates, after which non-typable strains could be pyocin-typed. For strict epidemiological studies, however, two methods should be used since many P. aeruginosa strains are of the same serological type (Appendix 3), and since results obtained may vary according to experimental conditions. Pyocin patterns have been known to be unstable due to variations in environmental conditions, or to acquisition of R-factors (23). In this study, 30 (19.8%) of the strains were randomly picked and retyped; constant pyocin patterns were obtained.

Analysis of the distribution of P. aeruginosa according to sample-types (Table 3) revealed that the largest percentage (67.7%) of positive samples were isolated from sediment, with the smallest percentage (33.8%) coming from water. Table 4 shows that mud and sand samples from both lotic and lentic areas had a high percentage of positives. The nature of the composition of sand, however, explains the finding that all of

the sand samples were positive. Sand particles are fine grains of rock that are constantly moving as a result of movement of the water column above them. This action promotes a more aerobic environment than would be found among stationary mud particles. Also, mud particles, unlike sand, retain available nutrients and result in a greater rate of metabolism, leading to an anaerobic environment. P. aeruginosa is capable of respiring under anaerobic conditions only in the presence of nitrate or nitrite (121). The fact that P. aeruginosa, when introduced as a faecal contaminant, may disappear rapidly from surface waters, has been observed by Hoadley and McCoy (96). Lighthart (100) found that the occurrence of Pseudomonas-like bacteria increased with depth, the greatest number being found in sediment samples. These findings, together with the results of this study, indicate that the sediment may be the reservoir for P. aeruginosa in natural aquatic environments. It should be noted, however, that the other sample types also showed a relatively high incidence of P. aeruginosa (Table 3).

Comparison of number of isolates from surface and middle (1 m below the surface) water (Table 4) showed that middle lotic water and surface lentic water both had a higher incidence of P. aeruginosa than surface lotic and middle lentic water, respectively. These findings could be explained by the physicochemical status of lotic and lentic areas in Pool 8, and by the physiology of P. aeruginosa. In lentic areas, still water would result in a high surface tension; this, together with the buoyancy of water, would cause a retention of organic nutrients on the surface. Also, the non-moving water in lentic areas would promote a greater oxygen content on the surface (17, 125). These factors would explain the greater concentration of organisms in the surface film

rather than the middle water of lentic areas. In lotic areas, however, current would result in an equal concentration of oxygen (35) and organic nutrients throughout the body of water, and lessening of the surface tension. Because of similar conditions (oxygen and nutrients) throughout the body of water, it would be expected that the organism would be equally distributed throughout this column of water. However, the findings of this study indicate that the middle lotic water had a higher concentration of P. aeruginosa than the surface water. A possible explanation for this apparent anomaly might be the phenomenon of negative phototropism. The organisms might have been positioning themselves at this depth to escape photo-oxidation, since no benefit could be gained from being at the surface. It has previously been shown that organisms are capable of exhibiting movement away from unfavorable conditions to optimum surroundings. Water samples were not taken below a depth of 1 m. In some areas (beaches and shallow areas), water depth was less than 1 m, leading to some water samples being taken immediately above the sediment. This is another factor that could explain, in part, the findings of this study. Sediment (containing P. aeruginosa) mixed with water might have led to a greater percentage of P. aeruginosa being isolated from the middle lotic water rather than from the surface water. Also, contamination of middle water by surface water while sampling could have contributed to the data obtained.

Plant parts above the surface of the water had a less incidence of P. aeruginosa than those below (Table 4). This finding can probably be accounted for by the fact that P. aeruginosa is susceptible to drying and has been infrequently recovered from habitats lacking moisture (52, 136).

A greater percentage of the bacteria from both predator and non-predator fish originated from the slime samples rather than from the feces (Appendix 1). Trust and Sparrow (154) isolated a greater number of Pseudomonas from the anterior regions of the alimentary tract of fresh-water salmonid fish than from the posterior portions of the tract. Apparently, existing unfavorable conditions in the posterior portions of the tract might preclude maximum survival of the bacteria. Potter and Baker (122) found that the number of gram-negative organisms per cm^2 of fish surface was high in comparison to the number per ml of water in the lake studied. They proposed the possibility that fishes play a passive role in the distribution of these microorganisms in a body of water. Fish coming in constant contact with material bearing Pseudomonas might result in prolonged colonization of the slime layer of these fish. Fish feeding-habits may influence the distribution of fish-borne P. aeruginosa; a greater number of predator fish were colonized than were non-predators (Table 5). Table 5 shows, furthermore, that many families had a 100% incidence of P. aeruginosa, indicating that some families might have ideal environments for maximum survival of the organisms, and that members of the family might more often come in contact with material bearing Pseudomonas. Also, possible differences in composition of fish slime might account for this finding.

