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# Presence and Distribution of *Salmonella* Species in some Local Foods from Baghdad City, Iraq

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**ABSTRACT**

A total of 353 local food samples were cultured for *Salmonella* species using mannitol broth and two selective enrichment media, tetrathionate brilliant green bile broth and selenite cystine broth. Fifteen *Salmonella* species and serotypes were isolated: *Salmonella paratyphi* B, *Salmonella typhimurium*, *Salmonella* 4,5,12:-:-, *Salmonella muenchen*, *Salmonella senftenberg*, *Salmonella lille*, *Salmonella alachua*, *Salmonella* 6,7:-:-, *Salmonella anatum*, *Salmonella enteritidis*, *Salmonella havana*, *Salmonella eppendorf*, *Salmonella emek*, *Salmonella california*, and *Salmonella* 1,3,19:-:-. *Salmonella* was recovered from 27% of food samples examined. The occurrence of some species was as high as 11% of the samples. *S. lille* and *S. alachua* were isolated and are reported for the first time in Iraq. Seventy food samples (19.8%) harbored one type of *Salmonella*. 11 (5.9%) harbored two types and four (1.1%) harbored three types. Isolation of *Salmonella* from foods is affected by types of enrichment media. Tetrathionate brilliant green bile broth was superior to selenite cystine for *Salmonella* recovery from most foods. Some species prefer certain enrichment media for growth and multiplication. Use of more than one type of selective medium increases the chance of isolation of more *Salmonella* species and serotypes.

After isolation of *Salmonella* by Gaffky in 1884 (*4*), the number of species and serotypes increased gradually, but it increased rapidly in the last 20 years to reach 1744 (*31*) by 1974. Thereafter, serotypes were added every year, based on classification of the Kauffman-White Scheme. Since Budd in 1856, on the basis of epidemiological evidence, suggested the infectious nature and transmission of typhoid fever, the concern about *Salmonella* is also increased because it affects health, food and economy of humans. The classical chain of *Salmonella* infection is feedstuffs-animal-foods-man.

The following criteria are used for tentative identification of *Salmonella* (*3,4,6,7,8,16*): gram negative rods; motile (except *Salmonella pullorum* and *Salmonella gallinarum*) by peritrichous flagella; fermentative to glucose, mannitol, maltose, and sorbose, except *Salmonella typhimurium*, *Salmonella paratyphi* C, *Salmonella enteritidis*, *Salmonella typhi* and *S. gallinarum* which do not produce gas; do not ferment lactose, sucrose, or salicin except *Salmonella arizona* (subgroup III) and *Salmonella houtenae* (subgroup IV); unable to liquefy gelatin except *Salmonella daressalam* (subgroup II), *S. arizona*, and *S. houtenae;* do not produce indol except *Salmonella panama*, *Salmonella eastbourne*, and some strains of *S. enteritidis*; do not produce urease; reduce trimethylamine oxide to trimethylamine; aerobic but some are facultatively anaerobic (*S. enteritidis*, *S. typhimurium* and *S. paratyphi* C); aerogenic except *S. typhi*; reduce nitrate to nitrite; utilize citrate as carbon source except *S. typhi* and *S. paratyphi* A; decarboxylate lysine except *S. paratyphi* A; do not grow in KCN medium except *S. houtenae*; positive in the MR and catalase tests; and negative in the VP and oxidase tests. The ultimate criteria in classification of the species are serological tests based on somatic and flagellar antigens, phase 1 and 2 (*4,17*).

Because *Salmonella* occurs in small number in food, compared to competing microorganisms, it is necessary to employ enrichment media for its isolation. Tetrathionate and selenite broth that are suitable for isolation of salmonellae from clinical materials have a definite short coming when used for isolation of the bacteria from food (*25*). Among the problems involved in isolation are: (*a*) Impairment of the selectivity of the media by the sample (*26*). (*b*) The physiological dormancy of *Salmonella* in food products; thus to increase its recovery from food products, it needs to be preenriched in non-inhibitory media, like mannitol broth, before employment of selective broth (*28,29*). In addition, the time of incubation in preenrichment medium is critical (*19,21, 24*). Examples of the continuing outbreaks of *Salmonella* infection from food are those of 1965 and 1966 caused by *S. newbrunswick* (*20,22*). After the reports on new isolation methods (*15,30*), a comparison study between four methods for detection of *Salmonella* in food appeared. Included were the conventional fluorescent antibody, rapid direct fluorescent antibody technique, microcolony fluorescent antibody and enrichment serology. The latter is simpler to perform, with fewer false-positive results and is more economical than other methods (*1,18,27*).

