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Prevalence and Antibiotic Susceptibility of Salmonella Species among Food Handlers in Khartoum- State (Sudan)

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Authors' contributions

This work was carried out in collaboration among all authors. Authors NEMAA and ANH designed the study, wrote the protocol and the first draft of the manuscript. Authors IG and MAIH performed the statistical analysis and managed the analyses of the study. Authors NEMAA, ANH and MAIH managed the literature searches and collected data. All authors read and approved the final manuscript.

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ABSTRACT

Aims/Objectives: To screen the prevalence of Salmonella fecal carriage among healthy foodhandlers and to identify the common species of Salmonella among study population and its antibacterial susceptibility in Khartoum state by taking stool samples.

Study Design: Cross-sectional study.

Place and Duration of Study: Khartoum state, Sudan, from January 2009 to December 2009. Methodology: Stool samples were collected, processed and cultured on suitable bacterial culture media. Presence of colonies resembling (non-lactose fermenting) Salmonella species were further

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studied to identify the bacterium by using appropriate biochemical tests. Confirmation and serotyping of isolates were done by using *Salmonella* antisera. Antibacterial susceptibility test for common antibacterial drugs were also studied.

Results: A total of 387 stool samples collected from apparently healthy food handlers werestudied. *Salmonella* fecal carriage among food handlers was 17 (4.4%) and the highest prevalence was noted in Umbada locality (5.1%). *Salmonella Paratyphi B* was the commonest 14 (3.6%), followed by *Salmonella* Typhi 2(0.5%) and *Salmonella* Cholerasuis 1 (0.3%). Our study revealed that 141 (36.4%) of food handlers were illiterate, 29 (7.5%) werepreschool, 134 (34.6%) were elementary school graduates 68 (17.6%) and 15 (3.9%) university graduates. Forty two (10.9%) of the studied cases mentioned that, they sometimes wash their hands by soap after defecation, while, 9 (2.3%) never washed theirhands. The study also, revealed that 3 (17.6%) of positive food handlers have had history ofprevious typhoid or gastroenteritis. The study demonstrated that *Salmonella* species isolated were sensitive to co-trimoxazole, ciprofloxacin, chloramphenicol, tetracycline, amoxicillin/ clavulanic acid. ceftriaxone and cefuroxime.

Conclusion: This study demonstrated the presence of considerable number of *Salmonella* fecal carriage among food handlers in Khartoum state. Most of them were illiterate and had poor compliance of hand washing after toilet use. Study also revealed that isolated *Salmonella* species were highly susceptibility to the common first line antibiotics used in Sudan.

Keywords: Salmonella fecal carriage; food handlers; prevalence of Salmonella; Salmonella sensitivity; Salmonella in Sudan; food handlers in Sudan.

1. INTRODUCTION

Salmonella infections most commonly begin with ingestion of bacteria in contaminated foodor water, or direct contact with animal and human carrier, where it causes enteritis, systemic infection, and enteric fever [1]. The type of infection depends on the serotype of Salmonella and host factors. It maintains a broad host range and, for unknownreasons, results in different diseases in different hosts. Humans are the only known carriers of Salmonella Typhi. Some individuals infected with Salmonella Typhior Salmonella Paratyphicontinue to harbor the bacterium in their tissues for variable length of time. This is defined asconvalescent carriers or healthy chronic carriers. Approximately, 1-4% of untreated typhoidinfections, caused by S. Typhi and 0.2-0.6% of Salmonella Paratyphibecome chronic carrierstate. The bacteria are most commonly present in the gallbladder, less often in the urinarytract, and are shed in feces and sometimes in urine [2].

Fecal carriage of *S.* Typhi *or S.* Paratyphi by a symptomatic food handlers are frequently documented as there servoir in foodborne *Salmonella* outbreaks, as they are contaminating food by their hands especially in ready to eat food [3,4]. It is well known that *Salmonella* can survive on the fingertips for several hours. Thus, slight breaches in hand hygiene by those infected, resulting in even microscopic fecal contamination of fingertips,could result in such

an outbreak [5]. This is why delicatessens, cafeteria and restaurants are most common places where contaminated foods were reported [6,7]. Therefore, consideration of the significance of fecal carriage of *Salmonella* by food handlers is important to public health.

