

## Screening for Cross-Reacting Capsular Polysaccharide K Antigens of *Escherichia coli* Using Antiserum Agar

GEORGE W. COUNTS\* AND MARVIN TURCK

Department of Medicine, University of Washington School of Medicine and Harborview Medical Center, Seattle, Washington 98104

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Agar plates containing antiserum against group B meningococcus or *Haemophilus influenzae* type b were used to determine the prevalence of cross-reacting K1 and K100 capsular polysaccharide antigens in 265 isolates of disease-causing *Escherichia coli*. K1 antigen was found in 22% of isolates from various sites. K100 antigen was found in only three isolates. This technique is a convenient method to detect specific *E. coli* K antigens for evaluation as possible factors important in the virulence of the organism.

Cross-reactions between bacteria of different genera have been well described. An *Escherichia coli* capsular antigen that cross-reacts with *Haemophilus influenzae* type b is a polysaccharide and is considered to be a K antigen (10, 13). *E. coli* K antigens that have been studied are similar to capsular antigens of other bacteria, in which the capsular polysaccharide seems important for the virulence of the organism. Such bacteria include *H. influenzae* type b, *Neisseria meningitidis*, and *Streptococcus pneumoniae*. *E. coli* K1 antigen is physicochemically and immunologically similar to the capsular polysaccharide of group B meningococcus (2, 5). The antigen that cross-reacts with *H. influenzae* type b is now called K100.

Hemagglutination inhibition, immunodiffusion, and immunoelectrophoresis have been used to detect and quantitate K antigens (1, 3, 9). A simple method to determine the presence of a specific K antigen-containing *E. coli* is to incorporate antiserum, either specific or cross-reactive, into agar used to culture the organism. Those isolates that contain the particular K antigen studied can be rapidly identified. The purpose of this study was to examine the antiserum agar technique as a screening tool for the detection of two K antigens, K1 and K100, in isolates of disease-causing *E. coli*.

Two hundred and sixty-five *E. coli*, isolated from blood, urine, stool, and other cultures, were tested. After purification, isolates were either tested immediately by plating on antiserum agar or stored on paraffin-sealed nutrient agar slants at room temperature. Agar plates were prepared by adding equine group B meningococcal antiserum (1/10, vol/vol) to Trypiti-

case (BBL) soy broth plus agarose (1.0%) and used to detect K1 antigen (12). Burro antiserum containing antibody against *H. influenzae* type b was similarly used to prepare plates to identify K100 antigen (13). After 18 to 24 h at 37°C, the plates were examined for halos of precipitation around individual colonies and along margins of confluent growth. The plates were stored at 4°C for an additional 24 to 48 h to provide for maximum definition of the halos.

Twenty-one percent of *E. coli* isolates from all sources contained K1 antigen (Table 1). The halos were easily visible using bright lighting and a dark background. Rarely did subsequent storage at 4°C reveal a K antigen-containing isolate that was not apparent after 37°C incubation, although in many cases the halos were more impressive after refrigeration. The cross-reactions were consistent and reproducible in K(+) and K(-) *E. coli* that were stored in the laboratory and used repeatedly over 1 year as controls in each experiment. However, it has been noted that the content of K antigen, measured by hemagglutination inhibition, may decrease with storage (7). There is complete correlation between results of K(+) and K(-) isolates, tested by immunodiffusion in our laboratory in 1973 (9), and current results using antiserum agar. The precipitation reaction is felt to be highly specific, with only K1(+) *E. coli* producing distinct halos on the meningococcal agar (12).

Efforts have been made to determine the contribution of *E. coli* K antigens to invasiveness of the organism for humans. Aside from the recognized association of K1 antigen and *E. coli* causing neonatal meningitis (8, 11), studies of K antigens and other *E. coli* diseases, although

TABLE 1. Prevalence of cross-reacting K antigens among *E. coli* isolates

Source (No.)	No. of <i>E. coli</i> isolates with cross-reacting K antigens in antiserum agar	
	K1 <sup>a</sup>	K100 <sup>b</sup>
Blood (127)	31 (24.4%)	1 (0.8%)
Urine (68)	20 (29.4%)	2 (2.9%)
Stool (22)	0	0
Other (48) <sup>c</sup>	6	0
Total (265)	57 (21.5%)	3 (1.1%)

<sup>a</sup> Antiserum against group B *Neisseria meningitidis*.

<sup>b</sup> Antiserum against *Haemophilus influenzae* type b.

<sup>c</sup> Includes sputum, wound, pleural, and abdominal drainage.

suggestive, do not clearly establish that K antigen contributes to virulence of *E. coli* in such diseases as bacteremia and urinary tract infections (1, 4, 6, 7). To establish such a role requires the evaluation of large numbers of *E. coli* by hemagglutination inhibition, immunodiffusion, or immunoelectrophoresis and perhaps studies in experimental animals. The antiserum agar technique is useful for screening large numbers of cultures, even using the agar for primary isolation, to examine the possible association of a specific K antigen and infection. Studies of K1 antigen are in progress in our laboratory. The K1 antigen detected by screening is quantitated by immunoelectrophoresis.

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