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Pyocin Sensitivity of *Neisseria gonorrhoeae* and Its Feasibility as an Epidemiological Tool

HAZEL D. SIDBERRY AND JERALD C. SADOFF*

Department of Bacterial Diseases, Walter Reed Army Institute of Research, Washington, D.C. 20012

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Pyocin inhibition of *Neisseria gonorrhoeae* and its feasibility as a gonococcal typing scheme were examined. Mitomycin C-induced pyocin lysates of *Pseudomonas aeruginosa* were able to selectively inhibit the growth of gonococcal strains. The particles associated with the inhibitory activity were non-dialyzable, heat labile, Pronase sensitive, trypsin resistant, and of large molecular weight by membrane and gel filtration techniques. The inhibitory activity was shown to be specific by absorption with sensitive and insensitive strains of *N. gonorrhoeae* and *P. aeruginosa*. Partial purification of pyocin lysates by ammonium sulfate precipitation followed by ultracentrifugation revealed phagelike particles consistent with high-molecular-weight R-type pyocines. These particles were associated with increased inhibitory activity and could be seen associated with the gonococcal cell surface. One hundred and six gonococcal strains could be differentiated on the basis of their sensitivity to 23 pyocin extracts. Thirty different patterns of pyocin inhibition were seen. Isolates from different body sites from the same patient could generally be identified as being similar strains. Strains isolated from known consorts had the same patterns. In general, agreement between pyocin typing and available epidemiological information was good.

No widely accepted, practical method of typing strains of *Neisseria gonorrhoeae* has yet been developed. The absence of a method for strain differentiation of gonococcal isolates is a major handicap in studying the epidemiology and immunology of the disease. Over the past several years, various approaches to gonococcal strain typing have been attempted. Attempts were made to schematically type gonococcal strains by serological methods based on antigenic structure (1, 6-10, 15, 20, 25, 30, 31), bacteriocin production (13), and nutritional requirements (5). Experimenting with both *N. gonorrhoeae* and *Pseudomonas aeruginosa* led us to the observation that mitomycin C-induced pyocin lysates could inhibit the growth of *N. gonorrhoeae*. Review of the literature revealed that Geizer had earlier noted anti-gonococcal activity associated with *P. aeruginosa* as well as other bacterial species, but the nature of this inhibition was not characterized (14). Toxic lipids produced by gonococci have been shown to be responsible for what was previously thought to be bacteriocin production (29). The phenomena described by Geizer, as well as the bacterial interference described by Kraus and Ellison (23) and Hipp et al. (18), may be due to toxic lipids or a variety of other mechanisms such as nutritional competition or bacteriocin production. Bacteriocin activity is usually limited to strains of the same or closely related species (24); however, some gram-positive bacteria, i.e., staphylococci and streptococci, produce bacteriocins with wider activity spectra (19, 28). This study explores pyocin inhibition of gonococcal strains and the feasibility of a gonococcal typing system based on this pyocin sensitivity.

MATERIALS AND METHODS

Microorganisms and media. *N. gonorrhoeae* (GC) strains isolated at Walter Reed Army Medical Center were kindly provided by Edmund C. Tramont and Herman Schneider; strains and epidemiological data from Indiana University were generously provided by George F. Brooks. All strains were confirmed by bacterial morphology with the Gram stain, oxidase reaction, and sugar fermentations. Strains were stored at −70°C in skim milk. The plate medium used for cultivation of *N. gonorrhoeae* was GC medium base (Difco Laboratories, Detroit, Mich.) plus 1% defined supplement (GCA) (27). The liquid medium (GCB) consisted of proteose peptone no. 3 (Difco Laboratories, Detroit, Mich.) plus 1% IsoVitaleX (BBL, Cockeysville, Md.).

Pyocin producer strains of *P. aeruginosa* (Ps) were a gift of Lloyd G. Herman. Pseudomonas strains were stored at 4°C in litmus milk and also maintained on Trypticase soy agar slants (BBL, Cockeysville, Md.).

Pyocin production. Pyocins were induced from 23 standard *P. aeruginosa* pyocin producer strains (A through T, W, V, X) by the method of Farmer and Herman (12). Eighteen-hour Trypticase soy agar plate cultures of *P. aeruginosa* were suspended in Trypticase soy broth (BBL, Cockeysville, Md.) to an optical density at 650 nm (OD_{650}) of 0.400 (Bausch & Lomb Spectronic 100). A 1-ml volume of this suspension was inoculated into 19 ml of Trypticase soy broth and incubated in a shaking water bath at 32°C for 3 h. Mitomycin C (Sigma Chemical Co., St. Louis, Mo.) was added to a final concentration of 1 μg/ml. The cultures were incubated for 5 h, shaken with 5 ml of chloroform, and centrifuged at 10,000 × *g* for 10 min. The clear upper layer was decanted and designated crude pyocin lysate. These lysates were stored at 4°C.

