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Bacteriophage Typing of Bacteriuric *Escherichia coli.** (30751)

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Investigations of *Escherichia coli* bacteriuria in the last few years have shown that several serological strains are responsible for the majority of urinary tract infections(1,2,3, 4). However, these studies have been based mainly on the O group antigens, and serological identification is difficult to perform and time consuming. The purpose of this study was to determine the feasibility of identifying bacteriuric *E. coli* with bacteriophages.

Materials and methods. Cultures. Urine specimens were collected by the clean-catch method at Mercy Hospital, Pittsburgh, Pa., and streaked on blood agar base (Difco) containing 5% human erythrocytes. Standard dilution loops were used to determine the quantitative bacterial count on the freshly obtained specimens. Representative colonies were picked from those plates containing 100,000 or more colonies per ml of urine. Any plate showing mixed infection was discarded. Biochemical tests were performed on the isolated cultures to ascertain that the organisms were *E. coli*(5). Stock cultures were maintained by streaking the surface and stabbing the butt of tryptose blood agar base (Difco) slants.

Isolation of phages. Attempts to isolate phages from *E. coli* by Fisk's method(6) were unsuccessful. Therefore, 10 ml quantities of raw sewage from the Allegheny County Sanitary Authority, Pittsburgh, Pa., were inoculated into 90 ml of brain heart infusion (BHI) broth (Baltimore Biological Laboratory) and incubated at 37°C for 26 hours. Cultures were then centrifuged at 3,000 r.p.m. for 30 minutes and the supernatant fluids filtered through S1 Seitz filter sheets #6. To determine phage activity, various concentrations of filtrate and cultures of *E. coli* obtained from various sources were plated. Phage action was revealed by zones of complete or semi-complete lysis, or by discrete plaques. To distinguish between phage activity and colicin activity, the method of Bailey and Glynn(7) was used and filtrates showing colicin activity were discarded.

Purification and propagation of phages. Areas with discrete plaques were picked and

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placed in 0.1 M ammonium acetate(8), filtered through S1 Seitz #6 filters, and replated on the susceptible strain of *E. coli*. This procedure was repeated 5 times for each phage and in this manner, 8 phages (A, B, C, D, E, F, G, H) were obtained which differed in their lytic activity for stock cultures of *E. coli*.

Experimentation showed that 0.2 ml of filtrate and 0.1 ml of an 18-hour BHI broth culture of a susceptible strain placed in 6 ml of BHI broth with 0.75% agar (Difco) gave the best results for the agar layer method of phage propagation(9). Phage suspensions were maintained at 4°C and prior to use, the routine test dilution (RTD) was determined.

Determination of phage patterns. A modification of the method of Wilson and Atkinson(10) for phage typing of *Staphylococcus aureus* was utilized. Prior to typing, a strain was incubated in BHI broth for 8 hours at 37°C and then swabbed on BHI agar plates. The RTD of the 8 phages was placed on the strain and plates were read after 6 hours' incubation at 37°C and again after 12 hours at room temperature. The only reactions recorded were those in which the degree of lysis was equal to, or greater than the amount of bacterial growth in the zone of the RTD.

Serological typing. The procedure used was a modification of the method of Edwards and Ewing(5). The typing sera for the O somatic groups had been prepared at the Communicable Disease Center, Atlanta, Ga., and were obtained from Dr. Donald Zangwill, Magee-Womens Hospital, Pittsburgh, Pa.

TABLE I. Phage Types of *E. coli* in Two Series of Bacteriuria Specimens.

Phage type	% of cultures Series-1	Series-2
ABCD	2	0
ABCDE	28	21
AH	2	0
BCDEFGH	2	0
BCDE	20	21
BCD	6	7
BDE	8	14
BE	4	0
DEG	2	0
DE	18	14
D	4	0
CD	2	0
E	2	0
BD	0	21

TABLE II. Production of Indole by *E. coli* and Susceptibility to Phage.

Indole production	No. of cultures	% typable
Positive	72	81
Negative	26	35
Total	98	69

Results. In Table I are shown the different phage types and a comparison of the percentages of different phage types found in cultures obtained from the same laboratory and typed by the same techniques, but at different times. The first series of isolates was obtained from June 1 to September 1, 1964, and the second series from February 1 to March 31, 1965. No patient from the first series was included in the second series. Four of the phage types—ABCDE, BCDE, BDE, DE—accounted for 74% of 54 typable bacteriuric cultures of series-1 and 70% of 14 typable cultures of series-2.

Several colonies from individual urine specimens were typed and in all cases, only one phage type was found. Several patients had 2 to 5 specimens collected over a 2-week period and in all instances, a strain with the same phage type was present in all specimens. Six cultures isolated from a patient over a 3-year period, 5 of which belonged to the same serological somatic group—075—while the other was serologically untypable, were obtained from Dr. Zangwill at Magee-Womens Hospital. These cultures were tested for their sensitivity to phage and all 6 cultures had the same phage type. In all, 69.4% of 98 isolates from urine were phage typable and 64.7% of 16 extraurinary isolates were phage typable.

As is shown in Table II, there appears to be a correlation between the ability to utilize tryptophan with the production of indole and susceptibility to phage. Edwards and Ewing reported that 99% of *E. coli* produce indole from tryptophan(5). Presumably, those strains which fail to produce indole should not be classified as *E. coli* and this would explain their resistance to the phages used in this study.

Forty selected cultures from urine, with 14

TABLE III. Comparison of Serological Groups and Phage Types.

