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Antibiograms, Serotypes, and Plasmid Profiles of *Pseudomonas aeruginosa* Associated with Corneal Ulcers and Contact Lens Wear

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***Pseudomonas aeruginosa* was isolated from the corneal scrapings of 11 of 14 patients with gram-negative corneal ulcers and from salt tablet-prepared saline solutions from 6 of these patients wearing soft contact lenses. Comparison of physiological properties, antibiograms, serotypes, and plasmid profiles for five of the patients indicated that the isolates from the ulcer and the saline solution of a given patient were of the same strain. Improper hygienic practices of contact lens wearers appeared to be a major factor in the epidemiology of pseudomonad corneal ulcers.**

Species of *Pseudomonas* are among the most virulent microorganisms associated with eye area infection (15). Investigations have demonstrated that pseudomonads are not a constituent of the normal eye flora but rather are transient opportunists that often enter the outer eye from contaminated ocular products (32, 33) or environmental sources (18). The presence of *Pseudomonas* spp. in the eye does not necessarily result in infection. Evidence indicates that the corneal surface must be impaired either by mechanical or chemical trauma, effects that could result from wearing contact lenses (13). Because *Pseudomonas aeruginosa* is ubiquitous in the environment, the immediate source of a *Pseudomonas* strain isolated from an eye infection is difficult to determine.

Numerous procedures, including the determination of general physiological properties, bacteriophage typing, pyocin typing, antibiograms, and serotyping, were used in epidemiological studies of *P. aeruginosa* (6, 10). Serotyping is recognized generally as one of the more reliable typing systems (5, 6, 19, 34); the Fisher scheme (F) and the International Antigenic Typing Scheme (IATS) are broadly used in the United States. Fisher et al. (12) developed an immunotyping scheme based on challenge protection in mice that yielded seven serotypes for *P. aeruginosa*. Brokopp et al. (6) used a standardized, commercially available system (Difco Laboratories, Detroit, Mich.) that was based on the O-antigen scheme (IATS) adopted by the Subcommittee on *Pseudomonadaceae* and Related Organisms of the International Committee of Systematic Bacteriology of the International Association of Microbiological Societies. Brokopp et al. (6) typed 96.3% of 725 isolates into the 17 O-antigen groups by this slide agglutination procedure. Brokopp and Farmer (5) compared the IATS and F systems and indicated the following serotype equivalents: F1 = IATS 6, F2 = IATS 11, F3 = IATS 2, F4 = IATS 1, F5 = IATS 10, F6 = IATS 8, and F7 = IATS 5.

The incidence of serotypes of *P. aeruginosa* (converted to IATS) from various sources is given in Table 1. Serotypes O:6 and O:11 are the most frequently reported, whereas serotypes O:12 to O:17 are considered quite rare (5, 20). In their review of *P. aeruginosa* associated with whirlpool baths, Ratnam et al. (26) reported that serotype O:11 is most common. Paired antigens may occur in some strains; for example, isolates that agglutinate in both O:7 and O:8 IATS antisera were reported (6, 31). In several studies, serotypes and antibiograms have been examined concurrently (9, 10, 20, 27). In general, antimicrobial agent susceptibility varied within a serotype and hospital isolates exhibited greater resistance to antimicrobial agents than did isolates from the environment.

TABLE 1. Incidence of serotypes of *P. aeruginosa* from various sources

Source of specimens	Reference	No. of isolates	Geographic location	Most common serotype (% of total isolates)
Clinical				
General	12	342	United States	O:6, O:11, O:2[a] (22, 18, 20)
	6	674	Md., Ala., N.C., Ill.; Brazil	O:6 (27)
	19	98	N.Y.	O:6, O:11 (25, 14)
	20	98	Athens, Greece	O:6, O:11 (29, 29)
	11	12	United States	O:11 (50)
Burns	10	36	Ohio	O:6[a] (50)
	9	202	Tex.	O:6[a] (38)
Cancer ward	34	467	Md.	O:6[a] (36)
Cystic fibrosis	27	21	Okla.	O:8, O:9 (35, 35)
Bacteremic	25	281	Miss., D.C.	O:6 (28)
Nonbacteremic	25	124	Calif.	O:6, O:11 (21, 21)
Eyes	32	6	Ga.	O:11 (57)
Drug users	28	25	Ill.	O:11 (92)
	4	42	Ill.	O:11 (76)
Water				
Whirlpools	26	2	United States	O:11 (100)
	30	4	Vt.	O:1 (100)
Mississippi River	24	152	Miss.	O:6[a] (34)

[a] Converted from Fisher to IATS.