Typhoid fever caused by *Salmonella enterica* serotype Typhi (*S. Typhi*) is a major cause ofmorbidity and mortality among children and adults in developing countries. It is estimatesthat 21.6 million illnesses and 216,500 deaths worldwide are due to *S.* Typhi infection annually [8,9].

Antibacterial resistance among Salmonella that may threaten human isolates isincreasing worldwide. Probable contributing factors are the use of antibiotics as growthenhancers in animal production and misuse of antibiotics [10]. To ourknowledge, there are very few published studies about Salmonella fecal carriage among food handlers in Khartoum. Therefore, this study was carried out to screen the prevalence of Salmonella fecal carriage among healthy food handlers and to identify the common speciesof Salmonella among study population and its antibacterial susceptibility in Khartoum stateby taking stool samples.

2. MATERIALS AND METHODS

Facility based descriptive cross-sectional study was designed by using different dependantand

independent variables. The study was carried out in Khartoum state which is the politicaland commercial center of Sudan. The total population in the year 2000 was estimated as 5million with an average annual growth rate of 6.29% [11]. Population growth is due to intensive movements and influx of internal refugees. Administratively, the state comprises seven localities.

Workers engaged in food premises (food handlers) in the study area represent the studypopulation irrespective to gender or age characteristics. Cashier personnel were excluded from being involved in the investigatory sample unless they are in a way or another dealing with food or water inside the premises. Microbiological study was carried out in public health laboratory, microbiology department, which is well equipped and work as a quality control centre.

2.1 Study Design and Sampling Technique

Cluster design was used to yield maximum precision. This design was based on the factthat, the variables under investigation will be generalized to all subjects under study irrespectto the wide diversity of hygiene conditions among food handlers. Simple random sampling techniques were adopted for the selection of the representative locality with a total of 12150 food handlers.

For calculation of the number of representative's individuals, the following formula was used:

$$n = \frac{N}{1 + N (e)^2}$$

Where n =the minimum desired sample size.

N = the total population

e = precision taken as 0.05

For calculating the number of individuals from the total population this gives:

$$n = \frac{12150}{1 + 12150(0.05)^2} = 399.9$$

390 food handlers from the total population were chosen and investigated by simple random techniques.

2.2 Data Collections

Data were collected using a questionnaire and direct interview. Collected data

include; educational status (illiterate, preschool, elementary school graduates, schoolgraduates and university graduates) based on Sudanese educational program; frequency ofhand washing after toilet use by using water and soap (always wash after defecation, notregular and never washed hands after defecation) based on their history; previousdocumented Salmonella infection based on culture or serological results: insurancestatus measured by having protective

2.3 Methodology

Stool samples were taken and cultured on suitable bacterial culture media (Xylose-Lysine Deoxycholateagar (XLD)-OXOID-UK), Deoxycholate Citrate Agar (DCA)-OXOID-UK) and Selenite-F broth (as an enrichment media-OXOID-UK) then processed for the isolation of Salmonella species [12]. Non-lactose fermenters suspected colonies were further studied by using appropriate biochemical tests to identify Salmonella species. Confirmation and serotyping of isolates were done by using Salmonella antisera (Mast Diagnostic-UK) [13]. Results were interpreted according to the guidelines of manufacturing company (Mast Diagnostic-UK).

Antibacterial susceptibility test for common antibacterialdrugsused (ciprofloxacin 5 μ g, amoxicillin/clavulanicacid 30 μ g, tetracycline 30 μ g, ceftriaxone 30 μ g, cefuroxime 30 μ g, cotrimoxazole 25 μ g and chloramphenicol 30 μ g) weredoneto the all of the isolated *Salmonella* species by using the Kirby-Bauer disk diffusion method. Results of reading were interpreted according to the guidelinesof Clinical and Laboratory Standards Institute CLSI (formerly NCCLS). *S. typhimurium* ATCC14028 was used as a control strain [14].

The data collected were analyzed by using statistical package for social sciences forpersonal computer (SPSS/PC). Frequencies were computed and chi-squared test was used. Statistical significance was set at P<0.05.

