

## Carriage Rate of *Salmonella* Serotypes in Hospital Patients and Comparison of Enrichment Media for their Isolation

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(Received for Publication April 27, 1971)

### Abstract

The efficiencies of Rappaport medium, strontium chloride, strontium selenite and selenite F broths for enrichment in the isolation of salmonellas from human stools were compared. The study showed that Rappaport medium when used in conjunction with strontium selenite broth gave the most satisfactory result, because they covered the isolation of a wider range of salmonellas. With these media, the carriage rate of *Salmonella* serotypes of 3,325 hospital patients who were admitted without complaints of diarrhoea or gastroenteritis or enteric fever, were tested. The result revealed that 5.6% of them carried *Salmonella* serotypes ranging from those that may produce food-poisoning to those which are causal agents of enteric fever. Moreover, patients who were infected with the liver-fluke, *Clonorchis sinensis*, had a higher carriage rate than those who were infected with other intestinal parasites or non-infected.

Enteric fever and gastroenteritis due to salmonellas are endemic in Hong Kong. A large number of *Salmonella* serotypes was isolated from stools of hospital patients with complaints of gastroenteritis (Huang and Chan-Teoh, 1964), Lo, Huang and Chan (1967) reported the recovery of 12 serotypes from the mesenteric lymph-nodes, faeces, bone-marrow and blood clot of healthy pigs slaughtered for human consumption. But, the human carrier rate of *Salmonella* serotypes other than

*Salmonella typhi* has not been reported.

An attempt to isolate salmonellas from stools of patients admitted to hospital, who had no complaint of gastro-intestinal disturbance during their stay, was carried out and the result is presented in this communication.

Iveson and Mackay-Scollay (1969) introduced two new enrichment media, viz. strontium chloride and strontium selenite broths for isolation of salmonellas from human and animal materials. They found

that strontium chloride medium was comparable to Rappaport medium for recovery of a wide range of salmonellas from human faeces and sewage and that strontium selenite medium was superior to selenite F medium for enrichment purpose particularly in the isolation of *S. typhi*.

These two new media were, therefore, included in the present study in order to compare with Rappaport medium and selenite F broth that were used routinely in our laboratory for isolation of salmonellas from stools.

### Materials and Methods

#### *I. Comparison of enrichment methods:*

The investigation involved the examination of a total of 1,206 human stool specimens, of which 812 were sent for routine parasitological examination, 216 were from gastroenteritis cases and 178 were from suspected enteric fever cases. Each stool specimen was inoculated into Rappaport medium (Rappaport, Konforti and Navon, 1956), strontium chloride broth, strontium selenite broth (Iveson and Mackay-Scollay 1969) and selenite F broth (Leifson, 1936) respectively, and was incubated at 37°C. It has been found that more salmonellas could be recovered from selenite F broth if it were incubated at 43°C (Harvey and Thomson, 1953; Georgala and Boothroyd, 1965). An additional selenite F broth culture incubated at 43°C was, therefore, included. Specimens were also plated directly onto S. S. agar for primary isolation. All the media were prepared according to their original formulae.

After incubation for 18–24 hr, the cultures from enrichment media were plated onto S. S. agar and suspicious colonies were identified in the usual way. Slide agglutination test was carried out with antisera obtained from the Burroughs Wellcome and Co. (London).

#### *II. Detection of salmonellas from carriers:*

This part of investigation covered the period from June 1969 to August 1970. Altogether 3,325 faecal specimens from different individuals submitted for routine parasitological examination from the Queen Mary Hospital of Hong Kong were examined. Stools from patients with clinical symptoms of diarrhoea or gastroenteritis or enteric fever and liquid stools were excluded. Specimens were inoculated into Rappaport and strontium selenite media respectively. All cultures were incubated at 37°C for 18–24 hr and then plated onto S. S. agar. Suspect colonies were identified as above mentioned.

The isolates were subjected to antibiotic sensitivity tests by the disc-method. 'Multodisks' (Oxoid) containing chloramphenicol, tetracycline, streptomycin (each 10 µg per disc), ampicillin (25 µg per disc), nalidixic acid (30 µg per disc) and nitrofurantoin (50 µg per disc) were employed. In addition, sensitivities to kanamycin, neomycin, gentamycin (each 10 µg per disc) and septrin (25 µg per disc) were tested with single discs supplied by the manufacturers.