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TABLE 2. Sources, serotypes, plasmid numbers, and selected biochemical tests of *P. aeruginosa* isolates

Isolate code[a]	Source[b]	IATS serotype	No. of plasmids	ONPG[c]	Gelatinase	N_2 gas	Pyorubin
A	Corneal ulcer	O:6	2	−	+	+	−
A-1	Saline squeeze bottle	O:6	2	−	+	+	−
B	Corneal ulcer	O:11	0	+	+	−	−
B-1	Lens case with saline	O:11	1	+	−	−	−
C	Corneal ulcer	O:6	1	−	+	−	−
C-1	Saline	O:6	1	−	+	−	−
D	Corneal ulcer	O:7,8	0	−	+	−	−
D-1	Saline	O:7,8	0	−	+	−	−
E	Corneal ulcer	O:11	0	−	+	−	+
E-1	Saline	O:11	0	−	+	−	+
F	Corneal ulcer	O:11	0	−	+	−	−
F-1	Lens case with saline	O:11	0	−	+	−	−
G	Corneal ulcer (large colony)	O:11	1	−	+	−	+
G-1	Corneal ulcer (small colony)	O:11	1	−	+	−	+
H	Corneal ulcer	O:5	0	−	−	+	−
I	Corneal ulcer	O:8	2	−	+	−	−
J	Lens case[d]	O:11	3	+	+	−	−
K	Gel cleaner[d]	O:6	5	−	+	+	−
L	Corneal ulcer	O:6	1	−	+	−	+
M	Corneal ulcer	O:6	0	−	+	−	−
O	ATCC 9027, human ear	O:1	2	−	−	+	−
P	ATCC 9027 C, human ear	O:1	1	−	+	−	−
Q	ATCC 15442, water, animal room	O:1	2	−	+	−	−
R	Oil-in-water emulsion[d]	O:15	3	−	+	−	−

[a] Isolates with the same letter code were from one individual.
[b] Saline, Tablet-prepared saline solution.
[c] ONPG, *o*-Nitrophenyl-β-D-galactopyranoside.
[d] Preserved products.

Recently, plasmid fingerprinting has been used for demonstrating the similarity of clinical and environmental isolates of bacteria in epidemiological studies. Isolates from the same strain contain the same number of plasmids with the same molecular weights and generally the same phenotype. Strains from different sources have different numbers of plasmids with different molecular weights. Plasmid fingerprinting has proved more accurate than other methods for indicating similar origins of isolates (29). Candal and Eagon (7) examined the plasmid profiles of *P. aeruginosa* found growing in industrial biocides. Plasmids were detected in all of the biocide-resistant isolates. The plasmid profiles of *P. aeruginosa* isolates from poloxamer-iodine solutions were identical to those of isolates from two of the three patients who developed peritonitis while on dialysis with systems disinfected with an iodine solution (23).

Information on plasmid profiles, serotypes, and antibiograms for *P. aeruginosa* associated with eye infections is not available. The incidence of pseudomonad infections noted among soft contact lens users supports the need for epidemiological investigations. This study compares the general physiological properties, antibiograms, serotypes, and plasmid profiles of *P. aeruginosa* from pseudomonad corneal ulcers and accessory contact lens products.

