Differentiation of *Proteus mirabilis* by Bacteriophage Typing and the Dienes Reaction

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A provisional typing schema based on sensitivity to 23 bacteriophages has been established for *Proteus mirabilis*. Seventy-three bacteriophages were isolated on strains of *P. mirabilis* (64), *P. vulgaris* (1), *P.morganii* (7), and *P. rettgeri* (1), but those isolated on *P. mirabilis* were the most useful in differentiating other strains of *P. mirabilis*. From the 73 phages studied, the best 23 were chosen by computer analysis for the provisional system, which was then used to study *P. mirabilis* infections in a 500-bed general hospital. All patient isolates for 19 months were saved and then compared by bacteriophage typing and the Dienes reaction in a retrospective study. There was evidence for only three instances of cross-infection or -colonization during this time. Bacteriophage typing was very sensitive in differentiating strains, since 200 strains were differentiated into 113 different lysis patterns and 94% were typable. The Dienes reaction was useful at times but often gave reactions that were difficult to read or that changed when the tests were repeated. The bacteriophages described by Schmidt and Jeffries were also evaluated and proved useful in combination with ours. The value of bacteriophage typing was clearly established, and work toward a standardized schema for *P. mirabilis* should continue.

Proteus mirabilis is one of the three most common species of *Enterobacteriaceae* isolated in clinical laboratories. It is well known as a pathogen in urinary tract infections and has been implicated in hospital outbreaks and cases of cross-infection (2, 4, 6, 8, 11, 15, 18, 20, 28, 31).

Several typing methods have been used to determine the epidemiology of hospital-acquired infections due to *P. mirabilis*. These include serological typing (5, 8, 19, 22, 24, 27), the Dienes reaction (2, 8, 9, 15, 26-29), bacteriocin production (27, 29), biotyping (2, 11, 15, 16, 18, 20, 29), antimicrobial susceptibility patterns (2, 6, 15, 19), and bacteriophage typing (2, 15, 17, 21, 23, 25, 29, 30). Each of these typing methods has been used with some success, either individually or in combination, but no method has gained wide acceptance.

Several typing systems based on bacteriophage sensitivity have been described for *Proteus* (15, 17, 21, 23, 25, 30), and the percentage of typable strains observed with these systems has varied from 48 to 84%. These studies suggested that a useful bacteriophage typing system could be developed if several difficulties could be overcome. The purposes of this study were to develop a bacteriophage typing schema

for *P. mirabilis* and to use it with the Dienes reaction (27) in determining the epidemiology of *P. mirabilis* in a 500-bed general hospital.

MATERIALS AND METHODS

Media. Trypticase soy agar (TSA), Trypticase soy broth (TSB), and motility test medium were obtained from BioQuest, Division of Becton, Dickinson & Co., Cockeysville, Md. MacConkey agar, triple sugar iron agar, Simmons citrate agar, and ornithine decarboxylase medium were from Difco Laboratories, Detroit, Mich. Hard TSA was prepared by adding 5 g of agar (Difco) to 1,000 ml of TSA. "Soft agar" for overlays contained 4 g of Oxoid ion agar no. 2 (Canalco, Rockville, Md.) in 1,000 ml of distilled water. All dilutions of bacteriophages and host strains were made in TSB and stored at 4 C, and all incubations were at 36 ± 1 C.

Proteus strains. Thirty-four *Proteus* strains, used in bacteriocin typing of *Proteus* (7), were obtained from A. G. Towers, Cross-Infection Reference Laboratory, Central Public Health Laboratory, Colindale, England. Four hundred isolates were from patients at a 500-bed community hospital and were isolated during 1970 to 1972. One hundred and seventeen additional strains of *P. mirabilis* were studied at the Center for Disease Control (CDC) and were from the following sources: 64 patient isolates sent by Henry Isenberg and Barbara Painter, Long Island Jewish Medical Center, New Hyde Park,

N.Y.; 14 patient isolates sent by Frank Kocka, Clinical Microbiology Laboratories, University of Chicago, Chicago, Ill.; and 39 isolates from the culture collection at CDC. All strains were stored in sealed tubes at room temperature without transfer.

