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Typing of *Pseudomonas aeruginosa*: comparison of the phage procedure with the pyocine technique

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SUMMARY

Two hundred and sixty strains of *Pseudomonas aeruginosa* were isolated from patients of the Asaf Harofe Government Hospital. The strains were typed by phage technique and 64 of them were also typed according to pyocine sensitivity. The two methods proved complementary, and reduced the number of untypable strains. Phage typing was performed with both routine test dilution (RTD) and more concentrated phage suspensions. The most reliable results were obtained at 100 RTD.

INTRODUCTION

Pseudomonas aeruginosa has become an agent of great importance in hospital-acquired infections during this decade. There may be several reasons for this, e.g. suppression of the normal intestinal flora by broad-spectrum antibiotics, therapeutic use of immunosuppressive drugs, the use of sophisticated medical instrumentation, and the presence of a larger number of at-risk individuals, especially infants and older people (Williams, Williams & Hyams, 1960; Ayliffe *et al.* 1965, 1966; Fierer, Taylor & Gezon, 1967; Hardy, Ederrer & Matsen, 1970). Many strains of *P. aeruginosa* are rather resistant to a wide range of antibiotic drugs (Lindberg, Curreri & Pruitt, 1970; Stone, Kalb & Joseph, 1971). In addition, *P. aeruginosa* seems to have an exceptional ability to survive and multiply in the hospital environment. *P. aeruginosa* has been recovered in hospitals from soaps, sinks, 'sterile' solutions, drinking water, and equipment for inhalation, as well as from food (Fierer *et al.* 1967; Moffet & Williams, 1967; Shooter, Gaga, Cook & Kumar, 1969; Weber, Werner & Matchnigg, 1971).

In order to locate successfully the source and vehicles of this infectious organism it is necessary to establish the relationship between all strains isolated. Three methods have been used to subdivide *P. aeruginosa* for epidemiological purposes: serotyping (Habs, 1957; Borst & De Jong, 1970), phage typing (Graber, Latta,

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Vogel & Brame, 1962; Sjöberg & Lindberg, 1968; Bergan, 1972), and pyocine typing (Wahba, 1963; Gillies & Govan, 1966; Govan & Gillies, 1969; Tagg & Mushin, 1971; Deighton, Tagg & Mushin, 1971).

The present communication is the first report from Israel on phage typing of *P. aeruginosa* strains. All isolates were from hospitalized patients. The results were compared with those obtained by pyocine typing.

MATERIALS AND METHODS

Organisms

Two hundred and sixty cultures of *P. aeruginosa* were isolated from 206 patients of the Asaf Harofe Government Hospital during the 9-month period from August 1971 to April 1972. The strains were identified by the following features: rapid positive oxidase reaction, production of pyocyanine and fluorescein, growth at 42° C. and absence of growth at 5° C.

Phage typing procedure

Phage typing was carried out with a set of 22 phages kindly supplied by Dr L. Sjöberg of the National Bacteriological Laboratory, Stockholm, Sweden. Growth of the propagating strains (PS) as well as propagation of phages was conducted on trypticase soy agar (TSA) as recommended by Sjöberg & Lindberg (1968). The routine test dilution (RTD) was determined as the highest dilution giving subconfluent lysis. All strains were examined at RTD and at 100 RTD, and half of them were also examined at 10 RTD and at 1000 RTD. Final phage patterns were obtained at 100 RTD. Phage reactions were estimated as follows: 5+ : confluent lysis; 4+ : almost confluent lysis; 3+ : more than 30 isolated plaques; 2+ : more than 20 isolated plaques; 1+ : less than 20 isolated plaques. Only reactions of 3+ and above were regarded as positive for typing purposes. Inhibition reactions were characterized by thinning of the bacterial growth in the area where the phage had been applied. Each strain was typed 2–3 times in order to confirm reproducibility.

Pyocine typing

The procedure described by Tagg & Mushin (1971) and Deighton *et al.* (1971) was used.

