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Serotyping of *Pseudomonas aeruginosa* Isolates from Patients with Cystic Fibrosis of the Pancreas

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Pseudomonas aeruginosa isolates (173) from 144 patients with cystic fibrosis (CF) of the pancreas in seven hospitals were serotyped with the agglutination systems of Homma (1974) and Fisher et al. (1969). The two systems were complementary. Strains from CF patients were much less likely to furnish a stable type on repetitive typing tests than strains from other patients. This was related to the frequent occurrence of mucoid *P. aeruginosa* strains. The 173 strains were divided among 11 Homma serotypes. A single Homma type (type 8) capable of mucoid growth comprised 104 (60%) CF strains. Eight serotypes were detected in 77 strains from 48 CF patients in one hospital; three strains were detected in one hospital CF unit; and two strains were detected in each of five hospital CF units. The CF serotype comprised from 50 to 93% of CF strains in the seven hospitals. These *P. aeruginosa* strains dissociated in vivo as judged by mucoid and nonmucoid colonies on primary culture plates and continued to dissociate during subcultures. Both colony types were the same serotype. The tendency to regard colonial phenotypes (mucoid, nonmucoid, rough) as separate strains was erroneous. Repetitive typing with the two systems gave better results than a single system. The mucoid *P. aeruginosa* strain is probably spread from patient to patient, rather than acquiring its mucoid characteristic de novo in the CF patient. It is not known why the mucoid CF strain has a peculiar predilection for CF patients, nor why it generally loses the quality in culture but retains it indefinitely in the patient.

Slide agglutination of *Pseudomonas aeruginosa* isolates collected from seven hospitals has been done using two sets of antisera: the set of seven sera described by Fisher et al. (5) and the set of 16 sera reported by Homma et al. (9). Two more sera have since been added to the Homma system for a total of 18.

Serotyping *P. aeruginosa* strains is difficult at best (8, 11) and the present work was an effort to improve the technique.

The majority of patients with cystic fibrosis (CF) of the pancreas (mucoviscidosis) suffer recurrent pulmonary infections caused by *P. aeruginosa* (H. Y. Reynolds, A. S. Levine, R. F. Wood, C. H. Zierdt, D. C. Dale, and J. E. Pennington, J. Clin. Med., in press).

It has been reported (12, 13) and was confirmed in the present study than an unusual rate of colonial dissociation occurs in *P. aeruginosa* isolates. Dissociation was believed to occur in vivo as well as in vitro because of dissociant colonies on primary culture. Laboratories may assume these to be separate infection strains and report them as such. This study further tested this assumption for *P. aeruginosa* from CF patients and explored evidence for a predominant strain of mucoid *P. aeruginosa* in these patients.

P. aeruginosa isolates from other sources produce slime (6) under specialized conditions of growth. The mucoid strains reported here are characterized by the large quantity of overt slime produced under standard growth conditions. After 3 to 4 days of incubation the slime may drip into the cover of an inverted culture plate, or in an upright plate may flow to cover the entire surface of the agar medium.

MATERIALS AND METHODS

Cultures. Hospital sources for *P. aeruginosa* isolates from individual CF patients and the individuals supplying the cultures were: Case Western Reserve University, B. Boxerbaum; Hahnemann Medical College and Hospital, D. S. Holsclaw; Children's Hospital Medical Center-Oakland, Calif., J. Kelly; Saint Joseph Hospital-Orange, Calif., M. J. Carson; Children's Orthopedic Hospital and Medical Center-Seattle, Wash., J. M. Doctor; St. Christopher's Hospital for Children (SCHC)-Philadelphia, Pa., N. N.

Huang; and Clinical Center, National Institutes of Health (CCNIH). Unless noted, each isolate was from a different patient.

Sera. Sera prepared from lipopolysaccharide fractions of seven strains (5) were kindly supplied by M. W. Fisher and H. B. Devlin. A set of 16 sera (9) prepared from boiled whole cell antigens, with the immune globulin isolated and exhaustively cross-absorbed, was kindly supplied by J. Y. Homma.

Agglutination. Overtly mucoid strains were often nontypable. This condition usually disappeared after four to seven biweekly transfers on plated media. A few strains remained too mucoid to type and were excluded from the study. Confluent growth from one plate of Mueller-Hinton agar that had been incubated for 18 h at 32 C was collected with a dry swab and transferred to 2.0 ml of 0.1 M phosphate buffer, pH 7.3. On a glass slide with raised ceramic lines (1 by 2 cm), 1 drop of this suspension was delivered by capillary (1 mm inside diameter) followed by 1 drop of antiserum. The slide was tilted by hand for 2 min and read for agglutination, with the aid of a concave mirror and spotlight.

