

## Original paper

# Prevalence of Salmonella Serotypes in Diarrheic and Non-Diarrheic Patients in Mosul-Iraq

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## Abstract

**Background:** Non-typhoidal salmonellosis is a worldwide distributed infection. It is a common enteric infection was caused by various *Salmonella* serotypes. It is considered as a foodborne disease, the direct transmission is commonly occurred between individuals. Many animal species, birds, and insects play an important role in transmission of infection to man.

**Aim of study:** This study aimed to investigate prevalence of enteric *Salmonella* infection with some influenced epidemiological parameters in patients in major three hospitals in Mosul city/Iraq.

**Materials and Methods:** A total 1028 stool samples were randomly collected from patients in three major hospitals (Al-Khansa, Ibin-Al Ether, and Al-Razy), from October/2000-to-April/2001. Culturing was achieved by using tetrathionate at 43 °C and *Salmonella-Shigella* agar at 37 °C for 24 hours of each. Isolated *Salmonella* colonies were serotyped in Central Health Laboratory in Baghdad.

**Result & Discussion:** Total isolation rate was 1.07%. Eleven salmonella isolates were identified: *S.typhimurium* (54.4%), *S.agona* (18.1%), and 9.09% for each of *S.anatum*, *S.worthington*, and the remaining non-serotyped species. High and low isolation rates were recorded in Jan.2001 and Nov. 2000 respectively. Salmonella isolation rate showed significant increase in patients of Al-khansa hospital (4.43%). A dramatic increase of *Salmonella* infection rate was recorded in diarrheic cases due to *Samonella* infection or concomitant enteric disease, similarly significant increase of *Salmonella* isolation rates (2.2%) were observed in children (6-to-10 years) due low education and little conciousness at this age, and in infants independent on breast feeding mainly due to contamination during preparation of milk bottles. Furthermore low infection rate recorded in males, and in those living in urban areas. A positive correlation was noticed between *Salmonella* infection and rearing of domestic animals in homes.

**Conclusion:** *S. typhimurium* was the dominant serovar. High isolation rates were recorded in diarrheic patients, in infant independent on breast feeding and children at primary school, people in rural areas were highly exposed to *Salmonella* infection than those in cities.

**Keywords:** Non Typhoidal *Salmonella*, enteric infection, human being, Epidemiology.

## Introduction

Non-typidal salmonellosis is distributed worldwide <sup>(1)</sup> constituting the main zoonotic <sup>(2)</sup> and food borne disease <sup>(3)</sup>, a posetive correlation between human and animals infections was recorded in Gambia

and Senegal<sup>(4)</sup>. Ninety four millions cases of gastroenteritis and 115,000 deaths globally each year were estimated<sup>(5)</sup> also WHO reported in 2013, tens millions of human cases occurring worldwide every year <sup>(6)</sup>. In addition transmission of the disease by direct contact between infected

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and non-infected individuals was reported<sup>(7)</sup>. Even though the non-typhoidal salmonellosis is usually a common enteric infection<sup>(8)</sup>, although most cases of non-typhoidal salmonellosis are mild but sometime infected people die, the severity of disease depends on host factors and strain of salmonellae<sup>(6)</sup>, a serious risk is initiated in certain sporadic cases particularly when salmonellae invade and/or are localized in vital organs or tissues<sup>(9)</sup>. It was reported that infants, elderly and immunocompromised patients are highly susceptible to bloodstream infection<sup>(10)</sup> and the case fatality rate in 2012 in Africa was estimated from 20 –to 25 %, beside that the most invasive strains were *S.typhimurium* and *S.enteritidis*<sup>(11)</sup>. Furthermore *Salmonella* species cause considerable losses in animal production and poses a threat to international food security<sup>(12)</sup>. Thus control of salmonellosis is a major goal of epidemiologists, but many difficulties were encountered as: large number of serotypes<sup>(13)</sup>, and their ability to infect many species<sup>(14)</sup>, also the difficulty in detection of asymptomatic (carriers)<sup>(15)</sup> and rapid increase of *Salmonella* resistance due to random uses of multiple antimicrobial drugs<sup>(16)</sup> so that some strains which are resistant to a range of antimicrobial drugs have emerged and are now serious public health concern, therefore routine antimicrobial therapy is not recommended for mild and moderate cases in healthy individuals<sup>(6)</sup>. The WHO in 1996 notified an increase of *Salmonella* infection in many continents<sup>(17)</sup> and such increase was correlated with increase in consumption of poultry products,. In Mosul many *Salmonella* serotypes were isolated from imported food-stuff<sup>(18)</sup>. In addition the contamination of locally processed poultry<sup>(19)</sup> and dairy products<sup>(20)</sup> by *Salmonellae* were reported. A previous study had revealed an isolation rate of 1.15% from stool of out and in-patients in several hospitals in Mosul<sup>(21)</sup>.

