Bacteriophage Typing of *Proteus mirabilis*, *Proteus vulgaris*, and *Proteus morganii*

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A bacteriophage typing scheme for differentiating *Proteus* isolated from clinical specimens was developed. Twenty-one distinct patterns of lysis were seen when 15 bacteriophages isolated on 8 *Proteus mirabilis*, 1 *P. vulgaris*, and 1 *P. morganii* were used to type 162 of 189 (85.7%) *P. mirabilis* and *P. vulgaris* isolates. Seven phages isolated on 3 *P. morganii* were used to type 13 of 19 (68.4%) *P. morganii* isolates. Overall, 84.1% of the 208 isolates were lysed by at least 1 phage at routine test dilution (RTD) or 1,000 × RTD. Fifty isolates, retyped several weeks after the initial testing, showed no changes in lytic patterns. The phages retained their titers after storage at 4°C for several months. A computer analysis of the data showed that there was no relationship between the source of the isolate and bacteriophage type. This bacteriophage typing system may provide epidemiological information on strains involved in human infections.

In recent years, *Proteus* has been recognized more frequently as a cause of many hospital-acquired infections (13). Since *Proteus* commonly develop resistance to antibiotics (27), such infections represent a serious therapeutic problem. The sources of these infections have not been established but may be due to autoinfection from the gut (10) or cross-infection in the hospital. Distinguishing between strains involved in epidemics and determining their source will remain nearly impossible until a simple and accurate typing system is available (27).

In the past, several methods have been used to differentiate strains of *Proteus*. These methods include serological typing (17, 20, 21), biochemical characterization (8, 10, 27), antibiotic susceptibility patterns (5, 9, 10), the Dienes reaction (2, 7, 22, 24), bacteriocin typing (6), and bacteriophage typing (10, 16, 18). Previous bacteriophage typing systems have not been sensitive enough, but most investigators concluded that additional research on a basic set of typing phages would improve sensitivity and result in a useful system.

The purpose of this study was to establish a collection of bacteriophages which could be used for the differentiation of *Proteus* strains in epidemiological studies.

**MATERIALS AND METHODS**

**Media.** The broth medium used in this study was nutrient broth (Difco). The solid medium, consisting of 0.5% peptone (Difco), 0.3% beef extract (Difco), 0.002 M CaCl₂, and 1.5% agar (Difco), was sterilized by autoclaving. The soft agar was the same except that the agar concentration was 0.75%. This agar medium is referred to as electrolyte-deficient nutrient agar (EDNA) since the electrolyte, sodium chloride, was omitted to prevent swarming of the organisms (14). All media were autoclaved at 121°C for 15 min, and all temperatures of incubation were 35°C.

**Cultures.** During 1971, 202 clinical isolates of *Proteus* were obtained from inpatients at Metropolitan Hospital in Detroit, and 8 additional strains were obtained from Hutzel Hospital in Detroit. Specimen type, age, and sex of the patient were noted whenever possible for each isolate. These isolates were identified as *Proteus* by a positive urease test in urea broth (Difco) and a positive phenylalanine deaminase test on phenylalanine agar (Difco). The species of each isolate was determined by the following additional biochemical tests: H₂S production on triple sugar iron agar (Difco), citrate utilization on Simmons citrate agar (Difco), ornithine decarboxylation in decarboxylase medium base (Difco) with 0.5% L-ornithine added, indole production at 24 h in tryptone broth (Difco) using Kovac reagent, gelatin liquefaction in nutrient gelatin (Difco), and acid production from glucose (1%), lactose (1%), mannitol (1%), sucrose (1%), and maltose (0.5%) in purple broth base (Difco). These media were prepared according to the manufacturer's instructions. Stock cultures were maintained on tryptic soy agar slants (Difco) at 4°C, and transfers were made every 3 or 4 months.

