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Epidemiological Patterns of Salmonella Carriers among Food Handlers in Shendi City- River Nile State- Sudan

A thesis submitted in accordance with the requirements of Shendi University for the degree of Philosophy Doctor of Public Health

By

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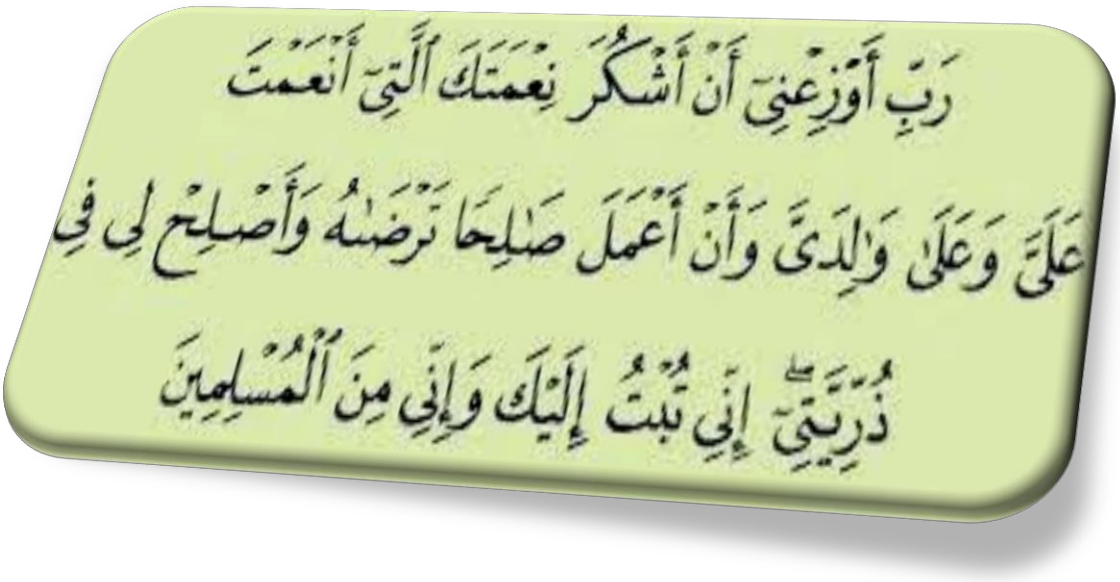

سورة الأحقاف الآية(15)

Dedication

To my Family,

To the soul of my father,

My mother,

My brother,

My wife,

My sons, Khalid and Ahmed

My daughter Arwa

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Abstract

A descriptive cross sectional study was conducted from July 2018 to June 2022 at different food services establishments in Shendi city-Sudan, The study aimed to recognize the patterns and Causes of Salmonella Carriers among food handlers in the Shendi city.

A total of 125 food handlers were selected from 14 different types of food service establishments at 13 different locations of collective types of food services establishments. Three hundred seventy five samples as 3 samples (Stool, urine and fingernail swab) from each food handler were collected for Salmonella investigation. Seven antibiotics were tested against the isolated Salmonella. A designed questionnaire and observational check list were used for collection of data that represents potential Causes for Salmonella carriers among food handlers.

The prevalence of Salmonella among food handlers was found to be (12.8%.) with pattern sites of positive Salmonella culture as (7.2%) from stool, (4.8%) from fingernail swabs and (3.2%) from urine. The isolates of Salmonella were identified and counted as *Salmonella typhi* (n=4), *Salmonella Paratyphi A* (n=5) and *Salmonella spp* (n=10). Salmonella carriers were detected among food handlers in 5 types of food service establishments as restaurants (25.5%), butchery (20.0%), grocery (16.7%), cafeteria (16.7%) and ovens (2.8%). There was no statistical significance between different types of food services establishments**.** Also, the cases were confined to 4 out of 13 locations in the studied population; City port (50%), Al-daim market(40%), Square 14 (16.7%) and Grand market (16%). There was no statistical significance between different work locations.

Multiple causes of Salmonella carriers among food handlers were found with statistical significant association such as health status, personal health, knowledge, hygiene practices and work place.

The antimicrobial test for Salmonella isolates showed variation in susceptible and resistant patterns with different in percentages. All Salmonella isolates showed resistant to ampicillin. *Salmonella typhi* showed susceptible pattern to cotrimoxazole (75%), ciprofloxacin, gentamicin and ceftriaxone (50%) and resistance to tetracycline(100%). *Salmonella Paratyphi A* isolates showed susceptible to gentamicin (100%) and chloramphenicol (80%) and resistance to ceftriaxone (40%). In addition all *Salmonella Paratyphi A* showed intermediate pattern level to ciprofloxacin (100%). *Salmonella species* other than *Salmonella typhi* and *Salmonella Paratyphi A* showed susceptible to chloramphenicol and gentamicin (80%), cotrimoxazole and ceftriaxone(70%),Tetracycline (60%) and ciprofloxacin (50%) .

The study concluded that carriers of Salmonella among food handlers exist in Shendi city with prevalence of 12.8% which is high. Multiple causes of Salmonella carriers among handlers were confirmed with significant association. The antimicrobial pattern of the isolated Salmonella indicated the presence of high percentage and multi resistance drugs. The study recommended further the health screening methods for the food handlers as part for pre-employment, routine health examination and training in food safety.

المستخلص

أجريت هذه الدراسة الوصفية المقطعية خلال الفترة من يوليو 2018 إلى يونيو2022 على متداولي الغذاء العاملين في منشأت خدمات الغذاء المتنوعة في مدينة شندي. هدفت الدراسة للتعرف على الأنماط الوبائية لدى حملة السالمونيلا وسط متداولي الغذاء في مدينة شندي.

حيث تم اختيار 125 من متداولي الغذاء من 14 نوع مختلف من منشأت خدمات الغذاء المختلفة المنتشرة في 13 موقع داخل مدينة شندي. تم جمع 375 عينة بواقع ثلاث عينات(براز- بول- مسحة اظافر) لكل واحد منهم لاستزراع السالمونيلا كما تم اختبار سبعة مضادات حيوية ضد السالمونيلا التي تم عزلها. وتم تصميم استبيان وقائمة ملاحظات وذلك لجمع بيانات تمثل المسببات لدى حملة السالمونيلا وسط متداولي الغذاء.

