SENSITIVITY OF *NEISSERIA GONORRHOEAE* TO PARTIALLY PURIFIED R-TYPE PYOCINES AND A POSSIBLE APPROACH TO EPIDEMIOLOGICAL TYPING

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A RAPID, inexpensive typing system for *Neisseria gonorrhoeae* would be of use in epidemiological studies, for distinguishing failures of treatment from cases of reinfection, and in the early recognition of strains that might have special pathogenic features such as those associated with disseminated infection (Knapp and Holmes, 1975; Morello, Lerner and Bohnoff, 1976; Schoolnick, Buchanan and Holmes, 1976; Eisenstein, Lee and Sparling, 1977). Previous efforts to type gonococcal strains have involved the serological detection of antigenic differences (Glynn and Ward, 1970; Maeland, Kristoffersen and Hofstad, 1971; Apicella, 1974; Johnston, Holmes and Gotschlich, 1976; Tramont *et al.*, 1976; Wang *et al.*, 1977), differences in nutritional requirements (Carifo and Catlin, 1973) and variations in sensitivity to bacteriocines (Flynn and McEntegart, 1972; Walstad, Reitz and Sparling, 1974; Knapp, Falkow and Holmes, 1975; Lawton *et al.*, 1976).

Observations on the production of pyocines and demonstration of sensitivity to these bacteriocines have been used as the basis of typing systems for *Pseudomonas aeruginosa* (Farmer and Herman, 1969; Govan and Gillies, 1969). Sensitivity to R-type pyocines has been reported for strains of *N. gonorrhoeae* (Morse *et al.*, 1976) and the possible use of pyocines as a typing tool has been investigated (Sidberry and Sadoff, 1977). Three types of bacteriocines have been described in *Ps. aeruginosa*: soluble bacteriocines (S-type) similar to the colicines of *Escherichia coli* (Ito, Kageyama and Egami, 1970), rod-type (R-type) particles resembling defective bacteriophages (Kageyama, 1964), and the flexuous F-type bacteriocines (Govan, 1974*b*). The R-type and F-type pyocines were chosen for the present study because they can be sedimented by ultracentrifugation and are visible in electron micrographs; these qualities allow their partial purification and separation from the S-type pyocines and other soluble inhibitory substances produced by *Ps. aeruginosa*.

Using 23 pyocine preparations, Sidberry and Sadoff (1977) obtained 30 sensitivity patterns among 106 strains of *N. gonorrhoeae* but the majority of the strains fell into two groups and further evaluation of the method with gonococcal isolates from several sources was suggested by the authors.

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MATERIALS AND METHODS

Micro-organisms and culture media. Strains of *Ps. aeruginosa* producing the R-type pyocines listed in table I were obtained from Dr John Govan of this department. Kageyama (1975) classified many R-type pyocines on the basis of their spectrum of activity, and strains from groups R1, R2, R3 and R5 were specifically chosen for use in this study. The Kageyama classification for the R-type pyocines produced by these strains is given when known. Strains of *Ps. aeruginosa* from which F-type pyocines were obtained were pp430, F1, R186, Ep520, Ep111, R34, R7095, Ep274, Ru84, and Ep 397. Strains of *Pseudomonas* were maintained by subculture on nutrient agar every 7 days. *N. gonorrhoeae* strains were supplied by the Center for Disease Control (CDC), Atlanta, Ga, USA and the Royal Infirmary, Edinburgh (RIE). Organisms from disseminated infections were obtained from Dr Joan S. Knapp, Neisseria Reference Laboratory,

TABLE I
Pyocine-producing strains of Pseudomonas aeruginosa and their corresponding indicator strains

Kageyama classification	Pyocine-producing strain	Pyocine indicator strain
R1	IS4	ISC
	IS6	ISC
	IS8	ISC
	ISD	ISC
	2285	ISC, IS6
R2	ISB	ISC
	R21	IS8
	pp430	IS8
R3	ISE	IS4
R4	...	...
R5	ISC	IS4, IS5
Unclassified	R205	ISC

... = Not available for examination.