Although official statistics on salmonellosis in Iraq are not available to us, enteric fevers and food poisoning outbreaks due to *Salmonella* are not uncommon. In a research project we are conducting on *Salmonella*, the bacterium has been frequently isolated from a wide variety of sources and from clinical cases for which tests were done in private laboratory work. Moreover, the frequency of isolation of *Salmonella* from poultry farms in Iraq is becoming alarming. This investigation was aimed at finding the occurrence and distribution of *Salmonella* species in local foods in the City of Baghdad,

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and to find the most appropriate culture media for isolation of the bacteria from such sources.

## MATERIALS AND METHODS

*Samples*

Three hundred and fifty three local food samples were collected in Baghdad, Iraq; they comprised raw chicken meat 47, raw vegetables (tomatoes, celery, lettuce, salad green) 43, baked cakes covered with cream 36, frozen kubba that was stuffed with meat and green raisins 34, raw milk 34, raw meat (beef and lamb) 33, home-made sweet cheese 26, non-carbonated soft drinks (orange and sherbert) 24, commercial olives exposed to house flies, other insects and dust 19, raw eggs 15, local hand-made sweet "Datly" exposed to air 14, home-processed raw cream 14, yogurt and house butter 8, fresh grapes and dates 6.

*Media*

All media used were dehydrated and prepared by Difco. For preenrichment from different food samples, mannitol broth was used (*11,14*). For selective enrichment media, tetrathionate brilliant green bile broth and selenite cystine broth were employed. For milk samples, neutral red lysine iron cystine broth (*15*) was employed. The differential media were brilliant green sulfa agar and xylose lysine desoxycholate agar.

*Physiological tests*

The following were employed: T.S.I. (Eiken Chemical Co. Ltd.); urea agar (Difco); lysine decarboxylase medium; gelatin liquefaction medium (Difco); mannitol motility medium (Difco); fermentation of glucose, lactose, salicin, sucrose and dulcitol; peptone water (Difco), MR-VP medium (Difco); Simmon citrate agar (Difco); malonate broth (Difco); potassium cyanide broth (Difco). The serological diagnostic media were tryptic soy agar and tryptic soy semisolid (Difco) using the Graigie tube method. Antisera were imported from Hoechst (W. Germany).

*Isolation of* Salmonella *from foods*

The following technique was employed to isolate *Salmonella* except when dairy products were tested. Ten g of food sample were added to 10 ml of mannitol broth and homogenized with a blender at 4000 rpm under aseptic condition. To the homogenate was added 90 ml of mannitol broth and the samples were incubated at 37 C for 18 h. One ml was transferred to 9 ml of tetrathionate brilliant green bile broth and then incubated at 37 C for 24 h. Another one ml was transferred to 9 ml of selenite cystine broth, incubated at 43 C for 24 h. One loopful from each of the above selective media was streaked on two differential media, brilliant green sulfa agar and Xylose lysine desoxycholate agar, and plates were incubated at 37 C for 24 h. Five *Salmonella*-suspect colonies were picked randomly from each plate for biochemical and serological tests. For milk and milk products, 10 ml or 10 g were placed in 100 ml of neutral red lysine iron cystine broth (*15*) which was incubated at 39 C for 24 h, and then processed as mentioned above.

Cold drinks were concentrated by centrifugation of 40 ml at 1000 *g* for 30 min in a refrigerated centrifuge, and then were reconstituted to 4 ml with physiological saline solution. One ml of the latter was transferred to 9 ml of tetrathionate brilliant green bile broth and another 1 ml to 9 ml of selenite cystine broth; they were incubated as above.

After tentative identification of the isolates, serologic groups and specific serotypes were determined. All isolates were confirmed at the National Salmonella Institute of Iraq. Further confirmation was obtained from Professor Le Minor at the Pasteur Institute in Paris.

