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Efficiency of cultures of rectal swabs and faecal specimens in detecting salmonella carriers: correlation with numbers of salmonellas excreted

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The importance of the asymptomatic carrier in the epidemiology of salmonellosis is undenied. Our knowledge of the carrier state itself, however, is grossly inadequate. As yet the carrier site of salmonellas exclusive of *Salmonella typhi* has not been adequately determined, and the patterns of salmonella excretion by asymptomatic carriers are undefined. Carriers are detected primarily by culturing faeces, and it is well known that a single faecal culture frequently may not detect an asymptomatic carrier of salmonellas. Frequently, three consecutive negative faecal cultures are accepted as sufficient evidence that a person is not excreting salmonellas (Mosher, Wheeler, Chant & Hardy, 1941). However, in a recent study at an institution for incurables, it was found that among thirty-six patients who had excreted *S. derby* for 11 months, recovery rates using rectal swab cultures varied widely (McCall *et al.* 1964). Over a third of the carriers could not be detected by culturing three consecutive daily rectal swabs. Speculative reasons for this inefficiency include inadequacy of taking rectal swabs as a method of obtaining faeces for culture, inability of laboratory techniques to detect salmonellas when low numbers are excreted, and intermittent excretion of salmonellas by the carrier.

Before the salmonella carrier state can be intelligently investigated, the efficiency of our methods of detecting carriers must be determined, and if necessary, improved. The excretion patterns of individual carriers also must be defined. The present study was designed to compare the efficiency of culturing rectal swabs and faecal specimens as methods of detecting salmonellas both in carriers of a short duration and in those harbouring the organisms for a prolonged period of time. The culture results are correlated with the excretion patterns of the carriers as depicted by determining the average number of salmonellas excreted per g. of faeces. Additionally, culture results following shipment and a delay in processing were evaluated.

MATERIALS AND METHODS

The subjects used in the investigation were residents at a home for incurables in Philadelphia, Pennsylvania, which was affected by an epidemic of *S. derby*

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infection. Infections among the residents were first discovered in June 1963. The outbreak within the institution lasted for some 18 months, thus providing the opportunity for studying both recently infected patients and persistent excreters of salmonella. All subjects were asymptomatic carriers at the time of the investigation. They suffered from a variety of chronic diseases, the most common of which were multiple sclerosis, cerebral palsy, Parkinson's syndrome, rheumatoid arthritis, and cerebral vascular disease.

The investigation was divided into two parts. The first part compared the efficiency of culturing rectal swabs and faecal specimens as methods of detecting salmonellas in known carriers, and correlated these results with the number of salmonellas excreted per g. of faeces (wet weight). Two groups of known carriers of salmonella were selected for this part of the investigation.

The first group consisted of six patients who had been negative for salmonella according to the results of four separate rectal swab cultures obtained during the preceding 4 months, but who were positive for the first time at the time of the investigation. Patients in this group were designated as asymptomatic carriers of short duration, and henceforth will be referred to as short-term carriers. Because none of these residents had experienced gastro-intestinal symptoms, the exact duration of infection could not be determined. This group consisted of one male and five females, the ages ranging from 30 to 90 years.

The second group used in the first part of the investigation consisted of eight patients who, 11 months after their initial positive culture, were still positive for salmonella. These eight subjects also had been positive at least once during culture surveys performed at the end of 6-, 7-, and 9-month intervals after their first positive culture. These results suggested that these patients had been chronic carriers since their initial infection. These subjects will be referred to as long-term carriers. There were three males and five females in this group, with ages ranging from 30 to 80 years.

All subjects with the exception of one short-term carrier who excreted *S. anatum*, were carriers of *S. derby*. Rectal swabs and faecal specimens were obtained from both groups. The rectal swabs were taken daily for 11 consecutive days, and faecal specimens were obtained when possible within 6 hr. of the rectal swabs.