RESULTS

Phage typing of 260 strains of *P. aeruginosa* at RTD gave 233 (88·9 %) typable and 29 (11·1 %) untypable strains. When typed at 100 RTD the number of typable strains increased to 240 (92·3 %). Half of the strains were also typed at 10 RTD and 1000 RTD. Typing at 10 RTD yielded less typable strains than did 100 RTD. Table 1 shows that typing at 100 RTD gave a better differentiation of the strains than did RTD. The phage pattern 73 (RTD) was composed of some strains untypable with pyocine and others of pyocine type 13−−. At 100 RTD this difference corresponded to strains reacting with phage 352 and C 188 respectively. On the

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Table 1. *Phage typing of* Pseudomonas aeruginosa *at different RTD values compared with pyocine typing*

Pyocine group	Phage patterns		
	RTD	10 RTD	100 RTD
UT	73	73	7/73/352
UT	73	73	7/73/352
UT	73	73	7/73/352
UT	73	7/73	7/73/352
13^{--}	73	7/73/C188	7/73/C188
13^{--}	73	7/73/C188	7/73/C188
13^{--}	73	7/73/C188	7/73/C188
13^{--}	73	7/73/C188	7/73/C188
31^{--}	44/1214/C11/M4	24/44/1214/C11/F8/M4	24/44/73/1214/C11/F8/M4
31^{--}	12/4/C11/M4	24/44/1214/C11/F8/M4	24/44/73/1214/C11/F8/M4
31^{--}	44/1214/C11/M4	24/44/1214/C11/F8/M4	24/44/73/1214/C11/F8/M4
31^{--}	7/73/352/M4	7/73/352/C18/M4	7/16/24/44/68/73/352/C18/F7/F8/M4
31^{--}	7/73/352	7/73/352/C18/F8/M4	7/16124/44/68/73/352/C18/F7/F8/M4
31^{--}	7/73/352/M4	7/73/352/C18/F8/M4	7/16/21/24/44/68/73/352/C18/F7/F8/M4
11^{--}	7/73/352/F8	7/73/352/C18/F8	7/24/44/73/109/1194/352/C11/C18/F8
34^{--}	21/68/73	7/21/68/73/352/C18/F8	7/21/68/73/352/C18/F8
3^{+-}	7/68/73	7/68/73/352/C18	7/44/68/73/352/C188/F8
3^{++}	44/1214	44/1214	44/1214/C11
10^{++}	24/1214/M4	24/1214/C11/M4	24/1214/C11/M4
10^{++}	44/1214/C11	44/1214/C11	44/1214/C11
10^{++}	44/1214	44/1214	44/1214/C11
10^{++}	44/1214	44/1214	44/1214/C11
1, UC($^{-+-+-}$)	44/1214	44/1214/C11	44/1214/C11
1, UC($^{-+-+-}$)	44/1214/C11	44/1214/C11	44/1214/C11
1, h	44/1214/C11	44/1214/C11	44/1214/C11
1, h	UT	44/1214/C11	44/1214/C11
1, h	UT	UT	44/1214/C11
1, h	UT	UT	44/1214/C11
5^{--}	UT	UT	21/44/1214
5^{--}	1214	44/1214	21/44/1214
5^{--}	1214	44/1214	21/44/1214
5^{--}	24/68/M4	21/24/68/73/M4	21/24/68/73/109/F8/M4
5^{--}	21/24/68/M4	21/24/68/73/M4	21/24/68/73/109/F8/M4
1, b	M6	M6	21/M6
1, b	M6	21/M6	21/M6
1, b	M6	21/M6	21/M6
1, b	M6	M6	21/M6

UT = untypable.

other hand, one of three strains reacting with pyocine 5^{--} was untypable with phages at RTD, whereas two others reacted with phage 1214. This difference disappeared when 100 RTD of the phage was used. Also some of the apparent lytic differences inside the pyocine 31 type disappeared at 100 RTD, and might have been artificial. By typing at 1000 RTD the number of typable strains was not increased above that obtained at 100 RTD, but in some cases almost all phages

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Table 2. *Comparison of phage typing at* 100 *RTD with pyocine typing correlated with clinical data* (Pseudomonas aeruginosa)