United States Public Health Service, Seattle, Washington; these included prototrophic strains and strains of different auxotypes from three geographic areas: Atlanta, Indianapolis, and Seattle. All RIE organisms had been identified as *N. gonorrhoeae* on the basis of Gram stain, oxidase reaction and sugar utilisation. On arrival at our laboratory each was retested for oxidase reaction and Gram staining. Strains were stored in skimmed milk at -20°C . The gonococcal strains were maintained on either Thayer Martin medium (TM) or modified New York City medium (MNYC; Young, 1978). When needed, colony types T1, T2, T3 and T4 were selected from GC Medium (Difco) with the aid of a stereoscopic microscope with a double system of substage lighting (Jephcott and Reyn, 1971).

Sensitivities to antibiotics were determined on clear GC Medium (Difco) with the following additions per 960 ml after autoclaving: 0.5% (w.v) ferric nitrate (British Drug Houses) 10 ml, GC supplement* 20 ml, 20% (w.v) glucose 10 ml. Antibiotics were added to GC agar at the following concentrations: penicillin G (Glaxo) sodium salt: 0.015, 0.03, 0.06, 0.125, 0.25, 0.5, 1, 2 units/ml; tetracycline hydrochloride (Achromycin, Lederle Ltd): 0.05, 0.1, 0.25, 0.5, 1 $\mu\text{g/ml}$; spectinomycin hydrochloride (Trobicin, Upjohn Ltd): 2, 4, 8, 16 $\mu\text{g/ml}$; chloramphenicol (Chloromycetin, Parke-Davis Ltd): 0.1, 0.2, 0.4, 0.8, 1.6 $\mu\text{g/ml}$; lincomycin hydrochloride (Upjohn Ltd): 4, 8, 16, 32 $\mu\text{g/ml}$.

* GC supplement: L glutamine (Koch Light Chemicals) 0.5 g, cocarboxylase (British Drug Houses) 0.01 g, water to 100 ml 20%.

Antibiotic-sensitivity testing. Visibly turbid suspensions (approximately 1×10^8 – 3×10^8 colony-forming units/ml) of gonococci were made in NaCl 0.85% (w/v) containing soluble starch 1% (starch-saline). Portions of the suspension were placed in the wells of a Steers replicator which delivered approximately 0.0025 ml of the suspension to the antibiotic plates (Steers, Foltz and Graves, 1959). The same suspensions were also used for the assay of sensitivity to pyocines. The plates were then incubated for 48 h at 37°C with 10% CO₂ in a Searle Qualitemp (80 TC) CO₂ incubator. Minimal inhibitory concentration (MIC) was recorded as the lowest concentration of antibiotic at which there were five or fewer colonies per spot inoculated.

Induction and preparation of pyocines. Pyocine preparations were obtained by a modification of the method of Govan (1974a). From an overnight nutrient-broth culture, 2 ml was added to 25 ml of sodium glutamate broth (SGB) which contained sodium glutamate 20 g, glucose (20% w/v solution) 20 ml, magnesium sulphate (MgSO₄ 7H₂O) 0.1 g, disodium hydrogen phosphate (Na₂HPO₄ 12H₂O) 5.63 g, potassium dihydrogen phosphate (KH₂PO₄) 0.25 g, calcium nitrate 10 mg, yeast extract 1 g, and distilled water to 1 litre. The sterile glucose in solution (Seitz filtered) was added to the cooled autoclaved medium before use (Kageyama and Egami, 1962).

The pseudomonads were incubated at 30°C in an orbital incubator (Gallenkamp) at 100 r.p.m. for 2.5–3 h. Mitomycin C (1.5 µg/ml) was added at this point and the cultures were incubated for another 2.5–3 h. The lysates were centrifuged at 10 000 *g* for 30 min. to remove bacterial cells and debris; each supernatant was decanted, shaken with chloroform 5% (v/v) and stored at 4°C. This preparation was the crude pyocine lysate.

The crude lysate (12.5 ml) was ultracentrifuged at 100 000 *g* for 3 h and the supernatant containing S-type pyocine activity and other soluble inhibitory substances was discarded. The pellet containing the R-type activity was resuspended in 10 ml 0.01M Tris buffer (pH 7.5) containing 0.01M MgCl₂·6H₂O and 0.01M MgSO₄·7H₂O (referred to hereafter as Tris buffer). This partially purified pyocine preparation was stored at 4°C.

