

Laboratory investigation of sewer swabs following the Aberdeen typhoid outbreak of 1964

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The sewer-swab technique was used by Moore (1948) to trace a *Salmonella paratyphi B* excreter and later to track down a *S. typhi* excreter (Moore, Perry & Chard, 1952). Other investigators, using Moore's methods, have successfully traced *S. typhi* carriers (Lendon & Mackenzie, 1951; Pilsworth, 1960).

A programme of sewer swabbing, based on Moore's method, was begun in Aberdeen during the closing stages of the typhoid outbreak in an attempt to locate possible undetected excretors of *S. typhi* among the population at risk. Although this was the prime purpose of the work, the survey developed into an investigation of the efficacy of techniques used. Some 2300 sewer swabs were examined between July 1964 and September 1966.

MATERIALS AND METHODS

Sewer swabs were prepared and collected as described by Moore (1948, 1950) and Moore *et al.* (1952). These, placed in sewers on Mondays and Tuesdays, were collected 7 days later.

Media

Throughout the investigation the following commercially available media were used with supplements as indicated: (a) Difco bismuth sulphite agar with added ferrous sulphate solution (B.S.A.) as recommended by Hobbs (1943); (b) oxoid s.s. agar with 1% saccharose added (s.s.s.A.); this medium was employed as it forms the basis of all our routine cultural work for enteric organisms, is easy to prepare and plates may be stored for several days without loss in efficiency; (c) oxoid mannitol selenite broth (M.S.B.) similar to the medium described by Hobbs & Allison (1945) for the isolation of *S. typhi*.

Cultural methods

On arrival in the laboratory, swabs were inspected and quarter-strength Ringer solution added to just immerse each swab. The content of each swab was then thoroughly expressed into the added fluid using a stout sterile glass rod.

Method 1

Serial tenfold dilutions of the swab fluid were made in quarter-strength Ringer solution from 1/10 to 1/10,000. Centrifuged deposit of 5 ml. of swab fluid was also prepared.

s.s.s.a. and B.S.A. plates were seeded with 1 drop each of swab fluid and its dilutions, and with 1-, 2- and 3-drop inocula of the centrifuged deposit. Inocula were spread in parallel lines with an angled wire (1 cm. spreading edge) without return to the original inoculum and without flaming the wire at any stage of the spreading. To the swab with its free fluid and to each of the dilutions, equal volumes of double strength M.S.B. were added; the mixtures were incubated for 18–24 hr. at 37° C. and then subcultured to s.s.s.a. and B.S.A. All s.s.s.a. plates were examined after 24 hr. and B.S.A. plates after 24 and 48 hr. at 37° C. Thus, a total of 26 plate cultures was examined for each swab.

Non-lactose-saccharose fermenting colonies from s.s.s.a. and black colonies from B.S.A. were transferred to Christensen's urea medium. Urease negative organisms were tested in glucose, mannitol and dulcitol broths (Kauffman, 1950) and spread on MacConkey agar and nutrient agar slopes. Organisms giving suggestive fermentation reactions were finally identified by slide agglutination with appropriate antisera. An attempt was made to select up to ten colonies from each plate culture, so that a total of 260 colonies might be examined from each swab.

Method 2

Four plates of B.S.A. were inoculated in series with 1 drop of expressed swab fluid and 1 drop each of 1/10, 1/100 and 1/1000 dilutions of the fluid in sterile heart infusion broth (Difco). Three tubes of 4 ml. single-strength M.S.B. were inoculated with 1, 2 and 3 drops of undiluted swab fluid, while a further 4 ml. of the fluid were added to an equal volume of double-strength M.S.B. An equal volume of double-strength M.S.B. was added to the swab and its remaining fluid. M.S. broths, after incubation for 18–24 hr., were subcultured to s.s.s.a. only. These procedures gave a total of nine plates per swab. Suspicious colonies were picked and dealt with as before.

Method 3

This followed method 2, except that plating after enrichment in M.S.B. was done on B.S.A. as well as s.s.s.a., increasing the total plates to be examined to 14. To conserve media and time, suspicious colonies were streaked on MacConkey agar only. Slide-agglutination tests with appropriate antisera were carried out and only on positive colonies were confirmatory biochemical tests done.

