Prevalence of *Salmonella* in Asymptomatic Food Handlers in Westlands, Nairobi, Kenya

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Abstract: *Salmonella are Gram-negative, facultative, intracellular, anaerobic bacteria that cause a wide range of diseases in diverse hosts. Ranging from gastroenteritis, enteric fever, bacteraemia and focal infections, these infections can lead to a convalescent lifetime carrier status/asymptomatic carriers. The aim of this study was to isolate, characterize and determine the prevalence of Salmonella species from stool samples of asymptomatic food handlers in Westlands Nairobi, Kenya. Being an epidemiological study, a cross sectional laboratory based design was employed. A total of 400 stool samples were used. The samples were cultured in both selective and differential media for isolation of Salmonella sp; serotyping and biochemical test was conducted for the confirmation of the isolated bacterial strains respectively. A total of 6 serotypes were isolated indicating a total prevalence of 2%, namely; Salmonella Paratyphi A, S. Paratyphi B and S. Enteritidis each with a percentage occurrence of 0.5%, S. Typhimurium, S. Typhisuis with a percentage occurence of 0.25%. Although a Salmonella prevalence of 2% in the developing countries can be considered as an insignificant figure, it indicates a probability of Salmonella infection being passed to the food consumers by the asymptomatic food handlers, posing a health risk to the unsuspecting food consumers.*

Keywords: Asymptomatic, Food handlers, *Salmonella,* Prevalence.

1. Introduction

There are two types of *Salmonella* associated with the human host, non-typhoid *Salmonella* and typhoid *Salmonella*. Non-typhoid *Salmonella* is more frequently experienced in developing countries as compared to the developed countries, it can affect as many as 21.5 million [19] individuals each year especially where unhygienic conditions are likely to occur. Salmonellosis caused by non-typhoid *Salmonella* is considered as one of the most widespread and common food borne diseases. At the moment, and in most countries chiefly in the sub Saharan regions, it represents a major public health burden. Although salmonellosis is a self-limiting infection, antibiotics may shorten the duration of illness [15]. Millions of human incidents are reported worldwide and is reported to cause thousands of deaths each year [19] especially among the young, immuno-compromised persons and the elderly whose immune systems are weak. Salmonellosis infections in humans are habitually contracted by the consumption of contaminated food of animal origin such as meat products, poultry products and milk. In addition a variety of other foods such as green vegetables planted using untreated organic manure or food handled improperly by infected persons have been indicated in the spread of *Salmonella* infection [15].

2. Literature Review

2.1 The organism

Salmonella belongs to the family enterobacteriaceae. Members of this genus are rod-shaped Gram negative and cause typhoid fever, paratyphoid fever and food borne illness [10]. Members of this genus have also been reported to produce hydrogen sulphide on Triple Sugar Iron Agar (TSI) except for *S. Paratyphi* A [4].

2.2 History

Salmonella bacteria were named after Daniel Elmer Salmon, an American veterinary pathologist, who together with Theobald Smith first isolated the bacterium from pigs [10]. Most cases of *Salmonella* infection involve undercooked meat, chiefly poultry meat [1], other sources other than meat have been implicated in infections by *S. enterica*.

2.3 Nomenclature

The taxonomy of *Salmonella* is considered complicated. Traditionally *Salmonella* was considered to have many serotypes that were initially said to be members of a single species *Salmonella enterica* but currently there are three recognized species, *S. enterica, the type species, S. bongori,* and *S. subterranean* with six main subspecies in *S. enterica* [18], *S. enterica* subsp. *enterica* (I), *S. enterica* subsp. *salamae* (II), *S. enterica* subsp. *arizonae* (IIIa), *S. enterica* subsp. *diarizonae* (IIIb), *S. enterica* subsp. *houtenae* (IV), *S. enterica* subsp. *indica* (VI), named according to where they were first isolated or the disease they are known to cause.
Based on the immuno-reactivity of O-lipopolysaccharide (LPS) and H-flagella protein antigens (Kauffman-White scheme), which are the most commonly used method of *Salmonella* classification, as of 2007, 2,557 serovars of *S. enterica* and 22 serovars of *S. bongori* have been recognized. All found in disparate environments and are associated with many different diseases [18]. Majority of human clinical isolates, include *Salmonella* serovariant's Enteritidis, Typhimurium, and Typhi.

2.4 Incidence of *Salmonella* infection among food workers

Foodborne illnesses are considered widespread and an ever growing public health crisis in both developed and developing countries. World health organisation (WHO) indicates that the global incidence of foodborne illness is difficult to estimate. According to WHO report in 2005, 1.8 million people died from diarrhoeal foodborne infections, a great proportion of these cases could be attributed to contamination of food and drinking water. In industrialized countries, the percentage of those suffering from foodborne infections yearly were reported to be up to 30% [18]. The numbers in developing countries have not been established, they are the majority who suffer the brunt of foodborne infections including those caused by parasites [18].

