Reduced Set of Phages for Typing *Escherichia coli*

M. GERSHMAN, G. MARKOWSKY,[1] and JACQUELINE HUNTER
Departments of Microbiology and Animal and Veterinary Sciences
University of Maine
Orono 04469

ABSTRACT

A phage typing set composed of 32 phages is described for differentiating *Escherichi coli.* Eight hundred sixty-six isolates from cases of bovine mastitis were used in this effort. Of these cultures 829, or 96%, were characterized successfully, and 178 phage types were observed. Thirty-seven isolates were not typable.

INTRODUCTION

Although mastitis is a costly disease plaguing the dairy industry, progress toward its control has lagged for numerous reasons. Important among these is that mastitis is caused by a number of microorganisms, some of which are yet to be characterized properly and placed in perspective.

Escherichia coli frequently is associated with mastitis. However, some strains live as saprophytes; therefore, separating these organisms into types can be of value in establishing the existence of unique pathogenic varieties.

One of the most significant properties of phage (bacterial viruses) is specificity for the host. By our exposing an isolate of *E. coli*, growing on the surface of an agar plate, to a standard set of dissimilar phages, a pattern will develop contingent on the susceptibility or resistance of the culture to the phages employed. A culture sensitive to a particular phage is lysed, and the destruction is manifested by an area devoid of bacterial growth. By this procedure, referred to as phage typing, types or strains can be characterized.

In keeping with our immediate interests, we developed a phage typing set of 53 distinct phages for differentiating *E. coli* (3). In recent months, to make our procedure more cost effective and less time-consuming, a combinatorial evaluation of our phages and phage patterns was conducted, and we determined that a reduced set of 32 phages would be more practical and could be used without compromising any significant attributes.

MATERIALS AND METHODS

Media

Nutrient agar and broth were used exclusively for testing phage filtrates and phage typing. Before use, agar plates were dried in an incubator for 2 h with lids partially opened. Nutrient agar, nutrient broth, and nutrient broth with .5% NaCl and .7% agar were used for phage propagation. In all of our procedures incubation temperature was 37°C.

Bacterial Cultures

Our reduced set of phages were isolated by 31 host cultures. Twelve came from Cornell University, Ithaca, NY; 9 from the National Animal Disease Laboratory, Ames, IA; and 10 from our own University of Maine Diagnostic Service, Orono. All of the cultures were of bovine origin, and some were implicated in mastitis. The 866 *E. coli* isolates used to ascertain the effectiveness of our typing phages were from quarter milk samples collected from mastitic cows in Iowa, Maine, Massachusetts, New Jersey, and Pennsylvania.

Phage Isolation and Propagation

Our initial collection of phages, from which our reduced set is derived, was isolated by 205 host cultures and 216 liters of sewage obtained from a local treatment plant during different seasons of the year.

Phages were isolated by enriching individual 100-ml, untreated sewage samples with 6 ml of a single, 1.5-h *E. coli* host culture. Turbidity of the host culture was barely evident and contained approximately 9×10^7 organisms/ml. After 18 h of incubation, brot
through a .45 μm membrane fil
for phage by spotting the filtrat
culture that was used in the enri
A lawn of this culture was p
evenly applying 2 ml of 1.5-h b
E. coli, containing a microb
similar to the one stated, over t
agar plate. The plate then was a
room temperature for 15 min a
a Pasteur pipette with a drop
filtrate under study. After the
absorbed thoroughly (approxim
min), the plate was inverted,
night, and examined the follow
isolated plaques appeared, the
three times by serial, single-pla
cases where phage activity was
permit single-plaque isolation
procedure was repeated with a
filtrates. Phages then were pr
method described by Swanstr
(4). In essence, this procedure i
a culture by a homologous phag
a soft, thin agar matrix resting o
of nutrient agar. A 60-ml am
agar was poured into a 15-cm
allowed to harden on a leveled su
broth with .5% NaCl and .7% ag
in 15-ml quantities, cooled
inoculated with a mixture of th
overnight agar slope suspended
and 2 ml of the phage to be prop
of the broth culture was adjust
concentration used for phage
combination was agitated gen
over the surface of the base la
harden, and incubated overnigh
10 ml of broth was added to th
soft-agar layer was removed
tongue depressor, transferred t
vigorously to break up the agar
and centrifuged at 60 × g fo
supernatant then was decanted,
a .45 μm membrane filter, a
phage content.

Testing of Phage Filtrates

The testing procedure invol
titration to determine the routi
(RTD) and a lytic pattern t
novelty, stability, and usefuln

Received July 1, 1983.
[1]IBM Thomas J. Watson Research Center, Yorktown Heights, NY 10598.

KOWSKY,[1] and JACQUELINE HUNTER
ology and Animal and Veterinary Sciences
University of Maine
Orono 04469

and less time-consuming, a com-
evaluation of our phages and phage
as conducted, and we determined
ced set of 32 phages would be more
nd could be used without com-
ny significant attributes.