## RESULTS AND DISCUSSION

Of 353 food samples, 95 samples (27%) harbored *Salmonella*. These were distributed in the following types of local food: Kubba and cakes 6.2%, meat 4.8%, raw milk 3.1%, chicken and sweets 1.7% of each, olives 1.4% (Table 1). The occurrence was extremely high in certain types of food, mainly kubba, cakes and meat. Due to the known difficulties encountered in isolation of *Salmonella* from food and the relative suitability of the preenrichment and enrichment media, these positive values might be lower than reality, and thus the negative samples may not mean the absence of this bacterium. Fifteen *Salmonella* serotypes were identified, of which *S. paratyphi* B was the most frequently encountered in food samples (11%). This was followed by the occurrence of *S. typhimurium*, *Salmonella* 4,5,12:-:- and *S. enteritidis* (Table 2). In other investigations (*2,9*), the most predominant *Salmonella* species and serotypes reported in foods were *S. typhimurium*, *Salmonella heidelberg*, *Salmonella thompson*, *Salmonella tennessee*, *Salmonella montevideo*, *Salmonella newport*, and *Salmonella oranienburg*. Among the species, *Salmonella lille* and *Salmonella alachua* were isolated for the first time in Iraq. The serological formulae for the isolated species are also shown in Table 2. *S. paratyphi* B was isolated from a wide variety of local foods and in a high percentage as shown in Fig. 1. *S. enteritidis* was found in raw milk and could be of high incidence, while *Salmonella* 4,5,12:-:- and *S. typhimurium* were common contaminants in other foodstuffs. This type of distribution emphasizes the

TABLE 1. *Presence of* Salmonella *in local foods in Baghdad.*

| Local foods | No. of samples | Positive samples: No. | Positive samples: Specific food (%) | Positive samples: Out of total (%) |
|---|---|---|---|---|
| 1. Kubba | 34 | 22 | 64.7 | 6.2 |
| 2. Cakes | 36 | 22 | 61.1 | 6.2 |
| 3. Meats | 33 | 17 | 51.5 | 4.8 |
| 4. Raw milk | 34 | 11 | 32.3 | 3.1 |
| 5. Sweets | 14 | 6 | 42.9 | 1.7 |
| 6. Chicken | 47 | 6 | 12.8 | 1.7 |
| 7. Olives | 19 | 5 | 26.3 | 1.4 |
| 8. Vegetables | 43 | 3 | 7.0 | 0.85 |
| 9. Cheese | 26 | 2 | 7.7 | 0.57 |
| 10. Cream | 14 | 1 | 7.1 | 0.28 |
| 11. Eggs | 15 | 0 | 0 | 0 |
| 12. Yogurt & butter | 8 | 0 | 0 | 0 |
| 13. Fruits | 6 | 0 | 0 | 0 |
| 14. Cold drinks | 24 | 0 | 0 | 0 |
| Total | 353 | 95 | | 27 |

TABLE 2. Salmonella *species isolated from local foods*[a].

| No. Salmonella | No. of positive samples | % Of total | Serological formula |
|---|---|---|---|
| 1. *S. paratyphi* B[b] | 39 | 11 | 1,4,5,12:b:1,2 |
| 2. *S. typhimurium* | 16 | 4.5 | 1,4,5.12:i:1,2 |
| 3. *Salmonella* 4,5,12:-:- | 16 | 4.5 | 4,5,12:-:- |
| 4. *S. enteritidis* | 13 | 3.7 | 1,9,12:gm:1,7 |
| 5. *S. muenchen* | 9 | 2.5 | 6,8:d:1,2 |
| 6. *S. senftenberg* | 7 | 2.0 | 1,3,19:gst:- |
| 7. *S. lille*[c] | 5 | 1.4 | 6,7:$Z_{36}$:- |
| 8. *S. anatum* | 2 | 0.57 | 3,10:eh:1,6 |
| 9. *S. alachua*[c] | 2 | 0.57 | 35:$Z_4Z_{23}$:- |
| 10. *S. emek* | 2 | 0.57 | 8,20:gms:- |
| 11. *Salmonella* 1,3,19:-:- | 1 | 0.28 | 1,3,19:-:- |
| 12. *S. california* | 1 | 0.28 | 4,12:gmt:- |
| 13. *S. havana* | 1 | 0.28 | 1.3,23:FG (S):- |
| 14. *S. eppendorf* | 1 | 0.28 | 1,4,12,27:d:1,5 |
| 15. *Salmonella* 6,7:-:- | 1 | 0.28 | 6,7:-:- |

[a]Total number of foods examined was 353,
[b]D-tartrate negative,
[c]Were isolated for the first time in Iraq.

