IDENTIFICATION OF *SALMONELLA TYPHI* IN FAECAL SPECIMENS BY AN ANTISERUM-AGAR METHOD

C. M. NOLAN*, ELIZABETH A. LABORDE†, R. T. HOWELL†
AND J. B. ROBBINS‡

*Department of Medicine, University of Arkansas College of Medicine,

†Division of Laboratories, Arkansas State Department of Health, Little Rock.

Arkansas 72201, and ‡Division of Bacterial Products, Bureau of Biologics,
Food and Drug Administration, Bethesda, Maryland 20014, USA

ISOLATION and identification of *Salmonella typhi* in faeces involves the use of several types of culture medium as well as biochemical and serological testing (Ewing and Martin, 1974). A simplified method of detecting faecal *S. typhi* would be of value, especially when the presence of other intestinal pathogens is unlikely. Two such circumstances are the search for asymptomatic *S. typhi* carriers associated with sporadic cases of typhoid fever, and the examination of stools from known typhoid carriers.

Recently, bacteria in mixed culture have been identified by means of immunoprecipitates that form about their colonies during growth on agar in which specific or cross-reacting antiserum has been incorporated. In the present study we assessed this technique, termed the antiserum-agar technique (Petrie, 1932; Bradshaw *et al.*, 1971), for the detection of *S. typhi* in faeces.

MATERIALS AND METHODS

Production of S. typhi strain Ty 2 antiserum. A donkey was immunised with the US standard typhoid vaccine, an acetone-inactivation preparation of *S. typhi* strain Ty 2 (Division of Immunology, Walter Reed Army Institute of Research, 1964) by a modification of the method of Alexander, Leidy and MacPherson (1946). Sterile sera or plasma obtained after immunisation were pooled, divided into portions and frozen at -20°C until used. This antiserum contained Vi, O, and H titres of 20 480, 640, and 10 140 respectively.

Antiserum agar was prepared by incorporating antiserum to *S. typhi* strain Ty 2 in SS agar (Difco Laboratories, Detroit, Mich.) or Blood-agar-base Infusion Agar (BAB Agar, BBL Division, Becton, Dickinson and Co., Cockeysville, Md). One hundred ml of antiserum were added to 1 litre of agar that had been rehydrated with distilled water, boiled, autoclaved (BAB agar only), and cooled to 50°C . After thorough mixing, the agar was poured into petri dishes, allowed to solidify, stored at 4°C and used within 4 days of preparation.

Laboratory study of S. typhi strain Ty 2 antiserum agar. Thirty-one laboratory strains of *S. typhi*, 24 other *Salmonella* isolates, 36 other Enterobacteriaceae, and 10 strains of *Pseudomonas aeruginosa* were cultured simultaneously on BAB agar, BAB agar containing antiserum (BAB-AS agar), SS agar, and antibody-containing SS agar (SS-AS agar). The Enterobacteriaceae and pseudomonads were isolated from hospital patients and the origins of the *S. typhi* isolates have been described previously (Nolan and Rosenfeld, 1977). The other *Salmonella* isolates were obtained from faecal specimens submitted routinely to the Arkansas State Department of Health. All plates were examined after overnight incubation at 37°C . Colony size and morphology were noted, and the antiserum-agar plates were examined for the presence of immunoprecipitates (haloes) in the agar surrounding individual colonies. Antiserum-agar plates were then stored at 4°C for 24 h and re-examined.

Clinical faecal specimens. During a 6-week period in April and May 1978, 141 consecutive faecal specimens submitted to the Bacteriology Laboratory of the Arkansas State Department of

Health were screened for the presence of bacteria that produced haloes during growth on SS-AS agar. These specimens were treated routinely for isolation of enteric pathogens (Ewing and Martin, 1974) including streaking on Bismuth Sulfite, SS and MacConkey Agars (Difco) and inoculation into Selenite Broth (Difco). In addition, each specimen was streaked on SS-AS agar directly and after overnight culture in selenite broth. Bacterial colonies on SS-As agar surrounded by haloes were identified by standard biochemical, fermentation, and serological reactions (Ewing and Martin, 1974). Halo-negative colonies that resembled *Salmonella* on SS-AS agar were similarly identified.