### Results

Table 1 shows that Rappaport medium is superior to all except for the isolation of *S. typhi*. Strontium chloride broth was comparable but a little inferior to Rappaport medium. Strontium selenite broth was superior to selenite F broth both in isolating *S. typhi* and other salmonella serotypes. Its superiority lies on the inhibition of *Proteus*. Selenite F broth incubated at 43°C did not give better result than that incubated at 37°C in the isolation of salmonellas from human stools.

From these findings, Rappaport medium was chosen in conjunction with strontium selenite broth for enrichment in the second part of the study.

Table 2 presented the number of *Salmonella* serotypes isolated from 3,325 non-diarrheal stools. Altogether 189 strains

belonging to 22 serotypes were isolated. The isolation rate was 5.6 per cent. All serotypes except *S. abortus-equi*, *S. san-diego*, *S. muenchen*, *S. newington* and *S. aberdeen* were reported before either from human or from animal origins (Huang and Chan-Teoh, 1964; Lo, Huang and Chan, 1967). The more frequently encountered serotypes were *S. derby* and *S. anatum*, which are of low pathogenicity and are commonly found from pigs in Hong Kong.

The seasonal variation in their isolation is indicated in Figure 1. As is expected, there was a higher rate of isolation in

**Table 2.** Distribution of *Salmonella* serotypes in carriers.

Serotypes	Number of cases	Percentage of cases
<i>S. paratyphi A</i>	1	0.5
<i>S. abortus-equi</i>	1	0.5
<i>S. paratyphi B</i>	9	4.8
<i>S. stanley</i>	4	2.1
<i>S. san-diego</i>	2	1.1
<i>S. derby</i>	42	22.1
<i>S. typhi-murium</i>	11	5.9
<i>S. paratyphi C</i>	3	1.6
<i>S. choleraesuis</i>	1	0.5
<i>S. thompson</i>	1	0.5
<i>S. tennessee</i>	3	1.6
<i>S. muenchen</i>	1	0.5
<i>S. new port</i>	9	4.8
<i>S. bovis-morbificans</i>	2	1.1
<i>S. sendai</i>	2	1.1
<i>S. typhi</i>	3	1.6
<i>S. enteritidis</i>	6	3.1
<i>S. anatum</i>	76	40.4
<i>S. newington</i>	1	0.5
<i>S. meleagridis</i>	4	2.1
<i>S. senftenberg</i>	6	3.1
<i>S. aberdeen</i>	1	0.5
Total	189	100.0

**Table 1.** Relative efficiency of different enrichment methods in the isolation of salmonellas from 1,206 stool specimens.

Methods	No. of strains of <i>S. typhi</i> isolated	No. of strains of <i>S. choleraesuis</i> isolated	No. of strains of other <i>Salmonella</i> serotypes isolated
Rappaport medium	0	5	71
Strontium chloride broth	1	4	57
Strontium selenite broth	20	0	53
Selenite F broth (37°C)	15	0	40
Selenite F broth (43°C)	14	0	36
Direct plating onto S.S. agar	9	1	24
Combination of all methods	21	6	81

the warmer months than in the colder ones. The peak incidence was in June, July and August.

Table 3 reveals the distribution of the isolates among the age groups. Though it has been reported that overt cases of *Salmonella* infection was prevalent in children under 10 years of age (Huang and Chan-Teoh, 1964), the carriage rate was slightly higher in the two extremes of the age groups, i. e. those under 10 years of age and those above 50.

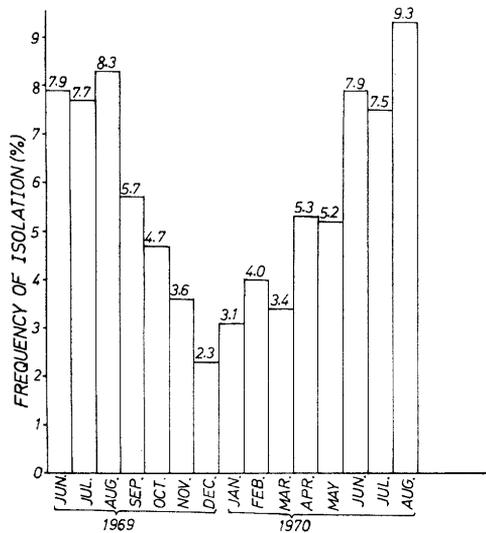


FIG. 1 SEASONAL PREVALENCE OF SALMONELLAS ISOLATED FROM FAECES

Table 3. Distribution of *Salmonella* isolates in age groups.