The opportunistic pathogen, P. aeruginosa, was abundant in Pool 8 in both the different sampling areas studied and in the various sample types of these sampling areas (Tables 6 and 7). Many authors feel that the prime reservoir of the organism reaching the environment and waters is the intestinal tract of man (27, 78, 80, 96, 126, 148). Hoadley (78), in a review of the organism and its relation to surface waters, cited

studies in which it was found that sewage was probably the major source of P. aeruginosa appearing in surface waters, with the organism being constantly present in raw sewage in numbers that vary from a few hundred to a few thousand per ml. In a study done in Navigation Pool No. 8 of the Upper Mississippi River, Claflin (35) determined that on the 2nd day after application of sewage sludge to experimental sandy soil plots, the average number of P. aeruginosa recovered was 11 per g. No recovery was noted after the 2nd day. P. aeruginosa was not recovered from water samples taken upstream and downstream from the experimental plots. A median MPN of 2,250 P. aeruginosa per ml was obtained over a period of one year from raw Madison, Wisconsin, sewage samples (78). Numbers of P. aeruginosa could be reduced by over 99% through secondary treatment of sewage. However, it has been demonstrated in the laboratory that growth in sewage occurs under aerobic conditions and numbers of organisms per ml can increase to a few thousand as noted in two trickling filters operated in series. Also, the effectiveness of chlorination of sewage in accomplishing vast reductions of the organism is debatable since many factors influence rate of kill by chlorine (58).

Eye, ear, nose, and throat ailments constitute the majority of illnesses reported among swimmers (78). Ear infections are increasing in incidence among swimmers, with P. aeruginosa being isolated in over 70% of cases of external otitis. Of all healthy ear canals, only 1 to 2% contain Pseudomonas by culture (71). There is therefore a high possibility that a relationship exists between the occurrence of P. aeruginosa in swimming waters and the incidence of outer ear infections among swimmers.

Data obtained in this study provides evidence that a potential

health hazard may exist in Pool 8 of the Upper Mississippi River. The presence of P. aeruginosa in these waters should be regarded as an indication of pollution. Grimes (69) in a study conducted in the vicinity of Crosby Island which is located at river mile 690.0 (12 km south of LaCrosse, Wisconsin), found a significant increase in counts of fecal coliforms after dredging operations in the Mississippi River navigation channel. Membrane filter fecal coliform densities gave a mean value of 44.2, 4 days prior to dredging, mean values of 94.0 and 67.3 on the first and last days of dredging (4-day period) respectively, and a mean value of 60.2 obtained 5 days after dredging. High counts were noted in the immediate area of the dredging, and were attributed to the release of sediment-bound fecal coliforms. In the same way, dredging might release sediment-bound P. aeruginosa, causing a re-distribution of the organism in the water column.

SUMMARY AND CONCLUSIONS

Pseudomonas aeruginosa is a unique, opportunistic pathogen that exhibits extreme biochemical versatility and resistance to antimicrobial drugs. It is therefore a problem organism that is being widely studied. In this investigation, it was of interest to determine the distribution of Pseudomonas aeruginosa in Navigation Pool No. 8 of the Upper Mississippi River, in terms of its abundance, the distribution of serological types, and the correlation between incidence of P. aeruginosa isolations and sample source. Comparison of serotyping and pyocin typing was also under investigation.

Acetamide broth, in conjunction with ultraviolet light, was used as the isolation medium. Confirmed P. aeruginosa cultures were typed using both serological and pyocin typing methods. Serological typing was accomplished with the 7 Fisher immunotypes; the 27 pyocin indicator strains of Farmer and Herman were used for pyocin typing.

Results indicate that Pseudomonas aeruginosa is abundant in this reach of the river, because it was isolated from 48% of the samples taken. Serological type 1 was predominant and it was hypothesized that the relative incidence of P. aeruginosa serotypes in clinical infections parallels their incidence in the natural environment. It was noted that some pyocin types tended to be associated with certain serological types, but the significance of this could not be determined.