The API system (Analytab Products, Inc., New York) of 20 biochemical tests for the identification of the *Enterobacteriaceae* (23, 29) was used in conjunction with the conventional tests. All inoculations and tests were carried out as recommended by the manu-
facturer. Nitrate reduction was determined by adding 1 drop each of sulfanilic acid (0.8% in 5 N acetic acid) and alpha-naphthylamine (0.5% in 5 N acetic acid) to the glucose cupule. Negative reactions were confirmed by testing for nitrate reduction in nitrate broth (Difco) in screw-cap tubes with the same test reagents. Negative tests were examined for 4 weeks. The presence of nitrate in negative tests was confirmed by the addition of zinc dust (8). The following American Type Culture Collection strains were used in the study: Escherichia coli 14849; Shigella dysenteriae 13313; S. flexneri 12661; S. boydii 9207; S. sonnei 9290; Edwardsiella tarda 15847; Salmonella typhi 19430; S. enteritidis 13976; S. enteritidis bioser Paratyphi-A 11511; Arizona hinshawii 13314; Citrobacter freundii 8090; Klebsiella pneumoniae 13883; Enterobacter aerogenes 13048; E. cloacae 13047; Serratia marcescens 13880; and Providencia alcalifaciens 9886. Also, 42 strains of Proteus mirabilis, 13 strains of P. vulgaris, 15 strains of P. morganii, 13 strains of P. rettgeri, 15 strains of Providencia alcalifaciens, and 13 strains of P. stuartii supplied by Betty R. Davis were used.

Bacteriophages. Eighteen phages were isolated from sewage by the enrichment technique of Adams (1). Samples of unchlorinated sewage effluent from four metropolitan Detroit sewage treatment plants were pooled before use in the enrichment technique. A 10-ml sample of pooled sewage was added to log-phase cultures of potential hosts in 50 ml of nutrient broth. After overnight incubation at 35 C, 50-ml samples of each enrichment were centrifuged at 5,000 x g for 15 min, and a few drops of chloroform were added to each supernatant. Phage activity was tested for by spotting each supernatant onto lawns of potential hosts on well-dried EDNA plates. After 6 to 8 h of incubation at 35 C, zones of complete or partial lysis, or individual plaques, were cut from the EDNA plates and placed in 4 ml of nutrient broth. Serial 10-fold dilutions were made from each supernatant. Two drops of a 4-h log-phase culture (3.5 x 10^4 colony-forming units) of the suspected host and 0.1 ml of each dilution were added to 3 ml of molten soft agar at 45 C. The mixture was poured onto an EDNA plate, and the plates were incubated for 6 to 8 h at 35 C. Eighteen plaques with morphological differences were picked with sterile capillary pipettes, and each was placed in 4 ml of nutrient broth. Three successive (single plaque) pickings from soft agar overlays were used to purify each of the 18 phages. Two additional phages, P1 and P5, were obtained from Diana Martin. All phage suspensions were stored at 4 C.

Propagation and titration of bacteriophages. A modification of the soft agar layer method of Swanson and Adams (26) was used for the propagation of the phages. Soft agar overlays were made, as previously described, with serial 10-fold dilutions of the cloned phages. The dilution giving nearly confluent lysis was determined and then used to prepare four identical soft agar overlays. Each overlay was covered with 5 ml of sterile nutrient broth, and, after standing for 4 to 6 h at room temperature, the broths were removed from the overlays and pooled. The pooled lysates were centrifuged at 5,000 x g for 15 min to remove the bacterial debris and were then stored at 4 C over a few drops of chloroform. Each phage preparation was assayed with soft agar overlays by using serial 10-fold dilutions of the lysates. The highest dilution which just failed to give confluent lysis on the propagator strain was defined as the routine test dilution (RTD) for the typing procedure (4). Only dilutions of 10^-3 or greater were used as RTD in the typing procedure.

Phages were designated by numbers and letters. Phage 4a, for example, was the first phage type isolated from the sewage enrichment of P. mirabilis strain number 4. Phage 4b was the second plaque type isolated. P. mirabilis number 4 was the propagator strain for both of these phages. For phages designated by fractions, the propagator strain is the top number and the strain used in the sewage enrichment from which the phage was isolated is the bottom number. The phages used for typing of P. mirabilis and P. vulgaris were propagated on P. mirabilis, except for phages 21b and 21c which were propagated on a P. morganii. All phages used for typing of P. morganii were propagated on strains of P. morganii.

Bacteriophage typing. A modification of the method of Blair and Williams (4) was used for the phage typing. Cultures to be typed were grown for 16 h in 4 ml of nutrient broth at 35 C and swabbed onto well-dried EDNA plates. After drying for 30 min, 1 drop of the RTD of each of the 20 phages and a sterile broth control was applied to the seeded plates. The simultaneous application (0.01 ml) of all phages at RTD and 1,000 x RTD was performed with an Accu-Drop Dispenser (Accu-Tech Corp., New York) according to the recommendations of the manufacturer. After the dried plates were incubated for 6 to 8 h at 35 C. Lytic reactions were recorded as 4+ (complete lysis), 3+ (semiconfluent lysis), 2+ (discrete plaques too numerous to count), and 1+ (isolated countable plaques). Any strain with no lysis by any of the phages at RTD was restested at 1,000 x RTD, and lytic reactions of 2+ or greater were considered positive (9).

