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Bacteriophage Typing as an Epidemiological Tool for Urinary *Escherichia coli*[1]

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Phage typing was used to identify strains of *Escherichia coli* isolated from urinary and nonurinary sources. When eight phages isolated in Pennsylvania were used to type 717 cultures from Missouri, 50.3% of 624 urinary isolates and 34.4% of 93 nonurinary isolates were typable. Strains from nonurinary sources were not found commonly in urine. When five additional phages isolated in Missouri were added to the original set of eight phages, 80.4% of 331 urinary isolates were typable. When this set of phages was used to type 552 urinary cultures isolated in California, Minnesota, Ohio, Pennsylvania, Virginia, and West Virginia, 82.0% of the cultures were typable. Some common phage types were found in high incidence among cultures from the different regions. No correlation was found between phage type and the pattern of resistance to antibiotics. Phage typing data were presented also on the number of strains in individual urine specimens and the recurrences of strains in patients with chronic bacteriuria. Of 97 fecal isolates, 75.2% of the cultures were typable, and the most common phage type was observed in high incidence among the urinary isolates from this region. When 75 cultures from nine other genera of enteric bacteria were typed, only the shigellae were lysed. In view of the information obtained by phage typing and the ease and speed with which it can be done, it is suggested that phage typing be considered a new tool in epidemiological studies of urinary tract infections by *E. coli*.

Identification of strains of *Escherichia coli* in epidemiological studies has been primarily by serological typing. These studies indicate that a small number of strains are responsible for the majority of infections of the urinary tract (6, 9), and these strains are different from those responsible for infantile diarrhea (3, 5). A method for studying bacteriuric strains by phage typing has been developed recently (2) and has been employed in this study. This paper is an extension of the use of phage typing for the identification of bacteriuric strains of *E. coli* and discusses the feasibility of phage typing in epidemiological studies of urinary tract infections.

MATERIALS AND METHODS

Bacteria. Cultures of *E. coli* were isolated over a period of 2 years from urine and nonurinary infections of patients at the University of Missouri Medical Center, Columbia. Nonurinary isolates were primarily from wounds and abscesses, sputum, blood, or peritoneal drainage. Also received for phage typing were urinary cultures of *E. coli* from six other geographical regions: California, Minnesota, Ohio, Pennsylvania, Virginia, and West Virginia. The indices of bacteriuria per milliliter of urine from all sources were 10^3 bacteria or greater (Mo.); 10^4 bacteria or greater (Ohio); and 10^5 bacteria or greater (Calif., Minn., Pa., Va., and W.Va.). Cultures were identified as *E. coli* by the biochemical tests usually recommended (3).

In one study, cultures of *E. coli* were isolated from the feces of healthy individuals, identified biochemically, and then phage-typed. In another study, cultures of enteric bacteria were obtained from the collection of the Department of Microbiology, University of Missouri, and from D. J. Hentges, in this same department, and phage typed. Cultures consisted of species from the following genera: *Alcaligenes, Citrobacter, Enterobacter, Escherichia, Klebsiella, Proteus, Pseudomonas, Salmonella, Serratia,* and *Shigella.*

Phages. Eight bacteriophages, A, B, C, D, E, F, G, and H, isolated from sewage in Pittsburgh by Brown and Parisi (2) were used in the first portion of this study. Five additional phages, I, J, K, L, M, were isolated from sewage in Columbia, Mo. Quantities (10 ml) of raw sewage or mixed liquor were inoculated into 90-ml quantities of Brain Heart Infusion (BHI; Difco) broth, incubated at 37 C for 24 hr, centrifuged, and filtered through a 0.45-nm membrane filter (Millipore Corp., Bedford, Mass.). To determine phage activity, filtrates were placed on BHI

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Agar (Difco) plates previously swabbed with indicator strains of *E. coli* grown in BHI broth for 18 hr at 37 C. Filtrates showing phage activity were diluted serially in 0.1 M ammonium acetate (1) and 0.1 ml of diluted filtrate mixed with 0.1 ml of the indicator strain in 2.5 ml of BHI broth containing 0.75% agar (Difco). This mixture was then layered on the surface of a BHI Agar plate. After incubation at 37 C for 4 to 6 hr, discrete plaques were picked with an inoculating needle and washed into 1 ml of ammonium acetate. This suspension of phages was then used for propagating by the agar layer method (10). This process of phage purification and propagation was repeated three times. Phages were stored at 4 C.