Age group (years)	Number of specimens	Salmonella strains isolated	Percentage of isolation
Under 10	297	23	7.6
11 - 20	239	11	4.6
21 - 30	471	24	5.1
31 - 40	548	28	5.1
41 - 50	587	25	4.2
51 - 60	540	35	6.1
61 & over	643	43	6.7
Total	3,325	189	5.7

There is no significant difference in the rate of isolation between the two sexes. Although, it appears that the males carried salmonellas more frequently than the females in the overall prevalence.

Since the specimens were submitted for routine parasitological examination, we tried to correlate the parasitic infection and the salmonella carriage rate. As illustrated in Table 4, the carriage rate was higher in those with clonorchiasis than those with other intestinal helminthic infection or those uninfected. It must be noted that none of the non-gastroenteritis patients admitted had clinical complaints of parasitological infection.

The isolates were sensitive to all the antibiotics tested except streptomycin and tetracycline. 9 out of 189 strains (4.7%) were resistant to both streptomycin and tetracycline, 4 (2.1%) were resistant to tetracycline and 29(15.3%) were resistant to streptomycin alone. Their sensitivities

Table 4. Correlation of *Salmonella* isolations with evidence of intestinal helminthic infection in 3,325 stools.

	No. of specimens examined	No. of specimens with <i>Salmonella</i> isolated	Positive rate	
Positive with eggs of	<i>Cl. sinensis</i>	512	45	8.8%
	<i>Cl. sinensis</i> and others	46	4	8.7%
	<i>Ascaris</i>	185	9	4.9%
	<i>Trichuris</i>	212	11	5.2%
	<i>Ascaris</i> or <i>Trichuris</i> and others	40	2	5.0%
	Other intestinal helminths	35	2	5.7%
No helminthic eggs found	2,295	116	5.0%	

to these antibiotics showed no difference with those *Salmonella* strains isolated from overt cases.

Of the two enrichment media used, Rappaport medium is superior to strontium selenite broth in isolating a wide range of *Salmonella* strains (Table 5). It has also been found that the isolation rate of *S. choleraesuis* with Rappaport medium was high (Iveson and Mackay-Scollay, 1969) but *S. typhi* was strongly inhibited

by this medium. However, 32 out of the 189 isolates would have been missed if strontium selenite broth had not been used. Judging from the findings, Rappaport medium if used in conjunction with strontium selenite broth for routine isolation of salmonellas from stools would give the most satisfactory result. For example, *S. choleraesuis* can be isolated from the Rappaport medium and *S. typhi* from the latter.

**Table 5.** Comparison of two enrichment media for the isolation of *Salmonella* serotypes from 3,325 normal stool specimens.

	Total No. of strains isolated	Rappaport medium		Strontium selenite broth	
		No. of strains isolated	No. of strains missed	No. of strains isolated	No. of strains missed
<i>S. paratyphi A</i>	1	1	0	1	0
<i>S. abortus-equi</i>	1	0	1	1	0
<i>S. paratyphi B</i>	9	8	1	5	4
<i>S. stanley</i>	4	3	1	2	2
<i>S. san-diego</i>	2	2	0	1	1
<i>S. derby</i>	42	36	6	28	14
<i>S. typhimurium</i>	11	9	2	5	6
<i>S. paratyphi C</i>	3	2	1	3	0
<i>S. choleraesuis</i>	1	1	0	0	1
<i>S. thompson</i>	1	1	0	0	1
<i>S. tennessee</i>	3	3	0	2	1
<i>S. muenchen</i>	1	1	0	0	1
<i>S. new port</i>	9	8	1	5	4
<i>S. bovismorbificans</i>	2	1	1	2	0
<i>S. sendai</i>	2	1	1	2	0
<i>S. typhi</i>	3	0	3	3	0
<i>S. enteritidis</i>	6	5	1	3	3
<i>S. anatum</i>	76	65	11	36	40
<i>S. newington</i>	1	1	0	0	1
<i>S. meleagridis</i>	4	3	1	2	2
<i>S. senftenberg</i>	6	5	1	3	3
<i>S. aberdeen</i>	1	1	0	1	0
Total	189	157 (83.1%)	32 (16.9%)	105 (55.5%)	84 (44.4%)

### Discussion

The large variety of *Salmonella* serotypes found can be attributed to the great amounts of food stuffs, especially pork, poultry and eggs, imported annually to Hong Kong. Serotypes such as *S. anatum* and *S. derby* are primarily of animal or fowl origin. For instance, Lo, Huang and Chan (1967) reported that a large number of the *Salmonella* serotypes they had isolated from the mesenteric lymph-nodes of imported pigs slaughtered for human consumption were also recovered from hospital patients in the period of their study.