Speciation of *Proteus* isolates. All isolates were phenylalanine positive and were identified at the University of Alabama according to Edwards and Ewing (10), from results of the following tests: hydrogen sulfide (H_2S) production on triple sugar iron agar, swarming on hard TSA, production of ornithine decarboxylase, production of indole, and growth on Simmons citrate. Of the isolates, 306 were identified as *P. mirabilis* and were typical: indole negative, swarm positive, ornithine positive, and citrate positive. The others were broken down as follows: 70 *P. morganii*, 21 *P. rettgeri*, and 7 *P. vulgaris*. Twenty-eight isolates did not fit the definition of any of the four species; these were called *Proteus* species.

Isolation of bacteriophages. The source of the bacteriophages was pooled raw sewage collected at the sewage treatment plant, Tuscaloosa, Ala. Samples (25 ml) were taken each day for 7 to 10 days and pooled. Four pools were used.

Bacteriophages were isolated by the enrichment technique of Adams (1). The host strain (1 ml of a stationary-growth-phase TSB culture) was added to 10 ml of TSB to which pooled raw sewage (2 ml) had been added. After overnight incubation, this enrichment mixture was treated with chloroform (10%, vol/vol) to kill the host cells and then assayed for bacteriophages. This was done by the soft-agar layer method described by Adams (1). Single, well-isolated plaques were picked for purification and cloned twice to insure purity.

High-titered preparations of the purified bacteriophages were obtained by inoculating 50 ml of TSB with an equal number of host cells and bacteriophages (1 ml). After these cultures were incubated overnight, the host cells were killed with chloroform (10%, vol/vol) and the bacteriophage titer was determined by the soft-agar layer method. The routine test dilution was also determined at this time. The routine test dilution was defined as the highest 10-fold dilution that gave semiconfluent lysis of its host strain. The bacteriophage suspensions were stored at 4°C over chloroform.

Twenty-six bacteriophages were isolated on the 26 different host strains of *P. mirabilis* obtained from Colindale. Phages were isolated on the hospital isolates as follows: 45 phages on 45 different isolates of *P. mirabilis*, 1 on *P. vulgaris*, 7 on 7 different *P. morganii*, and 1 on *P. rettgeri*. Thus, 82 phages were isolated.

Bacteriophage typing. Bacteriophages (at routine test dilutions) were dropped onto lawns of *P. mirabilis* isolates with the Accudrop applicator (12) (Sylvania Co., Milburn, N.J.). Lawns were prepared by flooding a dry hard TSA plate with 3 ml of a 1:10 dilution of a stationary-phase (24-h) TSB culture of the strain to be tested. Excess fluid was withdrawn with the aid of a safety pipettor, and the plates were dried with their tops off for 1 h, after which the

bacteriophages were applied. After the bacteriophage drops had dried, the plates were incubated 18 to 24 h and observed for lysis. An area of lysis with 10 or more plaques was defined as positive. The lysis patterns were converted to the code proposed by Farmer (13) (Table 1). This number was designated as the bacteriophage type.

Selection of bacteriophages for the final typing set. Eighty-two bacteriophages were tested against 306 *P. mirabilis* isolates in a series of four experiments. Results of these four experiments were analyzed by computer (14), and a provisional typing set, consisting of the best 23 bacteriophages, was selected.

Retrospective study in a 500-bed general hospital. The hospital is a city-county hospital and provides primary care to patients in about five counties. It has seven floors and 15 nursing units. The average patient is generally in the hospital for only a short time and has a private room; two patients share some rooms, but there are no wards.