Assay of pyocine activity against Ps. aeruginosa and N. gonorrhoeae. Pyocine activities of the crude lysates and the partially purified preparations were assayed by spotting doubling dilutions on nutrient-agar (NA) plates and MNYC plates and allowing the spots to dry. The NA plates were then flooded with the appropriate standard strains of *Ps. aeruginosa* (indicator strains) known to be sensitive to the particular test pyocine. Sensitive and resistant gonococcal strains, identified in pilot studies, were tested on MNYC.

Partially purified pyocine lysates were diluted 1 in 2 in Tris buffer and spotted with a dropper pipette calibrated to deliver 0.02 ml on plates of GC, MNYC or TM medium and allowed to dry. Overnight plate cultures of gonococci were suspended in starch-saline and used to flood the plates to produce a confluent lawn. The plates were allowed to dry and incubated overnight at 37°C aerobically with 10% CO₂. Some gonococci grew more slowly and were incubated for an additional 24 h. Results were recorded as + = inhibition, ± = inhibition with some overgrowth, – = no inhibition. *Ps. aeruginosa* indicator strains were included in each test as well as the gonococcal strains CDC486 or CDC046, which were sensitive to most pyocines tested, and strain 879 which was resistant to most pyocines tested. The sensitivity of a gonococcal strain to each pyocine was examined at least three times. Preparations were assayed regularly to monitor levels of active pyocines. Those with low titres usually gave equivocal or negative results. Table III includes representative data of pyocine titres and sensitivities of sensitive strains CDC486, CDC046 and resistant strain 879.

Serotyping of meningococci and sulphadiazine sensitivity testing were done by Dr R. J. Fallon, Department of Laboratory Medicine, Ruchill Hospital, Glasgow.

RESULTS

Preliminary studies

None of the F-type pyocines listed (see *Methods*) inhibited any of 84 gonococcal strains against which they were tested in pilot studies. Several of the R-type pyocines were able to inhibit 94.5% (104/110) of the gonococcal

strains selectively. Inhibition was observed on clear GC, TM, or MNYC or media; the darker background of the TM and MNYC plates facilitated readings and these media were less subject to contamination. Addition of 1% soluble starch or 2% bovine serum albumin to the GC plates did not affect inhibition of gonococci by the pyocines.

Comparison of sensitivities of different indicators to the test pyocines

Two types of zones of inhibition corresponding to those found for soluble (S-type) and rod-type (R-type) pyocines were observed with the gonococci. The S-type zones resembled those found with soluble bacteriocines (Fredericq, 1957) or other inhibitory substances that diffuse readily into agar away from the site of inoculation. Large zones of this type were observed with crude lysates. Typical R-type zones—smaller, discrete zones of inhibition limited to the site of inoculation (Ito *et al.*, 1970)—were found with the partially purified ultracentrifuged preparations. Table II illustrates the differences in inhibition produced by crude lysates and the partially purified pyocines. These were titrated against the appropriate *Pseudomonas* indicators and two gonococcal strains, one that had appeared to be resistant to pyocines tested in the pilot studies and one that appeared to be sensitive to many of the pyocines. Ultracentrifugation separated the heavier R-type particles from the S-type pyocines, pigments, and other soluble substances that had an inhibitory effect on the gonococci. The pyocines in table II were selected as examples of this effect because the partially purified pyocines of Kageyama groups R2 and R3 did not inhibit gonococci under the conditions used in the survey but the crude lysates did. These observations are of importance when our results are compared with those of other workers.

The concentration of the gonococcal inoculum usually made little difference to the inhibition observed with a series of twofold dilutions of pyocines up to 1 in 8192. Undiluted gonococcal inocula and tenfold dilutions up to 1 in 100

TABLE II

Titration of crude lysate and ultracentrifuged preparations against Ps. aeruginosa and N. gonorrhoeae

R-type pyocines from Kageyama group strain		Titre of the stated pyocine, preparation in tests with					
		<i>Pseudomonas</i> indicator		sensitive gonococci		resistant gonococci	
		Crude pyocine	Ultracentrifuged pyocine	Crude pyocine	Ultracentrifuged pyocine	Crude pyocine	Ultracentrifuged pyocine
R2	ISB	1024	128	128	1*	8	0*
	R21	8192	4096	128	4	8	0
	pp430	8192	8192	32	1	8	0
R3	ISE	8192	8192	32	1	8	0