وجدت الدراسة أن معدل حملة السالمونيلا وسط متداولي الغذاء(12.8%) مع نموذج التواجد الموجب للسالمونيلا في عينات الاستزراع (براز7.2%- بول3.2%- مسحة اظافر4.3%). المعزول من جنس السالمونيلا تم تعريفها وعدها السالمونيلا التيفية (4) ،السالمونيلا نظيرة التيفية(5)، أنواع السالمونيلا الأخريات (10). نموذج تواجد الحملة في أنواع منشأت الغذاء المطاعم (12) ، الجزارات (1) ،البقالات (1)، الكافتيريات (1)، الافران(1). انحصرت حالات حملة السالمونيلا في 4 مواقع مدخل المدينة (1) سوق الديم (2)، سوق مربع14 (1)، السوق الكبير(12). الدراسة وجدت مسببات متعددة لدى متداولي الغذاء ذات ارتباط ذو دلالة احصائية معنوية مثل الحالة الصحية، النظافة الشخصية، الممارسات الصحية، المعرفة بصحة وسلامة الغذاء والحالة الصحية لمنشأة خدمة الغذاء. تم اختبار7 من مضادات الجراثيم على انواع السالمونيلا المعزولة واظهر الاختبار اختلافات في نموذج الاستجابة والمقاومة لها طبقا لنوع

السالمونيلا. كل السالمونيلا المعزولة اظهرت مقاومة للامبسلين (100%). السالمونيلا التيفية كانت الاستجابة للكونتريموكزاول (75%)، السيبروفلوكزاسين، الجنتامايسين والسيفترياكزون (50%) وكانت المقاومة للتتراسايكلين (100%).

السالمونيلا نظيرة التيفية(أ) كانت الاستجابة للجنتامايسين (100%) والكلورومفينكول (80%) ونموذج متوسط للسيبروفلوكزاسين (100%). أنواع السالمونيلا الاخرى كانت الاستجابة للكلورومفينكول والجنتامايسين (80%)، ولكل من الكونتريموكزاول والسيفترياكزون(70%)، التتراسايكلين(60%) والسيبروفلوكزاسين (50%). خلصت الدراسة الى وجود حملة السالمونيلا وسط متداولي الغذاء بمعدل 12.8% في مدينة شندي وهو معدل عالي. واثبتت احصائياً قوة ارتباط المسببات لديهم. نموذج مضاد الجراثيم على أنواع السالمونيلا أشار الى وجود نسبة عالية ومتعددة من المقاومة لديها. أوصت الدراسة بإعادة تقييم طرق فحص متداولي الغذاء كجزء قبل التعيين والفحص الصحي المنتظم لهم وتدريبهم في مجال سلامة الغذاء.

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List of Abbreviations

Abbreviation		Explanation
ACC	-	Ampicillin Chloramphenicol and Co-trimoxazole
AOR	-	Adjusted Odd Rate
CAC	-	Codex Aliment arias Commission
CDC	-	Centre for Disease Control
CI	-	Confidence Interval
CLED	-	Eystine Lactose Electrolyte Deficient
CLSI	-	Clinical and Laboratory Standard Institute
DALY	-	Disability adjusted life years
DCA	-	Deoxy Cholate Agar
ESBL	-	Extended Spectrum Beta Lactamase
I.V	-	Intra Venous
L	-	Liter
MDR	-	Multiple Drugs Resistance
N / A	-	Not Applicable
NARMS	-	National Antimicrobial Resistance Monitoring System
NCCLS	-	National Committee for Clinical Laboratory Standards
OR	-	Odds Ratio
SPSS	-	Statistical Package for Social Science
SSA	-	Salmonella Shigella Agar
UNICEF	-	United Nations International Children Emergency Fund
WHO	-	World Health Organization
XLD	-	Xylose Lysine Deoxy cholate

1. INTRODUCTION

1.1. Background:

Food handlers play a significant role in food safety along the food service system. Food handlers are one of the sources of food contamination and they may inoculate several pathogenic microorganisms through different routes. *Salmonella* is one of the pathogenic microorganisms that can contaminate food through several routes and from different sources. Infected and asymptomatic food handlers are an important source of food contamination with species of *Salmonella*. Carriers sites of Salmonella included infected excreta and hands of the carriers.

Several factors correlated to food handlers may contribute to contaminate food causing epidemics and increase prevalence of food infection and poisoning with *Salmonella.* Detection of carriers of salmonella among food handlers and treatment with the susceptible antimicrobial is important and strict commitment with the standard measures for food handlers is one of the basis of prevention and control of epidemics of *Salmonella*.

1.2. Rationale:

The epidemics of *Salmonella* infection is a public health problem all over the world. Globally *Salmonella* estimated to be responsible for infection of 6 million people and more than 600,000 deaths annually [1] [2] ,Mortality due to *Salmonella* infections is mainly a health problem in developing countries, but morbidity due to acute *Salmonella* infections also has important socio-economic impact in industrialized nations [3].There is increasing in prevalence of food infection and food poisoning with species of *Salmonella.* Food handlers Carriers of Salmonella are considered one of the main sources of epidemics of Salmonella infection and food poisoning.

Studies on food handlers carriers of Salmonella in Sudan is rare and no previous study was conducted on causes correlated to carriers of salmonella among food handlers in Shendi city.

1.3. Importance of the study:

The study provided practical and scientific value to public health through knowledge of the prevalence of *Salmonella* carriers among food handlers, the causes associated with their presence, the species of *Salmonella* and the antimicrobials pattern will provide scientific value to establish plans for prevention and control of *Salmonella* infection and food poisoning.

1.4. Research question and hypothesis:

The research question was whether the level of self-hygienic, work place hygiene and health hygiene knowledge may have association with the risk of sustaining *Salmonella* in food handlers at the different food service establishments of Shendi city. It is hypothesized that the higher level of hygiene of food handlers either personally or / and work place would reflect in less risk of *Salmonella*. This would reflect through visible reduction in their positive sample sites of *Salmonella* in cultures. Therefore, the specific null hypothesis of the study is that there is no significant association between causes among food handlers carriers *Salmonella* confirmed.

1.5. Objectives of the study:

1.5.1. General Objective:

To determine patterns and causes of Salmonella carriers among food handlers in Shendi city.

1.5.2 . Specific Objective:

1. To determine Prevalence of *Salmonella* species among food handlers in the different food services establishment's.
2. To analyze the different causes and their association with Salmonella carriers among food handlers.
3. To determine the patterns of Salmonella carriers isolation among food handlers in Shendi city.
4. To determine the antimicrobial patterns of Salmonella isolation.

2. LITERATURE REVIEW

2.1. Concept of related terms of the study:

2.1.1. Epidemiology:

Is the study of the distribution and determinants of health-related states or events in specified populations, and the application of this study to the control of health problems,[4] Epidemiology is concerned with the frequency and pattern of health events in a population.

2.1.2. Distribution:

Distribution of disease occurs in patterns in community. Distribution patterns are described by time, place and person that may lead to: the generation of hypotheses about causative (or risk) factors that may suggest or lead to control measures for disease or preventive measures [5] .

2.1.3. Determinant:

Any factor, whether event, characteristic, or other definable entity, that brings about a change in a health condition or other defined characteristic[5]

2.1.4. Frequency:

Refers not only to the number of health events such as the number of cases of meningitis or diabetes in a population, but also to the relationship of that number to the size of the population. The resulting rate allow epidemiologists to compare disease occurrence across different populations [5].