However, in the last decade increased *Salmonella* infection was observed among diarrheic children in Mosul<sup>(22)</sup>. The aim of this study is to investigate the prevalence of *Salmonellae* in stool of diarrheic and non-diarrheic patients with reference to some epidemiological parameters in Mosul city.

## Materials and Methods

Stool specimens were randomly taken from 1028 out and inpatients (had no specific criteria), in AL-Razy, Ibin AL-Ether and AL-Khansa hospitals ,at Mosul in the period from October/2000-to-April/2001. Approval from the directorate of fundamentalist hospitals were taken, according to the request from Tikkrit University to support project of MSc student. Samples were collected three times monthly in the beginning, mid and end of each month .Swabs were prepared and brought to laboratory in 10 ml of buffered pepton water.

One ml of buffered pepton water was transferred in to 10 ml of enrichment tetrathionate broth and incubated at 43 °C for 24 hours. One loopful of the latter was streaked on selective *Salmonella-Shigella* agar, and incubated at 37 °C for 24 hours<sup>(23)</sup>. According to apparent characteristics of *Salmonella*, colonies were selected for three biochemical tests: triple sugar iron, Simmon's citrat , and urease production test. Bacteria giving rise to positive reactions in respects to the first and the second tests and negative result to the third test, were suspected to be *Salmonella* species. Isolates were kept on nutrient agar slants and serotyped serologically by using specific antisera, in the Central Health Laboratory in Baghdad. Data was obtained from patients include influencing factors recorded in special informative chart designed underneath. Statistical analyses were done by using chi-square test<sup>(24)</sup> result regarded significant at  $p \leq 0.05$ .

Table 1. Chart of patients

| Patient's Name | Sex |   | Age | Feeding pattern |                 | Site of residence |       | Animal rearing in homes |
|----------------|-----|---|-----|-----------------|-----------------|-------------------|-------|-------------------------|
|                | ♂   | ♀ |     | Breast          | Bottle/or mixed | Rural             | Urban |                         |
|                |     |   |     |                 |                 |                   |       |                         |

## Results

Total isolation rate of salmonella organism revealed 1.07% of 1028 stool samples. Eleven isolates were identified : *S.typhimurium* (54.4%), *S.agona* (18.1%)

and (9.09%) for each of *S.worthington*, *S.anatum*, and non- serotyped *Salmonella* species (Fig-1) .High and low isolation rates were recorded in Jan.2001(1.96%), and Nov. 2000 (0.65%) respectively (Table-2).