Assay of pyocin activity. Pyocin lysates diluted 1:2 in Geys balanced salt solution (Microbiological Associates, Inc., Bethesda, Md.) were spotted on GCA by use of a Lidwell replicator, which delivered 0.025 ml (12), and allowed to dry. Eighteen-hour plate cultures of *N. gonorrhoeae* to be tested were diluted in GCB to an OD_{650} of 0.400, diluted 1:100, and flooded over the plates to yield a confluent lawn. Excess fluid was then drawn off. The plates were allowed to dry and were incubated at 37°C in candle jars for 18 h. Only completely clear zones of inhibition were considered positive. Zones of inhibition with light overgrowth were recorded as ±. For quantitative studies, activity was recorded as the reciprocal of the highest dilution of pyocin able to produce complete inhibition. Pyocins were tested for activity against *P. aeruginosa* by a similar technique (2). All assays were performed in duplicate.

Absorption studies. Eighteen-hour plate cultures of *P. aeruginosa* (Ps P, Ps X, Ps W, Ps I) or *N. gonorrhoeae* (GC 120, GC 263, GC 134, GC 230) were harvested and washed in Geys balanced salt solution. A 1-ml volume of pyocin lysate was layered on the pellet and absorbed overnight at 4°C. For native complex absorptions, equal volumes of native outer membrane complexes (at a concentration of 4 mg of protein per ml), prepared by the method of Zollinger et al. for isolation of *N. meningitidis* outer membrane complexes (32) (J. C. Sadoff and B. L. Brandt, Abstr. Annu. Meet. Am. Soc. Microbiol. 1974, P140, p. 168), were mixed with pyocin lysates and absorbed overnight at 4°C. Mixtures were clarified by centrifugation at 12,000 × *g* for 15 min at 4°C and sterilized with chloroform. Serial twofold dilutions of the water phases were then assayed for activity.

Enzymatic digestions. Pyocin lysates were incubated for 18 h at room temperature with a 2-mg/ml final concentration of either Pronase (B grade; Calbiochem, La Jolla, Calif.) or trypsin (Worthington Biohemicals Corp., Freehold, N.J.) in 0.02 M tris(hydroxymethyl)aminomethane (Tris; Fisher Scientific Co., Fair Lawn, N.J.) buffer (pH 7.4). The lysates were then assayed for activity. Untreated pyocin lysate controls in Tris buffer were incubated under similar experimental conditions. Controls for the ability of enzymes, alone and at these concentrations, to inhibit growth were also included.

Kinetics of pyocin activity in liquid media. Eighteen-hour plate cultures of *N. gonorrhoeae* (GC 550) or *P. aeruginosa* (Ps I, Ps B) were suspended in 3.8 ml of broth, 0.2 ml of pyocin lysate (final dilution, 1:20) was added, and the mixture was incubated at 37°C in a shaking water bath. Control cultures were incubated under similar conditions. OD_{650} readings were taken at varying times. Colony-forming units were determined, after vigorous vortexing, by sampling, serial 10-fold dilutions, and plate counts in duplicate at each dilution.

Partial purification of pyocin. Crude pyocin lysates were purified by a modification of the Govan method (16). After mitomycin induction and chloroform treatment, 500 ml of lysate was treated with 30 ml of 1 M $MnCl_2 \cdot 4H_2O$ (added slowly during agitation), and the pH was adjusted to 7.5 with 1 N sodium hydroxide. The precipitate containing viscous material was removed by centrifugation at 2,400 × *g* for 15 min. Ammonium sulfate was added to 70% saturation and allowed to stand overnight at 4°C. The precipitate containing pyocin activity was collected by centrifugation at 2,400 × *g* (4°C, 30 min). The precipitate was dissolved in 25 ml of 0.01 M Tris buffer (pH 7.5) containing 0.01 M $MgCl_2 \cdot 6H_2O$ and 0.01 M $MgSO_4 \cdot 7H_2O$, and dialyzed overnight at 4°C against 4 liters of the same buffer. A portion of this pyocin preparation was then ultracentrifuged at 100,000 × *g* for 3 h. The gelatinous pellet was gently dissolved in Tris buffer (designated partially purified pyocin) and used for electron microscopic studies. A second portion of the redissolved, dialyzed ammonium sulfate-precipitated material was passed over a Sepharose 4B (Pharmacia, Uppsala, Sweden) column equilibrated with Tris buffer. The fractions were assayed for pyocin activity against *N. gonorrhoeae* and *P. aeruginosa*. The column was calibrated by passage of blue dextran.