2.1.5. Pattern:

Refers to the occurrence of health-related events by time, place, and person. Time patterns may be annual, seasonal, weekly, daily, hourly, weekday versus weekend, or any other breakdown of time that may influence disease or injury occurrence, Place patterns include geographic

variation, urban/rural differences, and location of work sites or schools Personal characteristics include demographic factors which may be related to risk of illness, injury, or disability such as age, sex, marital status, and socioeconomic status, as well as behaviors and environmental exposures. Characterizing health events by time, place, and person are activities of descriptive epidemiology [5] .

2.1.6. Health states or events:

Anything that affects the well-being of population behaviors related to health and well-being [5] .

2.1.7. Carriers:

A carrier is a person who harbors the pathogenic microorganism without suffering from any disease from it. Carriers are often a risk to the public health as they have the potential to infect others. Many outbreaks have known to occur due to carriers [6].

2.1.8. Prevalence:

Frequency of existing cases. Existing cases are called **"**prevalent cases**"** [4]

2.1.9. Food Hygiene:

Conditions and measures necessary for the production, processing, storage and distribution of food designed to ensure a safe, sound, wholesome product fit for human consumption [7] .

2.1.10. Food Safety:

Assurance that food will not cause harm to the consumer when it is prepared and/or eaten according to its intended use [8].

2.1.11. Personal Hygiene:

Those protective measures primarily with the responsibility of individuals which promote health and limit the spread of infectious disease chiefly

those transmitted by direct contact. Such measures encompasses washing hands with soap and waters and keeping the body and cloths clean by sufficiently frequent soap and water bath **.**

2.1.12. Food Safety Education:

Education dealing with the practices that keep food safe from environmental and bacterial contamination **.**

2.1.13. Food-borne disease outbreak:

The occurrence of two or more cases of a similar illnesses resulting from ingestion of a common food [9]**.**

2.1.14. Salmonellosis:

Clinical disease of man and animals resulting from infection by Salmonella species other than Salmonella typhi or Salmonella. Paratyphi A,B and C [10].

2.1.15. Zoonosis:

Any disease and/or infection which is naturally transmissible directly or indirectly between animals and humans [11] .

2.1.16. Zoonotic agent:

Any virus, bacterium, fungus, parasite or other biological entity which is likely to cause a zoonosis [11] .

2.2. Overview of food safety and hygiene:

Food safety and hygiene can be defined as the various conditions and practices that preserve the food quality in the prevention of contamination and food borne diseases [12] .

The World Health Organization defines food safety as the conditions and measures that are necessary during production, processing, storage, distribution and preparation of food to ensure that it is safe, sound, and

wholesome and fit for human consumption [13]. Through the food chain from farm to table food contamination may occur .Food safety is used as a scientific discipline describing handle, preparation, and storage of food in ways that prevent food-borne illness [13].

The World Health Organization stated five key principles of food hygiene as:

1. Prevent contaminating food with pathogens spreading from people, pets, and pests.
2. Separate raw and cooked foods to prevent contaminating the cooked foods.
3. Cook foods for the appropriate length of time and at the appropriate temperature to kill pathogens.
4. Store food at the proper temperature.
5. Use safe water and safe raw materials [14] .

2.3. Food handlers:

Food handlers may introduce pathogenic microbes to the food during the process of preparation, distribution and serving [15]. food handlers are the source of contamination and eventual health consequences [16]. Research findings from the food industry suggest that hands may play the role of a vehicle in the transmission of enteric pathogens. [17].

Workers may also carry the microbial pathogens on their skin, hair, digestive systems or respiratory tracts, Personal hygiene, health status, knowledge and practice of the food handlers contribute to affect the incidence of food borne diseases. These pathogens on food handlers are associated with poor personal hygiene and improper hygiene practices. Food handlers should maintain a high level of personal cleanliness and wear suitable protective clothing, head gear and footwear. People involved in food handling should refrain from smoking, spitting, chewing and

sneezing or coughing over unprotected food. Personal effects like jewelry, pins and other adornments should not be brought into food handing areas. A food handler implicated to be a carrier of a disease illness should be excluded from working in any food establishment [7].

2.4. Salmonella:

The first person who discovered the microorganism was the American scientist Salmon [4]. The first person who was recognized to be asymptomatic carrier of this microorganism was the famous Mary Mallon (1906-1915) or (Typhoid Mary),she was presumed to have infected 51 persons through her work in a kitchen and laundry in New York City, United States [18].

Salmonella is a Gram negative bacillus bacteria (rod-shaped), 2-3 x 0.4-0.6 µm in size, motile with whip-like flagella and facultative anaerobic intracellular pathogen [19]. Recent taxonomic studies have organized the genus *Salmonella* into two species, *S. enterica* and *S. bongori*, containing more than 2500 antigenically distinct subtypes or serotypes. [20]. The common species which cause illness in humans and animals is restricted to a number of species most importantly *Salmonella enterica serotype typhi* and *Salmonella enterica serotype enteritidis* which both account for approximately half of the number of *Salmonella* infections [21] [22]

2.5. Food borne disease with Salmonella:

Salmonella can stay alive in undercooked food and food contamination occurs when the food is handled by people with poor personal hygiene especially poor hand hygiene [23] *Salmonella* can grow fast on unfreeze food.[24]. Hence the safe food is the food handled with food safety precautions; (Clean, Cooking and Cold) abbreviated as 3Cs[25] Transmission of *Salmonella* occurs through ingestion of food or drinks contaminated with urine or feces of infected person; as it can be

transmitted to human via person to person transmission or through animals contact. Infection with *Salmonella* will develop a variety of illnesses like enteric fever, gastroenteritis or asymptomatic chronic carrier state [26].

Food borne with Salmonella can conveniently divided into two main groups these are:

First group typhoid and paratyphoid fevers, and second group Non typhoid fever. Group one typhoid and paratyphoid fevers caused by *Salmonella typhi* and *Salmonella Paratyphi A,B,C* respectively, with serotypes practically occur only in man. Enteric fever is a synonym for the syndrome classically associated with *Salmonella typhi, Salmonella Paratyphi A, B and C*. However, it may also be used to describe the syndrome of systemic. Typhoid fever is a disease caused by *Salmonella enterica serotype Typhi* (*S. typhi*),The disease is a major public health problem, particularly in developing countries. Typhoid fever causes 16 million cases with 600,000 deaths worldwide annually [27]. Humans are the sole reservoir and asymptomatic carriers may occur. The infectious dose is low ($<10^3$ organisms) and the incubation period may vary from one to six weeks, depending upon the dose of the organism. The natural mode of transmission is by ingestion of food or water contaminated by feces or urine of patients or asymptomatic carriers [27]**.**

The second group which is the non-typhoid enteric fever or enteric infections of a zoonotic character, caused by *Salmonella* species other than *Salmonella typhi, Salmonella Paratyphi A, B and C*. The term Salmonellosis is used to define the clinically manifest diseases of man and animals resulting from infection by *Salmonella* other than *Salmonella typhi* and *Salmonella Paratyphi A,B and C* [10] .