Method 4

This followed method 3 but two parallel series of M.S.B. in double-strength final concentration (Harvey & Price, 1964) were used. The additional set of broths contained sufficient streptomycin sulphate to yield a final concentration equivalent to 8 μ g./ml. of streptomycin base.

Since there was only one swab, duplication here was impossible and therefore the swab in its remaining free fluid was cultured in double-strength M.S.B. without streptomycin. These modifications increased the number of plates to be examined from 14 to 22 per swab. The addition of streptomycin to double-strength M.S.B. to

a concentration of 5 $\mu\text{g./ml.}$ was recommended by B. Moore (personal communication) to enhance the selectivity of that medium for the Aberdeen strain of *S. typhi*. Further investigation in this laboratory showed that the concentration of streptomycin could be increased to 8 $\mu\text{g./ml.}$ without detriment to the isolation of the Aberdeen strain of *S. typhi*.

Minor modifications of technique were introduced from time to time during all methods. Thus, the plate spreading described gave way in the last months of method 1 to spreading for separate colonies with a bacteriological loop. This in itself improved the isolation rate and was therefore used throughout the remainder of the programme.

RESULTS

Method 1 techniques were used to examine 1063 swabs over a period of about one year. Swabs were brought to the laboratory at a rate which finally reached approximately 30 per week. At the end of this period *S. typhi* had been isolated from 60 of the 1063 swabs. Table 1 gives the over-all comparative success in swab positivity of each procedure, direct and indirect, incorporated in method 1.

Table 1. *Analysis of S. typhi isolations using method 1*

	Total swabs examined	1063
	Total swabs positive	60
(a) Swabs positive if each technique had been used alone:		
	Direct culture on s.s.s.a. of swab fluid and dilutions	20
	Direct culture on B.S.A. of swab fluid and dilutions	15
	Direct culture on s.s.s.a. of centrifuged swab fluid deposit	17
	Direct culture on B.S.A. of centrifuged swab fluid deposit	15
	After enrichment of swab fluid and dilutions with recovery on s.s.s.a.	50
	After enrichment of swab fluid and dilutions with recovery on B.S.A.	11
(b) Swabs positive by one or more techniques:		
	Swabs positive by direct culture only	8
	Swabs positive after enrichment only	21
	Swabs positive by both direct culture and after enrichment	31
	Direct cultures positive on B.S.A. only	3
	Direct cultures positive on s.s.s.a. only	4
	Direct cultures positive on both media	1
	Cultures after M.S.B. enrichment positive on	
	B.S.A. only	1
	s.s.s.a. only	19
	Both media	1

In assessing the results, it was apparent that success, in the main, had been obtained in the enrichment cultures with s.s.s.a. as recovery medium. Of the 10 positive swabs in the first 450, eight were positive on s.s.s.a. after enrichment only, one was positive after enrichment on both s.s.s.a. and B.S.A. and one by direct plating on s.s.s.a. as well as on both s.s.s.a. and B.S.A. after enrichment. No swab was positive by direct plating on B.S.A.

Spreading for single colonies was now introduced. Of the next 525 swabs in method 1, 30 were positive. Although direct plating on s.s.s.a. and enrichment followed by s.s.s.a. were still more productive, positive results with B.S.A. for

direct plating now began to appear but, of the eight instances of positivity with B.S.A., only in one swab was this the only method positive.

The disappointing results with B.S.A. led to closer attention being paid to its preparation. It was found that, for best results, the medium had to be made in volumes no greater than 500 ml., required constant swirling during preparation to give uniform dispersal of the flocculent precipitate, required cooling to 50° C. with continued swirling even during pouring into plates and was best used with a dry surface and with minimal delay.

The remaining 88 swabs of the 1063 examined by method 1 yielded 20 which were positive. A more even distribution of positivity was now apparent, 14 swabs were positive using both s.s.s.a. and B.S.A. media. In a further three the credit for recovery goes to B.S.A., two of these were positive by direct culture and the third on B.S.A. after enrichment in M.S.B.