This was a retrospective study. All isolates of *P. mirabilis* obtained from patients from September 1970 through March 1972 were saved as they were isolated. Tabulated data during this time suggested that *P. mirabilis* was not causing clusters of infections related by time and space. A total of 200 isolates was collected during the study period. Information, such as date, source, specimen, and hospital location, was provided with 191 of the 200 isolates, but a nurse epidemiologist was not available to make follow-up investigations. No isolations of swarming *Proteus* were reported from environmental samples during this period. All 200 isolates were typed during April and May 1973 with the set of 23 bacteriophages. In this study we defined "colonization due to *P. mirabilis*" to include both colonization and infection. Since our study was conducted from a bacteriological rather than a clinical standpoint, we found it impractical to differentiate between infection and colonization. We use the term "cross-colonization" when two patients appear to have acquired

TABLE 1. Simplified method for reporting bacteriophage lysis patterns^a

Phage reactions	Code
+++	1
++-	2
+ - +	3
- + +	4
+ - -	5
- + -	6
- - +	7
- - -	8

^a The reactions are divided into triplets and converted to numbers from the table. If the number of reactions is not evenly divisible by three, the following symbols are used to code the remaining reactions: ++ = A; +- = B; -+ = C; -- = D; + = E; - = F. Example: lysis pattern +-+ (=3) +++ (=1) -+- (=6) -+- (=6) -+- (=6) +++ (=1) --- (=8) - (=F) would be coded: 316 661 8F.

the same strain without bacteriological evidence of a common source.

Dienes reaction. The Dienes reaction was used to compare all isolates that had identical bacteriophage lysis patterns. Isolates were inoculated 6 cm apart on a hard TSA plate and incubated. After 18 to 24 h the waves of swarming were observed and the Dienes reaction was recorded (Fig. 1). Several of the strains had lost the ability to swarm when they were tested. One to four passages through motility medium restored the ability to swarm in 50% of these. When a Dienes reaction was difficult to read, the test was repeated on TSA with 5% (vol/vol) sheep blood. Subsequent studies on the Dienes reaction were done at CDC. All untypable isolates that were related by time or place of isolation were also compared by Dienes reaction.

Bacteriophages described by Schmidt and Jeffries (25). These 15 bacteriophages (13/3a, 36a, 36/34, Fr 2, 29/34a, 29/366, 34a, 34/36, Fr 5, 46, 4a, 31/39, 39, 21b, and 21c) were obtained from W. C. Schmidt and C. D. Jeffries, Department of Microbiology, Wayne State University School of Medicine, Detroit, Mich. Fourteen of the phages formed plaques on their host strains when tested on TSA. However, phage 13/3a did not form plaques because it is inhibited by NaCl in this medium (W. C. Schmidt, personal communication). Thus phage 13/3a was excluded from further study, since it was not active when used on our standard medium. These Schmidt-Jeffries phages (S-J phages) were studied at the CDC after most of the study had been completed.

RESULTS

Lysis of *P. mirabilis* by bacteriophages. Phages isolated on *P. morganii* and *P. rettgeri* rarely lysed *P. mirabilis*; there were only five

positive reactions out of 700 tests, so none of these eight phages was considered further. The one phage isolated on *P. vulgaris* lysed 56 of 255 isolates of *P. mirabilis*, but it was not included in the final typing set of 23. The 73 phages isolated on *P. mirabilis* varied in their ability to lyse strains of this species. Phage PM45 lysed only 1 of 255 strains of *P. mirabilis* and that was the strain on which it was originally isolated. In contrast, phage PM276 lysed 62.2% of the isolates it was tested against. The percentages of *P. mirabilis* lysed by the 73 phages are shown in Table 2. Almost one-half of the phages lysed fewer than 5% of the strains and thus would be of limited value in a sensitive typing system. Table 3 shows the final set of 23 phages chosen, with the aid of computer analysis (14), as the most sensitive in differentiating isolates of *P. mirabilis*. Figure 2 shows two isolates with identical lysis patterns and one isolate with a different pattern.

Dienes reaction. Figure 1 shows a positive and negative Dienes reaction. After a few hours all three *Proteus* isolates began to migrate from their original points of inoculation (shown as dots) as a series of "waves of swarming." In Fig. 1 there is a zone of inhibition between the last waves of isolate 1 and isolate 3. This is defined to be a positive Dienes reaction (Dienes⁺) and in the past has been used to mean "isolate 1 and isolate 3 are probably not the same strain in an epidemiological or genetic sense." In contrast, isolates 1 and 2 have no zone of inhibition between the last waves of swarming. This is de-

FIG. 1. Dienes reaction on blood agar (left) and hard TSA (right); isolates 1 and 2 are Dienes⁺ with isolate 3 but are Dienes⁻ with each other.