* 1 = Undiluted preparation; 0 = no inhibition.

were tested. A more dilute suspension occasionally gave a clearer zone of inhibition; strains were retested with diluted suspensions when only faint reactions ($\pm$) were obtained with undiluted preparations. The partially purified pyocine preparations were diluted in twofold steps to 1 in 8192 and each dilution was spotted on to appropriate plates for assay of inhibitory activity against a known standard sensitive strain of *Ps. aeruginosa*, a sensitive gonococcal strain and a resistant gonococcal strain. The titres of inhibitory activity for the pyocines of Kageyama groups R1, R2, R3, and R5 and the pyocine that is unclassified are outlined in table III.

TABLE III
Comparative sensitivities of *Ps. aeruginosa* and *N. gonorrhoeae* to partially purified pyocines

R-type pyocines from		Titre of the partially purified pyocine preparation in tests with		
Kageyama group	strain	<i>Ps. aeruginosa</i> indicator	sensitive gonococci	resistant gonococci
R1	IS4	8192	1024	0
	IS6	8192	1024	0
	IS8	8192	1024	0
	2285	8192	1024	1
	ISD	1024	128	0
R2	ISB	128	0	0
	pp430	8192	1	0
	R21	4096	4	0
R3	ISE	8192	1	0
R4	...	...	...	...
R5	ISC	2048	1024	0
Unclassified	R205	8192	1025	0

Footnote as in table II.

Group R1. Preparations of pyocines in this group were potent in tests against the sensitive *Pseudomonas* indicator strain, and less potent against sensitive gonococci strain CDC486 or CDC046, usually by three dilution tubes. There was only a slight inhibition of the "resistant" strain 879 by the undiluted strain 2285 pyocine.

Group R2. Although the titres for pyocines pp430 and R21 on sensitive strains of *Ps. aeruginosa* were similar to those for pyocines for group R1, the sensitivity of gonococci to these pyocines was dramatically lower and not reliably reproducible. The titre for pyocine ISB determined with *Ps. aeruginosa* for this set of tests was lower than that of pp430 or R21 and no inhibition of gonococci was noted. Slight inhibition of the sensitive gonococcal strain was observed with another preparation of pyocine ISB that had a higher titre on the sensitive *Pseudomonas* indicator, but this was only with the undiluted pyocine.

Group R3. The only example of this group, pyocine ISE, yielded results similar to those observed with pyocines of group R2—high titres on tests against the sensitive *Ps. aeruginosa* indicator strain but little or no reproducible activity against gonococci tested.

Group R4. Our collection did not include a member of this group.

Group R5. Results obtained for pyocine ISC were similar to those found for pyocines of group R1—high titres on sensitive *Ps. aeruginosa* strain and slightly lower titres for sensitive gonococci.

Unclassified. Pyocine R205 was not classifiable in any of the Kageyama groups. Its titres on sensitive strains of *Pseudomonas* and gonococci were similar to those found for groups R1 and R5.

At the higher dilutions of each pyocine, areas of inhibition of growth became increasingly turbid. Morse *et al.* (1976) made similar observations and suggested that this was evidence for a non-replicating inhibitory substance. Not all strains were sensitive at the same dilutions of the pyocines; therefore a dilution of 1 in 2 was chosen for the screening procedure. This dilution would provide a large number of pyocine particles and correspond to the dilution used by Sidberry and Sadoff (1977).

Typing of Neisseria spp. with partially purified pyocine preparations

Isolates from different sites of individual patients as well as isolates from known consorts were tested for sensitivity to pyocines and antibiotics. A typical set of results is presented in table IV. Sensitivities to penicillin G, lincomycin, tetracycline, chloramphenicol, and spectinomycin were determined for each isolate with the same culture suspension that was used for determining pyocine sensitivities. These “matched” isolates usually had very similar pyocine patterns and identical patterns of sensitivities to the above antibiotics. We observed no correlation between patterns of pyocine sensitivities and sensitivity to any particular antibiotic.