Salmonellosis is the major foodborne illness in most countries [18]. Studies by [8] evaluated the impact of surveillance on the detection of outbreaks in restaurants from the year 1997 to 2004, 110 out of 4,976 (2.2%) confirmed *Salmonella* cases reported were identified as food workers. In the United States, approximately 40,000 cases of salmonellosis are reported every year though the actual number of infections may be thirty or more times greater if the milder cases were diagnosed and reported [3]. In a separate study [9] isolated *Salmonella* from 106 of 331,644 (0.032%) faecal samples from food handlers, with *S.* Serovar *enteritidis* being the most common among the food handlers. A different study by [20], found 29 out of the 305 (9.5%) asymptomatic food handlers sampled from a hospital cafeteria to be *Salmonella* carriers. The twenty nine isolates were grouped into five serotypes; Agona, Derby, Enteritidis, Infantis and Senftenberg. Through these studies, *S. enteritidis* has been indicated as the major cause of foodborne illnesses. This serotype is associated with significant morbidity and mortality worldwide and is reported to be responsible for approximately 17% of all human *Salmonella* infections in the USA [21].

These results indicate that food workers should be considered an important source of *Salmonella* transmission, and those identified through surveillance should raise a high index of suspicion of a possible outbreak at their place of work, hence food service managers need to be alert to *Salmonella*-like illnesses among food workers to facilitate prevention and control efforts.

3. Materials and Methods

3.1 Sample collection

Feacal samples were obtained from asymptomatic food handlers in Westlands division, Narobi. The study employed a cross sectional laboratory based study design involving stratified random sampling. The study population was identified and listed according to the defined strata; the required sample size was determined by fisher *et al,* and the appropriate representation in each stratum was done using random numbers for each stratum. Samples from consenting food handlers were collected in clean sealed polypots and transported to the laboratory at 4° C.

3.2 Isolation of Salmonella by enrichment and differential method

The populations of *Salmonella* in a stool sample maybe too low for the samples to be routinely cultured, necessitating subjection to enrichment culture was used to enrich the samples. Samples were then isolated on Mac Conkey agar and Xylose Lysine Deoxycholate (XLD) agar, which are both selective and differential media for bacterial identification [14][10] most colonies were able to grow within 24 hours of incubation on the differential media.

3.3 Characterization of the isolates

Serotyping is based on the immunologic reactivity of two surface structures polysaccharide O antigen; flageline protein and the VI antigen which are specific to *Salmonella* this separates the unconfirmed cases of *Salmonella* from other enteric bacteria. Biochemical tests that were used comprised the Triple sugar test (TSI) and Indole, Methyl Red, Voges-Proskauer and citrate tests (IMViC) [4]. IMViC reactions are a set of five useful reactions that are commonly employed in the identification of members of family enterobacteriaceae.

4. Results

4.1 Identification of the isolates

400 willing asymptomatic food handlers in the food industries were sampled for the study. Of the collected samples, 8 (2%) turned out to be positive for *Salmonella* while 392 (98%) turned out to be negative for *Salmonella*. Initially, after the primary isolation 14 samples were picked indicating positive *Salmonella* samples but after serotyping, 8 samples reacted positive for *Salmonella*.

Table 1: *Salmonella* serotypes isolated from asymptomatic food handlers

O antigen group	Isolate serotype	Number of isolates
A	*Salmonella* ser. Para*typhi* A	2
B	*Salmonella* ser. Typhimurium	1
B	*Salmonella* ser. Para*typhi* B	2
C$_1$	*Salmonella* ser. Typhisuis	1
D	*Salmonella* ser. Enteritidis	2
Total		**8**

From a sample of 400, 8 isolates were isolated. *S.* Para*typhi* A, *S.* Para*typhi* B and *S.* Enteritidis had the highest rates of isolation at 2 each while the remaining *S.* Typhimurium *and S.* Enteritidis had one isolate each. Serotyping of the O antigen was used in the identification of *Salmonella* in this study the isolates were grouped into four O (somatic) antigens grouping as shown in the first column of Table 1 above.

Table 2: Prevalence of *Salmonella* isolates

Serotypes	Frequency	Percentage
Salmonella Para*typhi* A	2	0.5
Salmonella Para*typhi* B	2	0.5
Salmonella Typhisuis	1	0.25
Salmonella Typhimurium	1	0.25
Salmonella Enteritidis	2	0.5

According to the above Table, frequency represents the number of isolates of the same kind isolated from the sample and converted into a percentage prevalence of the isolate among the isolated isolates. Among the asymptomatic food handlers tested, a prevalence of 2% was observed.

Figure 1: *S*. Para*typhi* B culture on XLD

Observation of small, pink colonies with or without black centres on XLD media plate as those above, indicated presence of *Salmonella* on the plated sample.