P. aeruginosa was isolated most frequently from sediment samples, while the smallest percentage was from water. These findings suggest that sediment may be the reservoir for P. aeruginosa in natural aquatic environments. In lentic waters, the bulk of the organisms were

concentrated in the surface film, whereas in lotic waters, more were isolated at 1 m below the surface. These depth distributions were possibly explained by current velocities, availability of oxygen and organic nutrients, surface tension, and negative phototropism. The bacterium was infrequently isolated from plant parts above the water's surface, and this was explained by its susceptibility to drying. Predator fish were more commonly infected by P. aeruginosa than were non-predator fish, and more of the organisms from fish were isolated from the slime layer than from the feces. Because of the high frequency in which the organism was isolated from Navigation Pool 8, it is possible that a potential health hazard exists for susceptible individuals who come in contact with these waters.

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APPENDIX

APPENDIX 1. Sample numbers and occurrence of P. aeruginosa listed according to area and source.

Sample No.	Occurrence[a]	Area	Source
123	-	F	Water--surface skim
80	+	F	" middle
158	+	F	" middle
161	+	F	" surface skim
81	+	F	" surface skim
183	-	F	" surface skim
19	-	F	" surface skim
178	-	G	" surface skim
103	+	H	" surface skim
177	+	H	" middle
182	-	D	" surface skim
49	-	I	" surface skim
157	-	D	" surface skim
16	+	E	" surface skim
199	-	F	" middle
185	-	F	" middle
186	-	F	" middle
187	-	F	" middle
188	-	F	" middle
189	-	G	" middle
190	-	G	" middle
191	-	H	" middle
192	-	H	" middle
193	-	D	" middle
194	+	D	" middle
195	-	I	" middle
196	-	E	" middle
197	+	E	" middle
198	-	E	" middle
8	+	B	" surface skim

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
18	+	B	Water--surface skim
28	+	B	" surface skim
47	-	B	" surface skim
130	+	C	" surface skim
134	-	C	" surface skim
156	-	C	" surface skim
227	-	A	" surface skim
228	+	A	" surface skim
229	-	A	" surface skim
230	-	A	" surface skim
231	-	A	" surface skim
232	-	A	" surface skim
233	-	A	" surface skim
234	+	D	" surface skim
235	+	D	" surface skim
236	-	D	" surface skim
237	-	D	" surface skim
238	-	E	" surface skim
239	-	E	" surface skim
240	-	E	" surface skim
241	-	E	" surface skim
242	+	E	" surface skim
200	+	B	" middle
201	-	B	" middle
202	-	B	" middle
203	-	B	" middle
204	-	C	" middle
205	+	C	" middle
206	-	C	" middle
207	+	C	" middle
208	-	C	" middle

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
209	-	A	Water--middle
210	+	A	" middle
211	+	A	" middle
212	-	A	" middle
213	+	A	" middle
215	+	A	" middle
214	-	A	" middle
216	+	D	" middle
217	-	D	" middle
218	+	D	" middle
219	-	D	" middle
220	-	E	" middle
221	-	E	" middle
222	-	E	" middle
223	-	E	" middle
224	-	E	" middle
110	+	F	Sediment--mud
54	+	F	" mud
144	+	F	" sand
93	+	F	" sand
148	+	F	" mud
132	-	F	" mud
125	+	G	" mud
85	+	G	" mud
138	+	H	" mud
128	+	H	" mud
139	-	D	" mud
50	+	D	" mud
135	+	D	" mud
146	+	D	" mud
87	-	D	" mud

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
100	+	I	Sediment--mud
143	-	I	" mud
109	+	D	" mud
147	+	E	" mud
83	-	E	" mud
150	+	E	" mud
310	+	B	" mud
311	+	B	" mud
313	-	B	" mud
312	+	B	" mud
321	-	C	" mud
322	-	C	" mud
327	-	C	" mud
334	-	D	" mud
335	+	D	" sand
336	+	D	" sand
142	+	F	Tripton--log
60	+	F	" plant
115	+	F	" coontail
42	+	F	" dock piling (creosol)
113	+	F	" wood
121	+	D	" log
184	+	D	" bird dropping
133	+	D	" floating leaf
155	-	E	" log
159	-	E	" log
226	-	C	" wood
302	-	F	" dock piling
337	-	D	" floating leaf
338	-	D	" bird dropping
243	-	A	Fish--log perch--slime