Phage typing. Routine test dilution (RTD) of each phage suspension in ammonium acetate was determined prior to use and used in the typing procedure. Cultures to be typed were grown in BHI broth overnight at 37 C and then swabbed on a BHI Agar plate. The RTD of each phage was placed in the phage applicator (Fig. 1) originally described by Zierdt, Fox, and Norris (15), and phages were applied to the surfaces of the inoculated plates. Plates were incubated at 37 C for 4 to 6 hr and then examined for lysis. A reaction was considered significant if the degree of lysis was equal to or greater than the amount of bacterial growth within the area of the drop of RTD.

Antibiotic sensitivity. Tryptic Soy Broth (Difco) was inoculated with a typical colony from MacConkey Agar (Difco) and incubated at 37 C for 2 to 6 hr. Approximately 0.2 ml of this culture was transferred to Tryptic Soy Agar (Difco) containing 2% sheep erythrocytes and distributed over the agar surface with a glass streaker. Multidisks (Consolidated Laboratories, Chicago Heights, Ill.) containing chloramphenicol (30 μg), erythromycin (5 μg), nitrofurantoin (100 μg), penicillin G (10 units), dihydrostreptomycin (10 μg), tetracycline (30 μg), and colistin (10 μg) were placed on the inoculated plates, and only the presence or absence of a zone of inhibition as determined by visual inspection was recorded for all chemotherapeutic agents except penicillin G and erythromycin.

RESULTS

Phage types with phages A through H. Results of typing 717 cultures from urinary and nonurinary sources in the University of Missouri Medical Center with the eight original phages isolated from sewage in Pittsburgh were obtained (Table 1). In all, 346 (48.3%) of 717 cultures were typable, but the remaining 371 (51.7%) were either nontypable or gave an ill-defined reaction designated as "indeterminate." Although 314 (50.3%) of 624 isolates from urine were typable, only 32 (34.4%) of 93 nonurinary isolates were typable. We found 41 different phage types in the urinary isolates and 20 phage types in the nonurinary isolates. This finding is not too surprising in view of the larger number of typable cultures from urine. It is interesting, however, that of 32 typable cultures from nonurinary sources, 11 (34.4%) represented eight phage types which were restricted to the nonurinary isolates and were not found in a much greater number of urinary isolates.

