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Elevated temperature technique for the isolation of salmonellas from sewage and human faeces

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SUMMARY

Modified Moore's swabs, placed in sewers for 5 days, were used to concentrate salmonellas from sewage. Duplicate cultures of swab strips in selenite broth were incubated at 41 and 37° C. respectively. *Salmonella* organisms were recovered consistently from the swabs when the enrichment broths were incubated at 41° C. However, when equal portions of the same swabs were incubated at 37° C., only 22 % of them yielded *Salmonella* organisms. These results indicate an advantage in incubating the selenite broths at 41° C. rather than 37° C. in attempting to isolate salmonellas from sewage.

One hundred and fifty faecal samples were examined for salmonellas by culture in selenite broths incubated at 41 and 37° C. Twelve (8 %) samples were positive at 41° C. compared to only 10 (6·7 %) positive samples at 37° C. This difference is not statistically significant to indicate an advantage of the elevated-temperature of incubation over the conventional temperature in attempting to isolate salmonellas from human faeces. Moreover, results of the recovery rates of *S. paratyphi* B, *S. typhi*, and *S. typhimurium* indicate that an incubation temperature of 37° C. is more appropriate for recovering salmonellas from artificially infected faecal samples than an incubation temperature of 41° C. This stresses the inability of laboratory studies to mimic conditions in nature.

INTRODUCTION

The need for rapid isolation of salmonellas from sewage, faeces and suspect foods has led to the introduction of several selective media. Various workers in this field have claimed the superiority of one or the other medium. Although elevated-temperature techniques have been mainly used to isolate thermophilic organisms (Wilson & Miles, 1964), a study of the literature will show that some authors have increased the selectivity of their media for mesophilic bacteria by raising the incubation temperature of their enrichment broths (Morris & Dunn, 1970; Wilson, 1938).

Harvey & Thomson (1953) increased the isolation rate of *Salmonella* from faeces cultured in selenite-enrichment broth by raising the incubation temperature. Spino (1966), using modified Moore's swabs, reported that *Salmonella* organisms were recovered consistently from surface waters of streams when the enrichment

broths were incubated at 41·5° C. Likewise, Morahan & Hawkesworth (1969) used modified Moore's swab technique to concentrate salmonellas from streams. The subsequent enrichment of gauze strips in enrichment broths at 41° C. yielded 10 *Salmonella* serotypes.

This present study was undertaken to evaluate the comparative efficiency of the elevated, 41° C., and the conventional, 37° C., temperatures of incubation of selenite-enrichment cultures on the isolation of salmonellas from sewage and human faeces. It is a part of a series of investigations into the occurrence and incidence of *Salmonella* serotypes in Lebanon. Isolations from humans and from animals have been previously reported (Nabbut & Jamal, 1970).

MATERIALS AND METHODS

Cultural methods

Modified Moore's swabs (Moore, 1948; Spino, 1966), having remained in sewers along the coast of the city of Beirut for 5 days, were removed from the sampling points and were placed in sterile beakers. They were collected at weekly intervals, brought to the laboratory and processed within 1 hr. of their collection. Strips, containing several layers of gauze cloth, were cut from the swab with flamed scissors, and added to flasks containing 300 ml. of selenite-enrichment broth. Approximately one-half of each swab was used to inoculate one enrichment broth and the other half to inoculate another enrichment broth. The two broth cultures were incubated for 24 hr. at 37 and 41° C. respectively. After incubation two SS plates were streaked from each enrichment culture and incubated at 37° C. for 24 hr. Non-lactose-fermenting colonies thought to be salmonellas were further identified by means of biochemical and serological tests according to standard methods (Edwards & Ewing, 1962).

Specimens of faeces submitted to the bacteriology section of the American University Hospital Laboratories, (AUHL) for bacteriological culture, were examined for the presence of salmonellas. Approximately 2 g. of each faecal specimen was added to 20 ml. of selenite broth in a universal bottle to give a 10 % suspension. This suspension was then shaken manually and 10 ml. volumes were then added to a pair of universal bottles one of which was incubated for 24 hr. at 37° C. whereas the other was incubated at 41° C. After incubation, 2 SS plates were streaked from each enrichment culture and incubated at 37° C. for 24 hr. Colonies resembling *Salmonella* were further identified according to standard procedures (Edwards & Ewing, 1962).