TABLE 2. Differences in the ability of the 73 bacteriophages (isolated on *P. mirabilis*) to lyse 306 isolates of *P. mirabilis*

Isolates lysed (%)	No. of phages
0-4.9	36
5.0-9.9	9
10.0-14.9	9
15-19.9	4
20-29.9	3
30-39.9	5
40-49.9	5
50-59.9	1
>60	1

fined to be a negative Dienes reaction (Dienes⁻), and its meaning has been "isolates 1 and 2 are probably the same strain."

We had intended to do the Dienes reaction on all pairs of isolates having the same bacteriophage lysis pattern (279 possible pairs); however, 21 of these test pairs had one member that did not swarm, so a comparison was impossible. Of the 258 Dienes reactions done, 242 were Dienes⁺ (meaning isolates were not the same strain, even though they had the same phage lysis pattern). Isolates with the same lysis pattern were almost always separated by time and space, so in this study the Dienes reaction was usually in agreement with epidemiological findings. Our conclusion was that the isolates had the same phage lysis pattern by chance alone; however, we could not conclusively exclude an undetected epidemiological link (such as colonization resulting from hospital food). Fifteen pairs had a negative Dienes reaction; but 12 of these were isolates from the same patient, so the result was expected (Table 4). However, three pairs with identical phage lysis patterns also had a negative Dienes reaction (indicating they are the same strain). Table 4 shows the pertinent data for these isolates.

Bacteriophage typing and the Dienes reaction in the study of infections due to *P. mirabilis*. The results from three critical hospital areas, the intensive care unit, the nursery, and a typical nursing unit, are shown in Table 5. In the intensive care unit there were no clusters of *P. mirabilis* isolates related in time. The results from bacteriophage typing were in agreement with this finding. Strains from different patients all had different lysis patterns. Three isolates were available from patient 228, and he seemed to have acquired a different strain in January 1972 from the one he had in November 1971 (Table 5).

In the newborn nursery *P. mirabilis* was not frequently isolated. There was only one instance where probable cross-colonization oc-

curred. Patients 225 and 226 acquired *P. mirabilis* with identical bacteriophage lysis patterns. However, their two isolates had a positive Dienes reaction, suggesting nonidentity. Isolates 225A and 225B from the same patient were Dienes⁺ originally but were Dienes⁻ when retested. Because of these apparent difficulties with the Dienes reaction, we placed more emphasis on bacteriophage typing, called isolates 225 and 226 the same strain, and counted this as a case of cross-colonization.

In nursing unit 2 South, *P. mirabilis* was seldom isolated. There were no proven cases of cross-colonization, but isolates 227 and 232 were both untypable. The sources of these isolates were two patients who shared the same room and whose stool cultures were both positive within 4 days. The Dienes reaction should have clarified this situation; instead, it produced equivocal results that could not be scored as positive or negative. Because of the epidemiological link and rarity of nontypable isolates, we considered these two isolates to be the same strain.

Cross-infection in the hospital's nursing units. The lysis patterns of all isolates in a particular nursing unit were compared with the others, and these data are summarized in Table 6. In several instances two isolates from different patients had the same lysis pattern

TABLE 3. Twenty-three bacteriophages chosen for the final typing schema

New phage designation	Old phage designation	<i>P. mirabilis</i> host strain	% of <i>P. mirabilis</i> strains lysed
R 1	1002	1002	3.8
R 2	1003	1003	49.7
R 3	1006	1006	43.4
R 4	1013	1013	4.4
R 5	1017	1017	38.8
R 6	1022	1022	36.3
R 7	1029	1029	7.2
R 8	11A	11A	15.7
R 9	27	27	5.4
R 10	29	29	9.8
R 11	41	41	5.1
R 12	48B	48B	13.8
R 13	73	73	10.3
R 14	75	75	35.5
R 15	81D	81D	36.1
R 16	82	82	8.4
R 17	134	134	19.3
R 18	135	135	23.2
R 19	173	173	51.5
R 20	193B	193B	17.0
R 21	195	195	44.4
R 22	196	196	28.3
R 23	275	275	17.8

FIG. 2. Isolates A and B have identical lysis patterns against 12 bacteriophages, but isolate C has a different pattern.

but a positive Dienes test; thus, the results on the identity of the two isolates conflicted. When results of the Dienes reaction conflicted with phage typing, we placed more emphasis on time and space relationships between the patients.