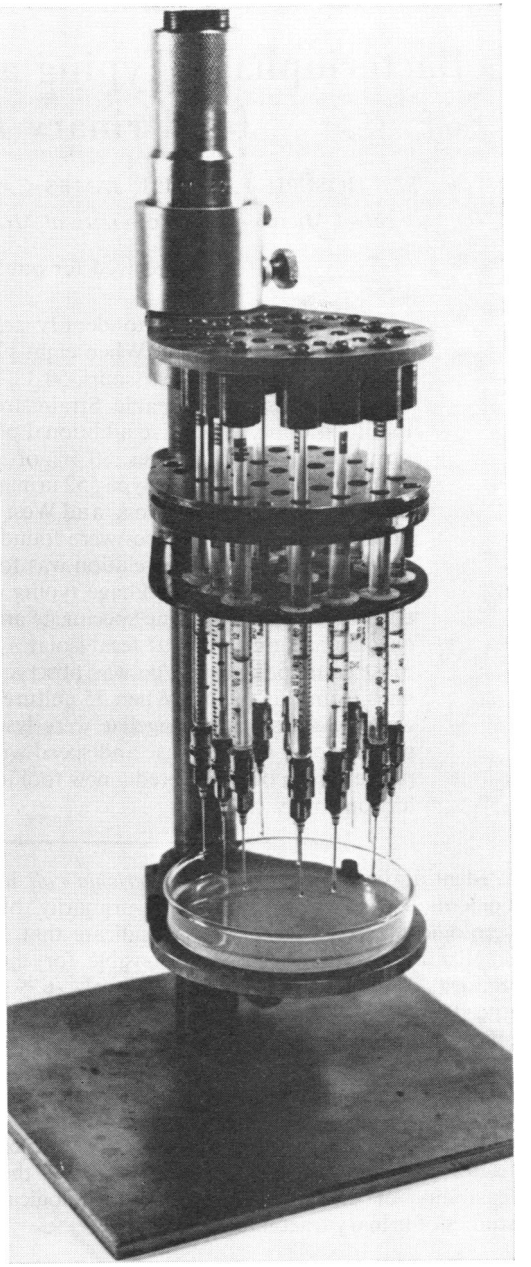

FIG. 1. *Mechanical phage applicator.*

Phage types with phages A through M. When

all 13 phages were employed in the typing of 331 urinary cultures isolated subsequently in the University of Missouri Medical Center, 266 (80.4%) of 331 cultures were lysed by 1 or more of the 13 phages (Table 2). Although 109 distinct phage types were observed among the 266 cultures, 9 phage types accounted for 40.2% of the typable cultures. To further determine the efficiency of these typing phages for urinary cultures from other geographical regions, typing of 552 cultures from six different states was attempted (Table 2). In all, 717 (81.2%) of 883 urinary cultures were lysed by 1 or more of the 13 phages. Also shown in this table are the phage types occurring most frequently in each region and the percentage of cultures with these phage types. Of the 11 different phage types shown in Table 2, 3 (IJLM, K, and ABCDEFGHKL) were found in two different regions, 2 (ABCDEFGH and GK) were found in three different regions, and 2 (ABCDEFGHK and GKL) were found in four different regions. Only phage types KL, ABCDEGK, EGK, and EG were found in high incidence in a single region.

Occurrence of multiple strains in a urine specimen. To determine whether more than a single strain of *E. coli* was present in a urine specimen, cultures from the urine of 26 different patients were obtained. Six separate colonies upon primary isolation were picked and phage typed. Of the 26 separate cultures, 16 clearly had only one strain present, 3 others probably had only one strain present, 6 clearly had two strains present, and 1 appeared to contain three strains.

Phage typing of cultures from patients with chronic bacteriuria. To determine whether strains with the same phage type were responsible for recurrences of bacteriuria, patients with chronic bacteriuria were chosen from the outpatient population. At each clinic visit, the urine was cultured and any organism identified as *E. coli* was phage-typed. Although the data are fragmentary, the urine from two patients had bacterial strains with the same phage type when two cultures were obtained 1 month apart. A third patient was followed more extensively. Eight urine cultures were obtained over a period of 7 months. Three distinctly different strains were identified through phage typing. Unfortunately, more than one colony was not picked for typing in each instance, so it was impossible to determine whether more than one strain was present in each urine specimen.

Relation of phage type to antibiotic resistance. Table 3 shows the number of phage types of strains resistant to the antibiotics tested. Although a number of phage types were associated with antibiotic resistance, no relation was found between a specific phage type and a particular pattern of antibiotic resistance. The pattern most commonly encountered was that of resistance to tetracycline and dihydrostreptomycin and was found in 87 (63.0%) of 138 cultures and consisted of 40 cultures comprising 14 phage types in addition to 47 untypable cultures.