Electron microscopic studies. Partially purified ultracentrifuged pyocins were fixed for 1 h at room temperature in 2.5% glutaraldehyde in 0.2 M cacodylate buffer (pH 7.2) and centrifuged at 100,000 × *g* for 3 h. A drop of the pellet was transferred to the surface of a collodion-coated grid; after 30 s excess fluid was removed. Grids were negatively stained with 2% sodium phosphotungstic acid and allowed to dry after removal of excess fluid (4). Some of the grids were lightly carbon coated. To observe the interaction of pyocin with *N. gonorrhoeae* (GC 265) or *P. aeruginosa* (Ps B), organisms from 18-h plate cultures were suspended in 3.5 ml of liquid medium to which 0.5 ml of partially purified pyocin was added, incubated at 37°C for 30 min, fixed in 2.5% glutaraldehyde in cacodylate buffer for 1 h, centrifuged at 5,000 × *g* for 45 min, transferred to collodion-copper grids, and processed as described above. Electron micrographs were taken on a Hitachi 11-C electron microscope at 75 kV, with a 300-μm condenser aperture and a 35-μm objective aperture.

RESULTS

Pyocin lysates were able to inhibit the growth of *N. gonorrhoeae*. These lysates were active in dilutions as high as 1:64 and could be

stored at 4°C for several months without appreciable reduction in activity (see Table 5). Heating at 37 or 56°C for 10 min reduced activity, whereas heating at 100°C for 10 min completely destroyed all pyocin activity for *N. gonorrhoeae* and *P. aeruginosa* (Table 1).

Treatment with 2 mg of Pronase per ml for 18 h at room temperature significantly reduced the activity of the pyocin lysates against *N. gonorrhoeae* and *P. aeruginosa*; trypsin had no effect under these conditions (Table 2). For certain strains, enzyme controls inhibited gonococcal growth, but never in a dilution of greater than 1:2. Control pyocins incubated under experimental conditions in Tris buffer without enzymes showed no loss of activity when compared with original lysates.

Following Sparling's suggestion to rule out the nonspecific toxic effect of certain lipids and phospholipids on GC (29), 2% (wt/vol) bovine serum albumin (Armour Pharmaceutical Co., Chicago, Ill.) or 1% soluble starch (Difco Laboratories, Detroit, Mich.) was added to the agar medium used for measuring inhibitory titers. Pyocin lysate preparations were unaffected by starch or albumin in terms of their activity for either *N. gonorrhoeae* or *P. aeruginosa*.

The pyocin lysate activity for *N. gonorrhoeae* was found to be non-dialyzable and was retained and concentrated with an XM-300 Amicon filter (Amicon Corp., Lexington, Mass.). When 70% ammonium sulfate precipitates of crude pyocin lysates were redissolved in Tris buffer and passed over a Sepharose 4-B column, activity against *N. gonorrhoeae* and *P. aeruginosa* was found in the exclusion volume.

A partial purification by ammonium sulfate precipitation followed by ultracentrifugation at 100,000 × *g* sedimented phagelike particles, visible by electron microscopy, which were associated with increased inhibitory activity for sensitive gonococci. Headless contractile tails, both extended and contracted, as well as particles suggestive of the ribonucleic acid polymerase-type particles described by Bradley (3) for mitomycin C-induced lysates of *P. aeruginosa*, were seen. Attachment of the phagelike particles to *N. gonorrhoeae* was similar to attachment to sensitive *P. aeruginosa* strains. Attachment of the particles to the numerous blebs on the surface of *P. aeruginosa* can be seen in Fig. 1. Attachment of contracted particles to the gonococcal surface is shown in Fig. 2 and 3.

The specificity of the pyocin lysate activity for *N. gonorrhoeae* was studied by means of specific absorptions. Results from one such study are shown in Table 3. Pyocin P had activity against four selected strains of *N. gonorrhoeae*, GC 120, GC 263, GC 134, and GC 230, and activity against *Pseudomonas* X, W, and I, but not against its parent strain, P. A sensitive *N. gonorrhoeae* strain, such as GC 120, was able to absorb all of the inhibitory activity of pyocin P for sensitive strains of *N. gonorrhoeae* and significantly reduce the titer for *P. aeruginosa*. A sensitive *Pseudomonas* strain, such as Ps X, was able to completely absorb activity for both species. An insensitive strain, such as the homologous *Pseudomonas* P, was unable to absorb pyocin activity against either *P. aeruginosa* or *N. gonorrhoeae*. It was noted that absorption with *Pseudomonas* P tended to increase activity toward gonococci, indicating probable release of pyocin from the absorbing organisms during the absorption process. Absorption of pyocin P with an insensitive gonococcus (strain 550) did not reduce the activity of pyocin P for sensitive gonococcal strains.

Specific activity of pyocin P could be reduced

TABLE 1. *Effect of temperature on pyocin activity*

Treatment	Indicator strain:			
	GC 120	GC 209	Ps W	Ps X
Pyocin M untreated	16[a]	16	4,096	4,096
Pyocin M, 37°C	4	4	256	512
Pyocin M, 56°C	4	4	256	1,024
Pyocin M, 100°C	0	0	0	0

[a] Reciprocal of highest dilution able to inhibit growth.