MATERIALS AND METHODS

agar and broth were used exclusively
phage filtrates and phage typing.
, agar plates were dried in an in-
r 2 h with lids partially opened.
gar, nutrient broth, and nutrient
.5% NaCl and .7% agar were used for
agation. In all of our procedures
temperature was 37°C.

tures

uced set of phages were isolated by
ltures. Twelve came from Cornell
Ithaca, NY; 9 from the National
ease Laboratory, Ames, IA; and 10
wn University of Maine Diagnostic
ono. All of the cultures were of
in, and some were implicated in
he 866 *E. coli* isolates used to
e effectiveness of our typing phages
quarter milk samples collected from
ws in Iowa, Maine, Massachusetts,
and Pennsylvania.

n and Propagation

al collection of phages, from which
set is derived, was isolated by 205
s and 216 liters of sewage obtained
l treatment plant during different
ne year.
ere isolated by enriching individual
reated sewage samples with 6 ml of
-h *E. coli* host culture. Turbidity of
lture was barely evident and con-
oximately 9×10^7 organisms/ml.

After 18 h of incubation, broths were passed through a .45 μm membrane filter and assayed for phage by spotting the filtrate onto the same culture that was used in the enrichment process. A lawn of this culture was prepared by our evenly applying 2 ml of 1.5-h broth culture of *E. coli*, containing a microbial population similar to the one stated, over the surface of an agar plate. The plate then was allowed to dry at room temperature for 15 min and spotted from a Pasteur pipette with a drop (.04 ml) of the filtrate under study. After the drop had been absorbed thoroughly (approximately 15 to 20 min), the plate was inverted, incubated overnight, and examined the following morning. If isolated plaques appeared, they were purified three times by serial, single-plaque passage. In cases where phage activity was too extensive to permit single-plaque isolations, the assaying procedure was repeated with a series of diluted filtrates. Phages then were propagated by a method described by Swanstrom and Adams (4). In essence, this procedure involved lysis of a culture by a homologous phage suspended in a soft, thin agar matrix resting on a thicker base of nutrient agar. A 60-ml amount of melted agar was poured into a 15-cm petri dish and allowed to harden on a leveled support. Nutrient broth with .5% NaCl and .7% agar was prepared in 15-ml quantities, cooled to 45°C, and inoculated with a mixture of the growth of an overnight agar slope suspended in 1 ml of broth and 2 ml of the phage to be propagated. Density of the broth culture was adjusted to equal the concentration used for phage isolations. This combination was agitated gently and poured over the surface of the base layer, allowed to harden, and incubated overnight. The next day, 10 ml of broth was added to the plate, and the soft-agar layer was removed with a sterile tongue depressor, transferred to tubes, shaken vigorously to break up the agar-phage complex, and centrifuged at 60 × g for 20 min. The supernatant then was decanted, filtered through a .45 μm membrane filter, and assayed for phage content.

Testing of Phage Filtrates

The testing procedure involved preliminary titration to determine the routine test dilution (RTD) and a lytic pattern to ascertain the novelty, stability, and usefulness of a phage isolate. The RTD, as defined by Anderson (1), is the highest dilution of phage that produces complete or confluent lysis on its propagating strain or a reaction approaching that order. Its use minimizes the occurrence of confusing crossreactions. The RTD's of our typing phages varied from 10^{-3} to 10^{-5}. These RTD's are listed in Table 1. They were established by titrating phage in 10-fold serial dilutions. Only phages with an RTD of not less than 10^{-3} were used.

The lytic pattern was determined by testing a phage, at its RTD, against all of the propagating strains that were used to maintain the phages that constituted our typing set. The lytic patterns of the typing phages are in Table 2. When RTD phage stocks were renewed, the lytic spectra of new and proceeding batches were compared to ensure that intrinsic properties were maintained. The phage pattern of each new subculture also was checked for similar reasons.

Storage

The RTD's were stored at 4°C and tested for potency at least once a week. A test dilution was satisfactory for phage typing as long as it produced confluent lysis on its propagating strain. In general, the test dilutions of the majority of phages retained their effectiveness for 4 to 6 wk and occasionally longer. In any event, longevity of a test dilution was not predictable, and frequent periodic checks were required.

Typing Technique

The mastitis isolates typed in this investigation each were inoculated lightly into 3 ml of broth and incubated for 1.5 h or until turbidity

TABLE 1. Routine test dilution (RTD) of typing phages.

Phage	RTD
2,3,4,6,7,9,10,11,12,13,15,16,17,18,19,21, 22,23,24,25,27,28,30,31,32	10^{-3}
1,5,14,20,26,29	10^{-4}
8	10^{-5}

TABLE 2. Lytic patterns of *E. coli* typing phages and their propagating strains.