Gastrointestinal symptoms are typical of this group of food borne salmonella. The mortality and morbidity of non-typhoid enteric fever is lower than in typhoid, and there are fewer complications [10]

2.6. Sites, detection and eradication of Salmonella carriers:

Sites of carriage of *Salmonella* may be feces, blood, gallbladder (bile), and urine. For poor personal hygiene *Salmonella* may be carried on hands[6] .

Carriers of Salmonella can be detected by culture of urine ,feces, or hand swabs. In addition or serology. Serological detection Vi antibody can be detected in serum for *Salmonella* carrier**.** Detection of carriers in society by 'sewer swab' technique.

Carrier state can be eradicated by long term antibiotic usage. Cholecystectomy may be sometimes required to eradicate carrier state of *Salmonella.* [6] . Persons suffering from a disease cable of transmitted by food or water, or who are carriers of an infectious organism, should not employ in food preparation or food handling. With respect to Salmonella transmission of particular importance are persons suffering from or who have recently been suffering from gastrointestinal illness or who have been in contact with such person. These should not handle food. It is preferable that personnel handling food receive necessary training and motivation in hygiene. [10]

2.7. Burden of food borne disease in developing countries:

Diarrheal disease is the second leading cause of burden of disease, measured as disability adjusted life years (DALYs), for all ages worldwide, after lower respiratory infections [28].

Diarrheal disease is the leading cause of morbidity around the world, particularly in developing countries. WHO estimates that in 2004 the incidence of diarrhea was estimated to be 4.6 billion cases among all age groups. that there were 2.2 million deaths caused by diarrheal disease among all age groups, 1.8 million in low-income countries alone, of who

1.5 million were children under 14 years of age [29]. *Salmonella* infections in the United States account for roughly 19,336 hospitalizations, 17,000 quality adjusted life years lost and $3.3 billion in total medical expenditures and lost productivity each year [30].

For human salmonellosis in the Netherlands, the costs are estimated to be between 32 and 90 million Euro per year [31]**.** Burden of disease from diarrhea is highest in low-income countries and, in these countries, is highest among children under 14 years of age, especially among those under 5 years of age (WHO/UNICEF 2009) [32]. Epidemiological data demonstrate that food is an important factor in transmitting pathogens that cause diarrheal illness [33]. Recent data from WHO indicate that globally food borne and waterborne diarrheal disease kills an estimated 2 million people annually [34]. Up to an estimated 70 percent of diarrheal episodes among young children could be due to pathogens transmitted through food [35-36] .This is because the amount of bacteria in contaminated food, when it remains at ambient temperature for extended periods, is much higher than in water; food provides a medium for exponential bacterial growth, while bacterial pathogens in water may survive for some time but will not increase significantly in the absence of other nutrients [37-38].

Food borne diseases are largely under-reported. [39]. Evidence of the health and economic burden of food borne disease has been stronger in developed countries than in developing. In developing countries, health statistics for many food borne diseases, when they are reported, are often recorded simply as “diarrheal diseases” because the specific pathogen is almost never identified. The recording of food borne disease as “diarrheal diseases” at the country level is carried over in WHO statistics, which also uses the term “diarrheal diseases” rather than specific foodborne pathogens.

For this reason, the exact proportion of diarrhea in developing countries caused by contaminated food remains unclear [40-44].

2.8. Antimicrobials for Salmonella:

Several studies in different countries stated the susceptible and resistance antimicrobials for Salmonella isolated from patients and food handlers. Chloramphenicol was introduced in 1984 as the first effective antibiotic in the treatment of typhoid fever. Resistant develop second years after its introduction. Amoxicillin and cotrimoxazole were effective alternative till its development of MDR Strains towards (ACC) at the end of 1980 and1990 [45].

Fluoroquinolone (ciprofloxacin and ofloxacin) and 3rd generation cepha (ceftriaxone and cefixame) and azithromycin come up as the 2nd line of treatment of for MDR strains. Resistance to two clinically important drugs, ceftriaxone (a cephem) and ciprofloxacin (a quinolone), has climbed in non-typhoidal *Salmonella* since 1996. In 2011, about 5% of *Salmonella* tested by CDC were resistant to five or more types of drugs [46]. Nalidixic acid and other antimicrobials respond effective then resistant strains developed. In the recent past 3rd generation cephalosporin's have gained importance for treatment of enteric fever due to their pharmacodynamics properties and low prevalence of resistance. Since 1990, non-typhoid Salmonella species other than Salmonella typhi and Salmonella Paratyphi A, B and C resistant to extended spectrum cephalosporin's have been developed in several countries. CDC has posted a series of interactive graphs that allow users to see the percentage of *Salmonella* human isolates resistant to various antibiotics tracked, by year, through the National Antimicrobial Resistance Monitoring System (NARMS). This graph includes the option to view all *Salmonella* isolates or any one of five of the

most common serotypes with resistance to antibiotics used to treat *Salmonella* infections [47] .

2.9. Previous studies:

A laboratory-based cross-sectional study was conducted in North West Ethiopia [48] among 220 food handlers from January 2015 to June 2016. Stool and fingernail samples from the subjects were cultured on bacteriological culture medium, and *Shigella and Salmonella* were isolated and identified following standard procedures. Antimicrobial susceptibility testing was performed for all isolates using the Kirby–Bauer disk diffusion method. The authors reported overall prevalence of Shigella and Salmonella in this study was 5.9%, with 3.6% of stool specimens testing positive for Salmonella species and 2.3% testing positive for Shigella species. Moreover none of the food handlers had positive cultures for Shigella or Salmonella in respect of their fingernail specimens. The isolation of either Shigella or Salmonella had a significant inverse relationship with the number of service years (P= 0.017). Furthermore, all isolates samples of Shigella and Salmonella were 100% susceptible to ciprofloxacin, norfloxacin, and gentamicin. However, all isolated pathogens were resistant to ampicillin (100%). The study highlight the importance of food handlers in the transmission of pathogens to the customers (students and the general population). In addition to Screening of food handlers, training for food handling and hand hygiene practices, and regular monitoring of the food handling practices should be done in order to avoid diseases that can be acquired through improper food handling, like bacterial infections [48] .

A cross-sectional study done in India [49], The authors aimed to detect intestinal carriage of *Salmonella species* and parasites as well as nasal and hand carriage of *Staphylococcus aureus* among asymptomatic food

handlers. The study investigated the personal hygiene practices followed by participants was recorded. A total of 300 asymptomatic food handlers participated in this study. The participants were asking to fill up a semi-structured questionnaire. Additionally nasal swabs and finger impressions were taken for laboratory investigations. The results revealed *Salmonella typhi* isolation from the stool culture of 2 (0.7%) food handlers. Moreover *Salmonella typhi* isolates were sensitive to ampicillin, cotrimoxazole, chloramphenicol, ciprofloxacin, and ceftriaxone but resistant to nalidixic acid [49] .