Phage typing of *P. mirabilis* from three different hospitals. Up until this point all work had been with isolates from a single hospital. At CDC we used the provisional set of 23 phages to type isolates from two additional hospitals and isolates from the CDC culture collection. Table 7 shows that the phages typed 116 of 126 of these isolates (92%). Of the recent clinical

isolates 78 of 82 (95%) were typable. In addition, we tested the S-J phage set with our standard conditions and found that 12 of the 14 phages lysed at least one of the strains, but only 25 of 126 strains (20%) were lysed by this set of phages. However, some of the S-J phages were quite useful in combination with our set.

DISCUSSION

Many hospital patients become infected or colonized with *P. mirabilis* during their stay in hospitals. There are several possible sources of this organism: the patient's own flora, the envi-

TABLE 4. Strains that had the same bacteriophage lysis pattern and a negative Dienes reaction

Isolate	Bacteriophage lysis pattern	Nursing unit	Room	Specimen	Date
34	418 645 5D	2-South	290	Wound	10-29-70
35	418 645 5D	6-Main	607	Abdominal wound	10-29-70
78A ^a	448 678 8D	1-Main	128	Stool	2-16-71
78B	448 678 8D	1-Main	128	Stool	2-16-71
137	418 645 5D	5-South	570	Urine	6-29-71
138	418 645 5D	1-Main	114	Abscess	7-3-71
139A	418 645 3D	3-Main	331	Stool	7-4-71
139B	418 645 3D	3-Main	331	Stool	7-4-71
139C	418 645 3D	3-Main	331	Umbilical cord	7-4-71
152A	448 788 8D	7-South	762	Urine	7-18-71
152B	448 788 8D	7-South	762	Urine	8-7-71
166B	585 645 1D	7-South	777	Wound	8-18-71
166C	585 645 1D	7-South	777	Wound	8-18-71
207	785 488 8D	2-South	288	Urine	10-4-71
208	785 488 8D	Out-patient	None	Stool	10-6-71
225A	448 888 3D	Nursery		Stool	11-8-71
225B	448 888 3D	Nursery		Stool	11-8-71
228B	448 678 8D	Intensive care	205	Rectum	1-12-72
228C	448 678 8D	Intensive care	205	Abdominal incision	1-19-72
245A	855 645 3D	4-Main	422	Stool	12-16-71
245B	855 645 3D	4-Main	422	Stool	12-16-71
263A	465 768 8D	3-South	378	Urine	1-30-72
263B	465 768 8D	3-South	378	Urine	1-30-72
270A	418 845 8D	3-South	367	Urine	2-9-72
270B	418 845 8D	3-South	367	Urine	2-9-72
276A	858 645 5D	Intensive care	207	Sputum	2-19-72
276B	858 645 5D	Intensive care	207	Stool	3-2-72

^a Multiple isolates from the same patient have the same number with an A, B, or C following it.

ronment, other patients, the hospital staff, and hospital food. Although several hospital outbreaks have been studied (2, 3, 7, 16, 18, 28, 32), relatively little is known about the ecology of colonization and infection with *Proteus*.

Perhaps more different methods have been tried for epidemiological surveillance of *Proteus* infections than for any other organism. The methods include serological typing, the Dienes reaction, bacteriocin production, antibiotic sensitivity, biochemical characteristics, and bacteriophage typing.

Serological typing has been used (5, 8, 19, 22, 24, 27), and the schema described by Perch (22), for both *P. vulgaris* and *P. mirabilis*, includes 49 "O antigens" and 19 "H antigens." Rustigan and Stuart (24) studied the biochemical and

serological relations of the genus *Proteus* but encountered heterogeneity. They concluded that serotyping of *Proteus* would be a tremendous task.