The second part of the investigation was designed to determine the effect on culture results of shipment and delayed processing of specimens. In addition to the fourteen subjects in the first part of the study, the group participating in this part included thirty additional 11-month carriers of *S. derby*. Daily rectal swabs were obtained in duplicate for 10 consecutive days from each of these forty-four known salmonella carriers. On the first day, both swabs were processed immediately. Thereafter, one swab was processed immediately and the duplicate swab was held in tetrathionate brilliant green (BG) broth at room temperature for 2 days. It was then shipped air mail from Philadelphia to the Communicable Disease Center, Atlanta, Georgia, for processing. The shipment delayed processing 1 day more.

Bacteriological methods

Rectal swab cultures were obtained using cotton-tipped swabs which were immersed in tetrathionate broth to which brilliant green dye was added to a final concentration of 1/100,000 (weight/volume). The swabs were inserted approximately 5 cm. into the rectum and rotated 3 to 5 times before withdrawing. Faecal specimens were collected in sterile plastic cups, when possible within 6 hr. of the time the rectal swabs were obtained, and were immediately refrigerated.

The same method for isolating salmonella was used for all specimens. Specimens were incubated in tetrathionate BG broth for 24 hr. at 37° C. Samples of the broth were then streaked on brilliant green agar with 8–10 mg. of sulphadiazine added per 100 ml. of agar, and the plates were incubated for 24 hr. Colonies suspected of being salmonella were picked to triple sugar iron agar and the identification confirmed serologically. An average of three colonies per plate were picked.

Estimation of the salmonella content of faecal specimens

The same faecal specimens were used to determine whether the specimen was positive for salmonella and to estimate the number of salmonellas present per g. of faeces (wet weight). To determine the most probable number (MPN) of organisms, a 10 % suspension using between 3 and 10 g. of faeces in tetrathionate BG broth was mixed for approximately 30 sec. in an electric blender, after which serial tenfold dilutions were inoculated in tetrathionate BG broth. Each dilution was made in triplicate. The broth was incubated for 24 hr and streaked on brilliant green agar plates which were then incubated for 24 hr. The presence of salmonellas in each faecal specimen was determined as previously described. When salmonellas were identified, the most probable number per g. of faeces (wet weight) was obtained using standard MPN tables (American Public Health Association, 1960). This method was used in preference to the technique of Miles & Misra (1938) which employs plate counts after dilution. Although we have found the two methods comparable when using pure cultures of *Salmonella in vitro*, *in vivo*, when the number of salmonellas is low and other types of bacteria are present, determining the MPN using pre-enrichment broth and a selective medium is more sensitive (Boring, unpublished data).

RESULTS

Efficiency of culturing rectal swabs versus faecal specimens, and the excretion patterns of asymptomatic carriers

In Tables 1*a* and *b* the results of cultures of rectal swabs and faecal specimens obtained from short-term salmonella carriers are listed. If the faecal specimen was positive, the number of salmonellas per g. of faeces is recorded. The differences in the ratio of positive cultures to the total number of cultures obtained from each individual ranged from 6/9 to 10/10 for rectal swab cultures, and from 6/7 to 9/9 for cultures of faecal specimens. Of fifty-six rectal swab cultures taken from the six short-term carriers, forty-six (82 %) were positive. Two consecutive rectal swab cultures negative for salmonella were obtained from two subjects. Of thirty-

six faecal specimens collected and cultured over a comparable period, thirty-four (94%) were positive for salmonella and from no patient were cultures of two consecutive faecal specimens negative for salmonella. The average number of salmonellas excreted per g. of faeces per subject ranged between 720 and 61,000. The average for all short-term carriers was 12,000.

Table 1. (*a*) *Culture results of successive daily rectal swabs and faecal specimens from short-term salmonella carriers*

	Subjects											
	1		2		3		4		5		6	
Day	S	F	S	F	S	F	S	F	S	F	S	F
1	+	ND	+	ND	+	ND	+	ND	+	ND	+	ND
2	+	ND	+	ND	−	ND	−	ND	+	ND	−	ND
3	+	+	+	+	+	+	ND	ND	+	ND	+	ND
4	+	+	+	+	+	+	+	ND	+	+	+	+
5	ND	+	ND	+	+	+	+	+	+	ND	+	ND
6	+	+	+	+	+	ND	+	+	+	ND	−	ND
7	−	+	+	+	+	+	+	+	+	ND	−	ND
8	+	+	+	+	−	+	+	ND	+	+	+	+
9	−	+	+	+	ND	−	+	+	+	+	+	ND
10	−	+	−	+	+	+	+	+	+	ND	+	ND
11	ND	−	ND	+	ND	ND	ND	ND	ND	+	ND	ND
Proportion positive	6/9	8/9	8/9	9/9	7/9	6/7	8/9	5/5	10/10	4/4	7/10	2/2

* All subjects excreters of *S. derby* except no. 2 who harboured *S. anatum*.
S, rectal swab; F, faecal specimen; +, positive; −, negative; ND, not done.