The carriage rate of salmonellas among hospital patients with no gastrointestinal disturbance was also high. Of the 22 serotypes isolated, 16 had been found in overt cases and 10 serotypes from pigs in Hong Kong (Huang and Chan-Teoh, 1964; Lo, Huang and Chan, 1967).

It must be noted that none of the patients whose faeces examined in the second series had direct evidence of gastrointestinal infection. The serotypes were uncovered only when the stools were submitted for routine parasitological examination. This condition may pose a problem in hospital infection, although most of the serotypes they carried may be passengers, yet many of them, such as the salmonellas of the typhoid-paratyphoid groups, *S. choleraesuis* and *S. sendai*, are well established causal agents of enteric fever.

The epidemiology of enteric fever in Hong Kong is not fully understood. Forrest *et al.* (1969) maintained that the disease was scattered so that neither water supply nor bulk food-stuffs could

be the main vehicles of infection. As is seen in the present study, 19 out of 189 strains (9.5%) of the isolates were established causal agents of enteric fever. Human carrier is, therefore, probably the main source.

A correlation of the carriage rate of salmonellas and parasitic infection supports the findings of McFadzean and Ong (1966) who reported that there was significantly higher incidence of typhoid carriers in cases of infection with the liver fluke, *Clonorchis sinensis*. This parasite is one of the precipitating factors of recurrent pyogenic cholangitis and contributes to the consistent intrahepatic carrier state of enteric pathogens. *C. sinensis* infection may be a factor although it could not be the sole factor associated with typhoid-paratyphoid carrier problem in Hong Kong. That *C. sinensis* is the most prevalent parasite found in the community has been reported by Huang *et al.* (1969).

Smith (1970) found that 4.3% of his salmonellas isolated from poultry in England and Wales in 1968 were resistant to antibiotics. Our findings were in accord with Smith's in terms of resistance to streptomycin and tetracyclines.

The moderately high percentage of streptomycin resistant strains in our cases is closely identical to that of Schroeder, Terry and Bennett (1968). This is perhaps a result of *in vivo* stepwise induction of resistance due to its wide clinical use. The number of incidence of transferred resistance was, however, low.

Many enrichment media have been

devised for isolation of salmonellas from contaminated materials. Each of them has its own merits and drawbacks. This problem had been critically reviewed by many authors and more recently by Iveson and Mackay-Scollay (1969). A satisfactory enrichment medium for that purpose must not only have the inherent property to inhibit the growth of other faecal contaminants but also be efficient to cover the isolation of a wide range of the *Salmonella* serotypes. In Hong Kong

where both *S. typhi* and *S. choleraesuis* are prevailing neither of the media described can be used singularly to achieve the result. The combination of Rappaport medium and strontium selenite broth is recommended. We support Iveson and Mackay-Scollay in that strontium selenite broth is definitely superior to selenite F broth as enrichment medium for isolation of *S. typhi* from contaminated materials as well as from clinical cases.

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入院患者のサルモネラ保菌率およびその分離のための増菌培地の比較

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摘 要

サルモネラ分離に際して Rappaport 培地, strontium chloride ブイヨン, strontium selenite ブイヨンおよび selenite F ブイヨンの4種の増菌培地を用い, その成績を比較した. その結果, Rappaport 培地に strontium selenite ブイヨンを併用すると, サルモネラの各種類の分離に最も満足すべき成績がえられた.

これらの培地を用いて, 下痢を全く伴わない患者, 胃腸炎あるいはチフス性疾患の患者 3,325名についてサルモネラ保菌率を検査した. その結果, 22 の血清型に属する 189株のサルモネラを分離した. 検出率は 5.6%である. その血清型は, 食中毒の原因と推定されたものから, チフス性疾患の原因菌に至るさまざまな型が含まれていたが, 最も多く分離されたのは *S. derby* と *S. anatum* であった.

また, *Clonorchis sinensis* に感染していた患者は, 他の腸寄生虫感染者あるいは非感染者より高率にサルモネラを保有していた.