TABLE 2. *Enzymatic treatment of pyocins*

Treatment	Gonococcal indicator strain:				*Pseudomonas* indicator strain:	
	GC 273	GC 210	GC 263	GC 209	Ps V	Ps W
Pyocin O control	8[a]	16	4	32	4,096	4,096
Pyocin O treated with Pronase	2	4	0	8	64	32
Pyocin O treated with trypsin	8	16	4	32	4,096	4,096
Pronase alone	0	0	0	0	0	0
Trypsin alone	0	0	0	2	0	0

[a] Reciprocal of highest dilution able to inhibit growth.

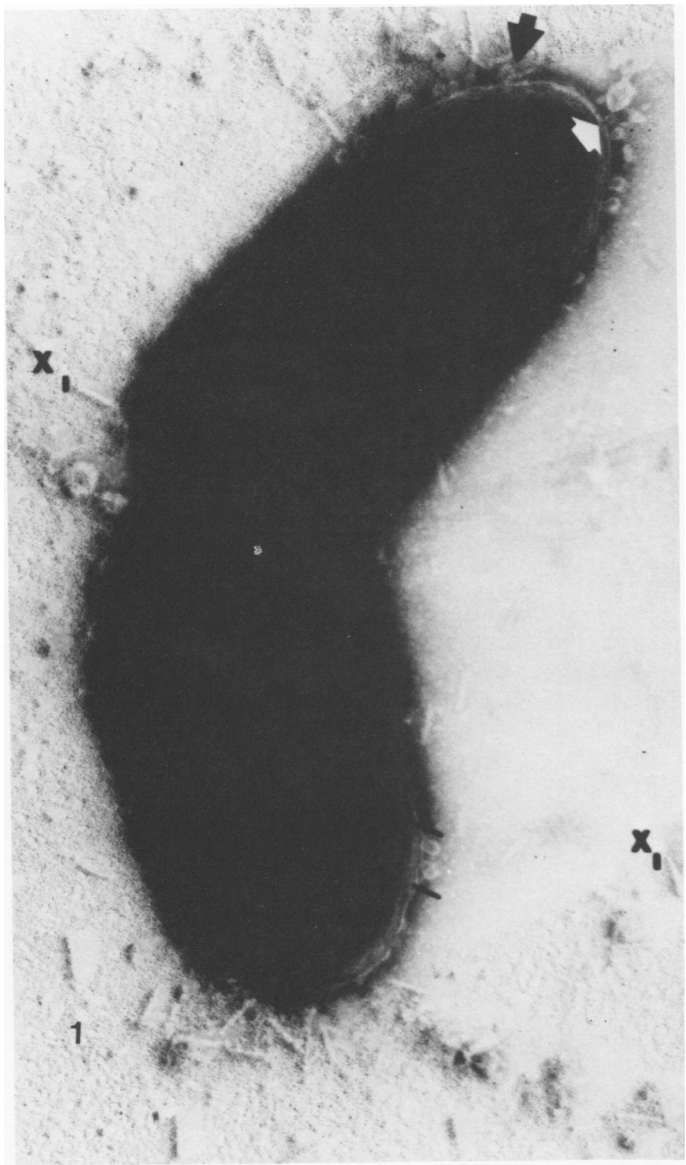

FIG. 1. *P. aeruginosa B after a 30-min contact with pyocin lysate J. Contracted particles (arrow) are visible attached to the bacterial surface. Uncontracted particles (X_1) and blebing (between brackets) of outer membrane can be observed.* ×75,000.

with native complex absorption. Absorption of pyocin P with native complex prepared from sensitive gonococcal strain 120 effectively reduced activity to both *N. gonorrhoeae* and *P. aeruginosa* (Table 3). Interestingly, the native outer membrane complexes from gonococci were more effective in removing activity against *P. aeruginosa* than against *N. gonorrhoeae*.

The kinetics and specificity of pyocin lysates were studied in liquid media. Pyocin lysates added to a resuspended, sensitive *N. gonorrhoeae* culture (GC 550) or *P. aeruginosa* culture (Ps K) caused a steady decline in OD readings, whereas ODs of untreated control cultures increased (Fig. 4 and 5). Pyocin G caused a three-log decline in the number of colony-forming units after 2 h of incubation