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
244	-	A	Fish--log perch--feces
245	-	A	" bullhead minnow--slime
246	-	A	" bullhead minnow--feces
247	-	A	" emerald shiner--slime
248	-	A	" emerald shiner--feces
249	-	A	" bullhead minnow--slime
250	-	A	" bullhead minnow--feces
251	-	A	" emerald shiner--slime
252	-	A	" emerald shiner--feces
253	+	A	" cyprinid
254	-	A	" spottail shiner--slime
255	-	A	" spottail shiner--feces
256	-	A	" spottail shiner--slime
257	-	A	" spottail shiner--feces
258	-	A	" river shiner--slime
259	-	A	" river shiner--feces
260	+	A	" sauger--slime
261	+	A	" sauger--feces
262	-	A	" brook silverside--slime
263	-	A	" brook silverside--feces
265	+	B	" perch--slime
266	-	B	" perch--feces
267	-	B	" perch--slime
268	-	B	" perch--feces
269	+	B	" largemouth bass--slime
270	+	B	" largemouth bass--feces
271	+	B	" largemouth bass--slime
272	-	B	" largemouth bass--feces
273	+	B	" black crappie--slime
274	-	B	" black crappie--feces
275	-	B	" black crappie--feces

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
278	+	B	Fish--bluegill--slime
279	-	B	" bluegill--feces
280	-	B	" bluegill--feces
281	+	B	" bluegill--slime
284	+	B	" spottail shiner--slime
285	+	B	" spottail shiner--feces
286	-	B	" silvery minnow--slime
287	-	B	" silvery minnow--feces
288	-	B	" silvery minnow--slime
289	-	B	" silvery minnow--feces
77	+	E	" white crappie--slime
73	-	E	" white crappie--feces
78	+	E	" bluegill--slime
68	+	E	" bluegill--feces
76	+	E	" pumkinseed--slime
74	-	E	" pumkinseed--feces
86	+	E	" white bass--slime
104	-	E	" white bass--feces
79	+	E	" warmouth bass--slime
65	+	E	" warmouth bass--feces
118	-	E	" yellow perch--slime
95	+	E	" yellow perch--feces
94	-	E	" yellow bullhead--slime
69	-	E	" yellow bullhead--feces
120	+	E	" white crappie--slime
89	+	E	" white crappie--feces
99	+	E	" gizzard shad--slime
107	+	E	" gizzard shad--feces
96	+	E	" black crappie--slime
84	-	E	" black crappie--feces
48	-	E	" black crappie--slime

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
15	+	E	Fish--black crappie--feces
56	-	E	" bluegill--slime
55	+	E	" bluegill--feces
52	-	E	" golden shiner--slime
58	+	E	" golden shiner--eggs
67	-	E	" bowfin--slime
66	+	E	" bowfin--feces
75	+	E	" bowfin--slime
59	-	E	" bowfin--feces
119	+	E	" northern pike--slime
117	-	E	" northern pike--feces
106	-	E	" northern pike--slime
98	+	E	" northern pike--feces
57	+	E	" northern pike--slime
90	+	E	" northern pike--feces
39	+	E	" yellow perch--slime
17	+	E	" yellow perch--feces
25	-	E	" spotted sucker--slime
9	+	E	" spotted sucker--feces
71	-	E	" spotted sucker--slime
33	-	E	" spotted sucker--feces
24	+	E	" spotted sucker--slime
4	+	E	" spotted sucker--feces
40	+	E	" yellow bullhead--slime
36	+	E	" yellow bullhead--feces
29	+	E	" golden shiner--slime
22	+	E	" golden shiner--feces
11	+	E	" carp--slime
26	+	E	" carp--feces
27	+	E	" carp--slime
5	+	E	" carp--feces

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
32	+	E	Fish--gizzard shad--slime
35	-	E	" gizzard shad--feces
34	-	E	" walleye--slime
30	-	E	" walleye--feces
2	+	E	" walleye--slime
37	-	E	" walleye--feces
23	-	E	" river carpsucker--slime
20	-	E	" river carpsucker--feces
31	-	E	" white bass--slime
38	+	E	" white bass--feces
13	-	E	" silver redhorse--slime
7	+	E	" silver redhorse--feces
14	-	E	" mooneye--slime
21	-	E	" mooneye--feces
1	-	E	" shorthead redhorse--slime
6	-	E	" shorthead redhorse--feces
3	+	E	" gizzard shad--slime
12	-	E	" gizzard shad--feces
176	+	F	Plant--duckweed
112	+	F	" coontail
124	-	F	" algae
141	-	F	" coontail
153	+	F	" algae
41	+	F	"
114	-	F	" moss
139	+	F	"
70	-	F	"
53	-	F	"
108	-	F	" water lily
61	-	G	"
91	-	G	"