RESULTS

Each laboratory strain of *S. typhi* produced haloes on SS-AS agar and BAB-AS agar (table I). No other salmonellas were halo-positive on BAB-AS agar but some produced haloes on SS-AS agar and this correlated with the O-serogroup classification. Of the 24 salmonellas, 14

TABLE I
Halo production on Salmonella typhi strain Ty 2 antiserum-agar by bacteria in pure culture

Species	Number of isolates		
	tested	producing haloes on	
		BAB-AS agar*	SS-AS agar†
<i>Salmonella typhi</i>	31	31	31
<i>Salmonella</i> spp.	24	0	12
<i>Escherichia coli</i>	9	0	0
<i>Klebsiella pneumoniae</i>	9	0	0
<i>Klebsiella aerogenes</i>	9	0	0
<i>Proteus mirabilis</i>	9	0	0
<i>Pseudomonas aeruginosa</i>	10	0	0

* Blood-agar-base infusion agar containing 10% (v/v) antiserum to *S. typhi* strain Ty 2.

† SS agar containing 10% (v/v) antiserum to *S. typhi* strain Ty 2.

possessed at least one of the two antigens carried by *S. typhi* (O antigens 9 and 12); these were *S. paratyphi* A (one strain), *S. typhimurium* (six strains), *S. heidelberg* (two strains), *S. enteritidis* (one strain), *S. javiana* (two strains), *S. berta* (one strain), *S. gallinarum* (one strain). Of these strains, 12 produced haloes on SS-AS agar but the two strains of *S. heidelberg* failed to do so. In contrast, none of 10 salmonellas that did not share serogroup antigens with *S. typhi* (*S. bareilly*, two strains; *S. norwich*, one strain; *S. infantis*, one strain; *S. thompson*, one strain; *S. newport*, four strains; *S. rubislaw*, one strain) produced haloes on SS-AS agar ($p < 0.001$, Fisher's exact test; Siegel, 1956).

It was impossible to distinguish *S. typhi* from other salmonellas on SS-AS agar by size or clarity of the haloes. In general, haloes were formed round all the colonies on a plate and were more clearly defined after storage of the plates at 4°C for 24 h. Of the 10 *Ps. aeruginosa* strains, five showed small precipitate spots in the agar under individual colonies after overnight growth on SS-AS agar but not on BAB-AS agar. These areas of precipitation remained circumscribed after storage at 4°C and could easily be differentiated from the larger haloes characteristic of *S. typhi*.

The antiserum-agar technique correlated well with isolation of *S. typhi* from faecal cultures (table II). The four specimens from which *S. typhi* was isolated by routine techniques were from known or suspected chronic typhoid carriers. *S. typhi* was isolated only on bismuth sulphite agar from the specimen that was falsely negative on antiserum agar; it did not grow on SS agar and all suggestive halo-negative colonies growing on SS-AS agar proved not to be *S. typhi* on

TABLE II

Study of 141 faecal specimens by the S. typhi strain Ty 2 antiserum-agar technique: correlation with standard procedures

Species (and O-sero-group)	Number of times isolated by	
	standard procedures*	antiserum-agar technique†
<i>S. typhi</i> (D)	4	3
<i>S. typhimurium</i> (B)	6	0
<i>S. norwich</i> (C ₁)	1	0
<i>S. newport</i> (C ₂)	2	0
<i>S. javiana</i> (D)	1	1
<i>Shigella sonnei</i>	5	0
Non pathogens	122	0

* See *Materials and methods*.

† SS agar containing 10% (v/v) antiserum to *S. typhi* strain Ty 2.

further testing. Therefore, the discrepancy resulted from failure of *S. typhi* to grow on SS-AS agar after primary streaking or subculture and not from failure of the organism to produce haloes on antiserum agar.

Cultures were obtained from three other suspected carriers from whom *S. typhi* was not isolated. With these three cultures, the antiserum-agar technique was also negative. Thus, results from antiserum agar were similar to those from routine methods in six of seven instances. Among other intestinal pathogens isolated from stool cultures, only *S. javiana*, a serogroup-D organism that shares two O antigens with *S. typhi*, produced haloes on SS-AS agar (table II). All *S. typhimurium* faecal isolates were halo negative although this serotype consistently produced haloes when pure cultures were streaked on SS-AS agar (table I).

DISCUSSION

This attempt to identify *S. typhi* in specimens of faeces by means of a serological reaction in the agar is not novel. Wassén (1930) found that motile bacteria migrating through agar were immobilised as they encountered homologous antiserum that diffused radially into the agar from an embedded strip of filter paper. He successfully used this principle to identify *S. paratyphi* in faecal specimens but failed in similar attempts with *S. typhi*.