Table 2. *Analysis of S. typhi isolations using method 2*

	Total swabs examined	45
	Total swabs positive	15
(a)	Swabs positive if each technique had been used alone:	
	Direct culture on B.S.A. of swab fluid and dilutions	14
	After enrichment of swab fluid and dilutions with recovery on s.s.s.a.	3
(b)	Swabs positive by one or more techniques:	
	Swabs positive by direct culture on B.S.A. only	12
	Swabs positive after enrichment on s.s.s.a. only	1
	Swabs positive by both direct culture and after enrichment	2

In examining B.S.A. plates difficulty still arose from the generalized blackening of the medium due to vigorous production of H₂S by sewage organisms. It was suggested by B. Moore (personal communication) that a snugly fitting filter-paper disk, moistened with a weak solution (1–2%) of lead acetate, should be inserted into the lids of B.S.A. plates. This proved very helpful by removing excess H₂S without any adverse effect on production of black halos by colonies of *S. typhi* or *S. paratyphi B*. This was introduced and used throughout methods 2–4.

In an effort to reduce the amount of work entailed for each swab using method 1, the procedures were cut and only the two which showed most promise were carried over as method 2. By this curtailed method, 45 swabs were examined and 15 were positive for *S. typhi*. In this series, B.S.A. certainly outscored M.S.B. enrichment followed by s.s.s.a. Only one positive would have been missed had the latter been omitted (Table 2).

Method 3 closely followed method 2 but plating after enrichment on both B.S.A. and s.s.s.a. was done for comparison of efficacy. A total of 72 swabs was covered by method 3 and 32 were positive. If B.S.A. had been the only solid medium used, 28 swabs would have been positive leaving only four which were positive only on s.s.s.a. after enrichment. Again, of the 28, four were positive on the B.S.A. only after M.S.B. enrichment (Table 3).

In the gross, however, direct cultures on B.S.A. were positive in 24 swabs, enrichment followed by B.S.A. in 14 and enrichment followed by s.s.s.a. in 15.

These results indicated a necessity to retain the use of s.s.s.a. as a recovery medium after enrichment, even although b.s.a. was also to be used, so that the isolation rate might be as high as possible.

Table 3. *Analysis of S. typhi isolations using method 3*

	Total swabs examined	72
	Total swabs positive	32
(a)	Swabs positive if each technique had been used alone:	
	Direct culture on b.s.a. of swab fluid and dilutions	24
	After enrichment of swab fluid and recovery on b.s.a.	14
	After enrichment of swab fluid and recovery on s.s.s.a.	15
(b)	Swabs positive by one or more techniques:	
	Swabs positive by direct culture on b.s.a.	11
	Swabs positive after enrichment only	8
	Swabs positive by both direct culture and after enrichment	13
	Cultures after m.s.b. enrichment positive on	
	b.s.a. only	4
	s.s.s.a. only	4
	Both media	0

Table 4. *Analysis of S. typhi isolations using method 4*

	Total swabs examined	110
	Total swabs positive	67
(a)	Swabs positive if each technique had been used alone:	
	Direct culture of swab fluid and dilutions on b.s.a.	33
	After enrichment of swab fluid in double-strength m.s.b. with:	
	Recovery on b.s.a.	28
	Recovery on s.s.s.a.	25
	After enrichment of swab fluid in streptomycin double-strength m.s.b. with:	
	Recovery on b.s.a.	40
	Recovery on s.s.s.a.	36
(b)	Swabs positive by one or more techniques:	
	Swabs positive by direct culture on b.s.a. only	6
	Swabs positive after enrichment only	34
	Swabs positive by both direct culture and after enrichment	27
	Cultures after double-strength m.s.b. enrichment positive on:	
	b.s.a. only	4
	s.s.s.a. only	0
	Both media	0
	Cultures after streptomycin double-strength m.s.b. enrichment positive on:	
	b.s.a. only	8
	s.s.s.a. only	4
	Both media	3
	Cultures after both m.s.b. enrichments both positive on:	
	b.s.a. only	3
	s.s.s.a. only	1
	Both media	2
	Other combinations of cultures positive after enrichments only	9

Method 4 yielded 67 swabs positive for *S. typhi* out of 110 examined. Use of streptomycin m.s.b. contributed positives which would not otherwise have been obtained but would have missed positives had it been used alone. Again the use

of S.S.S.A. as a recovery medium after enrichment also contributed positive results which would have been missed if it had not been used (Table 4).

