Bacteriophage Types and O Antigen Groups of
Escherichia coli from Urine

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Urinary strains of Escherichia coli from seven geographical regions were typed serologically for O-specific antigens and with phages capable of lysing the majority of urinary isolates. The O antigen groups 4, 6, 75, 1, 50, 7, and 25 were the common ones found. Of the 454 cultures tested, 66.1% were phage typable and 65.2% were serotypable with the 48 antisera employed. Also, 71.6% of the cultures for which an O group could be determined were phage typable. Furthermore, of those seven O-antigen groups implicated in urinary tract infection, 80.2% exhibited a phage pattern. Various phage types were found within an O-antigen group, and, although one phage type associated a high percentage of the time with one O-antigen group, no correlation was observed between other O-antigen groups and phage types. Studies with bacteriuric patients by phage typing showed the presence of two strains of E. coli within an O-antigen group. Serogrouping and phage typing of fecal isolates of E. coli revealed the presence of some O-antigen groups and phage types also found as predominant types among urinary isolates. Phage typability correlated highly with hemolysis of human erythrocytes. Elevated temperatures of incubation and a chemical curing agent were used to enhance typability of cultures refractory to the typing phages. Phage typing, due to its rapidity, ease, and ability to distinguish strains of E. coli within an O-antigenic group, is suggested as a possible method by which a better insight into the epidemiology of urinary tract infections may be obtained.

Serological identification of strains of Escherichia coli involved in urinary tract infections has been used since the scheme was devised by Kauffmann (10). Rantz (15), using this scheme, established that certain serotypes were predominant in the majority of urinary tract infections. Today, the fact that O-antigen groups 4, 6, and 75 are found most commonly in these infections, and that O-antigen groups 1, 50, 7, and 25 are found less frequently is well established (18). This method of identification has not, however, provided any answer as to why certain strains are found predominantly in these infections.

The introduction by Brown and Parisi (3) of a bacteriophage typing scheme for urinary E. coli and its subsequent refinement by Parisi et al. (13) offers the possibility that the means of identifying urinary E. coli might become as simplified as the identification of strains of staphylococci by phage typing. The purpose of this investigation was to determine whether phage typing was a more specific method of identification than serological typing, and whether phage typing would provide us with a better insight into the epidemiology of urinary tract infections.

MATERIALS AND METHODS

Bacteria. Cultures of E. coli isolated from urinary sources were obtained from seven geographical regions (California, Minnesota, Missouri, Ohio, Pennsylvania, Virginia, and West Virginia) between 1965 and 1970. Additional cultures from fecal sources were collected at the University of Missouri in 1968. Cultures were identified as E. coli by the use of biochemical tests (4).

Phages and phage typing. The eight bacteriophages, A, B, C, D, E, F, G, and H, isolated from sewage in Pittsburgh, Pa., by Brown and Parisi (3) and the five additional phages, I, J, K, L, and M, isolated subsequently from sewage in Columbia, Mo., were used in this study. Phages A through M were propagated by the semisolid agar technique as described by Eisenstark (5) and harvested by the method of Bradley (1). The method of phage typing was the same as reported previously (13) except that 0.5 ml of an overnight culture was inoculated into 10
ml of Brain Heart Infusion (BHI; BBL) broth and incubated in a shaking water bath at 37 C for 6 hr prior to typing. The various typing reactions are shown in Fig. 1. Complete lysis within a drop was recorded as 4+ (zone A), a 3+ reaction consisted of a few isolated colonies within the drop, and a 2+ (zone B) reaction consisted of an area which was approximately half-lysed. Reactions weaker than 2+ (zone C) were not recorded and cultures with such reactions were designated "nontypable."