Computer analysis of results. The phage typing patterns of P. mirabilis and P. vulgaris were considered as a single group, whereas the patterns of the P. morganii were considered separately. All data from the biochemical tests, phage typing, and source identification were analyzed for correlations by the CONSTAT program of the Wayne State University Computing and Data Processing Center by using an IBM S/360 full duplex model 67 computer.

RESULTS

Distribution and biochemical reactions of isolates. Of the 210 isolates, 184 (87.7%) were P. mirabilis, 19 (9.0%) were P. morganii, 5 (2.4%) were P. vulgaris, and 2 (0.9%) were P. rettgeri. The sources of 193 isolates obtained from documented sources are shown in Table 1. In general, the results from conventional biochemical
Table 1. Distribution of 193 isolates obtained from clinical specimens

| Type of specimen | Strains from females (%) | Total | Strains from males (%) | Total | Sub-totals (%) | Total |
|------------------|--------------------------|-------|------------------------|-------|----------------|-------|
| Urine            | 96                       | 49.8  | 23                     | 11.9  | 119            | 61.7  |
| Stool            | 23                       | 11.9  | 24                     | 12.4  | 47             | 24.3  |
| Vaginal swab     | 13                       | 6.8   | 8                      | 4.0   | 21             | 6.8   |
| Wound            | 0                        | 0     | 2                      | 1.0   | 2              | 1.0   |
| Other            | 3                        | 1.6   | 9                      | 4.8   | 12             | 6.2   |

Tests were in agreement with those reported by previous investigators (8). Agreement between the results from the API system and conventional tests was 100% except in the case of the citrate, sucrose, and nitrate reduction tests. All but one of the organisms had negative citrate tests, and only a few had positive sucrose reactions in the API system. None of the *P. mirabilis* had positive sucrose reactions. Also, 19 nitrate-positive strains (by conventional methods) were negative in the API system.

**Phage typing.** The phage lysis patterns were determined by testing the *P. mirabilis*, *P. morganii*, *P. vulgaris*, and *P. rettgeri* isolates at RTD and 1,000 × RTD. In addition, 42 *P. mirabilis*, 13 *P. vulgaris*, 15 *P. morganii*, and 13 *P. rettgeri* from the Enterobacteriology Unit of the Center for Disease Control (CDC), Atlanta, were tested at RTD and 1,000 × RTD. To determine the host specificity of the phages, 16 species of the family *Enterobacteriaceae* were tested. Since the genus *Providencia* is most closely related to the genus *Proteus*, 13 strains of *P. stuartii* and 15 strains of *P. alcalifiaciens* were tested. No *P. rettgeri*, *Providencia*, or other *Enterobacteriaceae* were lysed by any of the phages at RTD or 1,000 × RTD. No attempt was made at isolating phages active on *P. rettgeri* since only two clinical isolates of *P. rettgeri* were obtained. Consequently, this species was not included in the present typing scheme. As shown in Table 2, 175 (84.1%) of the 208 isolates tested were typed at RTD or 1,000 × RTD: 149 (71.6%) were typed at RTD and 26 (12.5%) were typed at 1,000 × RTD.

The lysis patterns of the 162 typable isolates of *P. mirabilis* and *P. vulgaris* are shown in Table 3. Group 1 was the largest, with 34.6% of all typable isolates, whereas groups 7 and 19 were next largest with 14.8 and 14.2%, respectively, of the typable isolates. Two of the *P. vulgaris* isolates were in group 1 and the other two *P. vulgaris* were in group 5. Phage 13/3a had the widest host range and lysed 120 (74%) of all typable strains. Phage Fr5 had the smallest host range and lysed only 2 (1%) of the typable strains. Phages 21b and 21c were the only ones which lysed isolates of both *P. morganii* and *P. mirabilis*, whereas phages 13/3a and Fr2 lysed isolates of *P. mirabilis* and *P. vulgaris*. All other phages shown in Table 3 lysed only *P. mirabilis* isolates.

Table 5 shows results of phage typing of 42 strains of *P. mirabilis* and 13 strains of *P. vulgaris* from the CDC. A new pattern of lysis, not observed for any of the clinical isolates, was observed for one isolate and thus constitutes an additional group, number 21 (Table 3).