Origin of isolate*	Ward	Date of isolation	Phage patterns	Pyocine group
U 1	44	14/9	21/F7	5^{--}
V 1	44	15/9	21/F7	5^{--}
P 2	93	3/9	21/24/68/73/109/F8/M4	5^{--}
P 2	93	8/9	21/24/68/73/109/F8/M4	5^{--}
P 2	93	9/9	21/24/68/73/109/F8/M4	5^{--}
R	42	26/8	21/44/1214	5^{--}
U 3	Amb.	4/11	21/44/1214	5^{--}
U 3	Amb.	9/11	21/44/1214	5^{--}
R	93	13/9	UT	1, h
P	93	19/9	44/1214/C11	1, h
R	37	22/9	44/1214/C11	1, h
R	15	12/10	44/1214/C11	1, h
R	28	8/11	44/1214/C11	1, h
R	40	31/8	44/1214/C11	1, h
R	37	1/11	44/1214/C11	1, h
R	37	10/11	44/1214/C11	1, h
R	15	12/10	44/1214/C11	1, UC($^{-+-+-}$)
R	37	13/10	44/1214/C11	1, UC($^{-+-+-}$)
P	42	7/10	44/1214/C11	17^{---}
R	27	17/8	44/1214/C11	17^{---}
R	28	23/8	44/1214/C11	10^{++}
R	40	16/9	44/1214/C11	10^{++}
B	44	30/9	44/1214/C11	10^{++}
R 4	15	5/10	21/68	10^{+-}
R 4	15	6/10	21/68	10^{+-}
R 4	15	7/10	21/68	10^{+-}
R 4	15	8/10	21/68	10^{+-}
U	93	30/8	UT	1, C
U	36	26/9	21/M6	1, b
U	36	28/9	21/M6	1, b
P	25	22/9	21/M6	1, b
B	37	22/9	21/M6	1, b
U	39	15/8	C18	1, b
U 5	36	23/12	C18/C188	1, b
P 5	93	9/12	C18/C188	1, b
P 5	93	10/12	C18/C188	1, b
P	30	9/12	C18/C188	14
B 6	26	26/9	7/73/C188	13^{--}
B 6	26	30/9	7/73/C188	13^{--}
B 6	26	1/10	7/73/C188	13^{--}
B	38	22/9	7/73/C188	12^{--}
R 7	42	13/9	7/73/352	UT
R 7	42	14/9	7/73/352	UT
R 7	42	15/9	7/73/352	UT
R	29	7/11	7/73/352	UT
R	28	31/12	UT	UT
P	42	7/9	2/7/21/68/73/352/C18/F8	UT
P	Opth.	7/9	2/7/21/68/73/352/C18/F8	34^{--}
U	43	4/8	2/7/21/68/73/352/C18/F8	3^{++}
U	30	25/10	2/7/21/68/73/352/C18/F8	3^{++}
R 8	30	5/9	7/16/21/44/68/73/352/C18/F7/F8/M4	29^{--}

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Table 2 (*cont.*)

Origin of isolate*	Ward	Date of isolation	Phage patterns	Pyocine group
R 8	30	6/9	7/16/21/44/68/73/352/C18/F7/F8/M4	29^{--}
U 9	30	3/11	7/16/24/44/68/73/352/C18/F7/F8/M4	31^{--}
U 9	30	16/11	7/16/24/44/68/73/352/C18/F7/F8/M4	31^{--}
U 9	30	31/2	7/16/21/24/44/68/73/352/C18/F7/F8 M4	31^{--}
U 10	40	5/9	24/44/73/1214/C11/F8/M4	31^{--}
U 10	40	6/9	24/44/73/1214/C11/F8/M4	31^{--}
U 10	40	7/9	24/44/73/1214/C11/F8/M4	31^{--}
P	36	10/9	UT	2^{--}
P	93	5/11	UT	2^{--}
R 11	40	7/9	UT	$UC8^{--}$
R 11	40	10/9	UT	$UC8^{--}$
U	93	30/8	UT	$UC^{--}(^{+++++-+-})$
P	42	14/11	UT	$UC^{+-}(^{++-++-++})$

Each number indicates a specific patient with more than one isolation.
* Key to column 1: B = blood, P = pus, R = respiratory, U = urine, V = vaginal.

gave strong lysis reactions at 1000 RTD. The number of inhibition reactions also increased considerably. Final phage patterns will therefore be presented as obtained at 100 RTD.

Pyocine typing was performed on 64 of the strains. The strains could be divided into 18 distinct types including one group of untypable strains. The same strains could be divided into 12 fairly distinct phage patterns and nine untypable strains. Differences in two or more lytic reactions were considered significant. By a combination of both procedures the 64 strains could be divided into not less than 26 types, as shown in Table 2. Of nine strains which were untypable with phage, eight were typed by the pyocine procedure. On the other hand, five of the six strains which were untypable by the pyocine method gave phage patterns. Only one strain was thus found untypable by both pyocine and phage procedures (Table 2). It should be pointed out that this strain was biochemically a typical member of *P. aeruginosa*.