In table V pyocine-sensitivity patterns for CDC and RIE strains are compared. There was a broader distribution of strains than that found by Sidberry and Sadoff (1977). Although there were differences in numbers per pyocine type in the two groups, there were only five small patterns—F, G, I, J and M—that were found among the CDC isolates but not the RIE ones.

Table VI presents pyocine-sensitivity patterns for strains from disseminated infections. There were no extraordinary sensitivities, e.g., to pyocines of Kageyama groups R2 or R3. Patterns of sensitivity were not associated with particular sites of isolation, penicillin sensitivity or auxotypes. The majority of strains from each of the three geographic regions had similar patterns.

Strains of *Neisseria meningitidis* from patients attending the Department of Venereology were also collected and several were tested for sensitivity to pyocines. Only two of those sent for typing were serotypable; strain 1042B was of serotype W135 and strain 1055A was of type Z'. The majority were serologically nontypable but were of different pyocine types. There were two patients from whom meningococci were isolated from genital sites. Strain 330B was

TABLE IV
Antibiotic and pyocine sensitivities of isolates from different sites of individual patients

Gonococcus isolate no.	Site	Inhibition by pyocines from producer strains										Minimal inhibitory concentration* of					
		ISD	2285	IS4	IS6	IS8	R205	ISE	R21	ISB	430	ISC	Pn	Lm	Cm	Tc	Sp
1045A B	Urethra Cervix	-	-	-	-	-	+	-	-	-	-	+	0.015	>32	0.8	0.1	8
1047A B	Urethra Cervix	+	-	+	+	-	+	-	-	-	-	+	0.015	>32	0.8	0.1	8
1048A B C	Urethra Cervix Rectum	+	-	+	+	+	+	-	-	-	-	+	0.015	>32	0.8	0.1	16
1049A B C	Urethra Cervix Rectum	+	-	-	+	-	+	-	-	-	-	+	0.125	>32	0.8	0.1	16
1051A B	Urethra Cervix	+	-	+	+	-	+	-	-	-	-	+	0.015	>32	0.8	0.1	16
1063A B	Urethra Cervix	+	+	+	+	-	+	-	-	-	-	+	0.015	>32	0.4	0.5	16
1078A B	Urethra Cervix	+	+	-	+	-	+	-	-	-	-	+	0.015	>32	0.4	0.25	16

+ = Inhibition; - = no inhibition.

* Minimal inhibitory concentration = $\mu\text{g/ml}$ except Pn = units/ml. Pn = penicillin G, Lm = lincomycin, Cm = chloramphenicol, Tc = tetracycline, Sp = spectinomycin.

TABLE V
Distribution of pyocine typing groups of *N. gonorrhoeae*

<i>N. gonorrhoeae</i> : pyocine- sensitivity pattern	Inhibition by pyocines from Kageyama group													Number of CDC strains (% of all CDC strains)	Number of RIE strains (% of all RIE strains)
	R1, strain						unclassified strain	R2, strain			R3, strain ISE	R5, strain ISC			
	ISD	2285	IS4	IS6	IS8	R205		ISB	R21	430					
A	-	-	-	-	-	-	-	-	-	-	-	-	3(4.8)	3(6.3)	
B	-	-	-	-	-	-	-	-	-	-	-	-	5(8.1)	1(2.1)	
C	-	-	-	-	-	-	-	-	-	-	-	-	1(1.6)	2(4.2)	
D	+	-	-	-	-	+	+	-	-	-	-	+	1(1.6)	1(2.1)	
E	+	-	-	-	-	+	+	-	-	-	-	+	2(3.2)	11(22.9)	
F	+	+	-	-	-	+	+	-	-	-	-	+	1(1.6)	0(0)	
G	+	-	+	+	-	-	-	-	-	-	-	+	3(4.8)	0(0)	
H	+	-	+	+	-	+	+	-	-	-	-	+	8(12.9)	7(14.6)	
I	+	-	±	-	±	+	+	-	-	-	-	+	1(1.6)	0(0)	
J	-	+	+	-	+	+	+	-	-	-	-	+	2(3.2)	0(0)	
K	+	+	+	+	-	+	+	-	-	-	-	+	5(8.1)	5(8.1)	
L	+	+	+	+	-	+	+	-	-	-	-	+	9(14.5)	6(12.5)	
M	+	+	+	+	+	+	+	-	-	-	-	+	2(3.2)	0(0)	
N	+	+	+	+	+	+	+	-	-	-	-	+	4(6.5)	10(20.8)	
O	+	+	+	+	+	+	+	-	-	-	-	+	15(24.2)	2(4.2)	
													Total 62	Total 48	

- = No inhibition; + = inhibition; ± = inhibition with some overgrowth. CDC = Center for Disease Control; RIE = Royal Infirmary Edinburgh.