Antisera and serological typing. Forty-eight individual O antisera of E. coli were received from the Center for Disease Control (CDC) in Atlanta, Ga. Forty-two O antisera were arranged into six pools for typing as recommended by Ewing and Davis (7), and the seventh pool (P) contained six antisera from numerous other recommended pools (Table 1). This pool was necessitated because of the unavailability in this study of the complete set of 147 O antisera and was satisfactory in that no cross-reactions occurred. Respective pools were prepared in 0.5% phenolized saline to give a final dilution of 1:50 of antiserum. For final antigenic determination, antisera were prepared individually in phenolized saline to a final dilution of 1:5. Cultures to be typed serologically were grown in the same manner as were cultures for phage typing. The procedure used for O antigen preparation and determination of E. coli was that recommended by Edwards and Ewing (4) except that 0.5% formalized saline was the diluent for the bacterial antigens.

Antibiotic sensitivity. Antibiograms of selected cultures of E. coli were determined according to the single high-potency disc procedure of Petersdorf and Sherris (14). The antibiotic discs (BBL) used and their concentrations were: ampicillin (10 μg), chloramphenicol (30 μg), kantrex (30 μg), neomycin (30 μg), streptomycin (10 μg), sulfathiazole (1.0 mg), demethylchlortetracycline (30 μg), and gentamicin (10 μg).

Hemolysis of human erythrocytes. Cultures of E. coli picked for their sensitivity or resistance to our phages were tested for their ability to cause hemolysis of human erythrocytes. These cultures were streaked for isolation onto Trypticase Soy Agar (BBL) containing 5% human blood and, after incubation at 37 C for 18 to 24 hr, were read for hemolytic activity.

Enhancement of phage typability. Two methods were used in an attempt to increase the percentage of phage-typable cultures. The first method consisted in growing phage-resistant cultures at 45 C for 18 to 24 hr in BHI broth prior to typing. Cultures were then typed and incubated at 45 C for 5 to 6 hr before recording the pattern of lysis.

The second method was the addition of ethidium bromide (Calbiochem, Los Angeles, Calif.) to the BHI broth used for growth of phage-resistant cultures of E. coli. Ethidium bromide was added to a final subinhibitory concentration of 100 X 10^-8 M, and the cultures were incubated for 18 to 24 hr prior to phage typing in the ordinary manner.

RESULTS

O antigen groups of urinary E. coli. Table 2 shows the predominant O group antigens of strains in the seven regions and indicates the

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**Table 1. Pool arrangement of O antisera of Escherichia coli**

| Pool group |
|------------|
| 1-A        | 1-B        | 2-A        | 2-B        | 3-A        | 5-A        | P          |
| 01ab       | 018ab      | 08         | 012        | 020ab      | 062        | 019ab      |
| 02ab       | 018ac      | 09         | 016        | 021        | 075        | 027        |
| 03         | 023        | 011        | 060        | 022        | 078        | 035        |
| 04         | 070        | 015        | 077        | 028ab      | 086a       | 058        |
| 06         | 0114       | 073        |            | 033        | 0126       | 074        |
| 07         | 0138       | 0125ab     |            | 081        | 0127a      | 0110       |
| 025        |            | 0125ac     |            | 083        |            |            |
| 026ab      |            |            |            | 028ac      | 0128ac     |            |
| 050        |            |            |            |            |            |            |
percentage of cultures which were serotypable from each region. The data indicate that O-antigenic group 6 was the most common group in six of the seven regions surveyed. In all, group 6 occurred in 89 (19.6%) of the 454 cultures typed. The next most common was O-antigenic group 75 which occurred in 41 (9.0%) of the cultures.

**Phage types of urinary E. coli.** Table 3 shows the predominant phage types and the percentage of typable cultures from each geographical region. In all, phage type GHK occurred in 35 (7.7%) of the 454 cultures typed, whereas phage type GHKL occurred in 34 (7.5%) of the cultures from these regions. Phage type E appeared in 34 (7.5%) of the cultures and was commonly found in all but one (California) of the regions.

