LABORATORY TECHNIQUES

Simple technique for detecting K1 antigen of *Escherichia coli*

D A Devine, A P Roberts, B Rowe

Abstract

The K1 antigen is a poor immunogen, its detection by serological means is difficult, and previously described methods using K1 specific bacteriophages require standardised suspensions of five different bacteriophages. A simple technique was developed which uses an unstandardised suspension of a single K1 specific bacteriophage applied with a 1 mm wire loop to bacteria streaked on to cystine lactose electrolyte deficient (CLED) medium. The technique correctly identified all 99 known K1 strains tested, including 14 strains negative with the serological method. Among 71 clinical isolates from urinary tract infections, the single bacteriophage method distinguished 30 K1 strains from 41 strains without this antigen. Suspensions of the bacteriophage were shown to remain fully active for at least two years when stored at 4°C. It is concluded that this technique required so little in materials, time, or equipment that it could be routinely used in most laboratories.

The K1 antigen of *Escherichia coli* is a poor immunogen, which hinders its detection by traditional serological means, so that its identification is largely confined to specialist laboratories. An easier and more reliable technique for detecting this antigen using K1 specific bacteriophages was reported by Gross, Cheasty, and Rowe,¹ but this involves the maintenance of five different bacteriophages, suspensions of which are standardised. We have been using a single K1 specific bacteriophage to detect this antigen in isolates of *E coli* from urinary tract infections. The purpose of this study was to determine whether this simple technique detected a sufficiently high proportion of K1 carrying strains of *E coli* to be reliable in routine use.

Methods

A K1 specific bacteriophage, which we designated ϕ K1GS, was originally isolated by Dr G Schmidt (Max-Planck Institute, Freiberg, West Germany) and was supplied to us by Dr C Hughes (Institut für Genetik und Mikrobiologie, 8700 Würzburg, West Germany). The K1 specific bacteriophages A,B,C,D and E were isolated by Gross, Cheasty, and Rowe.²

To propagate them 20 ml of nutrient broth (Oxoid No 2, CM67) was inoculated with the appropriate propagating strain of *E coli* and incubated for four hours at 37°C. Two drops of bacteriophage suspension were added by Pasteur pipette and incubation continued for a further 15-18 hours. The culture was then centrifuged and the supernate passed through a 0.22 μ m membrane filter. The resulting bacteriophage suspension was dispensed into Bijou bottles and stored in the dark at 4°C.

The strain of bacteria used to propagate ϕ K1GS was *E coli* 20896 (02:K1) supplied by Dr C Hughes. Bacteriophages A-E were propagated using *E coli* SC735 (02:K1:H4, Division of Enteric Pathogens, Colindale).

The *E coli* strains tested included 71, which had been isolated from suprapubic aspirates of urine at Charing Cross Hospital, and 99 known K1 strains, which had been isolated from a variety of sources and collected at the Division of Enteric Pathogens, Colindale. Of the Colindale strains, 60 had been included in previous studies^{2,3} and had been tested against each of the bacteriophages A-E individually and by the immunoprecipitation "halo" technique using equine meningococcal antiserum⁴ (46 positive, 14 negative) while the remaining 39 had been tested against a pool of the bacteriophages A-E.

Test strains of *E coli* were inoculated into 4 ml of peptone water (Oxoid CM9) and incubated at 37°C for four hours. A single streak of each culture was then made on to cystine lactose electrolyte deficient agar (CLED; Oxoid CM301) and allowed to dry (about five minutes). Up to six strains were tested on each plate. Once the streaks were dry, a small volume of bacteriophage suspension was placed on the centre of each using a 1 mm loop of 22SWG nickel-chrome wire. This size of loop allowed a volume of bacteriophage suspension to be delivered which gave a zone of lysis of about 3-4 mm with susceptible strains and caused minimal disruption of the bacterial streak during application. After incubation for 15-18 hours the presence of K1 was indicated by a distinct reduction in density of the bacterial growth where the bacteriophage suspension had been placed. Positive results were graded from + + + +, a completely clear zone of lysis, to +, a turbid plaque. A description of each grade is given with examples in the figure. The propagating strain, 20896, was used as a positive control and a strain of *E coli* 04:K5 as the negative control.

Using this technique, the 71 strains isolated

Department of
Medical Microbiology,
Charing Cross and
Westminster Medical
School, London
D A Devine
A P Roberts
Division of Enteric
Pathogens, Central
Public Health
Laboratory, Colindale,
London
B Rowe

Correspondence to:
Dr A P Roberts, Department
of Medical Microbiology,
Charing Cross and
Westminster Medical
School, London W6 8RP,
England.

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Five strains of *Escherichia coli* showing different grades of response to K1 specific bacteriophages.

++++ completely clear zone of bacterial lysis;
 +++ up to 20 colonies in an otherwise clear zone;
 ++ numerous small colonies in zone;
 + reduced but confluent growth giving a "turbid" zone;
 — no zone of bacterial lysis.

from suprapubic aspirates of urine were initially tested against bacteriophage ϕ K1GS and subsequently against bacteriophages A-E. The 99 Colindale strains were tested with the present technique using bacteriophages ϕ K1GS and E; although they were known to carry the K1 antigen, they were examined "blind" by one of us (DD), who was under the impression that a proportion were K1 negative. The effect of age on the bacteriophage suspension was assessed by testing 30 known K1 strains against freshly prepared, two year old and four year old suspensions of ϕ K1GS on the same day.