Phage types of fecal E. coli. When 97 fecal cultures of *E. coli* were typed with all 13 phages, 73 (75.2%) were lysed by one or more phages. In all, there were 48 distinct phage types; the most common phage types and the percentage

TABLE 1. *E. coli from urinary and nonurinary sources typed with phages A through H*

Determination	Source of culture	
	Urinary	Nonurinary
No. typed	624	93
No. typable	314	32
Percentage typable	50.3	34.4
No. of phage types	41	20
No. of phage types restricted to that source	29	8
No. of cultures belonging to restricted phage types	67	11
Percentage of typable cultures belonging to restricted phage types	21.3	34.4

TABLE 2. *Urinary E. coli from different geographical regions typed with phages A through M*

Source of culture	No. typed	Percentage typable	Phage types occurring most frequently and percentage of cultures with that phage type
Calif.	96	79.2	ABCDEFGHKL(8.3), GKL(8.3), ABCDEFGH(7.3), K(6.3), GK(5.2)
Minn.	100	92.0	GK(13.0), ABCDEFGHK(9.0), ABCDEGK(5.0)
Mo.	331	80.4	ABCDEFGH(6.3), IJLM(5.1), K(4.8), KL(4.5)
Ohio	90	84.4	GKL(11.1), EGK(6.7), IJLM(5.6)
Pa.	73	87.7	ABCDEFGHK(13.7), GKL(12.3), ABCDEFGH(6.8)
Va.	104	73.1	GK(8.7), ABCDEFGHK(6.7), GKL(5.8)
W.Va.	89	75.3	ABCDEFGHKL(11.2), ABCDEFGHK(7.9), EG(5.6)

TABLE 3. *Relation of phage type to antibiotic resistance*

Pattern of resistance to	No. of phage types	No. of typable cultures	No. of untypable cultures
Te[a]	4	10	10
S	4	7	8
F	3	4	3
CS	1	1	2
Te, S	14	40	47
F, S	1	1	0
F, Te, S	1	1	1
C, Te, S	1	1	2

[a] Abbreviations: Te, tetracycline; S, dihydrostreptomycin; F, nitrofurantoin; CS, colistin; and C, chloramphenicol.

of cultures with these phage types were IJLM (5.2), GKL and BEFGHI (4.1), and ABCDEFGHI and ABCDEFGHKLM (3.1). Interestingly, phage type IJLM which was the most common phage type among the fecal cultures was also one of the most common phage types among the urinary cultures from Missouri, and phage type GKL which similarly occurred in high incidence among the fecal cultures was also a common phage type among the urinary cultures from other regions (Table 2).

Phage types of other enteric bacteria. Of a total of 75 cultures comprising the following number of distinct strains or species from within the following genera (*Alcaligenes*, 3; *Citrobacter*, 1; *Enterobacter*, 3; *Escherichia*, 13; *Klebsiella*, 8; *Proteus*, 4; *Pseudomonas*, 3; *Salmonella*, 27; *Serratia*, 2; and *Shigella*, 11, only members of the genera *Escherichia* and *Shigella* were lysed by 1 or more of the 13 phages in the typing set. Yet, all 11 shigellae, which consisted of different strains of *S. flexneri* and *S. sonnei*, were typable. The phage types and the number of cultures with each phage type were: GHIJKLM, 4; GIJKLM, 2; IJLM, 1; and M, 4. All four phage types have been observed among those recorded for *E. coli*, and no differentiation with regard to strain or species could be made by phage typing.

DISCUSSION

The results show that strains of *E. coli* in the urine can be distinguished by phage typing. Although serological typing has been valuable in the identification of enteropathogenic strains of *E. coli*, serological identification of bacteriuric strains is neither as complete nor as readily accomplished. Whereas enteropathogenic strains can be identified by their O, H, and K antigens, serological identification of bacteriuric strains has been based mainly on the O-group antigens (6). Furthermore, antisera against bacteriuric strains are not available commercially and, at best, serological identification is time-consuming and difficult to perform. Another method of identification, it seems, would be desirable.