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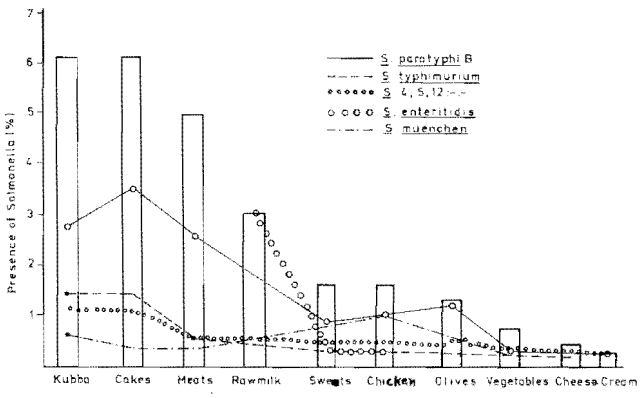


Figure 1. *The presence of* Salmonella *in some local foods.*

importance of the investigation of the source of contamination.

Eleven *Salmonella* serotypes were isolated from meats; they were mainly *S. paratyphi* B and the other 10 serotypes were *S. typhimurium, S. senftenberg, S. lille, Salmonella* 4,5,12:-:- *S. emek, S. alachua, S. california, S. anatum, S. muenchen* and *Salmonella* 6,7:-:-.

Since the classical chain of infection reported is feedstuff-animals-foods-man (*13*), strict bacteriological inspection and control is of vital importance. Eight different *Salmonella* serotypes were isolated from the commonly used food in Iraq, kubba; they were *S. paratyphi* B, *S. typhimurium, Salmonella* 4,5,12:-:-, *S. muenchen, S. senftenberg, S. lille, S. alachua* and *Salmonella* 1,3,19:-:-. The frequent isolation of *Salmonella* from kubba samples could be attributed to food handlers, contaminated water, kitchen utensils, beside the meat and other stuffings used. The last factor is confirmed by the isolation of the same serotypes from meat and kubba samples; furthermore, the species isolated were only found in meat, as in the presence of *S. senftenberg* and *S alachua.*

Cake samples contained six *Salmonella* serotypes; *S. paratyphi* B, *S. typhimurium, Salmonella* 4,5,12:-:-, *S. muenchen, S. havana* and *S. eppendorf.* A high contamination rate of cake samples (Table 1) may be due to food handlers or one of the constituents like cream and egg. The role of egg in the contamination of cake mixes is illustrated by some human outbreaks (*5*).

One *Salmonella* serotype was isolated from 70 (19.8%) food samples, two serotypes from 21 (5.9%) and three serotypes from 4 (1.1%), as shown in Table 3. Reports on frequency of *Salmonella* multiple infections are increasing (*23*).

TABLE 3. *Number and percent of local foods*[a] *harboring one or more* Salmonella *serotype.*

| Salmonella | Number | % |
|---|---|---|
| One serotype | 70 | 19.8 |
| Two serotypes | 21 | 5.9 |
| Three serotypes | 4 | 1.1 |

[a]Total food samples = 353.

The isolation of *Salmonella* from foods was found to be affected by types of selective enrichment medium used. As seen in Table 3, *S. paratyphi* B was isolated from 32 samples by using tetrathionate brilliant green bile broth but from only 20 samples using selenite cystine broth. However, the incubation temperature for the former was 37 C while for the latter it was 43 C. The high temperature used for selenite cystine broth has less inhibitory effect on *Salmonella* and the Arizona group than on other competing gram-negative rods (*12*). A *Salmonella* isolate recovered with one selective enrichment medium may not be recovered with another (*10,12*). When the identification of 20 colonies from each food sample (10 colonies from each of the two selective media) was evaluated, tetrathionate brilliant green bile broth revealed the highest number of *Salmonella* colonies (55.3%) in comparison to selenite cystine broth (44.7%), Table 5. Needless to say, there is an overlap in isolation of serotypes by selective media that were used. It seems that some *Salmonella* serotypes don't grow or at least are not enhanced in tetrathionate brilliant green bile broth, as noticed with *Salmonella* 6,7:-:-, *S. havana* and *S. eppendorf.* The reverse seemed true with *S. california* and *Salmonella* 1,3,19:-:-. It is evident from Tables 4 and