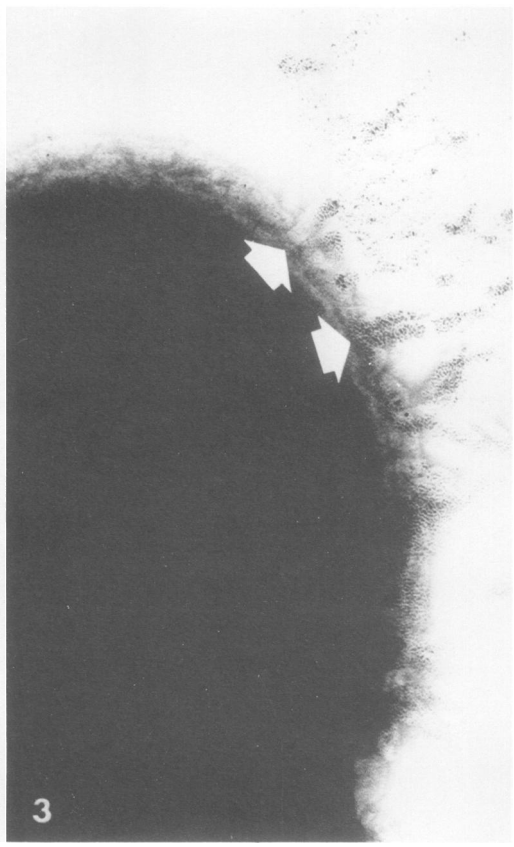

FIG. 2. *N. gonorrhoeae 265 after a 30 min contact with pyocin lysate G. Uncontracted particles (X_1) and contracted particles (X_2) are shown. Many contracted particles (between brackets) appear attached to the bacterial surface. ×75,000.*

FIG. 3. *N. gonorrhoeae 265 after a 30-min contact with pyocin lysate G. Two particles (arrows) appear absorbed to the gonococcal surface. ×75,000.*

TABLE 3. *Absorption of pyocin P*

Treatment	*N. gonorrhoeae*				*P. aeruginosa*			
	GC 120	GC 263	GC 134	GC 230	Ps P	Ps X	Ps W	Ps I
Pyocin P unabsorbed	8[a]	4	16	4	0	1,024	128	256
Pyocin P absorbed with GC 120[b]	0	0	0	0	0	64	2	4
Pyocin P absorbed with GC 120 native complex[b]	4	2	8	2	0	64	2	4
Pyocin P absorbed with *Pseudomonas* X	0	0	0	0	0	0	0	0
Pyocin P absorbed with *Pseudomonas* P	32	32	64	16	0	1,024	512	1,024

[a] Reciprocal of highest dilution having activity.

[b] Pyocins were absorbed with live, washed organisms or native complex as described in the text.

and a four-log decline after 6 h. Sensitive *P. aeruginosa* demonstrated a four-log decline in viability after only 15 min of incubation. When *P. aeruginosa* B and *N. gonorrhoeae* 550 were incubated with pyocins, they were not sensitive to G and P, respectively, by the standard plate assay; OD readings continued to increase (Fig. 4 and 5). Likewise, at 2 h there was no

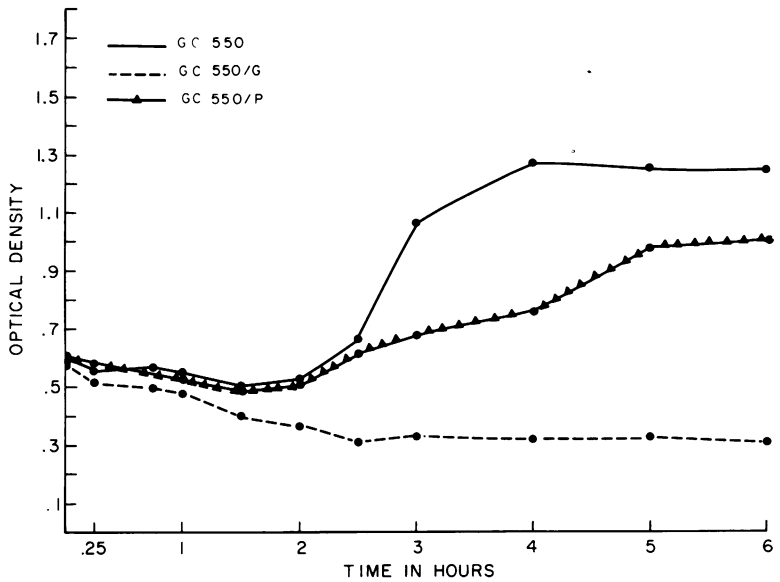

FIG. 4. *Effect of pyocins on the growth of N. gonorrhoeae. Strain GC 550, which is sensitive to pyocin G and insensitive to pyocin P, was resuspended in broth. Pyocin G added (GC 550/G, - - - -); pyocin P added (GC 550/P,▲); broth added (GC 550, ——) and incubated for 6 h.*