Propagating strain	Phage															
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	CL[a]	–	SCL	–	–	SCL	OL	–	SCL	–	–	–	–	–	SCL	–
2	–	CL	SCL	–	–	–	OL	–	SCL	–	–	–	–	–	SCL	–
3	–	–	CL	–	–	SCL	CL	–	SCL	–	–	–	–	–	–	–
4	SCL	–	SCL	CL	–	SCL	OL	–	–	–	–	–	–	–	SCL	+++
5	SCL	–	OL	–	CL	SCL	–	–	+++	–	–	–	OL	–	SCL	CL
6	–	–	SCL	–	–	CL	–	–	–	–	–	–	SCL	–	OL	–
7	–	–	SCL	–	–	SCL	CL	–	SCL	–	SCL	–	–	–	–	–
8	–	–	SCL	–	SCL	SCL	–	CL	SCL	–	–	–	SCL	–	+++	–
9	–	–	SCL	–	–	OL	OL	–	CL	–	–	–	–	–	CL	–
10	–	+++	–	–	–	–	–	–	–	CL	CL	++	–	–	–	–
11	–	–	SCL	–	–	SCL	SCL	–	–	–	CL	–	–	–	+++	–
12	–	–	SCL	+	–	SCL	SCL	–	–	++	SCL	CL	–	–	++	SCL
13	–	–	CL	–	–	CL	OL	–	CL	–	–	–	CL	–	CL	CL
14	–	–	OL	SCL	–	SCL	–	–	–	–	–	–	–	CL	–	SCL
15	–	–	SCL	–	–	CL	SCL	–	SCL	SCL	CL	–	–	–	CL	–
16	–	–	SCL	–	–	SCL	SCL	–	CL	–	–	–	–	<SCL	<SCL	CL
17	–	–	SCL	–	–	SCL	CL	–	SCL	–	SCL	–	–	–	–	–
18	–	–	–	–	–	–	–	–	–	–	–	–	–	SCL	–	–
19	–	–	CL	–	–	CL	–	–	–	<SCL	CL	SCL	–	–	SCL	+++
20	–	–	SCL	–	–	SCL	OL	–	–	–	–	–	–	–	<SCL	<SCL
21	–	–	–	–	–	OL	CL	–	CL	–	–	–	–	–	CL	OL
22	–	–	–	–	–	–	–	CL	–	CL	–	–	–	–	SCL	<SCL
23	–	–	SCL	–	–	SCL	SCL	–	SCL	–	–	–	–	–	CL	–
24	–	–	–	–	–	+++	CL	–	CL	–	–	–	–	–	–	–
25	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
26	–	SCL	–	–	–	–	SCL	–	–	<SCL	OL	–	–	–	–	–
27	–	–	SCL	–	–	–	SCL	–	–	–	–	–	–	–	<SCL	–
28	–	–	SCL	–												
29	–	–	–	–	–	SCL	CL	–	SCL	–	–	–	–	–	CL	–
30	–	–	–	–	–	–	<SCL	–	–	–	–	–	–	++	–	–
31	–	–	CL	–	–	–	OL	–	CL	–	–	–	–	–	CL	–

Propagating strain	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	SCL	–	–	–	–	OL	<SCL	<SCL	–	–	OL	<SCL	–	–	–	CL
2	SCL	–	–	–	+++	SCL	<SCL	SCL	–	–	OL	OL	–	–	–	CL
3	SCL	–	–	–	–	SCL	<SCL	<SCL	–	–	SCL	CL	–	–	–	–
4	SCL	–	<SCL	–	CL	OL	<SCL	<SCL	SCL	–	OL	CL	<SCL	SCL	–	CL
5	SCL	CL	–	–	–	–	–	–	SCL	–	SCL	SCL	–	–	–	CL
6	SCL	OL	–	–	–	–	–	–	<SCL	–	OL	<SCL	–	–	–	CL
7	SCL	–	SCL	–	++	SCL	<SCL	<SCL	–	CL	SCL	CL	–	–	–	CL
8	SCL	CL	SCL	–	SCL	–	–	–	–	–	<SCL	<SCL	–	–	–	SCL
9	SCL	SCL	–	–	SCL	OL	<SCL	<SCL	<SCL	–	OL	OL	–	–	–	CL
10	–	–	+	–	–	++	–	–	–	CL	–	–	–	–	–	–
						<SCL	++	+++	–	–	OL	OL	–	–	–	SCL
																SCL