TABLE VI
Pyocine typing of gonococci from disseminated infections

Auxotype	Source	Strain number	Site of isolation	Penicillin MIC (units/ml)	Inhibition by pyocines from Kageyama group											
					R1, strain			unclassified strain R205	R2, strain			R3, strain ISE	R5, strain ISC			
					ISD	2285	IS4		IS6	IS8	ISB			R21	430	
Protrophic	{Seattle Indianapolis Atlanta}	1071	Cervix	0.5	+	+	+	+	+	+	+	+	+	+	+	
		6302	Joint	0.015	-	-	-	-	-	-	-	-	-	-	±	
		6354	Cervix	0.125	-	-	-	-	-	-	-	-	-	-	±	
		7422	Urethra	0.03	+	+	+	+	+	+	+	+	+	+	+	
		7423	Blood	0.125	-	±	±	±	±	-	-	-	-	-	+	
<i>pro</i> ⁻	{Seattle Indianapolis Atlanta}	1947	Joint	1.0	+	+	+	+	+	+	+	+	+	+	+	
		6355	Cervix	0.015	-	-	-	-	-	-	-	-	-	-	+	
		7416	Cervix	0.015	-	-	-	±	±	-	-	-	-	-	+	
		7425	Blood	0.015	-	-	-	-	-	-	-	-	-	-	+	
		7432	Blood	0.06	-	-	-	±	±	±	±	-	-	-	±	
<i>arg</i> ⁻	Atlanta	7502	Blood	0.015	-	-	-	-	-	-	-	-	-	-	-	
		7503	Joint	0.015	-	-	-	-	-	-	-	-	-	-	-	
		7406	Cervix	0.015	-	-	-	-	-	-	-	-	-	-	+	
		Seattle	1567	Joint	0.015	+	-	-	-	-	-	-	-	-	-	+
			<i>arg</i> ⁻ , <i>hyx</i> ⁻ , <i>ura</i> ⁻	867	Joint	0.015	-	-	-	-	-	-	-	-	-	-
879	Cervix	0.015		+	-	-	-	-	-	-	-	-	-	-	+	
1241	Throat	0.015		-	-	±	±	+	+	-	-	-	-	+		
1255	Joint	0.015		+	+	+	+	+	+	+	+	+	+	+		
1341	Cervix	0.015		+	±	+	+	+	+	±	±	-	-	+		
1350	Urethra	0.015		+	-	±	±	±	±	+	+	+	+	+		
1362	Cervix	0.015		+	+	+	+	+	+	+	+	+	+	+		
1385	Blood	0.015		+	+	+	+	+	+	+	+	+	+	+		
<i>arg</i> ⁻ , <i>hyx</i> ⁻ , <i>ura</i> ⁻	Seattle	1402	Blood	0.015	+	±	±	±	±	+	+	+	+	+		
		1560	Blood	0.015	+	±	±	±	±	+	+	+	+	+		

+ = Inhibition; - = no inhibition; ± = inhibition with some overgrowth.