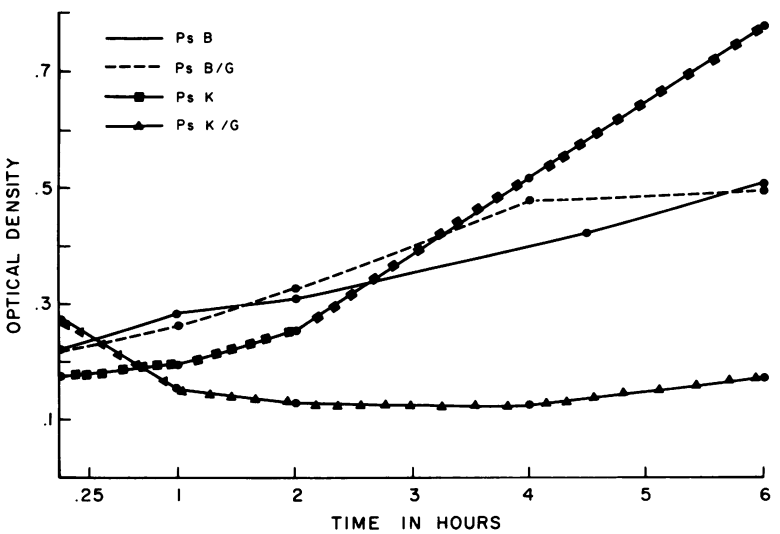

FIG. 5. *Effect of pyocin G on the growth of P. aeruginosa. Pyocin G was added to resuspended sensitive strain Ps K (▲) and insensitive Ps B (- - - -) and incubated for 6 h. Control cultures Ps B (——) and Ps K (■) had no pyocins added.*

decrease in colony-forming units. By 6 h, however, strain GC 550 demonstrated a two-log decrease in colony-forming units compared with the control culture. Pyocin activity for *N. gonorrhoeae* in liquid media appears to be specific. Resuspended plate cultures of *N. gonorrhoeae* and *P. aeruginosa*, however, have different kinetic responses to pyocins.

Preliminary studies indicated that gonococcal strains could be differentiated on the basis of their sensitivity to pyocin extracts. Twenty-three pyocin producer strains were selected, and pyocin lysates were prepared from each. A total of 106 strains was tested. It was found that pyocins D, F, L, W, V, and X had no activity against >96% of the strains tested. On

the other hand, pyocin lysates, A, B, C, E, and G, had activity against at least 98% of the gonococcal strains tested.

The remaining extracts were able to inhibit some gonococcal strains, but not others, and formed the basis of the typing scheme (Table 4). Inhibition patterns remained stable. When the same gonococcal strains were tested several months apart with different pyocin preparations, the same inhibition patterns were found, although occasional reactions became ± (Table 5). Similarly, when subcultured gonococcal strains were tested with the same pyocin preparations several months apart, the patterns were the same. Pyocin activity for *N. gonorrhoeae* was stable for at least 4 months at 4°C.

TABLE 4. *Distribution of pyocin activity*

Pyocin	No. of strains +	No. of strains ±	No. of strains −	% +	% ±	% −
A	53	2	0	96	4	0
B	103	3	0	97.2	2.8	0
C	106	0	0	100	0	0
D	1	0	105	0.9	0	99.1
E	103	2	1	97.2	1.9	0.9
F	3	0	103	2.8	0	97.2
G	102	3	1	96.3	2.8	0.9
H	5	7	43	9.1	12.7	78.2
I	5	5	96	4.7	4.7	90.6
J	5	12	87	4.8	11.6	83.6
K	61	17	28	58	16.0	26
L	0	1	53	0	1.9	98.1
M	76	3	27	71.7	2.8	25.5
N	41	5	7	77.4	9.4	13.2
O	70	6	30	66	5.7	28.3
P	57	8	40	54.3	7.6	38.1
Q	4	2	100	3.8	1.9	94.3
R	45	24	37	42.5	22.6	34.9
S	60	11	35	56.6	10.3	33.0
T	52	13	41	49.1	12.3	38.7
W	1	3	102	0.9	2.8	96.3
V	0	3	103	0	2.8	97.2
X	0	2	104	0	1.9	98.1

Thirty different patterns of pyocin inhibition were seen when 106 strains of *N. gonorrhoeae* were tested. The majority of strains typed (51) fell into a single pattern, 19 other strains gave a second pattern, and the remaining 36 strains could be classified into 28 different individual patterns. Since most of the gonococcal strains tested were provided by G. F. Brooks from an Indiana study involving multiple isolations from a limited number of patients, it was reasonable that a large number of strains were identical.

It was felt that the usefulness of pyocin typing of *N. gonorrhoeae* as an epidemiological tool could be evaluated by comparing strains from different body sites from the same patient and by comparing strains from known patient consorts. All tests were performed without prior knowledge of the epidemiological data. Twenty strains isolated from various body sites from seven different patients (initials used to identify patients are fictitious and are used only to facilitate the description of results) were examined. Representative data are shown in Table 6. In general, strains from different body sites of the same patient demonstrated similar patterns of sensitivity to pyocins. Minor variations, especially with equivocal reactions, were noted. Occasionally, inexplicable results were seen, such as for patient CM where the vaginal (GC 492) and urethral (GC 493) cultures gave patterns different from the cervical (GC 494) and pharyngeal (GC 495) isolates.