	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
1	SCL	–	–	–	–	OL	<SCL	<SCL	–	–	OL	<SCL	–	–	–	CL
2	SCL	–	–	–	+++	SCL	<SCL	SCL	–	–	OL	OL	–	–	–	CL
3	SCL	–	–	–	–	SCL	<SCL	<SCL	–	–	SCL	CL	–	–	–	–
4	SCL	–	<SCL	–	CL	OL	<SCL	<SCL	SCL	–	OL	CL	<SCL	SCL	–	CL
5	SCL	CL	–	–	–	–	–	–	SCL	–	SCL	SCL	–	–	–	CL
6	SCL	OL	–	–	–	–	–	–	<SCL	–	OL	<SCL	–	–	–	CL
7	SCL	–	SCL	–	++	SCL	<SCL	<SCL	–	CL	SCL	CL	–	–	–	CL
8	SCL	CL	SCL	–	SCL	–	–	–	–	–	<SCL	<SCL	–	–	–	SCL
9	SCL	SCL	–	–	SCL	OL	<SCL	<SCL	<SCL	–	OL	OL	–	–	–	CL
10	–	–	+	–	–	++	–	–	–	CL	–	–	–	–	–	–
11	SCL	–	–	–	–	<SCL	++	+++	–	–	OL	OL	–	–	–	SCL
12	++	–	–	–	–	OL	–	–	–	–	<SCL	CL	–	–	–	SCL
13	SCL	CL	<SCL	–	<SCL	SCL	–	SCL	–	–	<SCL	<SCL	–	–	–	CL
14	SCL	–	–	–	<SCL	–	–	–	<SCL	–	–	–	–	–	–	–
15	SCL	–	CL	–	++	SCL	–	SCL	–	SCL	SCL	SCL	–	–	–	SCL
16	SCL	CL	–	<SCL	<SCL	<SCL	–	–	OL	–	SCL	SCL	–	–	–	CL
17	CL	–	SCL	–	+	SCL	–	+++	–	SCL	SCL	CL	–	–	–	CL
18	–	CL	–	–	–	–	–	–	–	–	–	OL	–	–	–	<SCL
19	SCL	–	CL	–	–	CL	–	–	–	SCL	–	<SCL	–	–	–	CL
20	SCL	–	–	CL	CL	<SCL	–	+++	–	–	OL	SCL	–	–	–	<SCL
21	–	SCL	–	–	CL	OL	<SCL	<SCL	–	–	SCL	CL	–	–	–	CL
22	+	–	<SCL	–	+	CL	–	<SCL	–	–	SCL	SCL	–	–	–	SCL
23	SCL	–	–	–	SCL	SCL	CL	<SCL	–	–	SCL	SCL	SCL	CL	–	SCL
24	–	<SCL	<SCL	+	+	SCL	SCL	CL	–	–	CL	CL	–	–	–	SCL
25	–	–	–	–	–	–	–	–	CL	–	–	–	–	–	–	–
26	<SCL	–	–	–	–	CL	–	–	–	CL	OL	OL	–	–	–	OL
27	SCL	CL	–	–	–	SCL	<SCL	<SCL	–	–	OL	CL	–	–	–	+++
28	–	–	–	–	+	SCL	SCL	<SCL	–	–	SCL	CL	CL	CL	–	CL
29	–	OL	–	–	SCL	SCL	–	–	–	–	SCL	CL	SCL	CL	–	SCL
30	–	–	–	–	–	–	–	–	–	–	–	<SCL	–	–	CL	OL
31	SCL	SCL	–	–	–	SCL	SCL	–	–	–	OL	SCL	–	–	–	CL

[a]CL, Confluent lysis; OL, opaque lysis (opacity due to secondary growth); SCL, semiconfluent lysis; <SCL, less than semiconfluent lysis; +++, 121 plaques; +++, 81 to 120 plaques; ++, 61 to 80 plaques; ++, 41 to 60 plaques; +, 21 to 40 plaques; +, 6 to 20 plaques; –, 0 to 5 plaques.

TABLE 3. Mnemonic for reporting phage types.

Triplet	Number or letter representation
−−−	0
+++	1
++−	2
+−+	3
−++	4
+−−	5
−+−	6
−−+	7
++	A
+−	B
−+	C
−−	D

again reflected a population of 9×10^7 organisms/ml. A small quantity of the broth culture then was flooded onto each of four 100 × 15 mm agar plates and handled in much the same way as described for preparation of bacterial lawns. However, after each plate was dried, it was refrigerated for 30 min before being spotted with single drops of the phages that constituted our set. Each plate was spotted with eight dissimilar phages by a 26 gauge needle and incubated overnight. The following day, cultures were examined by hand lens and viewed through the bottom of the plate. Susceptibility to a phage was demonstrated by areas of clearing that ranged from isolated plaques to confluent lysis. Phage activity was recorded on reactions described in the legend of Table 2.

TABLE 4. Phage types isolated.

Culture no.	Phage pattern	Mneumonic
1	1/3/5/6/7/15/16/17/22/23/24/27/28/32	345 072 017 5C
2	1/3/6/7/9/13/14/15/16/18/22/28/32	373 013 050 5C
3	1/7/9/21/22/23/24/25/27/32	303 000 713 0C
4	1/7/9/21/22/23/24/25/27/28/32	303 000 713 5C
5	1/16/28/30	300 005 000 3D
6	1/21/25/29/30	300 000 705 4D
7	1/25/28	300 000 005 5D
8	1/25/29	300 000 005 6D
9	1/28	300 000 000 5D
10	2/3/6/7/9/11/12/15/16/17/22/23/24/26/27/28/32	473 472 014 5C
11	2/3/6/7/9/12/13/16/17/18/21/22/23/27/28/32	473 751 717 5C
12	2/3/6/7/9/15/16/17/22/23/24/27/28/32	473 072 017 5C
13	2/3/6/7/9/15/16/17/22/23/24/27/32	473 072 017 0C
14	2/3/6/9/11/12/24/27/28/32	477 400 077 5C
15	2/3/6/9/14/16/17	477 062 000 0D
16	2/6/7/9/15/22/25/27/28/32	673 070 053 5C
17	2/7/9/12/17/22/23/24/27/28	603 706 017 5D
18	2/7/9/15/18/22/23/27/28/29/30/32	603 077 027 1C
19	2/9/11/12/18/19/20/21/28/32	607 407 100 5C
20	2/9/11/16/17/19/20/22/24/26/27/32	607 602 234 0C

(continued)

TABLE 4. (continued) Phage types isolated.