The typing results for 15 strains of *P. morganii* from the CDC are shown in Table 6. Six of the strains were in three of the groups established in Table 4, but five different lysis patterns not previously seen were observed for eight of the strains. One of the isolates was not lysed by any of the phages. These new patterns of lysis for the CDC strains of *P. morganii* are shown in Table 7. These patterns constitute new groups, numbers 10 through 14. In all, 70 strains of *P. mirabilis*, *P. vulgaris*, and *P. morganii* from the CDC were tested: 12 (17.1%) were not lysed by any of the phages and 58 (82.9%) were typed. This figure is comparable to that for all *Proteus* (84.1% typed) (Table 2).

To test the stability and reproducibility of the typing system, we tested 50 clinical isolates selected at random several weeks after the initial testing. No changes in phage lysis pat-

Table 2. Distribution of typable and untypable strains of 208 clinical isolates of *Proteus* sp.

| Species        | No. strains | RTD (%) | 1,000 × RTD (%) | Totals (%) | Untypable strains (%) |
|----------------|-------------|---------|-----------------|------------|-----------------------|
| *P. mirabilis* | 184         | 136 (73.8) | 22 (11.9)      | 158 (85.7) | 26 (14.3)            |
| *P. morganii*  | 19          | 11 (57.9)  | 2 (10.5)       | 13 (68.4)  | 6 (31.6)             |
| *P. vulgaris*  | 5           | 2 (40.0)   | 2 (40.0)       | 4 (80.0)   | 1 (20.0)             |
| **Totals**     | **208**     | **149 (71.6)** | **26 (12.5)** | **175 (84.1)** | **33 (15.9)**        |
TABLE 3. Lysis patterns of 162 isolates of P. mirabilis and P. vulgaris

| Group no. | Lysis by phage: | No. in group | %   |
|-----------|----------------|--------------|-----|
|           | 13 6a 24 36 36a 36b Fr2 |               |     |
| 1        | +    |               | 56  | 34.6 |
| 5        | +    |               | 11  | 6.8  |
| 8        | +    |               | 10  | 6.1  |
| 9        | +    |               | 2   | 1.2  |
| 7        | +    | +            | 24  | 14.8 |
| 6        | +    |              | 1   | 0.6  |
| 4        | +    | +            | 1   | 0.6  |
| 3        | +    | +            | 2   | 1.2  |
| 2        | +    | +            | 9   | 5.5  |
| 10       | +    | +            | 2   | 1.2  |
| 11       | +    |              | 1   | 0.6  |
| 15       | +    |              | 2   | 1.2  |
| 17       | +    | +            | 2   | 1.2  |
| 16       | +    | +            | 1   | 0.6  |
| 20       | +    | +            | 4   | 3.6  |
| 18       | +    | +            | 6   | 14.2 |
| 19       | +    | +            | 25  | 14.2 |
| 21       | +    |              | 1   | 0.6  |

Table 4: Lysis patterns of 12 clinical isolates of P. morgani

| Group no. | Lysis of phage: | No. in group |
|-----------|----------------|--------------|
|           | 16 21c 26 34 21c 32a 32b Fr2 |               |
| 1        | +    |               | 1   |
| 2        | +    | +            | 2   |
| 3        | +    | +            | 1   |
| 4        | +    | +            | 1   |
| 5        | +    | +            | 1   |
| 6        | +    | +            | 3   |
| 7        | +    | +            | 1   |
| 8        | +    | +            | 1   |
| 9        | +    | +            | 1   |

Table 5: Results of phage typing of 42 P. mirabilis and 13 P. vulgaris from the CDC

| Group no. | No. strains in group | Total (%) |
|-----------|----------------------|-----------|
| 1         | 33                   | 60.0      |
| 3         | 1                    | 1.9       |
| 5         | 2                    | 3.6       |
| 7         | 2                    | 3.6       |
| 9         | 4                    | 7.3       |
| 10        | 1                    | 1.9       |
| 21        | 1                    | 1.9       |
| Untypable | 11                   | 20.0      |

* The group numbers refer to those established in Table 3.

Patterns were observed for any of the 50 isolates.