TABLE 4. *Effect of selective enrichment media on the isolation of* Salmonella *serotypes from local foods.*

| Salmonella serotype | No. of samples | Positive samples (1)[a] No. | (1)[a] % | (2)[b] No. | (2)[b] % |
|---|---|---|---|---|---|
| *S. paratyphi* B | 39 | 32 | 82.0 | 20 | 51.3 |
| *S. typhimurium* | 16 | 10 | 62.5 | 13 | 81.3 |
| *Salmonella* 4,5,12:-:- | 16 | 9 | 56.3 | 9 | 56.3 |
| *S. muenchen* | 9 | 8 | 88.9 | 5 | 55.6 |
| *S. senftenberg* | 7 | 3 | 42.9 | 4 | 57.1 |
| *S. lille* | 5 | 2 | 40 | 3 | 60 |
| *S. anatum* | 2 | 2 | 100 | 1 | 50 |
| *S. emek* | 2 | 1 | 50 | 1 | 50 |
| *S. alachua* | 2 | 1 | 50 | 1 | 50 |
| *S. enteritidis*[c] | 2 | 2 | 100 | 1 | 50 |
| *S. california* | 1 | 1 | 100 | 0 | 0 |
| *S. havana* | 1 | 0 | 0 | 1 | 100 |
| *S. eppendorf* | 1 | 0 | 0 | 1 | 100 |
| *Salmonella* 1,3,19:-:- | 1 | 1 | 100 | 0 | 0 |
| *Salmonella* 6,7:-:- | 1 | 1 | 0 | 1 | 100 |

[a](1) Tetrathionate brilliant green bile broth at 37 C.
[b](2) Selenite cystine broth, at 43 C.
[c]Eleven isolates of *S. enteritidis* were picked up in neutral red lysine iron cystine broth.

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TABLE 5. *Enhancement of two selective enrichment media on the growth of* Salmonella *serotypes.*

| Serotypes | Isolation of *Salmonella* colonies from: (1)[a] | % | (2)[b] | % | Total isolates |
|---|---|---|---|---|---|
| *S. paratyphi* B | 124 | 66.0 | 64 | 34 | 188 |
| *S. typhimurium* | 42 | 51.2 | 40 | 48.8 | 82 |
| *Salmonella* 4,5,12:-:- | 32 | 50.8 | 31 | 49.2 | 63 |
| *S. muenchen* | 27 | 60.0 | 18 | 40.0 | 45 |
| *S. senftenberg* | 11 | 64.7 | 6 | 35.3 | 17 |
| *S. lille* | 3 | 21.4 | 11 | 78.6 | 14 |
| *S. alachua* | 1 | 9.1 | 10 | 90.9 | 11 |
| *Salmonella* 6,7:-:- | 0 | 0.0 | 10 | 100.0 | 10 |
| *S. anatum* | 5 | 71.4 | 2 | 28.6 | 7 |
| *S. enteritidis* | 3 | 50.0 | 3 | 50.0 | 6 |
| *S. havana* | 0 | 0.0 | 5 | 100.0 | 5 |
| *S. eppendorf* | 0 | 0.0 | 5 | 100.0 | 5 |
| *S. emek* | 3 | 75.0 | 1 | 25.0 | 4 |
| *S. california* | 3 | 100.0 | 0 | 0 | 3 |
| *Salmonella* 1,3,19:-:- | 1 | 100.0 | 0 | 0 | 1 |
| Total | 255 | 55.3 | 206 | 44.7 | 461 |

[a](1) Tetrathionate brilliant green bile broth, 37 C.
[b](2) Selenite cystine broth, 43 C.

5 that the multiplication rate of specific types differs in both media, and generally tetrathionate enrichment was superior to selenite cystine for *Salmonella* recovery from most foods. This coincided with Mohr's results (*18*), but the use of more than one type of selective medium increases the chance of isolation of more species.

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