The Dienes phenomenon was first described in 1946 by Louis Dienes (9). It has been suggested that isolates are the same strain if they give no line of demarcation (Dienes⁻) as two advancing swarms meet. Those that have a mutual line of inhibition are different strains. Story (28) and de Louvois (8) concluded that swarming isolates that were Dienes⁺ were different but that the opposite was not always true. This test has been applied along with other typing systems in investigating cross-infections with *P. mirabilis*.

Epidemiological differentiation by antibiotic

TABLE 5. Comparison of isolates by bacteriophage typing in three nursing units of the hospital^a

Nursing unit	Date	Patient code	Room no.	Specimen	Bacteriophage lysis pattern
Intensive care unit	12- 7-70	51	202	Trachea	418 445 5D
	6-14-71	128	203	Suture site	418 645 3D
	7-12-71	144	204	Wound	448 868 3D
	10-19-71	214	203	Urine	418 845 8D
	11-14-71	228A	205	Sputum	448 545 3C
	12- 7-71	243	203	Sputum	418 545 3C
	12-22-71	247	203	Sputum	418 845 8D
	1- 8-72	255	203	Trachea	858 611 8D
	1-12-72	228B	205	Rectum	448 678 8D
	1-19-72	228C	205	Incision	448 678 8D
	2-17-72	274	208	Trachea	858 611 5D
	2-25-72	279	203	Sputum	858 845 5D
	2-29-72	280	200	Urine	448 434 8D
	2-19-72	276A	207	Sputum	858 645 5D
	3- 2-72	276B	207	Stool	858 645 5D
Nursery	10-24-71	216		Cord	417 613 8D
	11-12-71	223		Cord	118 645 5D
	11- 8-71	225A		Stool	448 888 3D
	11- 8-71	225B		Stool	448 888 3D
	11-11-71	226		Stool	448 888 3D
	11-20-71	235		Stool	418 545 1C
	11-27-71	240		Stool	415 745 5D
2-South	1- 4-72	254		Rectum	888 888 8D
	10-29-70	34	290	Wound	418 645 5D
	11-11-70	38	274	Urine	148 665 4D
	6-10-71	124	272	Urine	418 545 3C
	10- 4-71	207	288	Urine	785 488 8D
	11-13-71	227	270	Stool	888 888 8D
	11-18-71	232	270	Stool	888 888 8D

^a Multiple cultures from the same patient have the same number with an A, B, or C following it.

TABLE 6. Detection of cross-colonization by bacteriophage typing and the Dienes reaction

Nursing unit	Isolates of <i>P. mirabilis</i>	In-stances of cross-coloniza-tion ^a	Isolates with same phage lysis pattern	Untypa-ble iso-lates
1-South	16	1	0	2
1-Main	17	0	0	1
Intensive care	13	0	2	1
2-South	6	1	0	2
3-South	19	0	4	1
3-Main	4	0	0	0
4-Main	5	0	0	0
4-South	11	0	0	0
5-Main	11	0	0	0
5-South	14	0	0	2
6-Main	11	0	4	1
6-South	2	0	0	0
7-Main	22	0	4	2
7-South	22	0	4	0
Nursery	8	1	2	1
Out-patient	5	0	2	0
Emergency room	5	0	2	2

^a This decision was based on phage typing, the Dienes reaction, and how closely the cultures were related in time.

TABLE 7. Phage typing of *P. mirabilis* isolates from three additional sources

Source	No. of iso-lates	No. typable	% Typable
CDC collection	44	38	86
Long Island Jewish Medical Center	68	64	94
University of Chi-cago	14	14	100

sensitivity has been useful in one outbreak (6) where the minimum inhibitory concentration of tetracycline was determined. However, France and Markham (15) could not differentiate strains by their antibiograms, and Adler et al. (2) could make only crude and qualitative comparisons with susceptibility data.