Culture no.	Phage pattern
21	2/9/23/24/28/32
22	3/5/6/7/9/15/16/17/18/
23	3/5/6/7/9/15/16/21/22/
24	3/5/6/7/15/16/17/18/22
25	3/5/8/13/14/15/16/17/1
26	3/5/8/13/15/16/17/18/2
27	3/6/7/8/15/16/17/22/23
28	3/6/7/9/10/11/15/16/19
29	3/6/7/9/11/12/15/16/1
30	3/6/7/9/11/12/15/16/1
31	3/6/7/9/11/12/16/22/2
32	3/6/7/9/11/14/15/17/2
33	3/6/7/9/11/15/16/19/2
34	3/6/7/9/11/15/16/19/2
35	3/6/7/9/11/15/17/22/2
36	3/6/7/9/11/16/17/19/2
37	3/6/7/9/12/15/16/17/2
38	3/6/7/9/13/15/16/17/1
39	3/6/7/9/13/15/16/17/1
40	3/6/7/9/13/15/16/17/1
41	3/6/7/9/13/16/17/18/2
42	3/6/7/9/15/16/17/18/2
43	3/6/7/9/15/16/17/18/2
44	3/6/7/9/15/16/28/32
45	3/6/7/9/16/17/28/29/3
46	3/6/7/9/16/17/28/32
47	3/6/7/9/17/27/28/32
48	3/6/7/11
49	3/6/7/11/12/15/16/17
50	3/6/7/11/12/15/22/27
51	3/6/7/11/15/16/17/22
52	3/6/7/11/15/16/17/23
53	3/6/7/11/15/16/17/23
54	3/6/7/11/12/15/16/17
55	3/6/7/11/12/15/16/17
56	3/6/7/12/15/16/17/22
57	3/6/7/13/16/17/18/28
58	3/6/7/15/16/17/22/23
59	3/6/7/15/16/17/22/23

ected a population of 9×10^7 or-
l. A small quantity of the broth
en was flooded onto each of four 100
agar plates and handled in much the
as described for preparation of
awns. However, after each plate was
was refrigerated for 30 min before
ted with single drops of the phages
ituted our set. Each plate was spotted
t dissimilar phages by a 26 gauge
d incubated overnight. The following
res were examined by hand lens and
hrough the bottom of the plate.
lity to a phage was demonstrated by
clearing that ranged from isolated
o confluent lysis. Phage activity was
on reactions described in the legend of

	Mneumonic
	345 072 017 5C
	373 013 050 5C
	303 000 713 0C
	303 000 713 5C
	300 005 000 3D
	300 000 705 4D
	300 000 005 5D
	300 000 005 6D
	300 000 000 5D
32	473 472 014 5C
	473 751 717 5C
	473 072 017 5C
	473 072 017 0C
	477 400 077 5C
	477 062 000 0D
	673 070 053 5C
	603 706 017 5D
	603 077 027 1C
	607 407 100 5C
	607 602 234 0C

(continued)

TABLE 4. (continued) Phage types isolated.