**Comparison of phage and serological typing.** As shown in Table 4, 300 (66.1%) of the cultures were phage typable, whereas 296 (65.2%) were typable with the 48 antisera used. Of the 296 cultures which were serotypable, 212 (71.6%) were also phage typable. Considering just those seven O-antigen groups most often implicated

### Table 2. Predominant O-antigen groups of urinary Escherichia coli and their percentage of occurrence

| Geographical region | No. typed | Per cent typable | Predominant O-antigenic groups | Per cent of occurrence |
|---------------------|-----------|------------------|--------------------------------|------------------------|
| California          | 35        | 77.1             | 09                             | 11.4                   |
|                     |           |                  | 075                            | 11.4                   |
| Minnesota           | 73        | 71.2             | 06                             | 24.7                   |
|                     |           |                  | 04                             | 11.0                   |
| Missouri            | 64        | 54.7             | 06                             | 14.1                   |
|                     |           |                  | 07                             | 7.8                    |
| Ohio                | 64        | 68.8             | 06                             | 25.0                   |
|                     |           |                  | 075                            | 9.4                    |
| Pennsylvania        | 79        | 67.1             | 06                             | 25.0                   |
|                     |           |                  | 04                             | 10.0                   |
|                     |           |                  | 075                            | 10.0                   |
| Virginia            | 75        | 57.3             | 06                             | 17.3                   |
|                     |           |                  | 075                            | 10.7                   |
| West Virginia       | 64        | 65.6             | 06                             | 18.7                   |
|                     |           |                  | 018ac                          | 14.1                   |

### Table 3. Predominant phage types of urinary Escherichia coli and their percentage of occurrence

| Geographical region | No. typed | Per cent typable | Predominant phage types | Per cent of occurrence |
|---------------------|-----------|------------------|-------------------------|------------------------|
| California          | 35        | 65.7             | GHK                     | 14.3                   |
|                     |           |                  | GHKL                    | 8.6                    |
|                     |           |                  | E                       | 9.6                    |
|                     |           |                  | ABCEFGHK                | 8.2                    |
|                     |           |                  | GHKL                    | 8.2                    |
|                     |           |                  | GHK                     | 8.2                    |
| Missouri            | 64        | 68.8             | E                       | 6.3                    |
|                     |           |                  | ABCEFGH                 | 6.3                    |
|                     |           |                  | GHK                     | 6.3                    |
|                     |           |                  | M                       | 4.7                    |
| Ohio                | 64        | 79.7             | GHK                     | 10.9                   |
|                     |           |                  | E                       | 7.8                    |
|                     |           |                  | GHKL                    | 7.8                    |
|                     |           |                  | GHKL                    | 13.9                   |
|                     |           |                  | E                       | 10.1                   |
| Pennsylvania        | 79        | 64.6             | GHKL                    | 8.0                    |
|                     |           |                  | M                       | 6.7                    |
|                     |           |                  | GHK                     | 5.3                    |
|                     |           |                  | E                       | 5.3                    |
|                     |           |                  | EGH                     | 5.3                    |
| Virginia            | 75        | 61.3             | GHKL                    | 9.4                    |
|                     |           |                  | M                       | 6.3                    |
|                     |           |                  | GHK                     | 6.3                    |
| West Virginia       | 64        | 48.4             | GHKL                    | 8.0                    |
|                     |           |                  | M                       | 6.7                    |
|                     |           |                  | GHK                     | 5.3                    |
|                     |           |                  | E                       | 5.3                    |
|                     |           |                  | EGH                     | 5.3                    |
|                     |           |                  | E                       | 9.4                    |
|                     |           |                  | GHK                     | 6.3                    |
in urinary tract infections, 166 (80.2%) exhibited a phage pattern, whereas 41 (19.8%) were nontypable. Of the 36 cultures which were rough and could not be serotyped, 23 (63.9%) were typhable with the phages.