A study conducted in a university located in Kuala Lumpur, Malaysia [50] to compare the knowledge, attitude, and self-reported practices (KAP) of food safety assessment and microbiological assessment of food handlers' hands as an indicator of hygiene practices in food premises. This study involved 85 food handlers working in a university located in Kuala Lumpur, Malaysia. The authors in this study investigated food safety KAP among food handlers (n= 67) using a questionnaire. They also collected hand swabs (n= 85) which were tested for the total aerobic count, coliforms, and *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Vibrio cholera* and *Vibrio parahaemolyticus*. The study reported that the food handlers had moderate levels of food safety knowledge (61.7%) with good attitude (51.9/60) and self-reported practices (53.2/60). It is noteworthy that the good self-reported practices were not reflected in the microbiological assessment of food handlers' hands, in which 65% of the food handlers examined had a total aerobic count 20 CFU/cm2 and Salmonella was detected on 48% of the food handlers' hands. The authors documented that that the food handlers had adequate food safety knowledge. However, perceived knowledge failed to be translated into practices at work [50] .

A study was conducted [51]. to determine the number of *Salmonella* carriers in a migrant cohort in several food establishments in three major cities in Peninsular Malaysia. The authors collected socio-demographic data and stool samples were collected and analyzed using standard methods of detection and isolation. Nevertheless, antimicrobial susceptibility tests of the positive samples were also performed. The study investigated a total of 317 migrant food handlers, originating from South and Southeast Asian countries, who were recruited voluntarily. The study reported nine (2.8%) stool samples were confirmed to be Salmonella positive. Moreover PCR serotyping and pulsed-field gel electrophoresis identified four serotypes as Typhimurium (n= 3), Corvallis (n = 2), Hadar (n = 1), Agona (n = 1) and two unknown serovars. Antimicrobial susceptibility tests revealed that all nine isolates were susceptible to amoxicillin–clavulanic acid, cefotaxime, ceftazidime, ceftriaxone, and gentamycin. However, seven isolates were found to be multidrug resistant to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole,sulfonamides,streptomycin and tetracycline. The study also highlights that carriers of non-typhoid Salmonella exist among migrant food handlers, which poses a health risk to consumers through food contamination. The study results indicate a need for authorities to enhance food safety awareness in the migrant workers and to reevaluate current health screening methods to include preventive measure such as mandatory stool screening as part of the pre-employment and routine health examinations.

A cross-sectional study [52] was conducted among food handlers working at Al Neelain University cafeterias in Khartoum. Five food-handlers (6.25%) were *Salmonella species* stool carriers. Four of these species (5%) were *Salmonella Paratyphi B* which were all sensitive to ciprofloxacin, cefoperazone, and gentamycin; and resistant to tetracycline and cefepime.

Likewise one *Salmonella species* (1.25%) was *Salmonella Paratyphi A* and this species was found sensitive to all antibiotics tested, and concluded that unhygienic conditions were detected among food handlers and food processors in the cafeterias investigated. Presence of resistant food pathogens; particularly *Staphylococcus aureus* isolates was confirmed .

A study [53] was conducted in JIRAPUPPER West region to determine if food vendor carriage constitute a major source of spread of the disease in the community. The study was conducted from January to May 2015. One hundred and seventy (170) stool samples obtained from asymptomatic food handlers were tested for Salmonella. 182 stool and 112 blood samples were collected from patients and tested for Salmonella. Salmonella was recovered from 4.1% (7/170) food handlers and 10.4% (31/294) of patients. Stool samples yielded more Salmonella isolates 12.1% (22/182) than blood samples 7.1% (8/112). Among the Salmonella isolates obtained from food handlers, *Salmonella typhi* accounted for 71.4% (5/7) whereas 28.6% (2/7) were other *Salmonella species.* There were 31 Salmonella isolates obtained from patients. Of the 31, Salmonella Typhi recorded 9 (29.0%) and *Salmonella species* recorded 22 (71.0%). There was a total of 38 Salmonellae isolated from the study group.

Abdominal pains, headache and diarrhea were the common complaints presented by patients. Most Salmonella isolates showed high resistant pattern towards the common antimicrobials used including ampicillin, tetracycline and chloramphenicol. All the Salmonella isolates were however, susceptible to ciprofloxacin, ceftriaxone, cefotaxime and ceftazidime. Salmonella isolates were tested for ESBL and they were all not ESBL producers. About 4.1% of food handlers in Jirapa were carriers of Salmonellae. These food handlers constitute a potential source for the

transmission of the Salmonella pathogen, and may account for one of the reasons for the high cases of Salmonella infections in Jirapa [53] .

A cross sectional study [54] was conducted among food handlers in Arba Minch University students' cafeteria from April- June, 2015. The authors collected Stool sample and examined them for pathogens following standard procedures. The authors in the study conducted biochemical tests were done to identify the species of pathogens and sensitivity test was done using Kirby- Baur disk diffusion technique.

A total of 376 food handlers were enrolled in the study with the response rate of 100% for data collected by questionnaire. About 7.4% were aged less than 20 years with majority (63.3%) lay in the working age group of 21-35 years. However, a total of 345 food handlers participated for stool examination of whom, stool cultures revealed 6.9% of Salmonella and 3% Shigella isolates. Furthermore finger nail status (AOR=0.033), hand washing practice after toilet (AOR= 0.006) and touching food with bare hands (AOR= $p < 0.001$) were independent predictors of infectious enteric diseases among the food handlers. All isolated pathogen were resistant to amoxicillin (100%), followed by clarithromycin (41%) and amoxicillin-clavulanic acid (35%). The study reported that a high prevalence of enteropathogens among the study participants. The study also revealed poor personal hygiene like poor practice of hand washing. Therefore, pre placement, in service training on personal and food hygiene should be provided to all food handlers with regular sanitary inspection to improve adherence of food handlers to personal hygiene and food safety practices[54] .

A research work done [55] aimed to determine the prevalence of salmonellosis among food handlers and its implications on the health of food consumers in Lagos, Southwest Nigeria The authors collected (235)

blood samples into EDTA bottles from the food handlers at various locations in Victoria Island (Lagos Island) and Bariga (Lagos mainland) which were analyzed using standard methods. In addition Questionnaires were distributed and collated among 235 participants (food handlers) to test their knowledge on food safety. The results showed that 74 participants (31.5%) of the studied population had either previous or recent Salmonella infection as indicated by IgG and IgM anti-Salmonella immunoglobulin while 161 (68.5%) had neither recent nor previous infection. Ninety three (93) respondents were males while One hundred and forty two (142) were females of active working age bracket of 11- 60 years old. Among 93 males screened, 26(28.0%) were infected, 67(72.0%) were not infected while 48(33.8%) females were infected out of 142 females screened for *Salmonella enterica serovar Typhi and Paratyphi* infections. The study documented that food handlers were not responsible for food borne infections but potential risk factors in Lagos, Southwest, Nigeria . This was shown with regression and T-test statistical analysis reviewed that since F cal>F tab (1.776>0.969) and t cal>t tab (6.5>5.0) [55].