Culture no.	Phage pattern	Mneumonic
21	2/9/23/24/28/32	607 000 040 5C
22	3/5/6/7/9/15/16/17/18/21/22/23/25/28/32	743 071 725 5C
23	3/5/6/7/9/15/16/21/22/23/25/28/32	743 075 725 5C
24	3/5/6/7/15/16/17/18/22/23/25/28/32	745 071 025 5C
25	3/5/8/13/14/15/16/17/18/28/32	766 011 000 5C
26	3/5/8/13/15/16/17/18/28/32	766 031 000 5C
27	3/6/7/8/15/16/17/22/23/25/27/28/32	772 072 023 5C
28	3/6/7/9/10/11/15/16/19/22/23/26/27/28/32	773 275 524 5C
29	3/6/7/9/11/12/15/16/17/18/22/23/24/27/28/32	773 471 017 5C
30	3/6/7/9/11/12/15/16/17/22/23/27/28/32	773 472 027 5C
31	3/6/7/9/11/12/16/22/23/27/28/32	773 405 027 5C
32	3/6/7/9/11/14/15/17/22/23/26/27/28/32	773 646 024 5C
33	3/6/7/9/11/15/16/19/21/22/23/24/27/28/32	773 675 317 5C
34	3/6/7/9/11/15/16/19/23/24/27/28/32	773 675 547 5C
35	3/6/7/9/11/15/17/22/23/27/32	773 676 027 0C
36	3/6/7/9/11/16/17/19/21/22/23/24/27/28/32	773 602 327 5C
37	3/6/7/9/12/15/16/17/22/23/24/27/28/32	773 552 027 5C
38	3/6/7/9/13/15/16/17/18/21/22/23/27/28/32	773 031 727 5C
39	3/6/7/9/13/15/16/17/18/21/22/23/28/32	773 031 720 5C
40	3/6/7/9/13/15/16/17/18/21/22/23/27/28/30/32	773 031 727 3C
41	3/6/7/9/13/16/17/18/25/28/32	773 051 005 5C
42	3/6/7/9/15/16/17/18/21/22/23/25/27/28/32	773 071 723 5C
43	3/6/7/9/15/16/17/18/28/32	773 071 000 5C
44	3/6/7/9/15/16/28/32	773 075 000 5C
45	3/6/7/9/16/17/28/29/30/32	773 002 000 1C
46	3/6/7/9/16/17/28/32	773 002 000 5C
47	3/6/7/9/17/27/28/32	773 006 007 5C
48	3/6/7/11	775 600 000 0D
49	3/6/7/11/12/15/16/17/22/23/24/27/28/32	775 472 017 5C
50	3/6/7/11/12/15/22/27/28/32	775 470 057 5C
51	3/6/7/11/15/16/17/22/28/32	775 672 050 5C
52	3/6/7/11/15/16/17/23/28	775 672 060 5D
53	3/6/7/11/15/16/17/23/28/32	775 672 060 5C
54	3/6/7/11/12/15/16/17/22/23/24/27/28/32	775 472 017 5C
55	3/6/7/11/12/15/16/17/22/23/27/28/32	775 472 027 5C
56	3/6/7/12/15/16/17/22/25/27/32	775 772 053 0C
57	3/6/7/13/16/17/18/28/32	775 051 000 5C
58	3/6/7/15/16/17/22/23/24/27/28/32	775 072 017 5C
59	3/6/7/15/16/17/22/23/28/32	775 072 020 5C

(continued)

TABLE 4. (continued) Phage types isolated.

Culture no.	Phage pattern	Mneumonic
60	3/6/7/15/16/17/27/28/32	775 062 007 5C
61	3/6/7/15/17/21/22/23/27/28/32	775 076 727 5C
62	3/6/7/15/17/25/28/32	775 076 005 5C
63	3/6/9/11/12/15/17/28/32	777 476 000 5C
64	3/6/9/11/15/16/17/22/23/24/26/27/28/32	777 672 014 5C
65	3/6/9/11/15/16/17/22/23/24/27/28/32	777 672 017 5C
66	3/6/9/11/16/19/22/23/26/28/32	777 605 526 5C
67	3/6/9/13/15/16/17/18/21/22/23/24/27/28/32	777 031 717 5C
68	3/6/9/13/15/16/17/18/21/22/24/25/28/32	777 031 735 5C
69	3/6/9/13/15/16/17/18/28/32	777 031 000 5C
70	3/6/9/15/16/17/18/21/22/23/27/28/32	777 071 727 5C
71	3/6/9/15/16/17/18/28/32	777 071 000 5C
72	3/6/9/15/16/17/18/31/32	777 071 000 0A
73	3/6/9/15/16/17/21/24/25/27/28/32	777 072 773 5C
74	3/6/9/15/16/17/32	777 072 000 0C
75	3/6/9/15/17/18/26/28/32	777 074 006 5C
76	3/6/9/15/28/32	777 070 000 5C
77	3/6/11/12/15/16/17/28/32	770 472 000 5C
78	3/6/11/12/24/27/28/32	770 400 077 5C
79	3/6/11/15/16/17/22/23/32	770 672 020 0C
80	3/6/16/18/31/32	770 003 000 5A
81	3/6/15/16/17/32	770 072 000 0C
82	3/6/15/16/17/32	770 072 000 0C
83	3/6/15/17/32	770 076 000 0C
84	3/6/15/18/28/31/32	770 077 000 5A
85	3/6/16/17/21	770 002 700 0D
86	3/6/16/17/32	770 002 000 0C
87	3/6/16/22/23/28/32	770 005 020 5C
88	3/7/9/11/12/24/27/28/32	703 400 077 5C
89	3/7/9/11/15/17/19/22/23/26/27/28/32	703 676 524 5C
90	3/6/9/11/16/17/19/22/23/26/27/28/32	703 602 524 5C
91	3/7/9/11/17/21/28/32	703 606 700 5C
92	3/7/9/15/17/18/22/23/27/32	703 074 027 0C
93	3/7/9/16/28/32	703 005 000 5C
94	3/7/9/17/18/21/22/23/24/27/32	703 004 717 0C
95	3/7/15/16/17/22/23/27/28/32	705 072 027 5C
96	3/7/20/22/27/28/32	705 000 657 5C
97	3/7/27/28	705 000 007 5D
98	3/7/28/29	705 000 000 2D

(continued)