**Association of O-antigen groups with phage types.** The O-antigen groups occurring most frequently, the predominant phage types found within each group, and their percentages of occurrence are shown in Table 5. Except in one case, no relation was found between a specific O-antigen group and a particular phage type. Several different phage types were present within a serological group, and only the most frequently occurring are shown in this table. The one possible exception, as mentioned, is O-antigen group 75, where phage pattern GHKL occurred in 27 (65.9%) of 41 cultures; only one culture with this O antigen group was nontypable. Furthermore, only 7 (1.5%) other cultures of the 454 typed exhibited this phage pattern.

**Phage and serological types of cultures from patients with chronic bacteriuria.** In a limited study to determine whether phage typing would provide a more specific method of identification than serological typing, three patients with chronic bacteriuria at the Medical Center were studied during different outpatient visits. Phage patterns, serotypes, and antibiograms were determined with four separate colonies picked per urine sample. Although all patients exhibited a homotypic infection in relation to the O-antigen group, with one patient, two phage types were detected within a serological group. None of the antibiograms differed.

**Phage typability and hemolysis of human erythrocytes.** Thirty-two (32.4%) of 97 cultures picked for their sensitivity to our phages lysed the red cells. On the other hand, only 5 (5.4%) of 92 cultures picked because of their resistance to our phages lysed the red cells.

**Table 5. Predominant O-antigen groups and commonly associated phage types**

| O-antigen group | No. of cultures | Commonly associated phage types | Per cent occurrence |
|-----------------|----------------|--------------------------------|-------------------|
| 1               | 9              | NT*                            | 44.4              |
| 4               | 30             | ABCEFGEHM                       | 22.2              |
| 6               | 89             | ABCEFGEHK                       | 26.7              |
| 7               | 18             | GHK                            | 16.7              |
| 6               | 89             | NT                             | 27.0              |
| 7               | 18             | NT                             | 20.2              |
| 18bc            | 14             | NT                             | 17.1              |
| 22              | 10             | NT                             | 20.0              |
| 23              | 8              | NT                             | 20.0              |
| 25              | 8              | E                              | 25.0              |
| 50              | 12             | GHK                            | 25.0              |
| 75              | 41             | NT                             | 25.0              |
| 75              | 41             | GHKL                           | 65.9              |
| 75              | 41             | GHK                            | 17.1              |

* Nontypable.

**Table 4. Comparison of phage typing and serological typing**

| Determination | No. | Per cent |
|---------------|-----|----------|
| Cultures typed ........................................................................ | 454 |         |
| Phage typable cultures ......................................................... | 300 | 66.1    |
| Serotypable cultures ............................................................ | 296 | 65.2    |
| Serologically typable cultures that were phage typable ................. | 212 | 71.6    |
| Cultures with O-antigen groups most often implicated in urinary tract infection that were phage typable | 166 | 80.2 |
| Rough cultures .................................................................. | 36  |         |
| Rough cultures nonserotypable but phage typable ........................ | 23  | 63.9    |

The identification of O groups of *E. coli* has implicated different strains in certain pathological conditions. That only certain O groups of *E. coli* are the cause of the majority of infections (infantile diarrhea and urinary tract infections).
has emerged from the previous work of many investigators (6, 18). The data in this study support the findings that only a few of the more than 145 O-antigen groups are found in patients with bacteriuria. O-antigen groups 4, 6, and 75 accounted for the majority of urinary tract infections encountered by Turck et al. (18), an observation corroborated by this work. Furthermore, 67.2% of the isolates belonged in the first 25 O-antigen groups, a figure which is in close agreement with the figure of 69.3% reported by Ewing and Davis (8).