A cross-sectional study [56] was conducted to determine the Salmonella carrier status among food handlers of Addis Ababa university students' cafeteria, Addis Ababa, Ethiopia. A structured questionnaire was used to collect socio-demographic data and risk factors. Stool samples were collected from 233 food handlers for bacteriological techniques (culture, biochemical tests) and drug susceptibility tests were conducted. *Salmonella species* was detected in 8(3.4%) of the examined food-handlers. Two of them were *S. enterica serovar typhi,* one *S. Paratyphi A* and the remaining five were other *Salmonella serovars*, interestingly all Salmonella positive food handlers were female study subjects. Among the risk factors, hand washing habit after toilet with and without soap had a statistically

significant association with carrier status of salmonellae (p=0.003) and odds ratio of 0.07 with a 95% confidence interval (0.008, 0.58). The antimicrobial susceptibility profile showed that all isolates were resistant to ampicillin and all isolates were resistant at least to one of the antimicrobials tested. The finding of 3.4% carriage rate of Salmonella species among food handlers could be a source of salmonellosis unless carriers are treated after periodic screening and applying other preventive measures like health education, food handling trainings. Antimicrobial resistance profile also reflects that it would be a serious problem in the near future [56].

A descriptive study done in Nigeria [57] aimed determining the prevalence and pattern of bacteria and intestinal parasites among food handlers in the Federal Capital Territory. The authors found that 42.3% of the stool specimen tested positive for salmonella, they recommended, food establishments should ensure compulsory and proper treatment of staff with active illness. Disposable rubber gloves, plasters and other first-aid items for management of minor cuts/injuries should be provided for use by the food handlers when necessary [57].

A study in Lagos [58] 45.found that the prevalence of *S. typhi* among healthy food handlers was 5.7%. It has been reported that food vendors are carriers of a variety of bacterial enter pathogens, including *S. typhi*. The author suggested that inadequate personal hygiene can facilitate the transmission of these pathogens via food to humans. The serving stage is a critical point in the street food industry, because enter pathogens can survive on the hands for over three hours. Therefore, carriers of *S. typhi* and other Salmonella species who handle food in bukkas may pose a risk to their customers [58].

3. MATERIALS AND METHODS

3.1. Study Design:

This was a descriptive Cross sectional study.

3.2. Study Duration: From July 2018 to June 2022.

3.3. Study Area:

Shendi City is located in the Southern of the River Nile State and on the East bank of the River Nile between Latitudes 16 ,41° north and Longitude 33 ,24° east, its principal suburb on the west bank is Matamma town.The city is bordered locally from Musiktab village to the north to Algilla village, on the South and bordered to the east lining with Albutana area. The city is about 170Km north from Khartoum, the capital of Sudan. The population of the city is 55.516 (2007) .The city occupies an area of 145.96 Km and with a dry desert climate. Al Tahadi Road connects Shandi city with Khartoum and Al Damer city, the capital of River Nile State.

Shandi is the center of Jaalin tribe and an important historic trading center. The city is considered one of the agricultural cities in the northern Sudan where there is the largest area of mango in Swan and there are several projects for irrigated agriculture from the Nile River affiliated to the public and private sectors. The spinning and weaving industry, soap factory, Fayet Dairy Factory are the important industries in the city.

The railway station in the city is no longer used for passenger travel although freight trains continue to use the tracks. Shendi University is considered one of the most important institutions in the city.

There are about 13 main locations in Shendi city where collective different types of food services establishments were present. (Appendix I)

3.4. Study Population:

A total of 714 of food handlers who worked in food service establishments with license according to the population and health

administration of Shendi locality[59]. The food service establishment's involved 14 types according to the license of food allowed to be handled within the establishment.

There are 302 of food services establishments distributed in the city as follows: 3 warehouse for potato refrigerators, 5 warehouse for Banana refrigerators, 18 Refreshment shop, 72 Restaurant, 25 Butchery, 2 Ice factories, 3 Pasta factories, 8 Cafe, 33 Grocer, 8 Confectioner, 24 Booth, 9 Cafeteria, 41 Grinders and 51 Oven .(Appendix II).

3.5. Inclusion criteria:

- Licenses to be working as food handlers in the shandi city from the population and health administration.
- Willing to participate in the study.

3.6. Exclusion criteria:

- History of taken medication to treat intestinal ailment for the past 3 months.

3.7. Study Variables:

3.7.1. Independent variables:

Includes the Causes of Salmonella carriers among food handler:

i. Socio-demographic factors like:
   - Gender.
   - Nationality.
   - Marital Status.
   - Educational Level.

ii. Work Characteristic of Study population like:
   - Type of work.
   - Work Experience.
   - Work Place
   - Food handler's income.

iii. Training and Knowledge of food handlers about food safety.

iv. Health Status of food handlers.

v. Hygienic Practices of food handlers like:

- Personal hygiene.
- Hand washing practices.
- Other hygienic practices during work.

vi. Workplace Health Condition.

3.7.2. Dependent variables:

Positive culture of *Salmonella* among food handlers.

3.8. Sample size:

The sample size of the food handlers was taken by using the following Richard –Geiger formula:

$$n = \frac{\left(\frac{z}{d}\right)^2 \times (0.50)^2}{1 + \frac{1}{N}\left[\left(\frac{z}{d}\right)^2 \times (0.50)^2 - 1\right]}$$

Where:

n= Total sample size.

N= Total Number of population sample.

Z= the value of the Standard normal variable corresponding to is % level significance (1, 96).

d= Marginal error (0.0054).

Accordingly, after calculation a sample size of 125 food handlers were considered.

Distribution of the sample of the food handlers:

Food handlers were distributed according to food services establishments' types. (Appendix III). The sample size of the food handlers (125) was

distributed among the various types of food service establishments using the following formula:

$$ns = \frac{n \times ws}{N}$$

Where:

ns= Sample Size in each type of food service establishments.

n= Total sample size.

ws= Number of food handlers in each food industries.

N= Total number of food handlers in the Shandi city.

3.9. Sample selection:

Stratified random sampling technique was followed for selection of the food handlers sample from the total sample size. The acceptance of the Selected food handlers was obtained (Appendix IV)

Collection of samples for microbiological examinations:

Stool specimen: a small amount of fresh stool sample (>3 g or 4 ml if diarrhea) collected in a clean and screw capped tight-lid sample container. Stool samples collected for Salmonella culture were refrigerated at 4°C with the Cary-Blair transport medium and transported to microbiology laboratory, Faculty of Medical Laboratory Science shendi university microbiology laboratory within 24 hrs. of collection for microscopic examination and culture.

Mid-stream urine specimens, stool specimens, and finger nail swabs were collected from each study participants.

Urine specimens: were collected in wide mouth, sterile sealed urine containers, stool specimens were collected in clean, dry, wide mouth containers with spoon for collection of the specimens,

Finger nail swabs: were collected from both hands using separate applicator stick tipped with cotton and moistened with sterile normal saline swabs were taken from under nails of all participants **.**

All specimens were labeled with patients name and data and transferred during two hour to the laboratory. All the samples were coded with secret numbers and sent to microbiology lab for microbial examination.

3.10. Laboratory techniques: Culture of specimens

A loop-full (0.001 ml) of well mixed centrifuged urine specimens were streaked onto the surface CLED agar.