TABLE 4. (continued) Phage types

Culture no.	Phage pattern
99	3/9/15/16/17/
100	3/9/25/29/32
101	4/5
102	4/5/28
103	4/7/8/10/16/1
104	4/7/16/20/22/
105	6/7/9/11/12/1
106	6/7/9/16/18/2
107	6/7/23/27/28
108	6/8/13/15/17/
109	6/9/15/18/27/
110	6/9/16/18/28/
111	6/9/16/18/32
112	6/15/19/28/32
113	7/9/13/18/21/
114	7/9/15/18/27/
115	7/9/16/18/27/
116	7/9/21/22/23/
117	7/10/11/27/28
118	7/11/12/22/2
119	7/11/15/27/2
120	7/11/19/27/2
121	7/12/16/28
122	7/15/18/28/3
123	7/15/21/22/2
124	7/16/21/22/2
125	7/16/18/28/3
126	7/17/21/22/2
127	7/18/25/32
128	7/18/28
129	7/18/28/31/3
130	7/21/28
131	7/22/27/28
132	7/22/28/29
133	7/23/27/28
134	7/24/27/28/2
135	7/27/28
136	7/28
137	8/17/25/28/2
138	9/11/16/17/2

TABLE 4. (continued) Phage types isolated.

Culture no.	Phage pattern	Mneumonic
99	3/9/15/16/17/28/29/32	707 072 000 2C
100	3/9/25/29/32	707 000 005 6C
101	4/5	020 000 000 0D
102	4/5/28	020 000 000 5D
103	4/7/8/10/16/17/19/20/23/27/29/32	052 502 267 6C
104	4/7/16/20/22/23/27/28/29/30	055 005 627 1D
105	6/7/9/11/12/16/22/23/27/28/32	073 405 027 5C
106	6/7/9/16/18/21/22/27/28/29/30/32	073 003 757 1C
107	6/7/23/27/28	076 034 003 1C
108	6/8/13/15/17/18/25/27/28/29/30/32	076 034 003 1C
109	6/9/15/18/27/28/32	077 077 007 5C
110	6/9/16/18/28/32	077 003 000 5C
111	6/9/16/18/32	077 003 000 0C
112	6/15/19/28/32	070 070 500 5C
113	7/9/13/18/21/22/23/24/27/28/32	003 057 717 5C
114	7/9/15/18/27/28/32	003 077 007 5C
115	7/9/16/18/27/28/32	003 003 007 5C
116	7/9/21/22/23/25/28/32	003 000 725 5C
117	7/10/11/27/28	005 200 007 5D
118	7/11/12/22/23/24/28/32	005 400 010 5C
119	7/11/15/27/28	005 670 007 5D
120	7/11/19/27/28/32	005 600 507 5C
121	7/12/16/28	005 705 000 5D
122	7/15/18/28/32	005 077 000 5C
123	7/15/21/22/23/27/28	005 070 727 5D
124	7/16/21/22/23/27/28	005 005 727 5D
125	7/16/18/28/32	005 003 000 5C
126	7/17/21/22/23/24/27/28/32	005 006 717 5C
127	7/18/25/32	005 007 005 0C
128	7/18/28	005 007 000 5D
129	7/18/28/31/32	005 007 000 5A
130	7/21/28	005 000 700 5D
131	7/22/27/28	005 000 057 5D
132	7/22/28/29	005 000 050 2D
133	7/23/27/28	005 000 067 5D
134	7/24/27/28/29/30	005 000 077 1D
135	7/27/28	005 000 007 5D
136	7/28	005 000 000 5D
137	8/17/25/28/29/30	006 006 005 1D
138	9/11/16/17/22/28/32	007 602 050 5C

(continued)

Mneumonic

775 062 007 5C
775 076 727 5C
775 076 005 5C
777 476 000 5C
777 672 014 5C
777 672 017 5C
777 605 526 5C
777 031 717 5C
777 031 735 5C
777 031 000 5C
777 071 727 5C
777 071 000 5C
777 071 000 0A
777 072 773 5C
777 072 000 0C
777 074 006 5C
777 070 000 5C
770 472 000 5C
770 400 077 5C
770 672 020 0C
770 003 000 5A
770 072 000 0C
770 072 000 0C
770 076 000 0C
770 077 000 5A
770 002 700 0D
770 002 000 0C
770 005 020 5C
703 400 077 5C
703 676 524 5C
703 602 524 5C
703 606 700 5C
703 074 027 0C
703 005 000 5C
703 004 717 0C
705 072 027 5C
705 000 657 5C
705 000 007 5D
705 000 000 2D

(continued)

TABLE 4. (continued) Phage types isolated.