S. paratyphi B was isolated from 186 sewer swabs during the entire programme and other salmonella serotypes were isolated from 42 sewer swabs (Table 5). *S. typhi* and *S. paratyphi B* were recovered together from 12 swabs in the programme but multiple isolations involving other salmonella serotypes did not occur.

Table 5. *Other salmonellas isolated from sewer swabs during programme*

Name	Number of swabs positive
<i>S. paratyphi B</i>	186
<i>S. thompson</i> var. <i>berlin</i>	28
<i>S. typhimurium</i>	7
<i>S. infantis</i>	2
<i>S. dublin</i>	3
<i>S. give</i>	1
<i>S. tennessee</i>	1
Total	228

Table 6. *Results obtained from sewers related to known excreters 'A' and 'B'*

Sampling sites	Method 1	Method 2	Method 3	Method 4
Domestic sewer A	19/40	4/4	12/12	20/20
Domestic sewer B	11/22	3/4	7/12	15/22
Road A				
15 in. sewer	1/15 (1)	3/5 (0)	4/11 (0)	10/23 (0)
18 in. sewer	0/6 (0)	2/5 (0)	4/11 (0)	8/23 (0)
21 in. sewer	2/41 (1)	1/6 (0)	Not sampled	Not sampled
Road B				
9 in. sewer	6/16 (8)	1/5 (5)	4/13 (10)	5/20 (10)
15 in. sewer	0/15 (3)	1/6 (4)	1/4 (3)	0/3 (3)

N.B. Numerator indicates number positive for *S. typhi* and denominator total number of swabs. Numbers in parentheses indicate number positive for *S. paratyphi B*.

DISCUSSION

Results obtained by the four methods in this programme are not directly comparable with each other because of variation in the sites sampled during each method. In method 1 a high proportion of sewers sampled were of large diameter and each served large areas of the city. These sewers were sampled to allow tracing back from larger to smaller sewers. Certain smaller sewers related to known excreters were included as technique controls in method 1. Consistent isolations of *S. typhi* were not obtained from large sewers, and even in the smallest sewers results were disappointing. Methods 2-4 arose from efforts to improve isolation rates.

In Table 6 comparison is made of results obtained from certain sites related to known excreters which were sampled by all four methods. Improvement in the reliability of methods is seen, especially in the individual domestic sewers. With larger sewers more than half the swabs were always negative.

Isolation of *S. typhi* from sewage had always been recognized to be difficult. Moore's successful tracing of a *S. typhi* carrier (1950) by the sewer swab technique appears to have been due largely to his use of B.S.A., especially in direct techniques. The relative inefficiency of enrichment cultures was noted and studied by Moore (1948, 1950) and also Pilsworth (1960). The latter used a brilliant green selenite broth with added sulphonamide to give some improvement in enrichment procedure. Harvey & Price (1964) found that selenite broth in double concentration enhanced the isolation rate of *S. typhi* from polluted culvert water. They found the method useful in separating *S. paratyphi B* and *S. typhimurium* from *S. typhi*, and applied it successfully in examining sewage effluent and abattoir lairage washings.

Experience in this programme confirmed the value of B.S.A. in direct culture of sewer swab washings, once initial technical difficulties had been overcome. There appeared to be little to choose between B.S.A. and S.S.S.A. as a recovery medium for use with M.S.B., although in the later stages of the programme B.S.A. was preferred by technical staff because of its greater differentiating power. In method 4, enrichment techniques outscored direct B.S.A. cultures, largely through use of streptomycin with double concentration of M.S.B.

In Table 6 the frequency with which *S. paratyphi B* was isolated from samples from 'B's' larger sewers is shown. This strain frequently outgrew *S. typhi* in enrichment cultures, and occurred occasionally when using streptomycin M.S.B. With direct cultures, the similarity of colonial appearances frequently led to all selected colonies being *S. paratyphi B*. It can be concluded that isolation of *S. typhi* is considerably hampered by the presence of *S. paratyphi B* in sewage. Isolation of *S. typhi* and *S. paratyphi B* together on only twelve occasions during the programme adds further support to this conclusion. Difficulty in isolation of *S. typhi* in the presence of *S. paratyphi B* and other salmonella serotypes was also commented on by Harvey & Price (1964). In considering results from 'A's' sewers, low isolation rates in the larger sewers are attributable to the larger population served by these sewers and greater dilution of *S. typhi*.