All of the tests were performed by one person (R.W.). Tests on individual cultures were repeated at least once, and strains with unusual types were tested enough times to confirm the type.

Bacterial suspensions used for agglutination reactions were used immediately. Such suspensions could not be successfully stored for reuse because of spontaneous autoagglutination.

RESULTS

Summarized in Table 1 are individual serotypes on 173 cultures from the seven widely separated hospitals. Of Homma serotypes, 104 cultures (60.1%) were type 8, with no other type exceeding 4% of the total. Thirty-eight (22%) isolates were Homma nontypable. Fisher serotypes were more widely distributed, and 83 (48%) were multitypes of two to four sera. Type 1 (12.6%) and type 6 (18.5%) comprised the bulk of the monotype strains, whereas 4, 6 (14.5%) and 1, 2, 4, 6 (19.1%) included most of the multitype strains. There were 28 (16.2%) nontypable strains.

In Table 2 serotypes are arranged according to hospital source with corresponding Fisher and Homma serotypes. Fisher sera, giving monotype reactions on *P. aeruginosa* strains from non-CF patients (unpublished data), gave 64.3% multitype combinations of types 1, 2, 4, and 6 with CF-derived cultures. Evidence for this may also be seen in Tables 3 and 4. Fisher sera were useful as adjuncts in typing because the multitypes were also characteristic of CF isolates and provided identification when Homma sera were nonreactive. Fisher sera reactions corresponding to Homma type 8 or Homma nontypable might be any of those seen in Table 1, as monotypes and multitypes of types 1, 2, 4, and 6, but not including types 3, 5, or 7.

When Fisher sera reactions were used to identify cultures nontypable with Homma sera, and these were added to Homma type 8 strains, the CF strain comprised an additional 34 cultures or 138 (80%) of the 173 cultures studied.

Table 3 shows typing of sequential isolates from four patients in the SCHC group. The typings are representative of the differences encountered and the benefit derived by using the two typing sets together. These differences are similar to the changes obtained on retyping

TABLE. 1. *Agglutination types for Homma and Fisher antisera of 173 P. aeruginosa isolates from 144 patients with CF of the pancreas*

Homma sera types	Fisher sera types														
	1	2	4	6	7	1, 4	1, 6	1, 2, 6	1, 4, 6	1, 2, 4, 6	2, 6	3, 7	4, 6	NT[a]	Percent (Homma)
1										3			1	3	4.0
2					1									1	1.2
4							1			1				2	2.3
5									1						0.6
6										1					0.6
7												1			0.6
8	19	2	3	15		1	5	3	7	17			16	16	60.1
10			3							1			1	2	4.0
13												3			1.7
15														1	0.6
16	1									1			1		1.7
1, 8				1											0.6
NT[a]		1		16					1	8	1	2	6	3	22.0
Percent (Fisher)	12.6	1.7	3.5	18.5	0.6	0.6	3.5	1.7	4.6	19.1	0.6	3.5	14.5	16.2	

[a] NT, Nontypable.

TABLE 2. *Agglutination types for Homma and Fisher antisera of 173 P. aeruginosa isolates from 144 patients with CF (by hospital)*

Hospital[a]	Agglutination type		No. of strains	% CF strains[b]	Hospital[a]	Agglutination type		No. of strains	% CF strains[b]
	Homma	Fisher				Homma	Fisher		
CHMC	8	1, 2, 4, 6	3[c]	80	SCHC	8	1, 2, 4, 6	10[c]	72
	8	1, 4	1[c]			8	1	6[c]	
	4	NT[d]	1			8	6	6[c]	
						8	NT	4[c]	
CWRU	8	NT	7[c]	82		8	4, 6	3[c]	
	8	4, 6	6[c]			8	1, 2, 6	3[c]	
	NT	6	1[c]			8	1, 4, 6	3[c]	
	2	7	1			8	4	2[c]	
	2	NT	1			8	1, 6	1[c]	
	10	4	1			NT	1, 2, 4, 6	7[c]	
						NT	6	5[c]	
HMCH	8	1	4[c]	92		NT	4, 6	4[c]	
	8	1, 6	3[c]			NT	2, 6	1[c]	
	8	1, 4, 6	1[c]			1	1, 2, 4, 6	3	
	8	4	1[c]			10	4	2	
	8	NT	1[c]			13	3, 7	2	
	8	2	1[c]			NT	3, 7	2	
	NT	4, 6	1[c]			1	4, 6	1	
	1		1			1	NT	1	
CCNIH	8	6	7[c]	90		5	1, 2, 4, 6	1	
	8	4, 6	6[c]			6	1, 2, 4, 6	1	
	8	1	5[c]			7	3, 7	1	
	8	NT	4[c]			10	4, 6	1	
	8	1, 4, 6	3[c]			10	1, 2, 4, 6	1	
	8	1, 2, 4, 6	1[c]			10	NT	1	
	8	2	1[c]			13	3, 7	1	
	8	4, 6	1[c]			15	NT	1	
	8	6	1[c]			16	1	1	
	NT	6	7[c]			16	4, 6	1	
	NT	1, 4, 6	1[c]			16	1, 2, 4, 6	1	
	NT	NT	3		SJH	8	1	3[c]	93
	1	NT	1			8	6	2[c]	
						8	1, 2, 4, 6	2[c]	
COHMC	8	1	1[c]	50		8	4, 6	1[c]	
	8	1, 2, 4, 6	1[c]			NT	6	2[c]	
	NT	6	1[c]			NT	1, 2, 4, 6	1[c]	
	4	1, 6	1			NT	4	1[c]	
	10	NT	1			NT	4, 6	1[c]	
	4	NT	1			4	1, 2, 4, 6	1[c]	