3. RESULTS

Three hundred ninety healthy food handlers within three localities were included in thisstudy. Three of them were excluded because they were not Khartoum-State residence. Distribution of food handlers within localities were in Umbada, 98 (25.3%), Bahry, 98 (25.3%) and Khartoum

191 (49.4%). Salmonella fecal carriage among food handlers was 17 (4.4%). Salmonella Paratyphi Bwas the commonest 14 (3.6%), followed by Salmonella Typhi 2(0.5%) and Salmonella cholerasuis 1 (0.3%) (Table 1).

According to the educational status, the number of illiterate food handlers were 141 (36.4%), followed by 29 (7.5%) preschool, 134 (34.6%) elementary school graduates, 68 (17.6%) secondary school graduates and 15 (3.9%) university graduates. Of the positive cases 6(35.3%) of them were illiterate, 1 (5.9%) preschool, 6 (35.3%) elementary, 3 (17.6%) secondary school and 1 (5.9%) university graduate.

Three hundred thirty six (86.8%) of them said that they always wash after defecation, while 42

(10.9%) were not regular and 9 (2.3%) never washed hands after defecation. None ofthe study group had a long finger nail.

Relationship between current *Salmonella* fecal carriage and previous history of typhoid infection were explained in Table 2, the differences was statistically significant (P>0.05).

It was seen that there is relationship between *Salmonella* species and having protectivecard. See Table 3.

The study demonstrated that *Salmonella* species isolated were all sensitive 17 (100%) to theall tested antibacterial drugs; co-trimoxazole, ciprofloxacin, chloramphenicol, tetracycline, amoxicillin/clavulanic acid, ceftriaxone and cefuroxime.

Table 1. The relationship between Salmonella species prevalence and Localities (n=387)

Locality	Total		S		Total					
		5	S. Typhi	S. P	S. Paratyphi B S. Ch			holeraesuis		
		Total	Total Prevalence		Total Prevalence Tota		Prevalence Total		Prevalence	
Umbada	98	1	0.010	4	0.041	0	0	5	0.051	
Bahry	98	0	0	3	0.031	0	0	3	0.031	
Khartoum	191	1	0.005	7	0.037	1	0.005	9	0.047	
Total	387	2	0.005	14	0.036	1	0.003	17	0.044	
$X^2 = 1.7 \ (P = 0.04)$										

Table 2. The relationship between *Salmonella* species and history of previous history of typhoid infection (n=387)

Total			_ т	Total					
		S. Typhi		S. Paratyphi B		S. Choleraesuis			
No	%	No	%	No	%	No	%	No	%
24	6.2	1	50	2	14.3	0	0	3	17.6
363	93.8	1	50	12	85.7	1	100	14	82.4
387	100	2	10	14	100	1	100	17	100
	No 24 363	No % 24 6.2 363 93.8	No % No 24 6.2 1 363 93.8 1	No % No % 24 6.2 1 50 363 93.8 1 50	S. Typhi S. Par No % No % No 24 6.2 1 50 2 363 93.8 1 50 12	S. Typhi S. Paratyphi B No % No % 24 6.2 1 50 2 14.3 363 93.8 1 50 12 85.7	S. Typhi S. Paratyphi B S. Cho No % No % No 24 6.2 1 50 2 14.3 0 363 93.8 1 50 12 85.7 1	S. Typhi S. Paratyphi B S. Choleraesuis No % No % No % 24 6.2 1 50 2 14.3 0 0 363 93.8 1 50 12 85.7 1 100	S. Typhi S. Paratyphi B S. Choleraesuis No % No % No % No 24 6.2 1 50 2 14.3 0 0 3 363 93.8 1 50 12 85.7 1 100 14

Table 3. The relationship between *Salmonella* species and having protective card (insurance card or insurance status) one year ago (n=387)

Having protective	Total		Salmonella species isolated							Total	
card (insurance card			S. Typhi		S. Paratyphi B		S. Choleraesuis		-		
or insurance status) one year ago	No	%	No	%	No	%	No	%	No	%	
Yes	263	68	1	50	5	35.7	1	100	7	41.2	
No	124	32	1	50	9	64.3	0	0	10	58.8	
Total	387	100	2	100	14	100	1	100	17	100	