Although certain antigen groups are found more frequently in infections of the urinary tract, this method of identification still has not answered the question of whether these particular groups are more virulent for man or whether they are more prevalent in his environment so as to be implicated in a greater number of infections. Also, due to the time required and the unavailability of reagents, it is not feasible, at present, for diagnostic laboratories to incorporate serological identification of *E. coli* routinely into the evaluation of a patient with chronic urinary tract infection. Thus, it would seem that phage typing might make the identification of various strains of *E. coli* simpler and even more definitive. It has been shown that phage typing can be used to identify urinary strains of *E. coli* (3). With the addition of five more phages to this original set, Parisi et al. (13) demonstrated the presence of different phage types of urinary *E. coli* in seven geographical regions and that lytic reactions were reproducible upon consecutive phage typing. The purpose of this present study was to see whether certain O-antigen groups correlated with specific phage types. Many phage types, as many as 27 in one instance, were present in one O-antigen group. Although one phage type, GHKL, associated a high percentage of the time with O-antigen group 75, other phage types within this group also were observed. This provides further evidence that various strains of *E. coli* do exist within O-antigen groups. The fact that generally one O-antigen group of *E. coli* is present in each episode of bacteriuria is well established (9, 11). Three patients with bacteriuria studied at the University Medical Center corroborated this fact. However, it was shown that different strains of *E. coli*, as identified by phage typing, do exist in patients even though O-antigenic determination indicated only one strain. Furthermore, in an earlier preliminary study (13), phage typing did serve to identify different strains from the same patient over a period of several months. These data warrant further investigation encompassing larger groups of patients who suffer from bacteriuria over longer periods of time. The presence of the same phage types within different O-antigen groups also may suggest that the use of the O antigen in the identification of *E. coli* in bacteriuria is not as specific as phage typing.

Comparison of phage typing with serological typing was favorable in that it was possible to identify as high a percentage of cultures with phage typing as with serological typing. Furthermore, it was demonstrated that the phages typed the majority of strains of *E. coli* implicated in urinary tract infections by serotyping. Phage typing also provided a means of identifying the majority of strains of *E. coli* which were rough and could not be classified by their O antigen.

Although no particular phage type or O-antigen group emerged as predominant in the isolates of *E. coli* from human feces, that O-antigen groups 1, 4, 6, and 25 were found in the feces shows that the feces is capable of harboring these O-antigen groups. Whereas phage types E, M, EGH, GHKL, and ABCEFGHK, which had been predominant phage types among the urinary *E. coli* from certain geographical regions, were noted in the fecal isolates, because of the small number of isolates and a lack of case histories of individuals providing these specimens, no conclusion can be drawn from these data. It is suggested, however, that phage typing may be a simpler and easier method for determining the role which the feces play in harboring those strains of *E. coli* which predispose to urinary tract infection.

The effects of plasmids on the phage pattern of the host cell and the practical importance this has on phage typing schemes for enteric bacteria have been reviewed recently (16). It was hoped that by the use of some practical method, cells resistant to the phages under normal conditions could be converted to a sensitive state, thus increasing the percentage of typable cultures. Bradley (2) observed that when cultures were grown at elevated temperatures of incubation, they lost their refractory nature to phages which they had at 37 C. This method was thus chosen to increase phage typability, and, interestingly, a significant number of cultures, refractory to our phages at 37 C, became sensitive at 45 C. That cultures exposed to ethidium bromide did not show as high a percentage of typability as those grown at 45 C and that all cultures which became typable at 45 C did not become typable after exposure to ethidium bromide indicates that these two methods may be dissimilar in their mode of action in altering bacterial sensitivity to phages. Antibiograms of the cultures which became typable under these experimental condi-
tions did not change, indicating that drug resistance and resistance of bacteria to phages are not related to each other. Interestingly, several cultures which had been typable at 37°C and incubated at elevated temperatures changed their patterns of susceptibility to phages. The results in which cultures became typable at elevated temperatures of incubation may be explained possibly by the observations of other workers. Some investigators hypothesize that lysogeny is interfered with in some manner (12, 19) or that the membrane-bound nucleases which control restriction are inactivated (17). Thus, the phages at elevated temperatures of incubation enter into a lytic cycle, whereas at normal temperatures of incubation (37°C) this lytic development does not occur. The fact that the O-somatic antigen structure of E. coli changes when the cells are grown at different incubation temperatures has been shown recently (T. F. Rhodes and H. C. Fung, Bacteriol. Proc., p. 111, 1970). Changes in the bacterial cell wall antigens may also represent an explanation for alterations in susceptibility to bacteriophages at elevated temperatures. Obviously, more work is needed in this area.