All nail swabs were cultured in Blood agar and MacConkey agar medium, incubated at 37 °c for 24hrs.

Stool specimens were inoculated in selenite F medium and incubated at 37°c for 48 hours, then sub cultured on XLD, DCA and SSA media, incubated again for 24 hours at 37°c [59]. Laboratory diagnosis of salmonellosis is performed by isolation of Salmonella from (stool , urine and fingernails swabs), and cultured on blood agar and MacConkey's agar and perform Microscopic Examination. The isolates of Salmonella were identified according to the standard procedure.

Normally, for detection of Salmonella the sample was processed in the following steps, Pre-culture, Selective enrichment culture, Isolation and Confirmation/Identification.

Identification of species of Salmonella :

Colonial morphology

The growing colonies were morphologically examined for size, color, edges, moisture and microsite in all media.

The presence or absence of hemolysis on blood agar was indicated by observation of the zone around the colonies.

The colonies were examined on MacConkey agar for presence or absence of lactose fermentation.

The colonies were examined on XLD, DCA and SSA media for presence or absence of lactose fermentation with or without black center.

Indirect Gram stain:

Dry smear is prepared by emulsifying a colony in a drop of physiological saline and spread evenly in clean dry slide, then allowed to dry, and then the smear was fixed by passing over the flame for seconds.

Crystal violet was added to cover the fixed smear for one minute, then washed by tab water, then Lugol's iodine was added for one minute and washed off by tab water, then decolorized by using acetone alcohol for 5-10 seconds and also washed off by tab water, finally Safranin was added for 2 minutes and washed off by tab water then allowed to air-dry and examined under microscope by oil immersion lens (x100) [59].

Biochemical tests:

Oxidase Test (Cytochrome oxidase test)

A piece of filter paper was placed in a clean petri-dish and 2 or 3 drops of freshly prepared oxidase reagent were added. By using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism was removed and smeared on the filter paper. The development of a blue-purple color within a few seconds was stated as positive oxidase test [60].

Urease Test :

The test organism is inoculated in a medium of Christensen's urea agar and incubated at 37 c for 24 hours, the development of pink-red color is stated as positive urease test [59].

Citrate Utilization Test :

The test organism is inoculated in a medium of Simmon's citrate agar and incubated at 37 c for 24 hours, the development of a blue color is stated as positive citrate test [59].

Indole Test :

The test organism Inoculated in a bijou bottle containing 3 ml of sterile tryptone water, incubated at 35–37 c for up to 48 h. 2-3 of Kovac's reagent were added after the incubation time, shaked gently and examined for development of a red color in the *surface layer* within 10 minutes, which was stated as positive indole test [60].

Motility Test :

A heavy inoculum of test organism was inoculated by stabbing technique into semisolid medium by sterile wire, then incubated for at 37 c for 24-48hrs. The test tube slant then examined for the presence or absence of growth along the line of the stab inoculation [59].

Kligler's Iron Agar :

The tested organism was inoculated into Kligler's iron agar medium using a sterile straight inoculating needle to the button of tube and zigzag on slope, then aerobically incubated at 37°c for 18 hours. The indicator of this medium is phenol red, glucose found in the butt and lactose in the slope, yellow color indicate fermentation, cracks in medium indicate gas production and black color indicate H_2S gas production [59].

3.11. Antimicrobial testing;

Antimicrobial sensitivity testing of isolated *Salmonella* species was performed on Mueller-Hinton agar plate by the Kirby-Bauer disk diffusion method. Isolates were tested for their susceptibility against Ciprofloxacin,

Gentamicin, Chloramphenicol, Tetracycline, Cotrimoxazole, Ampicillin, and Ceftriaxone.

Standardized inoculum conforming to 0.5 McFarland standard turbidity of each isolate was inoculated on Mueller –Hinton agar plates using a sterile cotton swab by streaking the swab over the entire sterile agar surface three times, and then onto each plate the selected antimicrobial agents were placed. All plates were aerobically incubated at 37°c for 18 hours [61].

The zone of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter .The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameter of each drug are interpreted using the criteria published by the clinical and laboratory standard institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) or those included in the US Food and Drug Administration (FDA) approved product insert for the disks [61].

3.12. Data collection:

a. Direct interviewing of targeted sample population through field visit.

b. Data collection by using questionnaire forms and by direct assessment of application of standard health measures. (Appendix V).

c. Each participant was sent with a request for Laboratory examination of urine, stool and finger nails swab samples.

3.13. Statistical Analysis of Data:

An assessment of normality distribution was checked with Shapiro-Wilk test and by visual inspection of boxplots [62]. Results were expressed as percentages (%) and numbers (n) and for numerical data; Mean ± standard deviation (SD), median, minimum and maximum values. To determine differences between the study groups the numerical data were compared

using t-test or the alternative non parametric Mann-Whitney U test for data not fitting normal distribution.

Categorical data were compared using Chi square test (alternatively; Fisher exact test when Chi square not suitable). Multivariate analysis with binary logistic regression was used including factors with significant associations with the dependent variable to verify its independent predictors. Post hoc analyses were subsequently performed using the Bonferroni multiple comparison procedure to evaluate significant. Statistical significance was set at ($p<0.05$). All statistical analysis was performed in SPSS (Version 23.0. IBM SPSS Statistics, USA).

Figures were generated using Microsoft office Excel 2007. Pie chart was sued to depict rates of different categories of a single variable. Also, strip chart were used for either depicting single variable with multitude of categories as well as cross-tabulations for the dependent variable within the different variables (factors).

3.14. Ethical Considerations:

1. Access to data will not be allowed except through authority in charge and permission protocol fulfilled. Ethical approval was obtained from Administration of Researches and Projects-Ministry of Health and Population- River Nile State Sudan. (Appendix VI).

2. Data collection was conducted after obtaining written consent, full explanation of the research objectives and expected results to the participants.

3. Data was dealt with security to maintain participants' privacy and kept in special record coded with secret numbers when dealing with statistical analysis.

3.15. Limitations of the study

During the course of the study some limitations were detected which include:

1. lack of serum immunoglobulin testing to investigate the previous infection.
2. Prolonged closer of different food services establishments due to Corona pandemic.
3. The non-license food handlers who could not be included in the study population.

4. RESULT

The present study was a descriptive cross sectional, investigated a randomly selected sample of 125 food handlers distributed on a different locations and a variety of food services establishments in Shendi city. The study population age ranged from 16 to 63 years, most of them were below 45 years (n=100/125; 80.0%). Males were the predominant gender (n=120/125; 96.0%). Majority of participants were Sudanese (n=119/125; 95.2%). Majority of participants marital status were married (n=98/125; 78.4%). Regarding the educational level (n=32/125; 25.6%) of the participants were illiterate, (n=45/125; 36.0%), were educated at primary level, (n=35/125; 28.0%) were secondary level educated and the rest (n=13/125; 10.4%) were university graduates.(table 1)

The laboratory result of positive Salmonella culture were found in 16/125 (12.8%) of the food handlers, and 19/375 (5.06%) of the total samples for culture (n=125 Stool samples, n=125 fingernails Swabs, and n=125 Urine samples), This confers a relatively high prevalence of Salmonella among food handlers in the city. (table 2).