(*b*) *Estimated salmonella content of faecal specimens from short-term carriers*

	Subjects					
Day	1	2	3	4	5	6
3	11,000	460	46	ND	ND	ND
4	11,000	4,600	2,100	ND	11,000	46
5	24	4,600	240	11,000	ND	ND
6	240	460	ND	460	ND	ND
7	240	150	240	4,600	ND	ND
8	46	4,600	7	ND	110,000	11,000
9	24	46	0	7,500	11,000	ND
10	46	15	2,400	46	ND	ND
11	0	9	ND	ND	110,000	ND
Average	2,500	1,700	720	3,400	61,000	5,500

* All subjects excreters of *S. derby* except no. 2 who harboured *S. anatum*.

Table 2*a* and *b* list the results of cultures of rectal swabs and faecal specimens obtained from the eight long-term carriers. The ratio of positive rectal swab cultures to total rectal swabs obtained per subject varied from 1/10 to 10/10. One subject had two consecutive negative rectal swab cultures, two had three con-

secutive negative swabs, two had four consecutive negative swabs, two had five consecutive negative swabs, one had eight consecutive negative swabs and one had nine consecutive negative swabs. The ratio of positive cultures of faecal specimens to total number of faecal specimens obtained from each individual varied from 1/3 to 7/7. Only one subject had two consecutive negative cultures of

Table 2. (*a*) *Culture results of successive daily rectal swabs and faecal specimens from long-term salmonella carriers**

	Subject															
	1		2		3		4		5		6		7		8	
Day	S	F	S	F	S	F	S	F	S	F	S	F	S	F	S	F
1	+	ND	+	ND	+	ND	−	ND	+	ND	+	ND	+	ND	+	ND
2	+	ND	−	ND	+	ND	−	ND	+	ND	−	ND	+	ND	−	ND
3	−	ND	−	ND	+	+	−	ND	+	ND	−	ND	+	ND	−	ND
4	+	+	+	+	+	ND	+	ND	+	+	−	ND	+	+	−	+
5	+	+	+	+	+	+	+	+	+	ND	−	−	+	+	−	ND
6	+	+	+	ND	+	+	+	+	+	+	−	ND	+	+	−	+
7	−	+	+	+	+	+	−	ND	+	+	−	ND	+	ND	−	ND
8	−	+	−	+	+	+	−	ND	+	ND	−	−	+	ND	−	ND
9	−	+	−	+	+	ND	−	−	+	ND	−	ND	+	ND	−	ND
10	−	+	−	+	+	ND	−	+	+	+	−	+	+	ND	+	+
Proportion positive	5/10	7/7	5/10	6/6	10/10	5/5	3/10	3/4	10/10	4/4	1/10	1/3	10/10	3/3	2/10	3/3

* All subjects excreters of *S. derby*.
S, rectal swab; F, faecal specimen; +, positive; −, negative; ND, not done.

(*b*) *Estimated salmonella content of faecal specimens from long-term carriers*

	Subjects							
Day	1	2	3	4	5	6	7	8
3	ND	ND	1,100	ND	ND	ND	ND	ND
4	11,000	460	ND	ND	1,100	ND	240	24
5	11,000	1,100	1,200	1,100	ND	0	240	ND
6	1,100	ND	460	39	24	ND	24	3
7	3	9	240	ND	9	ND	ND	ND
8	24	1,100	11,000	ND	ND	0	ND	ND
9	110	240	ND	0	ND	ND	ND	ND
10	11,000	460	ND	46	100	240	ND	4
Average	4,900	530	6,900	300	310	80	170	10

* All subjects excreters of *S. derby*.

faecal specimens. The total ratio of positive rectal swab cultures to the total number of rectal swabs taken was 46/80 (58 %), whereas, the total ratio of positive cultures of faecal specimens to the total number of faecal specimens obtained was 32/35 (91 %). The average number of salmonellas excreted per g. of faeces per subject varied between 10 and 6900. The average for all long-term carriers was 1700.