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
126	-	G	Plant--
82	-	G	" pondweed
101	-	G	" pondweed
102	-	G	" Sagittaria latifolia
151	-	G	" water lily
64	+	G	" S. latifolia--stem
136	+	G	" water lily--root
140	+	G	" S. latifolia--root
131	+	G	" Sagittaria
51	+	G	" Sagittaria
160	-	H	" grass
129	+	D	" grapevine
175	-	D	" duckweed
111	+	D	" S. latifolia--root
44	-	D	" S. rigida--root
179	+	I	" algae
63	+	I	" cattail--root
181	+	E	" algae
122	-	E	"
180	+	E	" algae
225	-	C	" algae
301	-	F	" algae
305	+	F	" algae
317	+	C	" lotus--underside
318	+	C	" lotus
319	-	C	" lotus--stem
320	-	C	" lotus--flower
323	-	C	" coontail
324	-	C	" coontail
325	-	C	" water lily
326	+	C	" water lily--underside

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
329	+	A	Plant--algae
330	-	A	" algae
333	+	D	" root
137	+	F	Synthetic--milk carton
97	-	F	" plastic
45	+	F	" fishing bobber
92	+	E	" styrofoam
304	-	B	" rubber
306	-	B	" plastic rope
307	+	B	" rope
308	+	B	" rope
309	+	B	" fiberglass boat
316	-	A	" styrofoam
43	+	F	Metal--cork
152	-	F	" cover
62	-	F	" lead
127	-	H	" wire
303	-	F	" chain
314	+	B	" container
315	+	A	" buoy
331	+	A	" barge
332	+	A	" barge
46	-	F	Misc.--rocks
149	+	F	" clam shells
170	+	F	" oil
154	-	F	" oil
264	+	A	" snail
276	+	B	" shrimp
277	-	B	" water scorpion
282	+	B	" snail
283	+	B	" snail

APPENDIX 1.--(Continued)

Sample No.	Occurrence[a]	Area	Source
290	+	B	Misc.--water bug
291	+	B	" water bug
72	+	E	" turtle
88	+	E	" turtle
105	+	E	" turtle
300	+	F	" oil
328	-	A	" rocks

[a] + indicates presumptive P. aeruginosa isolates in acetamide broth that fluoresced under ultraviolet light.

APPENDIX 2. Morphological and biochemical characteristics of presumptive *P. aeruginosa* isolates.

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H_2S	Indole
2	-	rods, single	+	+	+	+	-	-
3	-	rods, single	+	+	+	+	-	-
4	-	rods, single	+	+	+	+	-	-
5	-	rods, single	+	+	+	+	-	-
7	-	rods, single	+	+	+	+	-	-
8	-	rods, single	+	+	+	+	-	+
9	-	rods, single	+	+	+	+	+	+
11	-	rods, single	+	+	+	-	-	-
15	-	rods, single	+	+	+	+	-	-
16	-	rods, single	+	+	+	+	-	-
17	-	rods, single	+	+	+	-	-	-
18	-	rods, single	+	+	+	+	-	-
22	-	rods, single	+	+	+	+	-	-
24	-	rods, single	+	+	+	+	-	-
26	-	rods, single	+	+	+	+	+	+
27	-	rods, single	+	+	+	+	-	-
28	-	rods, single	+	+	+	+	-	-
29	-	rods, single	+	+	+	+	-	-
32	-	rods, single	+	+	+	+	-	-
36	-	rods, single	+	+	+	-	-	-
38	-	rods, single	+	+	+	+	-	-
39	-	rods, single	+	+	+	+	-	-
40	-	rods, single	+	+	+	+	-	-
41	-	rods, single	+	+	+	+	-	-
42	-	rods, single	+	+	+	+	-	-
43	-	rods, single	+	+	+	+	-	-
45	-	rods, single	+	+	+	+	-	-
50	-	rods, single	+	+	+	+	-	-

APPENDIX 2.--(Continued)