In the present experiments *S. typhi* consistently produced haloes during growth on agar containing antiserum to the vaccine strain *S. typhi* Ty 2. In his original description of this phenomenon, Petrie (1932) showed that the haloes were precipitates formed as bacterial capsular material diffused into the agar from a colony growing on the surface and reacted with its specific antibody which was incorporated into the agar. More recent studies (Bradshaw *et al.*, 1971; Schneerson *et al.*, 1972; Craven *et al.*, 1978) confirm that the capsular polysaccharide antigens of encapsulated bacteria such as *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, and even *Escherichia coli* bearing "K" polysaccharide capsules (Schneerson *et al.*, 1972) give this precipitin reaction. The comparable antigen of *S. typhi* is the Vi antigen, which is also capsular and is a polysaccharide (Wong and Feeley, 1972). Antibody to the Vi antigen was present in high titre in the antiserum prepared for the present study and a Vi antigen-antibody reaction probably accounted for halo production by *S. typhi* on antiserum agar. Other *Salmonella* spp. only rarely produce Vi antigen, but some tested in the present study produced haloes on SS-AS agar. Halo production in these instances was probably caused by reactions between O-serogroup antigens 9 and 12 and their antibodies, which were demonstrated to be present in moderately high titre in the antiserum used. This explanation is supported by the discrepancy between halo production by *Salmonella* isolates on SS-AS agar and BAB-AS

agar (table I). The former, but not the latter, contains a high concentration of bile derivatives. It is possible that the bile salts in SS-AS agar improved the diffusion of lipopolysaccharide in the agar; this improvement may have allowed the formation of haloes around colonies that possessed somatic antigens in common with *S. typhi*. If this is so, haloes would not be expected around these strains on BAB-AS agar and none was seen.

Nonspecific halo production relating to the common salmonella O antigens could perhaps be prevented if another Vi-producing organism, such as *Citrobacter diversus* (Wong and Feeley, 1972), was used as the immunogen for antiserum production. The somatic antigens of this bacterium should not crossreact significantly with those of *S. typhi*. Alternatively, this problem could be solved by use of an antiserum against purified *S. typhi* Vi antigen. However, it has not yet been possible to prepare *S. typhi* Vi antiserum of sufficiently high titre for use in an antiserum-agar procedure (unpublished observation).

Despite the lack of specificity noted in the study of salmonellas in pure culture on antiserum agar, the results of the study of clinical faecal specimens were encouraging. Compared with the routine method for isolating *S. typhi*, the antiserum-agar method showed a sensitivity of 75% (one false negative among four positive cultures) and a specificity of more than 99% (one false positive among 120 negative cultures). We do not think that the antiserum-agar method should replace the traditional methods of isolating *S. typhi* from faecal specimens. However, its usefulness in other particular clinical situations has been demonstrated. It has been used for the rapid determination of meningococcal serogroups in a recent epidemic in Finland (Sivonen, Renkonen and Robbins, 1977). In addition, group-specific meningococcal carrier rates in a military population were obtained within 48 h by use of an antiserum-agar method (Craven *et al.*, 1979).

Similarly, an antiserum-agar method for identifying *S. typhi* in faeces might be clinically useful. Where typhoid fever occurs sporadically, over 80% of cases are associated with an undiagnosed asymptomatic carrier of *S. typhi* (unpublished observation). It is often necessary to perform stool cultures on as many as 10 family members and other intimate contacts of an index case in order to diagnose a carrier. The antiserum-agar method could provide a simple method for screening numerous faecal specimens in these circumstances. The technique could also be used as a screening device by public-health laboratories that must carry out periodic stool cultures during routine surveillance of large numbers of known typhoid carriers.

SUMMARY

An antiserum-agar technique was evaluated as a method for detecting *Salmonella typhi* in faeces. Thirty-one laboratory strains of *S. typhi* produced immunoprecipitate haloes during overnight growth on SS agar and blood-agar-base infusion agar (BAB) containing donkey antiserum to a vaccine strain of *S. typhi*. Other salmonella species sharing O serogroup antigens with *S. typhi* also produced haloes when streaked in pure culture on SS-antiserum agar but not on BAB-antiserum agar. One hundred and forty-one consecutive faecal specimens were cultured on SS-antiserum agar. Results with this method were concordant with those of established isolation techniques on specimens from six of seven suspected carriers of *S. typhi*.

Ten other salmonellas were isolated from the faecal specimens but only *S. javiana*, like *S. typhi* a serogroup-D organism, yielded false-positive haloes on antiserum agar. The antiserum-agar technique offers promise as a means of screening for *S. typhi* in faecal cultures.

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