TABLE VII
Sensitivity of *N. meningitidis* to R-type pyocines

Serotype	Strain number	Site	Sulphadiazine MIC (mg/litre)	Inhibition by pyocines from Kageyama group									
				R1, strain			unclassified strain	R2, strain			R3, strain	R5, strain	ISC
				ISD	2285	IS4	IS8	ISB	R21	430	ISE		
W135	1043B	Throat	5-10	-	-	-	-	-	-	-	-	-	-
Z'	1055A	Throat	1-5	+	-	-	+	-	-	-	-	+	+
Nontypable	339B	Cervix	1-5	+	-	-	+	-	-	-	-	+	+
	1056A	Throat	1-5	-	-	-	-	-	-	-	-	+	+
	1057A	Throat	0.1-1	+	-	+	+	-	-	-	-	+	+
	1058A	Throat	0.1-1	+	-	+	+	-	-	-	-	+	+
	1071A	Throat	0.1-1	+	-	-	+	-	-	-	-	+	+
	1093A	Throat	1-5	+	+	+	+	-	-	-	-	+	+
	1096A	Throat	1-5	+	+	+	+	-	-	-	-	+	+
	9102A	Cervix	5-10	±	±	-	±	-	-	-	-	+	+
	B	Urethra	1-5	±	±	±	±	-	-	-	-	+	+
	C	Rectum	1-5	±	±	±	±	-	-	-	-	+	+
	D	Throat	1-5	±	±	±	±	-	-	-	-	+	+

+ = Inhibition; - = no inhibition; ± = inhibition with some overgrowth.

found in a cervical culture and strains 9102 A, B, C, and D from cervix, urethra, rectum, and throat respectively. The genital isolates from these two patients were non-typable serologically and had different pyocine types. Three of the four isolates from patient 9102 had similar patterns of pyocine sensitivity and similar sensitivities to sulphadiazine (1–5 mg/ml). Strain 9102A was less sensitive to sulphadiazine (5–10 mg/ml) than the other three isolates and had a different pyocine-sensitive pattern (table VII). Further studies are in progress with a larger group of meningococci from different serological groups and sources.

DISCUSSION

These results are additional evidence that tests for sensitivity of gonococci to R-type pyocines may provide a rapid, simple, inexpensive method for typing *N. gonorrhoeae* (Sidberry and Sadoff, 1977). Isolates from consorts or from different body sites of individual patients could generally be identified as being similar strains. Patterns of antibiotic sensitivities for these matched isolates agreed with the findings for pyocine sensitivities. Sensitivity to pyocines appears to be a stable characteristic. A fresh local isolate and a strain from a disseminated infection, both subcultured twice a week for over a year, have maintained their original patterns of sensitivity to the pyocines tested.

Preliminary results with organisms from several sources (CDC collection) and local isolates indicated that there is a broader distribution among the pyocine sensitivity types of gonococci observed than that found by Sidberry and Sadoff (1977) with their isolates from limited geographical locations. Using 23 pyocine lysates they found 30 types; but a majority of their strains 48% (51/106) fell into one group and 18% (19/106) into a second group. We found 15 types in tests with 11 pyocines and there are several more types to be defined with the additional pyocines being used in our present studies.

Table VIII presents an analysis of our results compared with those of Sidberry and Sadoff (1977). The analysis is based on the schematic representation of pyocine receptors in *Ps. aeruginosa* proposed by Kageyama (1975). It was observed in our study that if a gonococcal strain was sensitive to any of the pyocines, it was sensitive to ISC, a pyocine of Kageyama group R5; the gonococcal strains in both studies were most often sensitive to members of this group. Fewer strains were inhibited by pyocines of group R1 and the proportions of strains inhibited by this group of pyocines were similar in both studies. Our results differed in that we were unable to detect reproducible inhibition by R2 and R3 pyocines among the gonococcal strains tested. Crude lysates of R2 and R3 pyocines produced large S-type zones of inhibition on the "sensitive" (CDC486) and "resistant" (879) standard strains of gonococci, but the ultra-centrifuged preparations did not inhibit either strain. Because the preparations used in Sidberry and Sadoff's studies were equivalent to our crude lysates, some of the inhibition by R2 and R3 pyocines that they observed with a few gonococcal strains may have been due to the presence of material other than the R-type pyocines.

TABLE VIII

Inhibition of N. gonorrhoeae by pyocines of Kageyama groups R1-R5; comparison of results with those of Sidberry and Sadoff (1977)

Percentage of <i>N. gonorrhoeae</i> isolates of indicated strain inhibited by pyocines of Kageyama group				
R3*	R4*	R2*	R1*	R5*
I=9.4	H=21.8	D= 0.9 F= 2.8 J =16.4 U=...	K =74 L = 1.9 M=74.5 N=76.8 O =71.7 P =61.9 Q = 5.7 R =65.1 S =66.9 T =61.4	A=100 B=100 C=100 E= 99.1 G= 99.1
(Sidberry and Sadoff, 1977)				
ISE=0	...	ISB=0 R21=0 430=0	ISD =84.5 IS4 =72.7 IS6 =67.3 IS8 =32.7 2285=42.7	ISC=94.5
(This study)				

* Arranged in the order of the receptor sites on the lipopolysaccharide fraction of *Ps. aeruginosa* suggested by Kageyama (1975); the receptor for R5 is nearest to and that for R3 is furthest from the cytoplasm.