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H_2S	Indole
51	-	rods, single	+	+	+	+	-	-
54	-	rods, single	+	+	+	+	-	-
55	-	rods, single	+	+	+	+	-	-
56	-	rods, single	+	-	-	+	-	-
57	-	rods, single	+	+	+	-	-	-
58	-	rods, single	+	+	+	+	-	-
59	-	rods, single	-	-	-	+	-	-
60	-	rods, single	+	+	+	+	-	-
63	-	rods, single	+	+	+	+	-	-
64	-	rods, single	+	+	+	+	-	-
65	-	rods, single	+	+	+	+	-	-
66	-	rods, single	+	+	+	+	+	-
68	-	rods, single	+	+	+	+	-	-
70	-	rods, single	+	-	-	+	-	-
72	-	rods, single	+	+	+	+	-	-
74	-	rods, single	+	-	-	+	-	-
75	-	rods, single	+	+	+	+	-	-
76	-	rods, single	+	+	+	+	-	-
77	-	rods, single	+	+	+	+	-	-
78	-	rods, single	+	+	+	+	-	-
79	-	rods, single	+	+	+	+	-	-
80	-	rods, single	+	+	+	+	-	-
81	-	rods, single	+	+	+	+	-	-
85	-	rods, single	+	+	+	+	-	-
86	-	rods, single	+	+	+	+	-	-
88	-	rods, single	+	+	+	+	-	-
89	-	rods, single	+	+	+	-	-	-
90	-	rods, single	+	+	+	+	-	-
92	-	rods, single	+	+	+	+	-	-

APPENDIX 2.--(Continued)

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H_2S	Indole
93	-	rods, single	+	+	+	+	+	+
95	-	rods, single	+	+	+	-	-	-
96	-	rods, single	+	+	+	+	-	-
98	-	rods, single	+	+	+	+	-	-
99	-	rods, single	+	+	+	+	-	-
100	-	rods, single	+	+	+	+	-	-
103	-	rods, single	+	+	+	+	-	-
105	-	rods, single	+	+	+	+	-	-
107	-	rods, single	+	+	+	+	-	-
109	-	rods, single	+	+	+	+	-	-
110	-	rods, single	+	+	+	+	-	-
111	-	rods, single	+	+	+	+	-	-
112	-	rods, single	+	+	+	+	-	-
113	-	rods, single	+	+	+	-	-	-
115	-	rods, single	+	+	+	-	-	-
118	-	rods, single	+	-	-	+	+	+
119	-	rods, single	+	+	+	+	+	+
120	-	rods, single	+	+	+	+	+	+
121	-	rods, single	+	+	+	+	+	+
123	-	rods, single	+	-	-	+	+	+
124	-	rods, single	+	-	-	+	+	+
125	-	rods, single	+	+	+	+	-	-
127	-	rods, single	-	-	-	+	+	+
128	-	rods, single	+	+	+	+	-	-
129	-	rods, single	+	+	+	+	-	-
130	-	rods, single	+	+	+	+	-	-
131	-	rods, single	+	+	+	+	-	-
133	-	rods, single	+	+	+	+	-	-
135	-	rods, single	+	+	+	+	-	-

APPENDIX 2.--(Continued)

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H_2S	Indole
136	-	rods, single	+	+	+	+	-	-
137	-	rods, single	+	+	+	+	-	-
138	-	rods, single	+	+	+	+	-	-
139	-	rods, single	+	+	+	+	-	-
140	-	rods, single	+	+	+	+	-	-
142	-	rods, single	+	+	+	+	-	-
143	-	rods, single	-	-	-	+	+	+
144	-	rods, single	+	+	+	+	-	-
146	-	rods, single	+	+	+	+	-	-
147	-	rods, single	+	+	+	+	-	-
148	-	rods, single	+	+	+	+	-	-
149	-	rods, single	+	+	+	+	-	-
150	-	rods, single	+	+	+	+	-	-
153	-	rods, single	+	+	+	+	-	-
158	-	rods, single	+	+	+	+	+	-
161	-	rods, single	+	+	+	+	+	+
170	-	rods, single	+	+	+	+	-	-
176	-	rods, single	+	+	+	+	-	-
177	-	rods, single	+	+	+	+	-	-
179	-	rods, single	+	+	+	+	-	-
180	-	rods, single	+	+	+	+	-	-
181	-	rods, single	+	+	+	+	-	-
184	-	rods, single	+	+	+	+	-	-
194	-	rods, single	+	+	+	+	-	-
197	-	rods, single	+	+	+	+	+	-
200	-	rods, single	+	+	+	+	-	-
205	-	rods, single	+	+	+	+	-	-
207	-	rods, single	+	+	+	+	-	-
210	-	rods, single	+	+	+	+	-	-

APPENDIX 2.--(Continued)