MATERIALS AND METHODS

Cultures. Corneal scrapings from patients with corneal ulcers were plated on chocolate agar and incubated at 37°C. When possible, eye area products in use at the time of infection were collected from the patients and selected materials were serially diluted and inoculated onto sheep blood agar and nutrient agar (BBL Microbiology Systems, Cockeysville, Md.). Dey Engly agar (Difco) was used for isolation with certain products containing preservatives. Primary isolates from corneal scrapings and samples of various ocular products were streaked on nutrient agar plates. Cultures of all gram-negative rods were selected for further study. Specimens from 11 of the 14 patients yielded pseudomonads. These isolates were studied in detail. The other specimens yielded *Serratia marcescens* and *Klebsiella oxytoca*. All isolates were lyophilized, and working cultures were maintained on nutrient agar slants.

Characterization of *Pseudomonas* isolates. All isolates were characterized by their Gram stain reaction and by biochemical reactions on the API 20E System (Analytab Products, Plainview, N.Y.), the Oxi-Ferm System (Roche Diagnostics, Div. Hoffman-La Roche Inc., Nutley, N.J.), the NF1 Trio-Tubes (Curtin Matheson Scientific, Inc., Houston, Tex.), and the AMS and Antimicrobial GNS Rapid systems (Vitek Systems, Inc., Hazelwood, Mo.). Growth was evaluated on Pseudosel and MacConkey agar (BBL) and on sheep blood agar at 42°C. Results from the rapid systems were confirmed by conventional tests for motility, oxidase activity, arginine dihydrolase, and urease (21). Two isolates obtained from the American Type Culture Collection were used as controls (ATCC 9027 and ATCC 15442). A second culture of ATCC 9027 secured through another laboratory at Georgia State University was also studied. Flagellar arrangement was determined for selected isolates.

Antimicrobial agent susceptibility testing. Antimicrobial agent susceptibilities were determined by the procedures of Bauer et al. (2) with the interpretive standards of Barry and Thornsberry (1). Antimicrobial discs (Difco) used were carbenicillin (100 μg), gentamicin (10 μg), tobramycin (10 μg), triple sulfa (300 μg), neomycin (5 μg), cephalothin (30 μg), mezlocillin (75 μg), tetracycline (30 μg), ampicillin (10 μg), streptomycin (10 μg), polymyxin B (300 U), cefamandole (30 μg), and amikacin (30 μg). Antibiograms were also determined with the Vitek system.