Culture no.	Phage pattern	Mneumonic
139	9/15/17/32	007 076 000 0C
140	9/16/17/32	007 002 000 0C
141	9/17/18/22/23/27/28/32	007 004 027 5C
142	9/18/25/28	007 007 005 5D
143	9/21/32	007 000 700 0C
144	9/23/28	007 000 060 5D
145	9/25/28/30/32	007 000 005 3C
146	10/23/28	000 500 060 5D
147	11/12	000 400 000 0D
148	11/12/19/22	000 400 550 0D
149	11/12/24/27/28	000 400 077 5D
150	11/15/28	000 670 000 5D
151	11/16/28	000 605 000 5D
152	11/19/32	000 600 500 0D
153	12/16/27/28	000 705 007 5D
154	12/18/22	000 707 050 0D
155	15	000 070 000 0D
156	15/16/17/28/29/30/32	000 072 000 1C
157	15/16/18/31/32	000 073 000 0A
158	16	000 005 000 0D
159	16/18/28/32	000 003 000 5C
160	16/28	000 005 000 5D
161	17	000 006 000 0D
162	17/24	000 006 070 0D
163	18	000 007 000 0D
164	18/21	000 007 700 0D
165	18/22/24/27/28/32	000 007 037 5C
166	18/23/25	000 007 065 0D
167	18/23/28/32	000 007 060 5C
168	18/28	000 007 000 5D
169	18/28/32	000 007 000 5C
170	18/31/32	000 007 000 0A
171	20	000 000 600 0D
172	22/26/27	000 000 054 0D
173	22/28	000 000 050 5D
174	23/28	000 000 060 5D
175	28	000 000 000 5D
176	29	000 000 000 6D
177	30	000 000 000 7D
178	32	000 000 000 0C

RESULTS AND DISCU

Only strong reactions, i.e
plaques per drop, were used
and reporting isolates, and
were made to correlate the
suspicious cultures, readings w
exacting detail. In the past
was recognized and reported o
phages to which it was susce
months we have adopted a
enables us to report any conce
or pattern resulting from an ex
phages by 10 digits and a lette
based on a mnemonic devised
Table 3 and is particularly
recording cultures with lengthy
With this system an isolate firs
strong + (+++ or above) or – re
phages. Then, from left to rig
signed to each type of triplet a
remaining two digits. Phage ty
13/16/17/18/21/22/23/27/28/
473 751 727 5C. In cases whe
responds to high numbered
commence with the first encou
istering a reaction. Phage type
5D. One hundred seventy-eig
were observed (Table 4), indica
coli types can be found in mast

Mneumonic
007 076 000 0C
007 002 000 0C
007 004 027 5C
007 007 005 5D
007 000 700 0C
007 000 060 5D
007 000 005 3C
000 500 060 5D
000 400 000 0D
000 400 550 0D
000 400 077 5D
000 670 000 5D
000 605 000 5D
000 600 500 0D
000 705 007 5D
000 707 050 0D
000 070 000 0D
000 072 000 1C
000 073 000 0A
000 005 000 0D
000 003 000 5C
000 005 000 5D
000 006 000 0D
000 006 070 0D
000 007 000 0D
000 007 700 0D
000 007 037 5C
000 007 065 0D
000 007 060 5C
000 007 000 5D
000 007 000 5C
000 007 000 0A
000 000 600 0D
000 000 054 0D
000 000 050 5D
000 000 060 5D
000 000 000 5D
000 000 000 6D
000 000 000 7D
000 000 000 0C

RESULTS AND DISCUSSION

Only strong reactions, i.e., 121 or more plaques per drop, were used in characterizing and reporting isolates, and when attempts were made to correlate the relationship of suspicious cultures, readings were compared in exacting detail. In the past, a phage type was recognized and reported on the basis of the phages to which it was susceptible. In recent months we have adopted a procedure that enables us to report any conceivable phage type or pattern resulting from an exposure to our 32 phages by 10 digits and a letter. This method is based on a mnemonic devised by Farmer (2) in Table 3 and is particularly convenient for recording cultures with lengthy representations. With this system an isolate first is delineated by strong + (+++ or above) or − reactions to the 32 phages. Then, from left to right a number is assigned to each type of triplet and a letter to the remaining two digits. Phage type 2/3/6/7/9/12/13/16/17/18/21/22/23/27/28/32 would become 473 751 727 5C. In cases where an isolate only responds to high numbered phages, readings commence with the first encountered triplet registering a reaction. Phage type 28 thus becomes 5D. One hundred seventy-eight phage patterns were observed (Table 4), indicating that many *E. coli* types can be found in mastitic milk samples.

Numerous *E. coli* types can be differentiated serologically. Phage typing is equally useful in delineating isolates, some of which may not respond adequately to serological procedures. Under such circumstances, phage typing could serve as an alternative.

Aside from relating an isolate to an outbreak, phage typing also can be used for surveillance and assessing strain distribution. Repeated typing revealed that our results were consistent and reproducible. Given the variety, ubiquitous distribution, and frequency of *E. coli* isolates, phage typing can contribute significantly to control of mastitis by helping the practitioner and health-related personnel to identify strains and to monitor the response of these isolates to theraphy.

REFERENCES

1 Anderson, E. S. 1962. The genetic basis of bacteriophage typing. Br. Med. Bull. 18:64.
2 Farmer, J. J., III. 1970. Mnemonic for reporting bacteriocin and bacteriophage types. Lancet 1:96.
3 Gershman, M., C. E. Merrill, and J. Hunter. 1981. Phage typing set for differentiating *Escherichia coli*. J. Dairy Sci. 64:2392.
4 Swanstrom, M., and M. H. Adams. 1951. Agar layer method for the production of high titer phage stocks. Proc. Soc. Exp. Biol. Med. 7:372.