The two excretors 'A' and 'B' received several courses of antibiotic therapy during method 1. It is reasonable to attribute some of the poorness of results to the antibiotics because the patient's weekly stool cultures were often negative at these times. 'A' and 'B' differed in their attitude to their infections. 'B', said to be a very particular person, used disinfectant liberally after visiting the toilet, during all methods in the programme. 'A' used disinfectant during method 1 but not during methods 2-4. Some of the negative results from 'B's' domestic sewer may have resulted from this use of disinfectant. The success rate with 'B's' sewers during method 4 was also lowered by 'B's' removal to hospital (and death) 5 weeks before the end of method 4. *S. typhi* was recovered from 'B's' domestic sewer on two occasions up to 10 days after his departure but no further isolations were obtained from 'B's' larger sewers.

S. paratyphi B was isolated at many different sites during the programme. Among the 186 sewer swabs positive for this organism a total of 18 different phage types occurred.

Type 2 was isolated from a sewer draining an institution housing a previously known excreter. Available local records gave no indication of possible sources of other strains. Types Beccles and 3b were traced to their sources without difficulty. With each the excreter was an elderly female. Type 3b var. 6 was isolated on one occasion from a large sewer some weeks after the Blackpool paratyphoid outbreak due to this strain. Approximately 1000 sewer swabs from our total examined were used in attempts to narrow down the origins of some of the *S. paratyphi B* strains.

CONCLUSION

The results suggested that the generalized swabbing programme in Aberdeen offered little prospect of tracing undetected *S. typhi* excreters. The small size of sewer needing to be sampled would have rendered the programme beyond the resources of all concerned. It is likely that the results were depressed partly by antibiotic therapy, use of disinfectants and by the presence of *S. paratyphi B*. In tracing unknown excreters of *S. typhi* it is less likely that antibiotics or disinfectants would be in use and better results might be attainable, but, in communities of any size, *S. paratyphi B* appears likely to impair results.

On balance, the results support the suggestion (Moore *et al.* 1952) that, in sewer swab surveys, sampling should begin at sewers draining 50 to 100 houses to give reasonable chance of success in tracing *S. typhi* carriers.

SUMMARY

Laboratory aspects of a programme of sewer swabbing during and after the Aberdeen typhoid fever outbreak, 1964, have been described. Results were discussed and reasons suggested for the relative insensitivity of methods employed in the isolation of *Salmonella typhi*. It was tentatively concluded that generalized sewer swabbing in a large city was not likely to be very helpful in tracing undetected excreters of *S. typhi* during and immediately after a major typhoid fever outbreak. The cost of the laboratory side of this investigation, for staff salaries, equipment, media, etc., was somewhat over £7000.

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REFERENCES

- HARVEY, R. W. S. & PRICE, T. H. (1964). The isolation of *Salmonella typhi* from selenite enrichment media. *Mon. Bull. Minist. Hlth* **23**, 233.
- HOBBS, B. C. (1943). Note on bismuth sulphite agar (Difco). *Mon. Bull. Minist. Hlth* **2**, 29.
- HOBBS, B. C. & ALLISON, V. D. (1945). Studies on the isolation of *Bact. typhosum* and *Bact. paratyphosum B*, III. *Mon. Bull. Minist. Hlth* **4**, 63.
- KAUFFMANN, F. (1950). *The Diagnosis of Salmonella Types*. Illinois: Thomas Springfield.
- LONDON, N. C. & MACKENZIE, R. D. (1951). Tracing a typhoid carrier by sewage examination. *Mon. Bull. Minist. Hlth* **10**, 23.
- MOORE, B. (1948). The detection of paratyphoid carriers in towns by means of sewage examination. *Mon. Bull. Minist. Hlth* **7**, 241.
- MOORE, B. (1950). The detection of typhoid carriers in towns by means of sewage examination. *Mon. Bull. Minist. Hlth* **9**, 72.
- MOORE, B., PERRY, E. L. & CHARD, S. T. (1952). A survey by the sewer swab method of latent enteric infection in an urban area. *J. Hyg., Camb.* **50**, 137.
- PILSWORTH, R. (1960). Detection of a carrier of *Salm. typhi* by means of sewer swabs. *Mon. Bull. Minist. Hlth* **19**, 201.