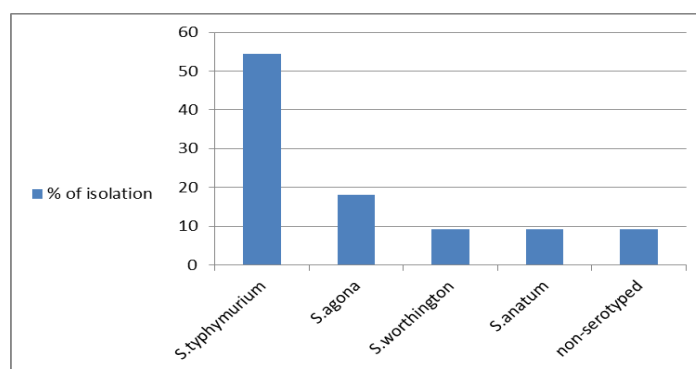


Fig1 .Percentages of Salmonella serotypes isolated from patients stool samples (N=1028).

Table 2. Salmonella serotypes in stool samples of (1028) patients with meteorological values.

|           | % of Salmonella Isolate | Environmental temp.Mean °C | R.H % | Rain Fall mm | Salmonella serotypes |          |             |           |                 |
|-----------|-------------------------|----------------------------|-------|--------------|----------------------|----------|-------------|-----------|-----------------|
|           |                         |                            |       |              | S. typhm             | S. agona | S. worthing | S. anatum | S.non serotyped |
| Oct. 2000 | —                       | 28.8                       | 45    | 12.4         | —                    | —        | —           | —         | —               |
| Nov.      | 0.65                    | 13.8                       | 60    | 46.7         | —                    | 1        | —           | —         | —               |
| Dec.      | 1.47                    | 9.2                        | 85    | 83.7         | 2                    | —        | —           | 1         | —               |
| Jan. 2001 | 1.96                    | 7.6                        | 81    | 25.9         | 2                    | 1        | 1           | —         | 1               |
| Feb.      | 0                       | 9.7                        | 72    | 37.9         | —                    | —        | —           | —         | —               |
| Mar.      | 1.28                    | 15.7                       | 72    | 82.5         | 2                    | —        | —           | —         | —               |
| Apr.      | 0                       | —                          | —     | —            | —                    | —        | —           | —         | —               |

Isolation of *Salmonella* was increasingly significant (  $p \leq 0.05$ ) in patients of Al-Khansa hospital (4.43%), but the lowest value was recorded in Al-Razy hospital (0.944) Fig.2.

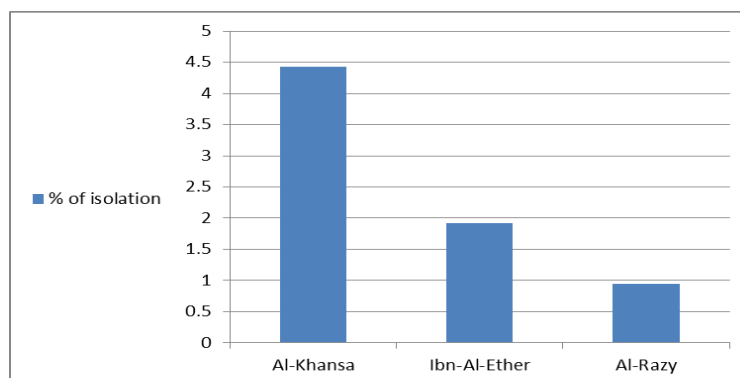


Fig 2. Isolation rates of *Salmonellae* in (1028) patients stool samples in three hospitals.

The diarrheic patients showed high rate of isolation (1.21%), whereas in non-diarrheic ones, the isolation rate was 0.45%, similarly significant ( $p \leq 0.05$ )

increase of isolation rate in diarrheic children (1 month-to-10 years) was observed (1.61%) tab-3.