Figure 2: Triple Sugar Iron agar slants used for the identification of *Salmonella* indicating the reaction of the different *Salmonella* isolates from samples.

The different types of colour changes in the slant and butt of a TSI agar slant indicates different results on presence and absences of *Salmonella* species. Black colour indicates presence of H_2S producing *Salmonella* species.

Figure 3: Citrate utilization test reaction results of the isolates from the asymptomatic food handlers.

Utilization of citrate as an alternative sugar source by isolate is indicated by the change of media colour from green to blue. Different species of enterobacteria give a varied outcome. *Salmonella* bacteria are citrate positive hence this test separates and identifies them from the enterobacteriace.

There were five different serotype isolates in the asymptomatic food handlers (Table 1). A plate with positive results is shown below (Figure 1). Biochemical test reactions that were observed for *Salmonella* positive samples are indicated in (Figure 2 and 3). The prevalence and type of isolates obtained are listed in (Table 2).

5. Discussion

In the above study, species that are known to be isolated from other animals such as *S*. Typhisuis was among the isolates isolated. *Salmonella* Typhisuis is normally isolated from pigs thus the name suis, and this can be explained to be as a result of human infection from handling infected pork or consumption of contaminated pork. Some of the serotypes isolated in this study are known to cause serious infections in man. Isolates such as *S*. Para*typhi* A and *S*. Typhimurium cause severe gastro intestinal infections, paratyphoid /enteric fever/salmonellosis and paratyphoid fever/*Salmonella* toxic food borne infection respectively [16]. Serovar Para*typhi* A is reported as the second most prevalent cause of typhoid, responsible for one third of cases or more in southern and eastern Asia [7].

Of the 400 samples obtained in the above study, 8 samples were found to be *Salmonella* positive. Representing 2% of the stool samples obtained from the asymptomatic food handlers. With a distribution of *S*. Para*typhi* A (0.5%), *S*. Para*typhi* B (0.5%), *S*.Typhimurium (0.25%), *S*. Typhisuis (0.25%) and *S*. Enteritidis(0.5%), this is in contrast to a research by [9], found of the 331,644 faecal samples from the asymptomatic food handlers a prevalence of 106 (0.032%). With *S*. serovar Infantis being the dominant

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serovar accounting for 48.1% of total isolates, followed by *S.* serovar Corvallis and *S.* serovar Enteritidis. In the study, *S.* serovar Infantis and *S.* serovar Corvallis were observed as not being dominant among symptomatic patients. Though this agrees with a study by [9] that showed a similar prevalence of *Salmonella* among the food handlers, 110 out of 4,976 which represent (2.2%) confirmed *Salmonella* cases reported were identified as food workers. The occurrence of *S.* Enteritidis in this study was observed to be higher among the asymptomatic food handlers, this concurs with other research on food handlers [9][13]. Similar results were also observed and noted that prior to 1997, *S.* Typhimurium predominated (prevalence of 75%) among cases of NTS bacteraemia in Kenya, and *S.* Enteritidis made up only 4·8% of cases by [5]. However, the study indicated that more recently, isolations of *S.* Enteritidis cultures have progressively increased to a occurrence of 40%, and the study attributed it to the changing lifestyles with more people rearing chickens for eggs as a supply of protein. Total *Salmonella* prevalence was relatively low among the food handlers at 2 %, indicating relatively low frequency of asymptomatic food handlers passing the infection to their customers according to this research. Though this does not rule out the possibility of infection of consumers by the asymptomatic food handlers, according to a research by [12], found out that more than half (62.2 %) of the food handlers washed their hands with water alone prior to eating as 27.7 % did not wash their hands at all times prior to food preparation. This possibility can predispose the consumers of food prepared by the asymptomatic food handlers to *Salmonella*, in case of a possibility of contamination of the food being prepared by faecal material in the unwashed hands of the asymptomatic food handler. All the serotypes had a prevalence of less than 1%. An asymptomatic food handler found co-infected with more than one *Salmonella* serotype was not observed in this study. The most prevalent O antigen group in this study was B (*S.* Para*typhi* B and *S.* Typhimurium), accounting for 37.5 % of the total *Salmonella* isolates isolated in this study. A total of 4 different groups were isolated in the study (*S.* Para*typhi* A in group A, *S.* Typhisuis in group C1 and *S.* Enteritidis in group D).

6. Conclusion

The prevalence of *Salmonella* serotypes isolated at 2 % can be considered as an insignificant figure. According to the above study, there was a chance of the *Salmonella* infection being passed to the food consumers. Making it mandatory to screen food handlers, education of the food handlers on proper work ethics and those diagnosed as carriers be given paid sick off to encourage openness in the approach of the treatment of the disease by the institutions in which they work for until they are proven safe by the public health officials to handle food for consumer consumption.

7. Scope for Future Study

Frequency of mandatory screening of food handlers by regulatory authorities should be standardized. Advisory services to all handlers, with appropriate treatment of carriers should be routine

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