A series of strains isolated from one contact source and his consorts over a 1.5-year period was examined (Table 7). Two strains isolated from ST 3 weeks apart in 1972 differed only in

TABLE 5. *Test of stability and reproducibility of pyocin preparations*[a]

Pyocin prepn			Inhibition pattern																		
Date induced	Date tested	GC strain	B	C	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	W	V	X
4/74	8/22/74	218	+	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
9/74	10/ 9/74	218	+	+	+	0	0	0	+	0	+	+	+	+	0	±	+	±	0	0	0
4/74	7/31/74	202	+	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
9/74	10/ 9/74	202	+	+	+	0	0	0	±	0	+	±	+	±	0	±	+	+	0	0	0
4/74	8/22/74	214	±	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
9/74	10/ 9/74	214	+	+	+	0	0	0	+	0	+	+	+	+	0	±	+	+	0	0	0
4/74	6/12/74	265	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
4/74	8/27/74	265	+	+	+	n	0	0	0	n	±	n	0	0	0	0	0	0	0	0	0
9/74	10/ 2/74	265	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

[a] +, Killing; ±, light overgrowth; 0, no killing; n, not tested.

TABLE 6. *Typing of strains from various body sites of the same patient*[a]

GC strain	Patient	Isolate site	Inhibition pattern																	
			B	C	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	W	V	X
212	AB	Cervix	+	+	+	0	0	0	+	0	+	+	+	+	0	±	+	+	0	0	0
214	AB	Urethra	+	+	+	0	0	0	+	0	+	+	+	+	0	±	+	+	0	0	0
203	BC	Cervix	+	+	+	±	±	±	0	0	0	0	0	0	0	0	0	0	0	0	0
211	BC	Urethra	±	+	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
218	CD	Urethra	+	+	+	0	0	0	+	0	+	+	+	+	0	±	+	±	0	0	0
219	CD	Vagina	+	+	+	0	0	0	+	0	+	+	+	±	0	±	+	+	0	0	0
287	EF	Cervix	+	+	+	+	+	±	0	0	0	0	0	0	0	0	0	0	0	0	±
279	EF	Rectum	+	+	+	+	±	±	0	0	0	0	0	0	0	0	0	0	0	0	+
161	GH	Cervix	+	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
162	GH	Nasopharynx	+	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
492	CM	Vagina	±	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
493	CM	Urethra	±	+	+	n	0	0	±	n	±	n	±	0	0	±	±	±	0	0	0
494	CM	Cervix	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
495	CM	Nasopharynx	±	±	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
316	KL	Cervix	+	+	+	0	0	0	0	0	+	+	+	+	0	+	+	+	0	0	0
317	KL	Joint	+	+	+	0	0	0	0	0	+	+	+	+	0	+	+	+	±	0	0
342	CM	Rectum	+	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
343	CM	Cervix	+	+	+	0	0	±	0	±	0	±	+	+	0	+	+	+	0	0	0
344	CM	Urethra	+	+	+	±	0	±	±	0	+	+	+	+	0	+	+	+	0	0	0
345	CM	Vagina	+	+	+	+	0	±	+	0	+	+	+	+	0	+	+	+	0	0	0

[a] +, Killing; ±, light overgrowth; 0, no killing; n, not tested.

TABLE 7. *Typing of single cohort of GC consort strains*[a]

GC strain	Date isolated	Inhibition pattern																		
		B	C	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	W	V	X
55-ST	10/18/72	±	+	+	n	0	0	±	n	+	n	+	+	0	+	±	±	0	0	0
85-ST	11/ 2/72	+	+	+	0	0	0	±	0	+	±	+	±	0	0	+	±	0	0	0
349-ST	9/17/73	+	+	+	+	±	+	+	0	+	+	+	+	0	+	+	±	0	±	0
343-CM	9/18/73	+	+	+	+	±	+	+	0	+	+	+	+	0	+	+	±	0	±	0
357-JT	9/19/73	+	+	+	+	±	+	+	0	+	+	+	+	0	+	+	±	0	±	0
484-ST	2/12/74	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
492-CM	2/19/74	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
527-ST	3/25/74	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
530-CM	3/26/74	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
533-JT	3/27/74	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
545-ST	4/19/74	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0
550-CM	4/23/74	+	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
553-JT	4/25/74	+	+	+	n	0	0	0	n	0	n	0	0	0	0	0	0	0	0	0

[a] +, Killing; ±, light overgrowth; 0, no killing; n, not tested.

their sensitivity to pyocin R and are probably similar. One year later, ST was reinfected with a second strain that appears different by pyocin typing from his original infecting strain. His consorts, CM and JT, were also infected with this second strain. Several months later in February 1974, ST was infected with a third strain differing by pyocin typing from his pre-

vious two. This strain was persistent in ST for 2 months either through treatment failure or reinfection and was consistently reisolated from his two consorts.

Eight additional sets of consort strains were tested for identity by pyocin typing (Table 8). Agreement between pyocin typing and available epidemiological information was good, in general, but there were some unexplained discrepancies. Patient GC 298, RO, by pyocin typing had a different strain from his two consorts. Strain GC 210 isolated from the nasopharynx of a male patient, OC, was similar by pyocin typing to strain GC 200 isolated from the cervix of his consort, SD, but strain GC 368 isolated 4 months later from the urethra of SD was different by pyocin typing from the original strains in that it was sensitive to pyocin V. Strain GC 129 isolated from a soldier just returned from the Far East and GC 130 isolated from his wife appeared clearly different by pyocin typing. Careful epidemiological investigation subsequently revealed that separate infections were likely.