Recovery of salmonellas from artificially infected faecal specimens

A group of 50 faecal specimens, obtained from the parasitology section of the AUHL, were screened to ascertain the absence of salmonellas. Each specimen was diluted with selenite broth to give a 10 % suspension which was then distributed, in 10 ml. volumes, into six universal bottles. Appropriate dilutions of a 24 hr. nutrient broth culture of *Salmonella typhimurium*, *S. typhi* and *S. paratyphi* B that contained about 20 organisms per ml. were separately added in 1 ml. volumes to two bottles of the faecal suspension respectively. Three of the artificially infected

10% faecal suspensions each containing one of the *Salmonella* species were incubated at 37° C. for 24 hr. whereas the other three bottles were incubated at 41° C. for 24 hr. The enrichment cultures were then streaked on SS plates with a 4 mm. platinum loop. After incubating the plates at 37° C. for 24 hr., colonies resembling salmonellas were tested by slide-agglutination with anti-O-serum of the particular species of *Salmonella* with which the enrichment broth faecal suspension had been inoculated.

Comparative growth of enteric bacteria at 37° C. and 41° C.

The amounts of growth of *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *S. paratyphi* B, *S. typhi* and *S. typhimurium* were compared at 37° C. and 41° C. in selenite broth. Each of the six organisms was grown in nutrient broth for 24 hr. at 37° C. and 0·1 ml. volumes of the appropriate dilutions of each organism were added to duplicate 10 ml. selenite broth in universal bottles. One of the bottles was incubated at 37° C. and the other at 41° C. for 24 hr. Each culture was then diluted serially to 10^{-11} in tubes containing 9 ml. of nutrient broth. All dilutions were incubated at 37° C. for 24 hr. Presence or absence of growth was determined by observing tubes for visible turbidity.

RESULTS

Sixteen *Salmonella* serotypes were isolated from all swab enrichment cultures incubated at 41° C. whereas only four (22%) of the corresponding swab cultures incubated at 37° C. yielded *Salmonella* organisms (Table 1). Incubation at 41° C. often resulted in an almost pure culture of salmonellas and in a marked reduction in the growth of coliform, proteus and pseudomonas organisms compared with incubation at 37° C.

Table 2 summarizes the comparative results from 12 (8%) out of 150 faecal cultures that were salmonella positive. Twelve (8%) *Salmonella* isolates were recovered when the faecal enrichment cultures were incubated at 41° C. compared to 10 (6·7%) isolates recovered from the corresponding faecal cultures incubated at 37° C.

The recovery rates of *S. paratyphi* B, *S. typhi* and *S. typhimurium* from 50 artificially infected faecal samples is shown in Table 3. It is observed that the total number of recoveries of the three *Salmonella* serotypes was always higher when the faecal enrichment cultures were incubated at 37° C. than when corresponding cultures were incubated at 41° C.

The effects of the incubation temperature on the growth of *S. paratyphi* B, *S. typhi*, *S. typhimurium*, *Proteus mirabilis*, *P. aeruginosa* and *Esch. coli* in selenite broths were determined. It is apparent from Table 4 that incubation at 41° C. is slightly inhibitory for all six organisms and more so for *Proteus mirabilis*, *P. aeruginosa* and *S. typhi*.

Table 1. *Effect of incubation temperature on the isolation of salmonellas from sewage using selenite enrichment broth*

	Salmonella serotypes isolated		Incubation temperature	
Group	Serotype	Number of isolations	37° C.	41° C.
B	*S. eppendorf*	1	+	+
B	*S. essen*	1	+	+
B	*S. paratyphi* B	1	+	+
B	*S. paratyphi* B	1	−	+
C1	*S. livingstone*	1	−	+
C1	*S. montevideo*	2	−	+
C2	*S. bovismorbificans*	1	−	+
C3	*S. sunnycove*	1	+	+
D1	*S. goeteborg*	2	−	+
E1	*S. amsterdam*	1	−	+
E1	*S. butantan*	1	−	+
E1	*S. fuhlsbuettel*	1	−	+
E1	*S. muenster*	1	−	+
E1	*S. nchanga*	1	−	+
E1	*S. sekondi*	1	−	+
M	*S. croft*	1	−	+
	Total	18	4[a]	18[a]

− = Salmonella not isolated.
\+ = Salmonella isolated.
a = The difference between 18 and 4 is statistically significant.