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H_2S	Indole
211	-	rods, single	+	+	+	+	-	-
213	-	rods, single	+	+	+	+	+	-
215	-	rods, single	+	+	+	+	-	-
216	-	rods, single	+	+	+	+	-	-
218	-	rods, single	+	+	+	+	-	-
228	-	rods, single	+	+	+	+	-	-
234	-	rods, single	+	+	+	+	-	-
235	-	rods, single	+	+	+	+	-	-
242	-	rods, single	+	+	+	+	-	-
246	-	rods, single	-	-	-	+	+	+
253	-	rods, single	+	+	+	+	-	-
260	-	rods, single	+	+	+	+	-	-
261	-	rods, single	+	+	+	+	-	-
264	-	rods, single	+	+	+	+	-	-
265	-	rods, single	+	+	+	+	-	-
269	-	rods, single	+	+	+	+	-	-
270	-	rods, single	+	+	+	+	-	-
271	-	rods, single	+	+	+	+	-	-
273	-	rods, single	+	+	+	+	-	-
276	-	rods, single	+	+	+	+	-	-
277	-	rods, single	+	-	-	+	+	+
278	-	rods, single	+	+	+	+	-	-
281	-	rods, single	+	+	+	+	-	-
282	-	rods, single	+	+	+	+	-	-
283	-	rods, single	+	+	+	+	-	-
284	-	rods, single	+	+	+	+	-	-
285	-	rods, single	+	+	+	+	-	-
290	-	rods, single	+	+	+	+	-	-
291	-	rods, single	+	+	+	+	+	-

APPENDIX 2.--(Continued)

Sample No.	Gram Reaction	Morphology	Oxidase	Growth at 41 C in		SIM medium		
				Acetamide Broth	BHI Broth	Motility	H S 2	Indole
300	-	rods, single	+	+	+	+	-	-
305	-	rods, single	+	+	+	+	-	-
307	-	rods, single	+	+	+	+	-	-
308	-	rods, single	+	+	+	+	-	-
309	-	rods, single	+	+	+	+	-	-
310	-	rods, single	+	+	+	+	-	-
311	-	rods, single	+	+	+	+	-	-
312	-	rods, single	+	+	+	+	-	-
314	-	rods, single	+	+	+	+	-	-
315	-	rods, single	+	+	+	+	-	-
317	-	rods, single	+	+	+	+	-	-
318	-	rods, single	+	+	+	+	-	-
326	-	rods, single	+	+	+	+	-	-
329	-	rods, single	+	+	+	+	-	-
331	-	rods, single	+	+	+	+	+	-
332	-	rods, single	+	+	+	+	-	-
333	-	rods, single	+	+	+	+	-	-
335	-	rods, single	+	+	+	+	-	-
336	-	rods, single	+	+	+	+	-	-

APPENDIX 3. Pyocin and Serological typing patterns of biochemically confirmed P. aeruginosa isolates.

Sample No.	Pyocin Typing		Serological Type
	Susceptible Indicator Strains	Pyocin Type (mnemonic)	
2	4, 15	858 878 888	auto-agglutinating
3	2, 20	688 888 688	1
4	12, 14, 15, 20, 27	888 748 687	1
5	2, 20	688 888 688	1
7	2, 7, 15, 20, 24, 27	685 878 677	1
8	18, 25, 27	888 887 883	nontypable
9	4, 15	858 878 888	auto-agglutinating
11	1, 5, 20, 27	568 888 687	rough
15	2, 9, 23	687 888 868	1
16	3, 5, 10, 11, 16	768 285 888	4
17	5, 7, 24	865 888 878	5
18	18, 21, 27	888 887 787	4
22	2, 20, 27	688 888 687	1
24	4, 15, 16	858 875 888	4
26	2, 20	688 888 688	1
27	9	887 888 888	nontypable
28	2, 5	668 888 888	nontypable
29	9	887 888 888	nontypable
32	2, 20	688 888 688	1
36	15, 20, 27	888 878 687	1
38	2, 15, 24, 27	688 878 877	1
39	6, 24	878 888 878	3
40	2, 5	668 888 888	nontypable
41	2, 5	668 888 888	nontypable
42	9	887 888 888	nontypable
43	2, 17, 18, 19	688 884 588	nontypable
45	2, 4, 9, 20, 22	657 888 658	7
50	6, 7, 10-18, 27	875 111 887	4
51	9, 15, 21	887 878 788	1

APPENDIX 3.--(Continued)