Results of phage typing 717 cultures of *E. coli* isolated in Missouri with phages A through H showed that 50.3% of the urinary isolates and 34.4% of the nonurinary isolates could be identified. Although this is not a high percentage of typability for the urinary cultures, this study showed that strains in the urine were not found frequently in nonurinary sources and strains from nonurinary sources were not found commonly in urine. The inclusion of phages I through M increased the sensitivity of our typing set considerably, not only for strains isolated in Missouri but also for strains from six distant geographic regions. We feel that 81.2% typability for 883 urinary strains from all regions is competitive with the serological typing percentages. Our findings are in agreement with those of Brown and Parisi (2), in that a relatively small number of phage types were responsible for the majority of cases of bacteriuria. Serological data also indicate that the majority of infections of the urinary tract are caused by *E. coli* in a few serological groups (5, 6, 9). Similarly, our findings for certain phage types occurring in high incidence in different geographic regions compare favorably with the serological data that, regardless of the geographic region, there was a consistency of the distribution of 0 groups responsible for urinary tract infections (6, 12). The preliminary study (2) and unpublished data from our laboratory comparing serological types with phage types show that there are several phage types within a serological group, and perhaps a more complete identification can be obtained by phage typing.

When multiple colonies were picked from a urine specimen and phage typed, in the majority of instances only one strain was present. This is in agreement with the serological data (13). Also, although the persistence of strains in two patients with chronic bacteriuria is consistent with the serological findings, the occurrence of three different strains in the third patient is not (8). It is obvious that long-term studies are needed with a large number of chronic bacteriuric patients in order to assess the value of phage typing in such instances.

We found no correlation between the phage type and the pattern of resistance to antibiotics. Although the routine use of antibiotics in therapy may possibly have been responsible for the predominance of strains found resistant to tetracycline and dihydrostreptomycin in our study,

Winterbauer, Turck, and Petersdorf reported (14) that treatment with broad-spectrum antibiotics did not result in the replacement of sensitive strains of *E. coli* by resistant strains. On the contrary, these investigators reported that antibiotic therapy increased the fecal carrier rate of certain serological groups which frequently cause urinary tract infections.

Phage typing cultures of *E. coli* from the feces of normal individuals in Missouri showed that the phage type occurring in highest incidence among the 97 fecal isolates was also one of the phage types occurring in highest incidence among the urinary isolates in Missouri. Further studies of fecal and urinary cultures may be valuable in determining the source of infection in individuals with a history of chronic bacteriuria. Although multiple colonies from fecal cultures were not picked for typing, the relatively large number of phage types observed supports the serological data that the fecal flora usually consists of strains of *E. coli* from several serological groups (11, 13).

The typability of all 11 cultures of shigellae and the similarities in phage type with escherichiae are further evidence of the serological (4) and genetic (7) relationships observed between these genera. Although we have no evidence that our phages, which were isolated from sewage, are indeed coliphages, the inability of our phages to lyse species from other genera of bacteria, and their typability for both escherichiae and shigellae indicate that our phages are probably coliphages.

Due to unknown properties of the bacterium or phage-bacterium interaction, the lytic reactions of some cultures are easier to read than of others. When 32 cultures of *E. coli* selected from all geographic regions were typed on 5 days consecutively, the typing data for 24 of the cultures were reproducible (not shown). For cultures where typing data were not consistent, some lytic reactions were either lost or reduced in intensity. One explanation for the loss of a lytic reaction is the subsequent overgrowth of a lysed culture. The reduction in intensity of a lytic reaction might be accounted for by the subjectiveness of the method in recording the degree of lysis. Discrepancies in the degree of lysis could result also from a reduction in phage titer or an alteration in phage receptor sites. However, we do not feel that these difficulties limit the usefulness of the phage typing procedure, but we do emphasize the need for rather strict adherence to the methodology of phage typing.

To date, our knowledge of the epidemiology of urinary tract infections is incomplete. Much remains to be learned concerning the origin, the mode of transmission, and the existence of a nosocomial population of strains causing infection of the urinary tract. We feel that the ease and speed with which phage typing can be done would justify its use in epidemiologic studies of infections by bacteriuric strains of *E. coli*.

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