The receptor site for the R-type pyocines is in the lipopolysaccharide (LPS) (LPS) of *Pseudomonas* (Ikeda and Egami, 1973; Govan, 1974a; Koval and Meadow, 1977) and a similar location has been suggested for *Neisseria* (Sidberry and Sadoff, 1977). Table VIII is based on Kageyama's (1975) proposed pyocine receptor sites for his groups R1-R5 in the LPS of *Pseudomonas*. The receptor for R5 is in the portion of the LPS nearest the cytoplasm. The other receptors, in order towards the most distal portions of the LPS, are R1, R2, R4, R3. The "R" numbers correspond to sites thought to be essential for sensitivity to each pyocine; e.g., mutants lacking the receptor for R3 were resistant to pyocines of the R3 group but sensitive to those of groups R1, R2, R4, and R5. If the receptors for R-type pyocines in *Pseudomonas* and in gonococci are in the LPS, this analysis suggests a similarity in the "deeper" portions of the LPS of *Neisseria* and *Pseudomonas*. Other workers have also found that colony types T1 and T4 of the same strain showed no difference in sensitivity to the pyocine R-type 611, 131 (Morse *et al.*, 1976), indicating that pili probably do not form part of the receptor sites for the pyocines examined. The receptors for F-type pyocines are not found in the LPS fractions of *Pseudomonas* (Govan, 1974b) and, significantly, we did not find any inhibition of gonococci with the 10 F-type pyocines tested.

Gonococci from disseminated infections did not have unique pyocine sensitivities differing from those found for strains from localised infections.

The sensitivity of *Salmonella typhimurium* to some bacteriophages depends on the presence of receptor sites in the O repeat units of the LPS. Mutants that have lost these outer portions, rough or R-mutants, are less virulent than the smooth wild type. They are also insensitive to bacteriophages that attack the wild type because they lack the receptor (Rapin and Kalckar, 1971). If there were a unique portion in the outer LPS of the strains from disseminated gonococcal infection that resulted in serum resistance, or increased resistance to phagocytosis, it might be detectable with pyocines. Sensitivity was anticipated to pyocines of Kageyama groups R2 and/or R3, but no reproducible sensitivities were observed.

These findings are encouraging although further investigation with an emphasis on purification, standardisation and storage of the pyocine preparations used at present is clearly needed. Testing of other pyocines of the various Kageyama groups would be of value. Partially purified preparations of R2, R3 and R4 pyocines from other sources need to be examined to determine whether there are LPS receptors for these groups or whether the inhibitory effects reported by Sidberry and Sadoff (1977) were due to material other than the R-type pyocines. Pyocines that are "unclassified" by the Kageyama scheme may be useful because we have evidence for the selective inhibition of gonococci by pyocine R205 in the present study. In addition, selection of gonococcal indicator strains will be necessary.

Preliminary experiments indicate that this system may be able to differentiate strains of *Neisseria meningitidis* that are nongroupable by present serological methods.

SUMMARY

Strains of *Neisseria gonorrhoeae* from a variety of sources were examined for sensitivity to 11 partially purified R-type pyocines from *Pseudomonas aeruginosa*. Selective inhibition of gonococci by pyocines of Kageyama groups R1 and R5 was observed. "Matched isolates", those from consorts or different body sites of individual patients, usually had very similar pyocine-sensitivity patterns and identical sensitivities to five antibiotics tested. This study included local isolates, strains from diverse geographic regions, and strains from disseminated gonococcal infections. It also proposed a relationship between pyocine-receptor sites in the lipopolysaccharide of *Ps. aeruginosa* and *N. gonorrhoeae*. Topics needing further evaluation are discussed.

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