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LITERATURE CITED

1. Bradley, D. E. 1963. The structure of coliphages. J. Gen. Microbiol. 31:425-445.
2. Bradley, S. G. 1968. Applied significance of polyvalent bacteriophages, p. 101-135. In W. W. Umbreit and D. Perlman (ed.), Advances in applied microbiology, vol. 10. Academic Press Inc., New York.
3. Brown, W. J., and J. T. Parisi. 1966. Bacteriophage typing of bacteriuric Escherichia coli. Proc. Soc. Exp. Biol. Med. 121:259-262.
4. Edwards, P. R., and W. H. Ewing. 1962. Identification of enterobacteriaceae, p. 61-91. Burgess Publishing Co., Minneapolis.
5. Eisenstadt, A. 1967. Bacteriophage techniques, p. 449-524. In K. Maramorsh and K. Koprowski (ed.), Methods in virology, vol. 1. Academic Press Inc., New York.
6. Ewing, W. H. 1963. Isolation and identification of Escherichia coli serotypes associated with diarrheal diseases, p. 1-25. CDC Publication, Communicable Disease Center, Atlanta.
7. Ewing, W. H., and B. R. Davis. 1961. O antisera pools for preliminary examination of Escherichia coli cultures, p. 1-9. CDC Publication, Communicable Disease Center, Atlanta.
8. Ewing, W. H., and B. R. Davis. 1961. The O antigen groups of Escherichia coli cultures from various sources, p. 1-21. CDC Publication, Communicable Disease Center, Atlanta.
9. Jackson, G. G., V. M. Kozi, and R. L. Jao. 1965. Relation of serogroup strain prevalence and Escherichia coli urinary tract infections, p. 150-158. In E. H. Kass (ed.), Progress in pyelonephritis. F. A. Davis Co., Philadelphia.
10. Kauffmann, F. 1947. Serology of the coli group. J. Immunol. 57:71-100.
11. Kunin, C. M., R. Deutscher, and A. Paquin, Jr. 1964. Urinary tract infection in school children: an epidemiological, clinical and laboratory study. Medicine 43:91-130.
12. Lieb, M. 1953. The establishment of lysogeny of Escherichia coli. J. Bacteriol. 65:642-651.
13. Parisi, J. T., J. C. Russell, and R. J. Merlo. 1969. Bacteriophage typing as an epidemiological tool for urinary Escherichia coli. Appl. Microbiol. 17:721-725.
14. Petersdorf, R. G., and J. C. Sherris. 1965. Methods and significance of in vitro testing of bacterial sensitivity to drugs. Amer. J. Med. 39:766-779.
15. Rantz, L. A. 1962. Serological grouping of Escherichia coli: study in urinary tract infection. Arch. Intern. Med. 109:537-42.
16. Richmond, M. H. 1970. Plasmids and chromosomes in prokaryotic cells, p. 249-277. In H. Charles and B. C. Knight (ed.), Organization and control in prokaryotic and euarkyotic cells. University Press, Cambridge, England.
17. Schell, J., and S. W. Glover. 1966. The effect of heat on host-controlled restriction of phage λ in Escherichia coli K (Pl). J. Gen. Microbiol. 45:61-72.
18. Turck, M., A. R. Ronald, H. Clark, R. H. Winterbauer, E. Atlas, F. Silverblatt, and R. G. Petersdorf. 1969. Studies on the epidemiology of Escherichia coli, 1960-1968. J. Infect. Dis. 120:13-16.
19. Zichichi, M. L., and G. Kellenberger. 1963. Two distinct functions in the lysogenization process: the repression of phage multiplication and the incorporation of the prophage in the bacterial genome. Virology 19:450-460.