TABLE 3. Antibiotic susceptibility patterns of *P. aeruginosa*

Isolate code[a]	Susceptibility (zone of inhibition [mm]) to[b]:												
	AN	AM	CB	MA	CR	GM	MZ	N	PB	S	TE	TM	SSS
A	S(18)	R(6)	S(24)	R(6)	R(6)	R(12)	S(24)	R(6)	S(16)	R(7)	R(12)	S(22)	I(16)
A-1	S(19)	R(6)	S(21)	R(6)	R(6)	I(13)	R(6)	R(6)	I(11)	R(7)	R(8)	S(17)	R(6)
B	S(19)	R(6)	S(19)	R(6)	R(6)	S(15)	S(20)	R(9)	S(14)	R(8)	R(10)	S(20)	S(18)
B-1	S(21)	R(6)	I(14)	R(6)	R(6)	S(17)	S(20)	R(9)	S(14)	R(9)	R(10)	S(21)	S(17)
C	S(20)	R(6)	S(20)	R(6)	R(6)	S(16)	S(20)	R(8)	S(13)	R(8)	R(12)	S(20)	I(15)
C-1	S(21)	R(6)	S(22)	R(6)	R(6)	S(18)	S(22)	R(10)	S(14)	R(10)	R(13)	S(22)	S(20)
D	S(18)	R(6)	S(22)	R(6)	R(6)	S(16)	S(17)	R(6)	S(13)	R(7)	R(6)	S(18)	R(11)
D-1	S(18)	R(6)	S(22)	R(6)	R(6)	S(18)	S(17)	R(6)	S(13)	R(9)	R(8)	S(19)	I(13)
E	S(20)	R(6)	S(19)	R(6)	R(6)	I(14)	S(18)	R(7)	S(13)	R(6)	R(10)	S(20)	R(12)
E-1	S(18)	R(6)	S(18)	R(6)	R(6)	I(14)	S(18)	R(7)	S(13)	R(6)	R(9)	S(20)	R(11)
F	S(17)	R(6)	S(19)	R(6)	R(6)	R(12)	S(21)	R(6)	S(14)	R(6)	R(12)	S(19)	S(19)
F-1	S(18)	R(6)	S(21)	R(6)	R(6)	I(13)	S(21)	R(6)	S(12)	R(6)	R(12)	S(18)	S(18)
G	S(20)	R(6)	S(20)	R(6)	R(6)	S(16)	S(18)	R(6)	S(13)	R(9)	R(10)	S(21)	S(16)
G-1	R(9)	R(6)	S(22)	R(6)	R(6)	R(10)	S(25)	R(6)	S(18)	R(6)	R(8)	S(15)	R(6)
H	S(19)	R(6)	S(20)	R(6)	R(6)	S(16)	S(22)	R(7)	S(13)	R(7)	R(11)	S(20)	R(12)
I	S(20)	R(6)	S(22)	R(6)	R(6)	R(12)	S(18)	R(7)	S(13)	R(9)	R(10)	S(21)	S(18)
J	S(19)	R(6)	S(19)	R(6)	R(6)	S(16)	S(20)	R(8)	S(14)	R(8)	R(6)	S(21)	S(17)
K	S(20)	R(6)	S(20)	R(6)	R(6)	S(18)	S(17)	R(7)	S(13)	R(9)	R(11)	S(22)	R(12)
L	S(19)	R(6)	S(19)	R(6)	R(6)	I(14)	S(19)	R(6)	S(14)	R(6)	R(11)	S(19)	I(13)
M	S(23)	R(6)	S(22)	R(6)	R(6)	S(17)	S(21)	R(8)	S(14)	S(17)	R(12)	S(22)	R(6)
O	S(18)	R(6)	R(11)	R(6)	R(6)	R(10)	I(13)	R(6)	S(13)	R(7)	R(6)	S(20)	I(13)
P	S(18)	R(6)	S(22)	R(6)	R(6)	S(15)	S(21)	R(8)	S(13)	R(8)	R(7)	S(20)	R(12)
Q	S(18)	R(6)	S(18)	R(6)	R(6)	I(14)	R(11)	R(6)	S(12)	R(6)	R(6)	S(20)	R(6)
R	S(19)	R(6)	S(20)	R(6)	R(6)	I(13)	S(17)	R(6)	S(13)	R(8)	R(10)	S(20)	R(6)

[a] Isolates with the same letter code were from one individual.

[b] Drugs: AN, amikacin; AM, ampicillin; CB, carbenicillin; MA, cefamandole; CR, cephalothin; GM, gentamicin; MZ, mezlocillin; N, neomycin; PB, polymyxin B; S, streptomycin; TE, tetracycline; TM, tobramycin; SSS, triple sulfa. Susceptibilities: S, susceptible; I, intermediate; R, resistant.

Serotyping and plasmids. Isolates were serotyped by slide agglutination with the *P. aeruginosa* Antiserum Set (Difco) as recommended by Brokopp et al. (6). Plasmid profiles were obtained by the procedures of Birnboim and Doly (3) and Cook et al. (8).

Case histories. Six patients (A to F), four myopic females and one myopic and one aphakic male, examined between July 1979 and October 1985 yielded isolates of *P. aeruginosa* from scrapings of their corneal ulcers and from tablet-prepared saline solutions (nonpreserved, 0.9% NaCl) for use in their heat disinfection regimen. Patient A, a 12-year-old female, wore extended-wear lenses (55% water content) and used periodic cleaning, but she did not heat disinfect the lenses before wear. Patient B, a 23-year-old female, wore daily wear, soft contact lenses without regular heat disinfection. Patient C, a 30-year-old female also wore daily-wear, soft contact lenses and reportedly used regular heat disinfection; however, she did not thermally disinfect the saline used as a wetting agent during wear. Details of the case histories of patients D to F, who wore daily-wear, soft contact lenses, were reported as cases 3 to 5, respectively, by Wilson et al. (33). All the patients (A to F) either used salt tablet-prepared saline as a wetting solution or did not follow a regular thermal disinfection regimen or both. Patients A to F were hospitalized for treatment of corneal ulcers. Details on contact lens patients G to M were not available.