Table 3. Isolation rates of *Salmonellae* in stool of(1028) diarrheic and non diarrheic patients.

| Cases  | Types of diarrhea | No. of Samples | No. of Isolates | % of isolates | Total samples | % of total isolates |
|--|-------------------|----------------|-----------------|---------------|---------------|---------------------|
| Diarrheic Children (1 month-to-12 years)     | Bloody            | 2              | 0               | —             | 560           | 1.60                |
|  | Non-bloody        | 558            | 9*              | 1.61          |               |                     |
| Diarrheic Adult patients                     | Bloody            | 9              | 0               | 0             | 262           | 0.381               |
|  | Non-bloody        | 253            | 1               | 0.395         |               |                     |
| Total diarrheic Patient                      | Bloody            | 11             | 0               | 0             | 822           | 1.216               |
|  | Non-bloody        | 811            | 10*             | 1             |               |                     |
| Non diarrheic Children (1 month-to-12 years) | —                 | 88             | 0               | 0             | —             | 0                   |
| Non diarrheic Adult                          | —                 | 118            | 1               | 0.847         | —             | —                   |
| Total non- diarrheic Patients                | —                 | 206            | 1               | 1.485         | —             | —                   |

\*significant  $p \leq 0.05$

The isolation rates of *Salmonellae* varies according to different ages of patients. It was highly increased in children aged from 1 month to 10 years (1.38%) as compared

to adult patient (0.526%) Fig-3. Furthermore the highest value( $p \leq 0.05$ ) of isolation rate was recorded in children aged 6-to-10 years (1.72-2.2%).

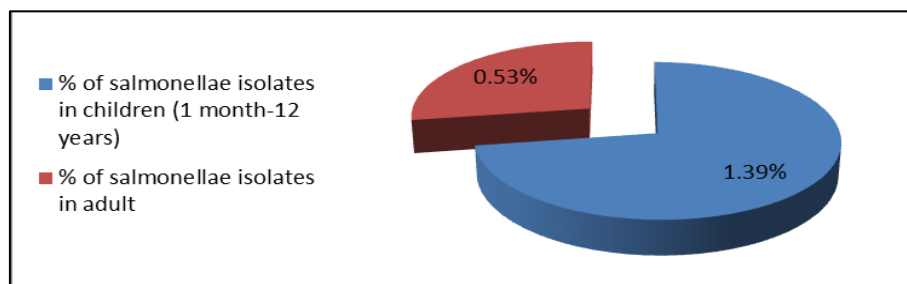


Fig 3. Percentages of *Salmonella* isolation in children and adult patients.

The pattern of milk feeding influences isolation rate of salmonella organism in infants .Isolation rate showed values of

0.88% and 2.6% in infants fed on breast and bottle milk feed or mixed respectively Fig.4.

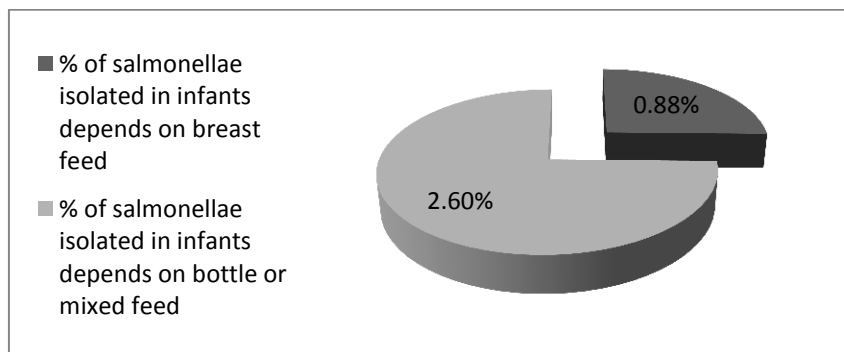


Fig 4. Percentage of salmonella isolation from stool samples(426 ) according to pattern of milk feed.

Sex variation in *Salmonella* isolation rate was observed in this study, where high isolation rate of 1.18% ( $p \leq 0.05$ ) was recorded in female patients compared with

male patients (0.99%), also female children (1 month to 12 years) showed slight non-significant increase in isolation rate Fig.5.