Computer analysis of data. All data in this study were analyzed by computer with a statistical analysis program. Analyses performed included correlations, scatter plots, histograms, two-way contingency tables, and normal probability plots. Correlations and two-way contingency tables showed a relationship between those Proteus in phage group 19 and the ability to reduce nitrates to nitrites. Phage group 19 had 3 nitrate-positive strains, 9 nitrate-delayed-positive strains, and 11 nitrate-negative strains using the conventional nitrate test. Phage group 10 and phage group 19 are lysed by phages 31/39 and 39, but group 10 is also lysed by phage 13/3a. Phage group 10 had 5 nitrate-positive strains, 1 nitrate-delayed-positive strain, and 3 nitrate-negative strains. A computer-generated normal probability plot based on phage groups and sex, age and speci-
Table 6. Results of phage typing of 15 strains of P. morgani from the CDC

| Group no. | No. strains in group | Total (%) |
|-----------|----------------------|-----------|
| 3         | 1                    | 6.7       |
| 7         | 4                    | 26.7      |
| 8         | 1                    | 6.7       |
| New groups* | 8                  | 53.3      |
| Untypable | 1                    | 6.7       |

*The group numbers refer to those established in Table 4.
* See Table 7.

men type showed a nearly straight line. Such a result indicates that the phage groups demonstrated no trends in their infection patterns and thus were evenly distributed between the two sexes, throughout all age groups and specimen types.

DISCUSSION

P. mirabilis accounts for between 70 and 96% of all infections due to Proteus (10, 12, 21). Of the isolates in this study, 87.7% were P. mirabilis, a figure which agrees with those reported by previous investigators. Most authors have shown the incidence of P. vulgaris, P. morgani, and P. rettgeri to be between 1 and 4% for each species. However, Adler et al. (2) observed P. morgani in 7.5% of the Proteus infections studied, whereas, in this study, a slightly higher rate of 9.0% was observed. The percentages of P. vulgaris and P. rettgeri seen in this study, 2.4 and 0.9%, respectively, are in agreement with previously reported figures (10, 12, 21).

Nearly half (49.8%) of the isolates were obtained from females with urinary tract infections. This contributed to the imbalance in distribution of hosts, since 69.9% of all the isolates in this study were obtained from females. The rate observed in this study would seem to be significant, though certainly not unexpected, since females would be more disposed than males to autoinfection of the urinary tract from the intestinal flora.

The biochemical reactions, except for the H₂S, citrate, and sucrose reactions, were in agreement with those of previous investigators (8). The differences observed were not surprising, since a collection of Proteus isolated in a single geographic area may vary biochemically when compared to a collection from another area. A study of 413 strains of Proteus in the Memphis, Tenn., area showed that wide deviation from the average percentages can be expected for several biochemical reactions (25). In most instances, the results from the Analytab were in agreement with the results from the conventional tests.

Many isolates in this study gave delayed positive reactions for the citrate, sucrose, and nitrate tests. Therefore, an incubation period longer than recommended for the Analytab may give more definitive results for these tests. When used as a diagnostic tool, the Analytab system permits identification of the four species of Proteus.

As shown in Table 2, 84.1% of the isolates were lysed by one or more of the phages: 71.6% were typed at RTD and 12.5% were typed only at 1,000 × RTD. These results are in agreement with previous phage typing studies of Proteus (2, 10, 15, 28). Vieu (28) was able to divide 82% of the 90 isolates tested into 10 preliminary phage types. Pavlatou et al. (15) divided 71% of 193 isolates into 14 phage types. Popovic and Ghioni (18) had less success with their phage typing system, as only 48% of the 100 isolates tested were typed. France and Markham (10) divided 81% of the 229 isolates they tested into 10 phage types. Adler et al. (2) used France and Markham’s (10) set of phages in a study and were able to type 72% of the 185 isolates tested, but 46% of these isolates were of 1 phage pattern. Izdebska-Szymona (11) divided 80% of 305 isolates into 17 groups with 9 phages.

The phages active on P. mirabilis and P. vulgaris gave semiconfluent and confluent lytic areas which were always clear and easily distinguishable. No background growth was present, and thus no hazy or clouded reactions were seen. This was also true when testing with the phages at 1,000 × RTD. In a few instances, phage 13/3a did produce very clouded areas when used at 1,000 × RTD, and these were interpreted as inhibition of growth rather than lysis of the strain being tested.

The phages active on P. morgani did not give
such obvious reactions. The plaques were very small and, in most cases, were hazy or clouded, even on the propagator strains. The differentiation between inhibition and lysis was slightly more difficult with the *P. morganii* phages than with those active on *P. mirabilis* and *P. vulgaris*. However, after gaining experience, this differentiation can be made easily, since the inhibitory reactions were generally much more clouded than actual lytic reactions. Dilution of phages in cases suspected of inhibition supported this observation. The phage preparations were stable when stored at 4°C over a few drops of chloroform. Even after several months, none of the phage lysates showed a decrease in titre greater than one-half log.