Sample No.	Pyocin Typing		Serological Type
	Susceptible Indicator Strains	Pyocin Type (mnemonic)	
100	2, 20	688 888 688	1
103	7, 26	885 888 886	2
105	21, 22, 26	888 888 756	4
107	2, 4, 5, 6	618 888 888	7
109	8, 26	886 888 886	3
110	3, 4	758 888 888	4
111	4, 9, 15, 20	857 878 688	1
112	3	788 888 888	nontypable
113	2, 4, 9, 19	657 888 588	7
115	2, 20	688 888 688	1
119	5, 6, 9	847 888 888	7
120	14, 16, 17	888 862 888	4
121	1, 2, 9, 11, 12	287 488 888	6
125	6, 8, 23, 26	876 888 866	3
128	2, 15, 20	688 878 688	1
129	5, 24, 25, 26	868 888 872	5
130	7, 10, 26	885 588 886	2
131	7, 10, 26	885 588 886	2
133	7, 10, 26	885 588 886	2
135	7, 10, 26	885 588 886	2
136	2, 4, 9, 19	657 888 588	7
137	7, 26	885 888 886	2
138	24, 25, 27	888 888 873	5
139	2, 15, 20	688 878 688	1
140	6, 19, 21, 22	878 888 358	7
142	5, 19, 21	868 888 388	7
144	6, 16, 20, 22	878 885 658	7
146	15, 20, 27	888 878 687	1
147	2, 20	688 888 688	1

APPENDIX 3.--(Continued)

Sample No.	Pyocin Typing		Serological Type
	Susceptible Indicator Strains	Pyocin Type (mnemonic)	
148	4, 9, 15	857 878 888	1
149	2, 20	688 888 688	1
150	4, 9, 15	857 878 888	1
153	7, 15, 26	885 878 886	2
158	19, 21, 22	888 888 358	7
161	4, 9, 15	857 878 888	1
170	3, 4, 16, 18	758 883 888	4
176	2, 20	688 888 688	1
177	3, 7, 26	785 888 886	2
179	7	885 888 888	2
180	4, 9, 15, 20	857 878 688	1
181	2, 15, 20	688 878 688	1
184	17, 23, 26	888 886 866	3
194	2, 9, 23	687 888 868	1
197	2, 4, 5, 6, 9	617 888 888	7
200	2, 15, 20	688 878 688	1
205	6, 16, 20, 22	878 885 658	7
207	4, 9, 15, 20	857 878 688	1
210	3, 7, 15	785 878 888	2
211	18, 21, 26, 27	888 887 784	4
213	6, 17, 26	878 886 886	3
215	5, 19, 21	868 888 388	7
216	6, 8	876 888 888	3
218	4, 9, 15	857 878 888	1
228	6, 17, 26	878 886 886	3
234	2, 20, 27	688 888 687	1
235	6, 26	878 888 886	3
242	10, 13, 17	888 556 888	4
253	2, 15, 20	688 878 688	1

APPENDIX 3.--(Continued)

Sample No.	Pyocin Typing		Serological Type
	Susceptible Indicator Strains	Pyocin Type (mnemonic)	
260	4, 9, 15	688 878 688	1
261	17, 26	888 886 886	3
264	7	885 888 888	2
265	12, 14, 20	888 768 688	1
269	3, 4, 16, 18	758 883 888	4
270	8, 15, 16, 17	886 872 888	4
271	27	888 888 887	5
273	3, 9, 15, 27	787 878 887	4
276	7, 15, 27	885 878 887	5
278	12, 14, 20	888 768 688	1
281	4, 7, 23	855 888 868	4
282	3, 4, 5, 9, 14, 15	727 848 888	4
283	2, 7, 15, 20	685 878 688	1
284	3, 4, 16, 18	758 883 888	4
285	7, 16, 26	885 885 886	2
290	17, 23, 26	888 886 866	3
291	25, 26, 27	888 888 881	3
300	4, 9, 15	857 878 888	1
305	5	868 888 888	nontypable
307	9, 20, 27	887 888 687	1
308	10, 18, 27	888 587 887	4
309	2, 5, 20	668 888 688	1
310	23, 26, 27	888 888 864	4
311	2, 5	668 888 888	nontypable
312	5, 24, 25	868 888 875	5
314	25, 26, 27	888 888 881	5
315	5, 11, 21, 27	868 688 787	4
317	4, 7, 15, 16	855 875 888	4
318	15, 20	888 878 688	1

APPENDIX 3.--(Continued)

Sample No.	Pyocin Typing		Serological Type
	Susceptible Indicator Strains	Pyocin Type (mnemonic)	
326	16, 20, 21	888 885 488	7
329	26	888 888 886	2
331	4, 7, 16, 17	855 882 888	4
332	7, 26	885 888 886	2
333	10, 18, 27	888 587 887	4
335	14, 15, 21, 27	888 848 787	1
336	7, 10, 20	885 588 688	1