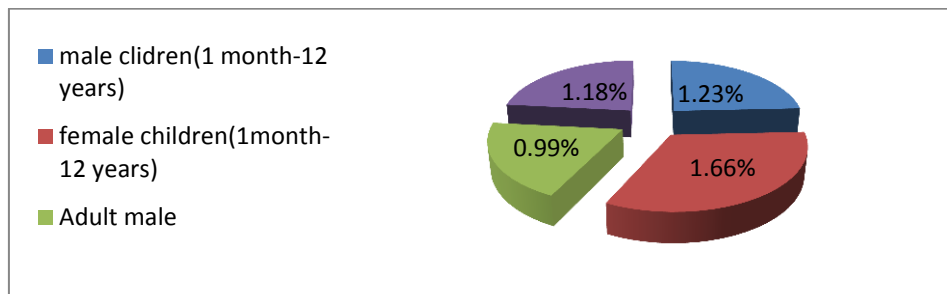


Fig. 5. percentage of *Salmonella* organisms in stool samples(1028) of both sexes.

Locality of living influences the isolation rate of salmonella organisms in Mosul. Patients living in rural areas showed considerable increase ( $p \leq 0.5$ ) in salmonella isolation rate (2.4%) as

compared to patients living in Urban areas (0.88%). Besides, correlation between infection and rearing of productive animals revealed that isolation rate had increased significantly ( $p \leq 0.5$ ) to 3.01% Fig.6,7.

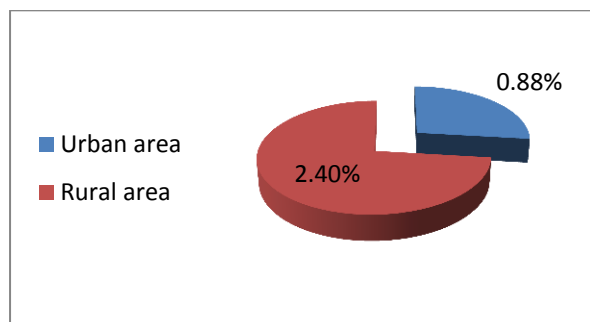


Fig.6 *Salmonella* isolation rate in Patients according to places of living

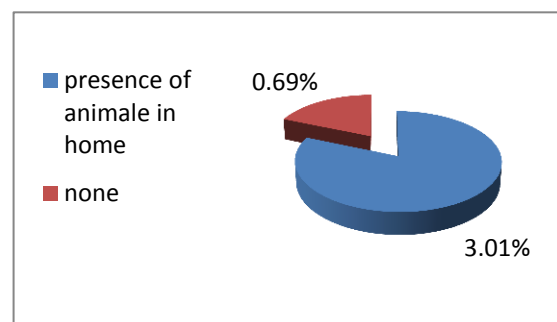


Fig.7 *Salmonella* isolation rate in Patients according to presence of animals in homes

## Discussion

Salmonellosis is a notifiable food borne disease, constituting a considerable public health importance and substantial economic losses. The human need for animal proteins greatly facilitates wide distribution of the diseases all over the world <sup>(25)</sup>. In this study isolation rate of *Salmonella* (1.07%) in Mosul was lower than recorded value (18.39%) in Baghdad <sup>(26)</sup>. Nonetheless, a study had revealed that typhoidal *Salmonella* infection (2.3%) in Baghdad <sup>(27)</sup> was higher than non-typhoidal infection in our study, Humphery <sup>(28)</sup>, reported an increase *Salmonella* infection in England and Wales in 1991-to- 1993. Variations in