Table 2. *Effect of incubation temperature on the isolation of salmonellas from* 150 *faeces using selenite enrichment broth*

	Salmonella serotypes isolated		Incubation temperature	
Group	Serotype	Number of isolations	37° C.	41° C.
B	*S. sandiego*	1	+	+
B	*S. typhimurium*	1	−	+
B	*S. typhimurium*	1	+	+
C1	*S. tennessee*	2	+	+
C2	*S. manhattan*	1	+	+
C3	*S. kentucky*	1	+	+
D1	*S. goeteborg*	2	+	+
E1	*S. anatum*	2	+	+
E1	*S. anatum*	1	−	+
	Total	12	10[a]	12[a]

\+ = Salmonella isolated.
− = Salmonella not isolated
a = The difference between 12 and 10 is not statistically significant.

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Table 3. *Effect of incubation temperature on total number of recoveries of* Salmonella paratyphi *B*, S. typhi *and* S. typhimurium *added* to* 10% *faecal suspensions in selenite broth*

Salmonella serotype	Number of samples examined	Total number positive when incubated at 37° C. and 41° C. Number	%	37° C. Number	%	41° C. Number	%
S. paratyphi B	50	32	64	29	58	17	34
S. typhi	50	20	40	20	40	5	10
S. typhimurium	50	35	70	34	68	25	50

* Approximately 20 organisms were added to 10 ml. of the faecal suspensions.

Table 4. *Effect of incubation temperature on the growth of* Escherichia coli, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi *B*, S. typhi *and* S. typhimurium *in selenite broth*

Organism	No./ml. of selenite broth	Incubation temperature 37° C.	41° C.
Escherichia coli	220	10^{-9}*	10^{-8}
	220	10^{-9}	10^{-8}
Proteus mirabilis	220	10^{-9}	10^{-5}
	200	10^{-9}	10^{-6}
Pseudomonas aeruginosa	900	10^{-9}	10^{-4}
	100	10^{-8}	10^{-4}
Salmonella paratyphi B	50	10^{-8}	10^{-9}
	100	10^{-9}	10^{-9}
S. typhi	260	10^{-8}	10^{-4}
S. typhi (different strain)	200	10^{-9}	10^{-7}
S. typhimurium	750	10^{-9}	10^{-8}
	200	10^{-9}	10^{-8}

* Values indicate the highest dilution to yield growth when subcultured to nutrient broth.

DISCUSSION

A comparison was made of the effects of different incubation temperatures on the isolation of salmonellas from sewage and human faeces. The enrichment of swab cultures at 41° C. was found to be appropriate for the isolation of salmonellas from sewage. This is in agreement with reports by other workers (Spino, 1966; Harvey & Price, 1968; Morahan & Hawksworth, 1969).

The elevated-temperature of incubation seems to suppress the competing Gram-negative bacteria and to permit *Salmonella* organisms to grow in a relatively pure culture, thus providing an advantage for recognizing the salmonellas. *Esch. coli*, *Pseudomonas aeruginosa*, *Proteus*, *Klebsiella*, *Aerobacter* and other enteric bacteria are normally found in sewage and are troublesome organisms in attempts to isolate salmonellas from sewage. The incubation of selenite-enrichment broth at 41° C. was found to be slightly inhibitory to *Esch. coli*, *Salmonella typhimurium*, and

more so for *Proteus mirabilis*, *P. aeruginosa* and *S. typhi* (Table 4). The elevated-temperature technique is, therefore, a factor contributing to the selectivity of the growth of *S. paratyphi* B and *S. typhimurium*, but not for the growth of *S. typhi*. It is recommended for the isolation of salmonellas from sewage and river water, because they contain relatively few salmonellas and a large number of Gram-negative competing organisms of faecal origin.

The elevated-temperature technique may have practical application in epidemiological studies by providing a greater yield of salmonellas from natural waters and sewage. The occurrence of salmonellas in various sewage outfalls and surface waters is important as evidence of an existing health hazard in the population from which the sewage is derived and is directly related to the degree of endemicity of salmonellosis found in a certain community.

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