No. of cultures	Serological group	Phage type	No. of strains with phage type
15	N.T.*	AH, BCD, BCDEFGH, BDE, BE, DE	1
		D	2
		BCDE	3
		ABCDE	4
5	Auto-aggl†	ABCDE, BCDE, F	1
		BDE	2
1	01 and 06	ABCD	1
6	04	BCD	1
		ABCDE	3
		BCDE	2
4	07	DE	4
1	011	BE	1
3	025	ABCDE, BDE, DE	1
5	075	CD	1
		BCDE, DE	2

* N.T.—not typable. † Auto-aggl—auto-agglutination.

different phage types, were tested against 10 somatic antisera (01, 2, 4, 6, 7, 11, 15, 25, 62 and 75) of strains occurring most frequently in urinary tract infections (Table III). Only 20 (50%) of the cultures tested gave positive serological reactions.

Discussion. The results show that strains of *E. coli* in the urine can be identified by bacteriophages. The phage types which occurred in urine specimens did not occur frequently in the extraurinary isolates and those phage types responsible for extraurinary infections were not found in high incidence in the urinary tract.

When 40 phage typable cultures were tested against the 10 antisera reported to identify the majority of strains isolated from urinary tract infections(11), only 20 gave positive reactions. In most instances, there were several different phage types within a serological group and a more complete identification was obtained. An advantage of phage typing was seen in which one culture from a series of cultures from a patient with chronic pyelonephritis over a 3-year period was untypable serologically but typable with phage. Thus, it was shown that the same strain was present in all specimens from the patient and the phage type did not change over the 3-year period. Also, it appears that phage typing has advantages over serological typing in that phage typing is faster and easier to perform.

The results from phage typing were in agreement with serological results which demonstrated relatively few strains of *E. coli* cause a majority of infections(1,2,3,4). In view of the high percentage of phage typable cultures and the infrequent occurrence of bacteriuric strains in extraurinary tract isolates, we feel that the replacement of 1 or 2 phages in our typing set with phages more specific for urinary tract isolates would justify their use in the identification of such strains.

Summary. A bacteriophage typing method of identifying strains of *E. coli* found in urinary tract infections has been developed. Eight phages were isolated from sewage and used to classify *E. coli* into 14 phage types. Certain types were found to cause a majority of the infections. Twenty of 40 phage typable cultures were typable serologically and no definite correlation was obtained. However, there were several phage types in each serological group. Several advantages in using phage to identify strains from urinary tract infections were pointed out.

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Antiviral Activity of Ammonium Picrate in Mice. (30752)

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In the course of screening compounds for antiviral activity in mice, ammonium picrate was found to be uniquely effective against the Columbia SK virus (encephalomyocarditis) infection. Results of experiments characterizing the antiviral properties of this compound are reported here.

Materials and methods. The efficacy of ammonium picrate was evaluated in Taconic Farms male albino adult mice infected subcutaneously or intracerebrally with aqueous dilutions of SK virus. Stock virus was the supernatant derived by centrifugation from a 10% aqueous suspension of homogenized pooled infected brain tissue. Infectivity of stock virus was such that 90 to 95% of the nontreated mice infected subcutaneously with 0.2 ml of a 10^{-6} dilution died, with a mean survival time of 4.5 days. The mortality of mice infected with a 10^{-7} dilution was approximately 50%. Ammonium picrate was lethal at 200 mg/kg given as a single dose by either intraperitoneal or subcutaneous injection; a dose of 100 mg/kg by these routes was tolerated with no overt ill effects. A single oral dose of 400 mg/kg administered by stomach tube was lethal; a dose of 200 mg/kg by this route was tolerated. Treatment with ammonium picrate by injection or by oral tubing was given at 24 hours and 2 hours before infection, and again at 24 hours after infection, unless otherwise specified. The activity of ammonium picrate in infected mice was measured routinely in terms of the number of animals surviving on the 21st day postinfection.

TABLE I. Effect of Ammonium Picrate on Survival of Mice After Subcutaneous Infection with Columbia SK Virus.

Drug dose* (mg/kg)	Survivors/total mice on 21st day: Intraperitoneal	Subcutaneous	Oral tubing
400	—	—	Toxic
200	Toxic	Toxic	19/19
100	94/100	19/20	19/19
50	66/80	13/20	17/20
25	48/100	5/20	8/20
12	24/80	6/20	4/20
6	—	4/20	7/20
0	6/120	2/20	2/20
ED_{50}, mg/kg:	22 (18–27)	29 (22–39)	26 (20–34)

* Dose given by indicated routes at 24 hr and 2 hr before, and at 24 hr after subcutaneous infection with 0.2 ml of 10^{-6} dilution of stock virus.

Results. Mice infected subcutaneously with a 10^{-6} dilution of stock virus responded to either parenteral or oral treatment with ammonium picrate (Table I). Graded dosage of compound produced a graded effect. Maximum tolerated dose levels protected 95 to 100% of the infected mice. All treated mice surviving the infection remained free of any symptoms of the infection or ill effects of treatment and were, on visual inspection, indistinguishable from the noninfected controls. The median effective dose (ED_{50}) for each route of treatment, based on at least 20 mice per dose level, was calculated by the method of Litchfield and Wilcoxon(1).

The effectiveness of ammonium picrate, at maximum tolerated intraperitoneal dosage, was tested against graded doses of SK virus injected subcutaneously. The results of this experiment show that the larger the infecting