*Salmonella* isolation rates were attributed to various environmental, economic, social, geographical and climatic conditions <sup>(29)</sup> as well as various culturing techniques used. It is well known that animal products are the main sources of spreading salmonellosis and previously low animals' protein consumption might be contributed in lowering *Salmonella* infection. On the other hand, *Salmonella* infection is often lower than the real value <sup>(30)</sup> as many patients have not been examined by physicians. Moreover, patient's habit to use medicaments randomly and microbiological investigation of stool of patients had been rarely performed, although stool culturing inevitably detect infected patients. Thus

culturing is frequently repeated for confirming diagnosis<sup>(31)</sup>. *Salmonella typhimurium* was the main dominant serotype (54.4%). The domination of *S.typhimurium* not restricted to human being, but also recorded in animals and their products<sup>(32, 20)</sup>. In Italy *S.typhimurium* was frequently isolated from children<sup>(33)</sup>. The wide distribution of *S.typhimurium* in the world may be attributed to its ability to surviving in various environmental conditions<sup>(34)</sup> and its resistance to multiple antimicrobial drugs<sup>(11)</sup>. Insignificant climatic effect on isolation of *Salmonella* recorded in our study in cold months. This in turn must be resulted in lowering the total isolation rate of *Salmonella* species. However, several studies revealed increment isolation rate of *Salmonellae* in warm months<sup>(35)</sup>. High isolation rate of *Salmonella* serotypes were observed in diarrheic patients (1.12%). This might indicate that a number of diarrheic cases were due to *Salmonella* infection or diarrhoea was attributed to other enteric pathogens as *E.coil* and rota virus...etc. the concomitant infection which would lead to a diminution of immune mechanism in gastrointestinal tract and increased excretion of *salmonellae*, it was reported that patients with AIDS had shown high infection rate with salmonellae<sup>(9)</sup>. Increased *Ssalmonella* discharge might be also attributed to unnecessary utilization of large quantities of antibiotics in both medical and veterinary aspects<sup>(14,16)</sup>. It was noticed that bloody diarrhea had not accompanied with an increase of *Salmonella* infection and this was in agreement with finding of other workers<sup>(36)</sup>. Age influenced *Salmonella* infection rate in children was observed (1.828%) particularly in age group of 6-10 years. Children at this stage are mostly in primary schools and because of inadequate health education and cultural consciousness, they are frequently exposed to infection also as they buy food from mobile sellers and unhygienic food shops. In addition lack of maintenance of water

tanks and cycles in their schools, as well as improper care in washing and cleaning of their hands and utensils. Previous study in Mosul had revealed high infection rate (4.01%, 5.34%) in children<sup>(22, 29)</sup>. Nevertheless, high infection rate was recorded in infants particularly in those depending on bottle feeding milk (2.6%) whilst salmonella infection was significantly lowered in infants depending on breast feeding. As such great chance of contamination occurs during preparation of milk in bottles<sup>(37)</sup>, also the milk powder might be already contaminated. Many workers considered lack of breast feeding as the serious risk factor in disease occurrence<sup>(38)</sup>. Fresh unpasteurized milk consumption assists in increasing *Salmonella* infection as it is easily contaminated<sup>(19)</sup>. Also it contains sufficient quantity of lipids, which in turn, protects *Salmonella* from gastric acidity<sup>(39)</sup>. Sex variation was markedly observed in *Salmonella* infection in our study, and this was in agreement with the finding of others<sup>(21)</sup> there was no clear explanation, but it might be due to immunological and social implications. High *Salmonella* infection rate was recorded in patients living in rural areas (2.4 %) as compare to those living in urban areas (0.88 %), which was also in agreement with other work<sup>(21)</sup>. Moreover, *Salmonella* infection rate was increased up to 3.01% in patients rearing animals in their homes (cities and villages). This has eventually reflected the role of animals and their products in spreading of *Salmonella* to human being<sup>(40,20,2)</sup>. These facts were supported by data obtained from AL-Khansa hospital where *Salmonella* infection rate was higher than in other hospitals. This is due to the fact that rural patients and those having animals in their homes were 47.8 % and 39.13 % respectively. In conclusion non typhoidal *Salmonella* infections were increased in infants fed on bottle milk or mixed (breast) and children at stage of primary school (6-to-10 years), moreover people in rural areas and those rearing

animals in homes also showed high isolation rates, thus community awareness for the dangerous of infectious disease is required, furthermore sanitation and hygienic measurements must be applied in all walks of life.

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