We examined a total of 260 strains of *P. aeruginosa* by phage lysis (Table 3). If again differences in two or more lytic reactions are accepted as significant, this strain collection can be divided into 22 patterns, including 22 strains resistant to lysis by phage. Seven strains gave different patterns on repeated typing, and were therefore designated in the table as miscellaneous.

Twenty-eight (48·2 %) of the strains with phage pattern 44/1214/C11 were characterized by the development of a brown colour which usually started from the lysed area. This brown colour started developing in the incubator and its intensity increased when the culture was kept at room temperature for 5–6 hr. This reaction was typical and persistent when the strains were grown on TSA, whereas on synthetic medium (Sutter, Hurst & Fennia, 1963) the brown colour did not develop. In this survey the brown pigment was not observed among other strains.

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Table 3. *Phage pattern distribution of* P. aeruginosa *isolated from* 206 *patients of Asaf Harofe Government Hospital from August* 1971 *to April* 1972

Serial No.	Phage patterns	No. of patients	Source of strains: Urine	Respiratory	Skin	Blood	Vaginal	Faeces	No. of strains	%
1	119	5	3	1	3	—	—	—	7	2·6
2*a**	1214	5	3	—	1	3	—	—	7	2·6
2*b**	21	12	—	7	3	—	5	—	15	5·7
3	21/F7	6	5	—	1	—	1	—	7	2·6
4	21/M6	12	4	5	2	2	1	—	14	5·3
5*	21/1214	2	2	—	—	—	—	—	2	0·7
6	21/31/C18	6	—	2	4	2	—	—	8	3·1
7	21/1214/109/1198	4	2	2	—	—	—	—	4	1·5
8	21/44/1194/C18/F8	3	2	—	1	—	—	—	3	1·2
9	21/44/1214	3	2	—	1	—	—	—	3	1·2
10	21/44/68/109/1194/C18/F8	1	—	—	—	1	—	2	3	1·2
11	21/24/68/73/109/F8/M4	3	1	1	3	—	—	—	5	1·9
12	24/44/73/1214/C11/F8/M4	2	3	—	—	1	—	—	4	1·5
13	C18/C188	19	7	8	6	—	1	—	22	8·2
14	7/73/352	7	3	6	—	—	—	—	9	3·4
15	7/73/C188	2	—	—	—	4	—	—	4	1·5
16	7/16/21/C18	8	5	1	2	—	—	—	8	3·1
17	7/21/24/44/109/1194	4	2	—	2	—	—	—	4	1·5
18	7/73/352/1214/C18/C21	3	6	—	—	—	—	—	6	2·2
19	7/16/21/24/44/68/73/352/C18/F7/F8/M4	18	17	8	2	1	1	—	29	11·0
20	2/7/44/68/73/352/C18/F8	4	2	—	2	—	—	—	4	1·5
21	44/C18/F7	5	—	—	5	—	—	—	5	1·9
22	44/1214/C11	45	13	27	17	—	1	—	58	22·4
23	Untypable	20	7	8	4	3	—	—	22	8·6
24	Miscellaneous	7	5	1	1	—	—	—	7	2·6
	Total	206	94	77	60	17	10	2	260	100·0
	%		36·1 %	29·6 %	23·0 %	6·5 %	3·8 %	0·8 %		

* 2a, 2b and 5 may belong to the same group.

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DISCUSSION

According to the observations reported above, it is profitable to perform phage typing at 100 RTD of phage. The number of typable strains was higher than at RTD, there was a better differentiation of the patterns, and the homogeneity in reactions within certain types improved (Table 1). Sjöberg & Lindberg (1968) also found that the use of elevated concentrations of phage suspensions increased the number of typable strains, but with 1000 RTD they found a high incidence of inhibition reactions.

Typing of *P. aeruginosa* strains by two methods, such as pyocine typing and serotyping, has been proposed by Matsumoto, Tazaki & Kato (1968). However, several authors found serotyping of limited value as most strains fell into relatively few types (Hobbs, Gowland & Byers, 1964; Farmer & Herman, 1969). A combination of pyocine typing and phage typing was used by Farmer & Herman (1969). They first treated the strains with mitomycin C in order to obtain more distinct typing. By this procedure differences in sensitivity to the bacteriophages were frequently revealed; thus two strains which were epidemiologically identical varied in phage reactions, and phage lysis could therefore not be considered for typing. The typing method used in this study helped to overcome the limitations of each method. The fact that almost all isolates which were untypable by one method could be typed by the other might also be of some practical interest.

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