Results

Of the 71 urinary strains tested, 41 were not lysed by any of the bacteriophages (table 1). The remaining 30 strains were all lysed by each of the bacteriophages, but with some strains the grade of response to the different bacteriophages varied between + and + + + +. The greatest number of + + + and + + + + responses were found with ϕ K1GS and E. Although the person examining them had been led to believe that some of the 99 Colindale strains were K1 negative, all were found to be positive with both ϕ K1GS and bacteriophage E (table 2): this was equally true whether the presence of K1 had originally been detected using the bacteriophages A-E individually or as a pool, and whether or not they were positive by the immunoprecipitation "halo" technique.

For 23 of 30 strains of K1 *E coli*, the response using freshly propagated or stored ϕ K1GS

Table 1 Grade of response of *E coli* isolates from urinary tract infections to K1 specific bacteriophages

Grade of response	Bacteriophage					
	ϕ K1GS	A	B	C	D	E
++++	6	8	2	12	5	17
+++	19	8	18	6	7	10
++	3	7	2	9	4	1
+	2	7	8	3	14	2
—	41	41	41	41	41	41
Total	71	71	71	71	71	71

Table 2 Grade of response of *E coli* strains isolated from various sources (Colindale strains) to K1 specific bacteriophages

Grade of response	Bacteriophage	
	ϕ K1GS	E
++++	37	27
+++	40	56
++	18	7
+	4	9
—	0	0
Total	99	99

differed by no more than one grade, with none giving a negative response. The results for those strains in which a greater difference occurred are shown in table 3. The activities of the fresh and two year old suspensions were similar, but the four year old suspension showed reduced responses with all seven strains, and in one case no response was detected.

Discussion

There has been increased interest in the prevalence of the K1 antigen of *E coli* since it was reported to occur with greater than expected prevalence in strains causing neonatal meningitis.⁴ In the past the most reliable technique for its detection has entailed the use of equine meningococcal group B antiserum, which is only available from Robbins' group in the United States of America. The isolation of bacteriophages specific for K1¹ has enabled more laboratories to look for this antigen, but five bacteriophages have to be tested by placing a drop of each from a Pasteur pipette on to a lawn culture of the *E coli* strain. Using the method described in this paper, one bacteriophage detected all those K1 strains of *E coli* which had previously been identified by bacteriophages A,B,C,D and E and by the halo formation test using meningococcal group B antiserum. Either ϕ K1GS or E were most suitable in that they gave strong positive results (+ + + and + + + +) with most strains tested; bacteriophages A,B,C and D detected K1 in the same isolates but gave a higher proportion of + and ++ responses. Each of the six bacteriophage suspensions also lysed 14 isolates which were K1 negative by the halo formation technique.

The technique described here has several advantages over previously used bacteriophage methods for detecting K1. It requires the propagation, maintenance, and use of only one bacteriophage and no standardisation of the

Table 3 Effect of length of storage on activity of ϕ K1GS against 30 strains of *E coli**

Strain No	Grade of response with ϕ K1GS stored for:		
	0 years	2 years	4 years
CX 0277	++++	+++	++
CX 0432	+++	++++	++
CX 0434	+++	+++	+
CX 0508	+++	++++	+
CX 0517	+++	+++	+
CX 0539	++	+	—
CX 0606	++	+++	+

*With 23 of the 30 strains tested the three results varied by no more than one grade and none became negative.

suspension is necessary. The results are very easy to read; about 80% of K1 strains gave a + + + + or + + + response, and even with the weakest response, +, there was a distinct reduction in bacterial growth. Phenomena which could make interpretation of the weakest responses difficult, disruption of the bacterial streak, and dilution of bacteria by the bacteriophage suspension, are avoided by the method of application we describe. We have shown that the suspension of ϕ K1GS can be stored at 4°C for two years without adverse affect on its activity. As our method uses little in terms of materials or time large numbers of strains can be tested, and the ease of maintenance together with the small volume of bacteriophage needed means it is a simple matter for any laboratory to

test *E. coli* isolates for the presence of K1, even if this is only required occasionally.

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- 1 Gross RJ, Cheasty T, Rowe B. Isolation of bacteriophages specific for the K1 polysaccharide antigen of *Escherichia coli*. *J Clin Microbiol* 1977;6:548-50.
- 2 Cheasty T, Gross RJ, Rowe B. Incidence of K1 antigen in *Escherichia coli* isolated from blood and cerebrospinal fluid of patients in the United Kingdom. *J Clin Pathol* 1977;30:945-7.
- 3 Gross RJ, Ward LR, Threlfall EJ, Cheasty T, Rowe B. Drug resistance among *Escherichia coli* strains isolated from cerebrospinal fluid. *J Hyg* 1983;90:195-8.
- 4 Sarff LD, McCracken JGH, Schiffer MS, Glode MP, Robbins JB, Ørskov I, Ørskov F. Epidemiology of *Escherichia coli* K1 in healthy and diseased newborns. *Lancet* 1975;ii:1099-104.