The phage susceptibilities of *P. mirabilis* and *P. vulgaris* (Table 3) compare favorably with the results reported by other investigators. Vieu's (28) scheme had one group of 46%, one group of 16%, and eight smaller groups between 1 and 8%. Pavlatou et al. (15) had a more equal distribution of strains in their phage typing scheme, with six groups between 9 and 17% and eight smaller groups between 0.7 and 5%. France and Markham (10) reported one phage group of 30%, one of 20%, one of 10%, and five groups between 1 and 5%. Izdebska-Szymona (11) reported 17 groups, the largest group being 30% of the total number of typable isolates. Because of the occurrence of large percentages of isolates within single phage types, Popovici et al. (19), Adler et al. (2), and Burke et al. (5) concluded that phage typing was not a particularly useful tool in epidemiological studies of *Proteus*. On the other hand, France and Markham (10) felt that the opposite was true.

The arrangement of isolates in Table 4 indicates that a more complete phage typing system for *P. morganii* probably would have many groups of approximately equal size. This trend was further substantiated by the results shown when 15 strains of *P. morganii* from the CDC were tested (Table 6, 7). Six strains fell into three of the groups established in this study, but eight strains fell into five new groups. In all, 12 clinical isolates and 14 CDC strains of *P. morganii* were divided into 14 groups, with the largest group containing 7 strains. The next largest group, number 11, contained four isolates. The remaining 12 groups contained one or two isolates each. The *P. morganii* typing system will be complete only after further research with many additional strains.

A large portion of the CDC strains of *P. mirabilis* and *P. vulgaris* fell into group 1 (Table 5). Several of the strains of *P. mirabilis* supplied by the CDC were actually those isolated several years ago by Perch (17). All of these strains except one fell into group 1 and thus contributed to the unusually high percentage of strains in that group. A new pattern of lysis not observed for any of the clinical isolates was observed for one organism and thus constitutes a new group, number 21 (Table 3). In all, 70 CDC strains of *Proteus* were tested and 82.9% were typable. This figure is comparable to that observed for the clinically isolated *Proteus*, 84.1%. The results of these phage typing studies on 70 strains of *Proteus* sp. from the CDC serve to point out that strains from widely separated geographical areas demonstrate many of the same lysis patterns shown by locally isolated strains. A lysis pattern not previously seen for *P. mirabilis* was observed in only one instance.

The phages used in this study did not lyse any of the other strains of *Enterobacteriaceae* tested and thus appear to be highly specific for the genus *Proteus*. Also, none of the 28 strains of *Providencia* tested were lysed by these phages. To test the stability and reproducibility of the typing system, 50 clinical isolates were selected at random and tested several weeks after the initial typing. In addition, many isolates have been subsequently tested every few weeks as a check on the stability of the typing system. No changes in the initial phage lysis patterns were observed for any of the isolates. This indicates that the typing system is stable and the results are reproducible.

All of the data in this study were analyzed by computer. Correlations and two-way contingency tables showed a relationship between those *Proteus* in phage group 19 and the ability to reduce nitrates to nitrites. Phage group 19 had only 3 nitrate-positive isolates, but 9 delayed nitrate-positive isolates and 11 nitrate-negative isolates using the conventional nitrate test. The relative significance of this observation has yet to be determined, but future research may provide an explanation for this unexpected correlation.

In an effort to determine whether the phage groups showed any trends, a computer-generated normal probability plot was constructed. The plot was nearly linear, which indicates that the phage groups showed no trends in their infection patterns and thus were evenly distributed between the two sexes, throughout all age groups and specimen types. This was confirmed by computer constructed scatter plots with the appropriate data. These scatter plots had a random distribution of phage groups. This observation is notable, since Vieu
observed an unequal distribution of phage groups among specimen types. He reported that strains from stools and skin lesions generally had phage lysis patterns that differed from strains isolated elsewhere.

Our study shows that a bacteriophage typing system which meets Anderson's (3) requirements can be devised for Proteus: (i) the typing patterns and the phage lysates are stable, (ii) the organism dealt with has been divided into an adequate number of groups, (iii) the technique is fairly simple and the results are reproducible and easy to read, (iv) the typing system could be standardized for general use, and (v) the results are available quickly, usually within 24 h of the receipt of the pure culture. We believe that further epidemiological trials would confirm the reliability of this system so that it might be made available for general use. Bacteriophage typing of Proteus is an important epidemiological tool because it provides a method by which various strains of Proteus causing human infections can be differentiated.

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