The comparative results of cultures of rectal swabs and faecal specimens collected from the six short-term carriers and the eight long-term carriers are summarized in Table 3.

In an effort to correlate the proportion of positive rectal swab cultures with the number of salmonellas excreted per g. of faeces, the number of faecal specimens with a given quantity of salmonellas was compared with the results of the rectal swab cultures obtained within 6 hr. of the time the faecal specimen was collected. The correlations appear in Table 4. When fewer than 100 organisms were

Table 3. *Comparison of rectal swabs and faecal specimens collected from short-term and long-term salmonella carriers*

	Number collected	Number positive	Per cent positive
Rectal swabs, short-term carriers*	56	46	82
Rectal swabs, long-term carriers†	80	46	58
Faecal specimens, short-term carriers	36	34	94
Faecal specimens, long-term carriers	35	32	91

* Represents carriers excreting salmonella for less than 4 months.
† Represents carriers excreting salmonella for 11 months.

Table 4. *Correlation of positive rectal swab cultures with number of salmonellas excreted per g of faeces**

No. of salmonellas / g of faeces (wet weight)	No. rectal swabs positive / total no. rectal swabs	Per cent positive
0	0/3	0
10	3/6	50
10–99	7/14	50
100–999	13/17	76
1,000–9,999	9/10	90
10,000–99,999	11/12	92
> 100,000	1/1	100

* Rectal swabs and faecal specimens collected within a 6 hr period on the same day.

present, 50 % or less of the rectal swab cultures were positive whereas twenty-one of twenty-three rectal swab cultures were positive when greater than 1000 salmonellas per g. of faeces were present. No rectal swab culture was positive when a faecal specimen was negative. In contrast, nineteen cultures of faecal specimens yielded salmonella when rectal swab cultures were negative.

Effect of shipment and delayed processing on recovery rates

No significant effect on recovery rates was noted when rectal swabs were shipped and processing was delayed for a period of 1 to 3 days. The results of shipment and delayed processing are listed in Table 5. Of the 440 rectal swabs processed immediately, 230 were positive. Of the remaining 440 rectal swab specimens simultaneously collected and processed after a 1, 2, or 3-day delay, 226 were positive.

Although no significant difference was noted in the total number of positive cultures obtained, in some cases only one of the two swabs from a patient collected simultaneously was positive. By processing both rectal swabs, one immediately and one following a 1-, 2-, or 3-day delay, twenty-one positive cultures were obtained which would have been missed if only one swab had been processed. Two swabs collected simultaneously, therefore, increased the efficiency of rectal swab cultures in detecting carriers by 9 %.

Table 5. *Effect of shipment and delayed processing on the recovery of salmonellas from known excreters*

Time of culture*	No. positive	No. positive in control
No delay	20	21
1-day delay	72	72
2-day delay	67	67
3-day delay	67	70
Total	226	230

* Includes shipment delay of 1 day.

DISCUSSION

In 1955, Thomson, using the quantitative technique of Miles & Misra (1938), studied the number of pathogenic bacilli excreted in the faeces of individuals suffering from acute bacterial gastroenteritis caused by *Shigella*, enteropathogenic *Escherichia coli*, and *Salmonella*. Most subjects with acute salmonella gastroenteritis excreted greater than 1×10^6 pathogens per g. of faeces (wet weight). In the same study, Thomson found that in asymptomatic excreters, the number of salmonellas excreted per g. of faeces tended to be slightly less. Of the six cases of asymptomatic salmonella infection studied, the numbers of salmonellas per g. of faeces were 5×10^6 to 5×10^7 in one case, 1×10^5 in three cases, and 1×10^4 in two cases. No mention was made of how long the patients had been excreting salmonellas, but they would seem to fit our category of short-term carriers. Because of the insensitivity of the Miles and Misra technique when estimating the salmonella content of faeces in mixed culture, individuals harbouring less than 10,000 salmonellas per g. of faeces might have been missed. By utilizing pre-enrichment broth and selective media, low numbers of salmonellas can be detected and estimated by determining the MPN.