 $X^2 = 1.67 (P = >0.05)$

4. DISCUSSION

Salmonellais recognizedworldwide importantfoodbornehumanpathogen, and isfound in theintestinal tract of both animals and humans [15]. Although, a symptomatic nontyphoidal Salmonellae (NTS) excretion in the general populationrarely cause spreading Salmonellae, but, food workers, generally, are considered to be potential sources [16]. When contamination of commercial food occurs, significant public health problems and economic loss normally follow. In many locations worldwide, routine screening for NTS carriedout and giving an antibiotic to a symptomatic food workers infected with NTS is a common practice [17].

The study showed that the prevalence of *Salmonella* fecal carriage among food handlersin Khartoum state was 4.4%. This is relatively smaller than the 6% prevalence reported in Irbid, Jordan [18]. However, it is to some extend higher than the 2.3%, 1.68% prevalence rates estimated in Ghana and Italy respectively [19,20]. Unpublished report of *Salmonella* fecal carriage among food handlers in Khartoum state during 2004 and 2005 showed that the prevalence rate was 0.13% and 0.26% respectively (Sudan Ministry of Health-statistical report, 2005).

The vast majority of *Salmonella* species isolated were *Salmonella* Paratyphi B (82.4%) followed by *Salmonella Typhi* (11.8%). This finding was not in line with the study done in Japan, by Hirose, et al. where they reported that the most frequent one was *S. enteritidis*, followed by *S. Litchfield*, *S. Thompson*, *S. Hadar*, *S. Typhimurium*, *S. İnfantis*, *S. Tennessee*, *S. Montevideo*, *S. Agona and* S. Braenderup [21].

Our study showed that 35.3% of *Salmonella* fecal carriage group were illiterates. Similar results were obtained by Khanum, et al. who, reported that 40% of *Salmonella* cases were adults who were uneducated [22].

In this study, 10.9% of food handlers mentioned that they sometimes wash their hands by soap after defecation and 2.3% never washed their hands, this, generally, may be considered a potential source of spreading of *Salmonella* species.

It was observed that 17.6% of food handlers had history of previous typhoid orgastroenteritis. It was also seen that 32% of study group have had no protective card (insurance card) a year ago, which indicates lack of health insurance. Having protective card provides card holders to free healthcare acsess, which is very diffucult and expensive. In our opinion, all of these might probably be responsible for the high prevalence reported in this study.

Studies showed that since the beginning of the 1990s, strains of Salmonella which are resistant to a range of antibacterial drugs, including firstchoice agents, have emerged and threatening to become a serious public health problem. This resistance results from the use of antibacterial drugs both in humans and animals. Multi-drug resistances (MDR) to critically important antibacterial drugs are compounding the problems [10,23]. Tenover et al. [23] revealed that the wide spread of overuse and misuse of antibiotics has been associated with emergence of resistance for these drugsworldwide. Antibiotic treatment results in a rapid cure and this helps to prevent further spread of Salmonella. Routine antibiotic susceptibility testing of isolated Salmonella species is justifiable and of great help to the physicians, to improve treatment and management of carriers [23].

Fortunately, in this study all isolated *Salmonella* species were highly susceptibility to the common first line antibiotics used. These results disagree with Malla, et al. in Nepal [24] and Mirza, et al. [25] which reported multidrug resistant *S.* Typhi to certain first line antibacterial drugs.

5. CONCLUSION

This study concludes presence of considerable number 7 (4.4%) of Salmonella fecal carriage among food handlers in Khartoum state. Most of food handlers were illiterate 141 (36.4) and had poor compliance with hand washing 51 (13.2%) after toilet use. Intensification of training programs for food handlers for primary health care and hygiene by using through mass media, schools, face to face is needed. Screening program for Salmonella and Shigella covering all food handlers must be carried out routinely to discover the carriers and treat them. Considering possible drug resistance, routine bacterial cultures and susceptibility tests are recommended.

CONSENT

Informed consent was taken and the results of the positive cases were reported to the doctors. Free medication was given.

ETHICAL APPROVAL

Study was approved by Alzaiem Alazhari university research and ethical committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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