[a] CHMC, Children's Hospital Medical Center; CWRU, Case Western Reserve University; HMCH, Hahnemann Medical College and Hospital: COHMC, Children's Orthopedic Hospital and Medical Center; SJH, Saint Joseph Hospital.

[b] Total Homma type 8 CF strain, 60.1%; overall CF strain, 80%.

[c] CF strain.

[d] NT, nontypable.

CCNIH strains at regular intervals (Table 4).

Both Homma and Fisher sera were often nonreactive with freshly isolated mucoid CF strains. After long passage, CF strains, even though nonmucoid, had a tendency to become nontypable again, particularly with Homma sera. The mucoid isolate from a specimen is regularly the same strain as the nonmucoid dissociants accompanying it on a primary culture plate. This refers specifically to dissociant colonies, whether from primary culture plates or from subcultured plates, because a few patients may carry more than one serotype of *P. aeruginosa*.

In the CCNIH series there was no patient from whom mucoid and nonmucoid isolates were separate serotypes. In the 48-patient SCHC series, from whom 77 cultures were submitted for study, three serotypes were isolated from one patient and two serotypes from

TABLE 3. *Agglutination types (Homma and Fisher systems) of P. aeruginosa isolates cultured at different times from CF patients*

Patient	Isolate	Homma	Fisher
1	1	8	6
	2	8	1, 2, 4, 6
	3	8	6
	4	8	6
	5	8	1, 2, 6
	6	8	1, 2, 4, 6
2	1	8	4, 6
	2	8	4
	3	NT[a]	1, 2, 4, 6
	4	NT	6
	5	NT	1, 2, 4, 6
3	1	8	NT
	2	8	NT
	3	8	NT
	4	8	6
4	1	NT	NT
	2	8	1, 2, 4, 6
	3	8	6
	4	8	4, 6

[a] NT, Nontypable.

TABLE 4. *Agglutination reactions (Homma and Fisher systems) repeated at monthly intervals of P. aeruginosa strains from CF PATIENTS (CCNIH)*

P. aeruginosa strain	Typing	Homa	Fisher
1	1st	8	4, 6
	2nd	8	1, 2, 4, 6
	3rd	NT[a]	4, 6
2	1st	8	4, 6
	2nd	8	1, 4, 6
	3rd	8	1, 4, 6
3	1st	8	1
	2nd	8	6
	3rd	NT	6
4	1st	8	1
	2nd	8	1
	3rd	8	1

[a] NT, Nontypable.

another. But since the strains were isolated at different monthly intervals, it is not known whether these patients were infected coincidentally with more than one serotype.

A few of the 173 tested strains were mucoid but typed as separate strains from the predominant strain. As stated, the mucoid condition was noted as cultures were received, but an unknown number had already lost the characteristic. There was one distinct mucoid strain serotype from Hahnemann Medical College and Hospital (homma type 1, Fisher nontypable), one from Children's Hospital Medical Center (Homma type 4, Fisher nontypable), one from SCHC (Homma nontypable, Fisher type 3, 7), and none from Case Western Reserve University, Children's Orthopedic Hospital and Medical Center, Saint Joseph Hospital, and CCNIH. Thus, including the Homma type 8 mucoid strain, there were four mucoid strain serotypes.

Of the 11 Homma nontypable strains from CCNIH, eight were a Fisher type or pattern that corresponded to Homma type 8. As stated, strains from CF patients often become nontypable with Homma sera, especially after a long series of transfers on plated media. Considering this fact and invoking the Fisher sera reactions, none of the 11 Homma nontypable strains could be judged to be a strain different from Homma type 8. By additional evidence of repetitive typing reactions of these strains, it appeared that the 11 Homma nontypable strains probably were identical to the Homma type 8 strain. One of the 41 CCNIH strains was Homma type 1, Fisher nontypable. This nonmucoid strain was distinct. Including the predominant strain, there were 11 Homma serotypes among the 173 cultures.