The bacteriocin typing system described by Cradock-Watson (7) has been useful in two studies (2, 7), but it is generally believed that additional indicators are needed to increase its sensitivity. In a study by Tracy and Thomson (29) none of the strains was typable by the Cradock-Watson method. The same 200 isolates

of *P. mirabilis* used in this study have been tested by Dianna Presley (personal communication) for bacteriocin production by a modification of the method described by Farmer (14), and 90% of the isolates were typable. Biotyping has been feasible only when atypical reactions occur (15, 16, 18, 20).

Several systems of bacteriophage typing have been developed. The one used by France and Markham (15) was 81% effective when used with biotyping, but the 229 isolates fell into only 10 phage types. This same set was evaluated by Adler et al. (2), who found that 72% of 185 isolates were typable, but 50% of them had the same lysis pattern. Popovici and Ghioni (23) typed 48% of 100 isolates tested. Izdebska-Szymona et al. (17) typed 80% of 305 isolates of *P. mirabilis*. Vieu (30) divided 82% of 90 isolates into 10 bacteriophage types. Pavlatou et al. (21) used 15 bacteriophages and divided 213 strains into 14 phage types. Schmidt and Jeffries (25) developed a typing schema for *P. mirabilis*, *P. vulgaris*, and *P. morganii*. With this system 84.1% of 208 isolates were lysed by at least one bacteriophage. The bacteriophage typing system we developed in this study was made up of 23 bacteriophages, and 94% of 200 *P. mirabilis* isolates were lysed by at least one bacteriophage. This is the highest percentage reported thus far. These isolates were divided into 113 different bacteriophage types, and 89 of these types consisted of only one isolate. This is in contrast to the poor sensitivity found by Adler et al. (2); they reported that 50% of the isolates were the same bacteriophage type. Likewise, the isolates tested by France and Markham (15) fell into 10 bacteriophage types. The 15 bacteriophages used by Schmidt and Jeffries (25) divided 189 isolates into 21 phage types.

We used the S-J phages along with ours to type strains from three new sources. However, all typing was done on TSA, a medium quite different from the electrolyte-deficient nutrient agar described by Schmidt and Jeffries (25). This no doubt accounts for the low percentage of typable strains observed with the S-J phage set. In addition, their most useful phage, 13/3a, was excluded because it was not active when tested on TSA. Thus the 20% typable observed with the S-J phages was a result of our specialized conditions, and their observed value of 86% typable better reflects the value of this phage set. Computer analysis of all the phages studied indicated that two of the S-J phages would be chosen in a final set of the best 12 typing phages. In the future we hope to establish a standardized phage typing system for *P. mirabilis* and distribute it to other interested investigators.

We found bacteriophage typing to be a very useful technique in determining the epidemiology of infections due to *P. mirabilis*. In the study hospital, *P. mirabilis* was frequently isolated, but bacteriophage typing indicated that most of these isolates were not related. Many colonizations/infections were probably endogenous, as others have shown.

The Dienes reaction was quite useful at times but sometimes gave reactions difficult to interpret. Most of the isolates not lysed by any of the 23 bacteriophages (and thus indistinguishable by bacteriophage typing) had a positive Dienes reaction, which indicated that they were different strains. These Dienes⁺ isolates were usually separated in time and location, so the Dienes reaction was in agreement with epidemiological data. Thus we gave more weight to the positive Dienes reaction and concluded that the isolates were different strains. In other instances where the Dienes reaction could have clarified the situation, the test could not be scored as positive or negative. Our conclusion is that the Dienes reaction can be useful in the clinical microbiology laboratory. However, the fact that it sometimes gives false-positive results when a strain is tested against itself warns that the results should not be taken as absolute. The Dienes reaction can be more useful if it is used in conjunction with other typing methods.

Typing methods have their place in the surveillance of *Proteus* infections. We plan to evaluate bacteriophage typing, antibiograms, and the Dienes reaction for differentiating strains of *P. mirabilis*. If documented outbreaks or unusual infection problems due to *P. mirabilis* or *P. vulgaris* occur, we can offer the above typing services to assist those doing the epidemiological investigation. However, before any cultures are sent, arrangements for *Proteus* typing should be made directly with one of us at CDC.

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