The results of this investigation indicate that the number of salmonellas per g. of faeces excreted by short-term carriers and by long-term carriers varies from day to day. The variations result in low numbers of salmonellas being excreted at times. Indeed, at times, salmonellas are either absent or cannot be detected by present bacteriological methods. That the low yield of positive rectal swab cultures obtained from known excreters was, at least in part, related to the low number of salmonellas sometimes excreted by these carriers, was indicated by the comparison between positive rectal swab cultures and quantitative faecal analysis. When

fewer than 1000 salmonellas per g. of faeces were present, recoverability of salmonellas by culturing swabs was poor.

Cruickshank & Swyer (1940), using MacConkey medium, demonstrated in symptomatic hospital patients that cultures of rectal swabs were superior to cultures of faecal specimens in detecting shigellas. Shaughnessy, Frierer & Snyder (1948) in a study of chronic salmonella carriers suggested that catharsis improved recovery rates, but there were no significant differences in the rates of recovery from rectal swabs and faecal specimens. In the present study, the superiority of culturing a faecal specimen of 3–10 g. over culturing a rectal swab was clearly demonstrated. Although the difference was greater in the long-term carriers, it was also significant in the short-term carriers. The higher yield of positive cultures in short-term carriers probably resulted from a greater number of salmonellas per g. being excreted, as was suggested by an average of 12,000 organisms per g. of faeces recovered from short-term carriers as opposed to 1700 per g. of faeces recovered from long-term carriers.

Although the rectal swab culture is valuable for determining the presence of salmonellas in large culture surveys, it cannot be relied upon to detect carriers unless several swabs are taken, the number depending upon the duration of the carrier state and the number of organisms excreted. In this study, three consecutive rectal swab cultures were needed to detect all six short-term carriers, and nine consecutive rectal swab cultures were needed to detect the eight long-term carriers of salmonellas. Two consecutive cultures of faecal samples would have detected all six short-term carriers and would have missed only one of the eight long-term carriers.

Although the second part of the study revealed that by collecting two rectal swabs simultaneously, the efficiency of rectal swab cultures in detecting salmonella carriers could be improved by approximately 9%, the results would still be inferior to culturing 3–10 g. of faeces.

Delayed processing and shipment of rectal swab specimens had no deleterious effect upon the total number of positive cultures obtained from the salmonella carriers. This is comforting, in that cultures frequently have to be shipped for analysis, thereby resulting in a processing delay of several days. Studies are needed to determine whether such delays in processing might affect the result obtained by culturing faecal specimens instead of rectal swabs, and whether cultures of faecal specimens are superior to rectal swab cultures in identifying symptomatic cases of salmonellosis.

SUMMARY

The comparative efficiency of cultures of rectal swabs and faecal specimens in detecting salmonellas in asymptomatic carriers was determined and then correlated with the number of salmonellas excreted per g. of faeces (wet weight). In six short-term salmonella carriers, 82% of rectal swab cultures taken daily for 11 consecutive days were positive for salmonellas, whereas 94% of faecal specimens cultured were positive. Similarily, in 8 long-term carriers, 58% of the rectal swab cultures were positive as compared with 91% of faecal specimens. The

inferiority of the results of rectal swab cultures correlated with their inefficiency in detecting salmonellas when fewer than 1000 salmonellas per g. of faeces were present.

The patterns of salmonella excretion as depicted by estimated salmonella content of faecal specimens showed that all carriers had irregular day-to-day variations. The average number of salmonellas excreted per g. of faeces by short-term carriers was greater than that by long-term carriers, although both groups revealed the same wide variations in number of organisms excreted.

The effect of delayed processing and shipment of specimens upon recovery of salmonellas was also studied. No deleterious effects were noted when specimens were shipped and processing was delayed for up to 3 days.

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