DISCUSSION

Old laboratory strains may provide patterns or types markedly different from the original. A few strains that were initially too mucoid to type and remained mucoid were excluded from the study. Washing and/or trypsin digestion did not make them typable. The mechanism is not understood for loss of slime production when CF strains are transferred.

The reasons are also unknown for the predominance of a single mucoid strain in the respiratory tracts of CF patients, or why that strain, almost unique among human pathogenic strains, produces excess or overt slime.

A mucoid isolate from a patient is regularly the same serotype as the nonmucoid variants accompanying it on primary isolation plates. If a premise is to be made that the mucoid isolate is more pathognomonic than the nonmucoid, it should not be made on the assumption that the mucoid isolate is a different strain of *P. aeruginosa* from the nonmucoid, unless this is established through painstaking investigation. If a mucoid strain is apparently isolated in the absence of a nonmucoid strain, or vice versa, there cannot be a ready assumption that this strictly characterizes the infecting strain. When

careful effort is made to examine hundreds of isolated colonies, dissociation into both mucoid and nonmucoid colonies of a range of colonial morphology is the expected finding.

Homma type 8 *P. aeruginosa*, although the dominant mucoid strain among CF patients, was seen only in one of 233 isolated from other patients at CCNIH. This infection was in a patient with Wegener's granulomatosis who visited the CF ward frequently.

There are, in many hospitals, mucoid strains that infect non-CF patients, but this occurrence may also be based on two differences in observation. The first is that "smooth and moist" is interpreted as mucoid by some bacteriologists, and second that there may be little critical reexamination of cultures more or less casually designated as mucoid.

Final strain identification particularly of CF strains may be based on a judgment, considering phenotypic colonial characters, repetitive typing, and use of different serotyping sets. It might be argued that such identification is not valid, since there may not be exact numerical serotype correspondence between cultures that are judged to be the same strain. This technique seems more valid, however, than the ready acceptance of different numerical serotypes as identifying distinctly different *P. aeruginosa* strains, based on a single set of sera and on a single typing. This practice provides data that is often erroneous and epidemiologically misleading, whether the technique is that of serotyping, pyocine typing, or bacteriophage typing. The literature on surveillance of *P. aeruginosa* infections is replete with questionable data, because of strain typing results based on one typing experience per strain.

We have used pyocine typing by pyocine production of unknown *P. aeruginosa* strains on an 18-strain test panel described by Jones et al. (10). In our hands there was insufficient stability in repetitive typing to justify continued use of this system. Chadwick (2) has reported similar difficulty.

Bacteriophage typing in our hands (13) required repetitive typing as well as judgment decisions to relate *P. aeruginosa* strains from CF patients. Four key phage lytic reactions were observed with CF strains, accompanied by a number of other phage reactions that were inconstant.

It is evident that the antigens reacting with Homma sera are different from those reacting with Fisher sera. Failure of a strain to agglutinate with one system is more often than not coupled with agglutination by the other system.

Homma et al. (9) have described methods for colony selection to provide more stability in phage, pyocin, and serotyping of *P. aeruginosa*. These methods have helped to stabilize bacteriophage and serotyping in our laboratory. There are however antigenic changes that are not reflected in colonial appearance.

The question of whether the *P. aeruginosa* strains infecting CF patients become mucoid during infection or are transmitted from CF patient to CF patient as a unique mucoid strain remains unanswered. The latter hypothesis seems to acquire some favor because the predominant mucoid strain is a single serotype. If the former hypothesis were operative, postinfection conversion of antigenically distinct strains to the mucoid form would result in an equal number of mucoid serotypes infecting CF patients. This does not seem to be the case.

Cetin et al. (1) reported two (0.8%) of 242 *P. aeruginosa* strains from patients to be encapsulated (mucoid). Elston and Hoffman (4) reported that eight (1.7%) of 475 strains from patients were encapsulated. The number from CF patients, if any, was not reported. Doggett (3) found 12 (2.1%) mucoid strains in 560 clinical cultures. It was not reported whether more than one of the 12 isolates were from the same patients. As suggested by the one mucoid *P. aeruginosa* isolation (CCNIH) from a non-CF patient, it is quite possible that non-CF patients acquire mucoid *P. aeruginosa* from CF patients.

The unique ability of the Homma serotyping set to furnish strong, stable reactions with the difficult CF cultures made possible an adequate technique for strain identification.

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