RESULTS

All the nonpreserved saline solutions, the contact lens cases with aged and often unidentifiable holding solutions, and the gel cleaner yielded greater than 10^6 bacteria per ml. Cultures from corneal ulcers and the preserved hard contact lens solutions from two patients were identified as *S. marcescens*. One corneal ulcer scraping and the holding solution from the contact lens case of that patient yielded *K. oxytoca*. The twenty isolates selected for more finite studies were typically motile, obligately aerobic, oxidase-positive, gram-negative rods. Representative isolates had one or two polar flagella. When two flagella were present, they appeared to have a common origin. Transmission electron microscopy revealed various numbers of pili on selected isolates. Strain differences among certain isolates in pigment production and hemolysis were evident, but the isolates from a patient and from the associate saline solution usually were similar. Two isolates (G-1 and K) were negative for arginine dihydrolase with the API 20E system, and isolate A-1 was negative with the Oxi-Ferm system. All isolates had positive arginine dihydrolase reactions with the Moeller test (21), with the rapid NF1 Trio-Tubes, and with the Vitek computerized system. Growth at 42°C and growth characteristics on selected agars verified identification of the *P. aeruginosa* isolates. The culture sources, their serotypes, the number of plasmids, and selected physiological properties are given in Table 2.

All isolates were resistant to ampicillin, cefamandole, cephalothin, neomycin, and tetracycline and were susceptible to tobramycin (Table 3). Except for intermediate reactions of A-1 and B-1, the isolates were susceptible to carbenicillin and polymyxin B. A single isolate, G-1, one of two morphologically distinct isolates from the same ulcer, was resistant to amikacin. Resistant, intermediate, and susceptible reactions to gentamicin and triple sulfa were evenly distributed among the isolates. The differences in susceptibilities for gentamicin were slight with the Kirby-Bauer method: the zones of inhibition for the seven resistant and intermediate reactions ranged only from 10 to 14 mm. All isolates except one were susceptible to gentamicin with the Vitek computerized system. The results from the antibiogram testing systems were in close agreement for the other

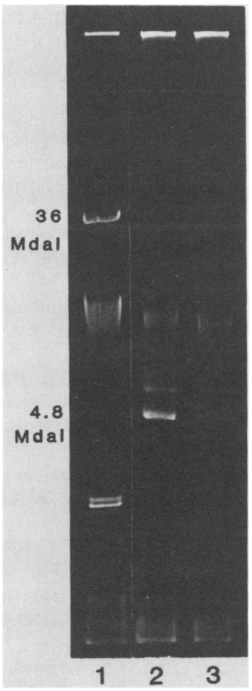

FIG. 1. Agarose gel electrophoresis of plasmid DNA. Lanes: 1, *Escherichia coli* V517 (control); 2, *P. aeruginosa*, A (ulcer); 3, *P. aeruginosa*, A-1 (saline solution).

antimicrobial agents (triple sulfa was not tested with the Vitek system).

Of the 20 eye-associated isolates, 16 were serotypes O:6 or O:11. Isolates from corneal ulcers and saline solutions of the same patients (paired sets) were identical serotypes. Of the 12 ulcer isolates, 3 were the rare serotypes O:5, O:7,8, and O:8.

Plasmids were demonstrated in 12 of the 20 eye-associated isolates. Of the six paired sets, five sets exhibited identity within a pair between the clinical specimen and the saline solution (Fig. 1). Three of the sets lacked plasmids, whereas in one set (A, A-1) two plasmids (approximately 5 and 10 megadaltons [MDa]) were present and in the other set (C, C-1) one plasmid (approximately 30 MDa) was found. For one of the paired sets (B, B-1), the isolate from the ulcer lacked plasmids, whereas the isolate from the solution had one large plasmid (approximately 100 MDa).