DISCUSSION

Pyocin inhibition of *N. gonorrhoeae* and the feasibility of a gonococcal typing scheme based on pyocin sensitivity were examined. The inhibitory activity appeared to fulfill Bradley's criteria for a high-molecular-weight bacteriocin (3). The particles associated with the activity were non-dialyzable, heat labile, Pronase sensitive, trypsin resistant, and of large molecular weight by membrane and gel filtration techniques. Phagelike particles with pyocin activity towards both species of bacteria were isolated by ultracentrifugation and visualized, by electron microscopy, attaching to sensitive *N. gonorrhoeae* and *P. aeruginosa*. The ultrastructure and distribution of these particles were very similar to those described by Bradley for the mitomycin C-induced pyocin lysates he studied (3). Their appearance and properties are consistent with R-type pyocins described by Ishii et al. (22) and others (16, 17). The possibility that some of the activity may have been due to bacteriophage rather than a bacteriocin has not yet been explored in detail. Negatively stained electron micrographs (unpublished) did demonstrate phagelike particles attached to gonococcal pili.

The reaction between pyocin and *N. gonorrhoeae* was found to be specific as demonstrated by absorptions with *P. aeruginosa* and *N. gonorrhoeae* organisms and outer mem-

TABLE 8. *Typing of consort strains*[a]

GC strain	Date isolated	Inhibition pattern																	
		B	C	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	W	V	X
195-JP	6/15/73	+	+	+	0	0	0	n	0	±	0	n	0	0	0	±	0	0	0	0
211-JA	6/23/73	±	±	±	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
179-NZ	6/ 7/73	0	±	+	n	0	0	0	n	±	n	0	0	0	0	0	0	0	0	0
298-RO	8/ 7/73	±	±	+	n	0	0	±	n	±	n	±	±	0	±	±	±	0	0	0
287-ED	8/ 2/73	+	+	+	+	+	±	0	0	0	0	0	0	0	0	0	0	0	0	+
279-BH	7/26/73	+	+	+	+	+	±	0	0	0	0	0	0	0	0	0	0	0	0	+
180-WE	6/14/73	+	+	+	n	0	0	+	n	+	n	+	+	0	±	±	±	0	0	0
182-TC	6/14/73	+	+	+	n	0	0	+	n	+	n	+	+	0	+	±	±	0	0	0
202-TC	6/26/73	+	+	+	0	0	0	0	±	+	±	+	+	0	+	+	+	0	0	0
165-LS	5/ 9/73	±	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
173-AA	6/ 5/73	±	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
152-CP	7/31/72	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
154-AP	8/31/72	+	+	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
129-WD	5/15/72	+	+	+	±	±	+	±	0	+	+	+	+	0	+	+	+	+	±	+
130-MD	5/16/72	+	+	+	±	±	+	0	0	0	0	0	0	0	0	0	0	0	0	0
161-TR	5/ 1/73	±	+	+	n	0	0	+	n	+	n	+	+	0	+	+	+	0	0	0
158-BL	5/ 1/73	±	+	+	n	0	0	±	n	+	n	±	±	0	+	±	±	0	0	0
210-OC	6/19/73	+	+	+	0	0	0	+	0	+	+	+	+	±	+	±	±	0	0	0
200-SD	6/26/73	+	+	+	0	0	0	±	0	+	±	+	±	0	±	±	±	0	0	0
368-SD	10/17/73	±	+	+	n	0	0	±	n	±	n	±	+	0	+	+	+	0	+	0

[a] +, Killing; ±, light overgrowth; 0, no killing; n, not tested.

brane preparations. The specific surface component responsible for pyocin receptor activity in *N. gonorrhoeae* is unknown but may be lipopolysaccharide, as appears to be the case in *P. aeruginosa* (11, 16, 21, 26).

The potential usefulness of pyocin typing for *N. gonorrhoeae* as an epidemiological tool must be tempered by the realization that susceptibility to standard pyocin preparations has had limited usefulness for the epidemiological study of *P. aeruginosa* itself. The preferred method is to induce pyocins from the unknown *Pseudomonas* strain and then test them against standard indicator strains (12). Nevertheless, a correlation between gonococcal pyocin types and epidemiological data in terms of matched consort strains was observed. Likewise, isolates from different body sites from the same patient could generally be identified as being similar strains.

The advantages of the system for epidemiological use are its simplicity and rapidity, as well as the stability of pyocin activity for GC. Although most strains in this study fell into a small number of groups, over 30 distinct types were identified in the 106 strains tested. Evaluation of the overall usefulness of the system awaits testing of more strains over a wider epidemiological range.

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