In general, those isolates from products containing preservatives (gel cleaner, rinsing and holding solutions, and oil-in-water emulsion) contained a greater number of plasmids and plasmids of larger molecular size (>75 MDa) than did those isolates from nonpreserved saline (Fig. 2). The plasmid profile for a given isolate appeared stable; identical profiles were obtained for three separate tests during a 6-month period.

DISCUSSION

P. aeruginosa appears to be one of the more common contaminants recovered from contact lens products and one of the more frequent etiological agents of corneal ulcers associated with contact lens wear (13, 22, 31). Wilson et al. (33) implicated *P. aeruginosa* as the etiological agent of corneal ulcers in five of six patients who used home-prepared saline. We obtained *P. aeruginosa* from the corneal ulcers of 8 of 11 patients wearing contact lenses and reexamined six isolates from three of the cases reported by Wilson et al. (33).

None of the isolates was resistant to carbenicillin, an antimicrobial agent to which most nosocomial isolates are resistant (5, 20). This lack of resistance suggests that the eye-associated pseudomonads are not nosocomial. Except for one set of isolates belonging to serotype O:7,8, additional data were required to document an association of the solution and ulcer isolates, particularly within the commonly occurring serotypes O:6 and O:11. Probable relatedness for isolates within a given set for five of the six saline-ulcer paired cultures was demonstrated by the similar plasmid profiles of the cultures. One set (B, B-1), of the common O:11 serotype, differed in a single large plasmid. The isolates in this set also demonstrated slight differences in gelatinase production and hemolysis on sheep blood agar at 35°C. These findings may indicate that the two isolates were from different sources. Colonization of clinical specimens by two or more *P. aeruginosa* strains was reported (27). Conversely, large plasmids also may occasionally be integrated into the chromosome (16). Such a phenomenon is suggested because isolates B and B-1 are *o*-nitrophenyl-β-D positive, a characteristic that is found only among 3% of *P. aeruginosa* serotype O:11 isolates (17).

Isolates from preserved solutions tended to contain a greater number of plasmids and plasmids of larger molecular size (approximately 100 MDa) than did isolates from nonpreserved saline. Large plasmids (>100 MDa) were found previously in preservative-resistant *P. aeruginosa* strains (23). Glassman and McNicol (14) found that pseudomonad-like bacteria from polluted regions of the Chesapeake Bay contained multiple plasmids that were larger than 30 MDa, whereas such bacteria recovered from cleaner sites contained small plasmids of approximately 3 MDa.

The failure to use a disinfection regimen routinely or the

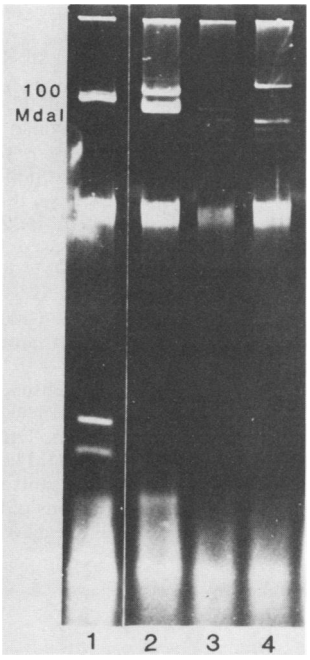

FIG. 2. Agarose gel electrophoresis of plasmid DNA of *P. aeruginosa* from preserved products. Lanes: 1, *E. coli* pDK9 control; 2, K (gel cleaner); 3, J (holding solution); 4, R (oil-in-water emulsion).

use of nonsterile saline as a wetting agent were common factors in the corneal infections with *P. aeruginosa* by six soft contact lens wearers. Our findings, like those of Wilson et al. (33), further implicate improper hygienic practices with tablet-prepared saline solutions in the epidemiology of corneal ulcers of contact-lens wearers. Moreover, for five of these six patients, similar physiological and morphological characteristics, antibiograms, serotypes, and plasmid profiles of isolates from the corneal ulcer and from the tablet-prepared saline solution of a given patient indicate that the isolates were from a common source.

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