The current study showed there is statistically significant between some of socio-demographic factors (unmarried status and lower educational level) with positive Salmonella culture result (P=0.007 and P=0.001 respectively). Meanwhile no statistically significant with other socio-demographic factors (age, gender and nationality). (table3)

Most of the participants, were working in restaurants (n=47/125; 37.6%) and ovens (n=36/125; 28.8%) while the rest of the Participants distributed over the other 12 types of food service establishment (figure 2)

The positive Salmonella culture results were reported among workers in 5 out of 14 services; restaurants (12/47; 25.5%), butchery (1/5; 20.0%), grocery (1/6; 16.7%), cafeteria (1/16; 16.7%) and ovens (1/36; 2.8%). There was no statistical significance difference between different types of food services establishments. (figure 3)

Work experience ranged between 1 and 29 years the mean 7.5 years (SD=6.8 years) and the median 5 years, This result indicated a significant difference between positive and negative Salmonella culture in favor for food handlers with more work experiences (P=0.008) .(figure 4)

Also, the cases were confined to 4 out of 13 locations in the studied population; City port (1/2; 50%), Al-daim market (2/5; 40%), Square 14 (1/6; 16.7%) and Grand market (12/75; 16%). There was no statistical significance between different work locations.(figure 5 & 6)

In the current study we assessed the relationship between 4 different elements of knowledge and training of food handlers and occurrence of Salmonella in the study population (food safety training, food borne infection, source of food contamination, and health requirement of work) was tested by The Fisher's and Pearson Chi-Square test, which indicated that there is significant association between positive Salmonella culture results with lack of training and knowledge.(table 5& figure 7)

In the current study the **Health status** of food handlers (fever, diarrhea, vomiting, stomachache, headache, blood in stool and medical examination during the recent period, received treatment) was tested by The Fisher's and Pearson Chi-Square test, which indicated that there is significant association between positive Salmonella culture results with recent gastrointestinal symptoms : fever, diarrhea, stomachache , headache and blood in stool, (P=0.044, P<0.001, P<0.001, P=0.044 and P=0.027

respectively).While no significant association with vomiting P=0.079, medical examination during the recent period P=0.220 and received treatment P=0.079.(table 6& figure 8)

The current study also appeared that the **personal hygiene practices** of food handlers (wearing of special clothes for the work, wearing of clean clothes and especial for the work, covering of the hair, non-cutting the fingernails, general body cleanliness, the presence of uncovered hand wounds, the presence of covered hand wounds, the presence of nose secretion and having a heath certificate) was tested by The Fisher's and Pearson Chi-Square test, which indicated that there is significant association between positive Salmonella culture results with uncovering of the hair, non-Cutting fingernails, unsatisfactory general body cleanliness, The presence of uncovered hand wounds and The presence of nose secretions (P=0.016, P<0.001, P<0.001, P<0.001 and P=0.02 respectively). While no significant association with Wearing of special clothes for the work P=0.067, Wearing of clean clothes and un-special for the work P=0.067, The presence of hand wounds covered P=1.000 and have a health certificate P=0.122 .(table 7 & figure 9)

Hand washing practices was tested by The Fisher's and Pearson Chi-Square tested found a significant association between most indicators of Hand washing practices (washing hands with water only, washing hands with water and soap, washing hands before starting work, washing hands after blowing nose, washing hands after touching any objects and positive Salmonella culture result (P=0.001, P=0.001, P<0.001 and P<0.001, P=0.022 respectively). While no significant association with other Hand washing practices indicators (washing hands after going toilet; P=0.128 and washing hands after touching body parts; P=0.125). (table 8 & figure 10)

Others work hygienic practices(Shaking hands while working, Biting nail with the teeth, un keeping food in the place away from contamination, Sneezing, coughing and Smoking on the food) were assessed among the food handlers and showed that there is statistical significant association with positive Salmonella culture results (P=0.047, P<0.001, P<0.001, P<0.001 P=0.003, respectively). (table 9 & figure11).

Additionally, in the current study the **workplace Health Conditions** factors of food handlers was tested by The Fisher's and Pearson Chi-Square test, which indicated that there is significant association between positive Salmonella culture results with negligene of sanitation of work surface, negligene of Cleaning of Instrument and Equipment, negligene of Cleaning of working Place, negligene of daily dispose of wastes, negligene of Using the sanitizer when washing utensils, Use of non-safe water and presence of Insect infestation (P=0.002, P<0.001, P<0.001, P<0.001, P<0.001, P=0.06 and P<0.001, respectively). While no significant association with The Building is in good condition; P=0.241, presence of a wash basin; P=0.508 and availability of refrigerator; P=0.243 . (table 10 & figure 12)

Part (I): Socio-demographic of the study sample.

Table No. (1): Socio-demographic Characteristics

No.	Factors	Frequency	Percentage%
1.	**Age**		
	15-24	18	14.4
	25-34	43	34.4
	35-44	39	31.2
	45-54	22	17.6
	55-64	3	2.4
2.	**Gender**		
	Male	120	96.0
	Female	5	4.0
3.	**Nationality**		
	Sudanese	119	95.2
	Non Sudanese	6	4.8
4.	**Marital Status**		
	Yes	98	78.4
	No	27	21.6
5.	**Educational Level**		
	University	13	10.4
	Secondary	35	28.0
	Primary level	45	36.0
	Illiterate	32	25.6
	Total	125	100.0

Part (II): Laboratory Analysis:

Table No. (2): Patterns of Sample Cultures for Salmonella.

Salmonella Culture		Samples			
		Urine	Fingernails Swab	Stool	Number of the samples
Negative culture		121(96.8%)	119(95.2%)	116 (92.8%)	356 (94.9%)
Positive culture	Salmonella SPP	3(2.4%)	1(0.8%)	6 (4.8%)	19 (5.06%)
	Salmonella Typhi	1(0.8%)	2(1.6%)	1 (0.8%)	
	Salmonella Paratyphi	0(0.0 %)	3(2.4%)	2 (1.6%)	
Total of samples		125(100%)	125(100%)	125(100%)	375 (100%)

The table showed that 125 food handlers participant with the prevalence of positive culture of the samples for Salmonella was (19/375; 5.06%) and distributed as follow (9/125; 7.2%) from stool , (6/125; 4.8%) from fingernail swab and (4/125; 3.2%) from urine .

In this study, stool culture was performed for 125 food handlers, confirm the presence of different species of Salmonella (n=9/125; 4.8%), sub typed into *Salmonella species* (n=6/125; 4.8%),*Salmonella typhi* (n=1/125;0.8%), and *Salmonella Paratyphi* (n=2/125; 1.6%).

Positive fingernails swabs Salmonella culture result taken from food handlers (n=6/125; 7.2%) sub types into, *Salmonella species* (n=1/125; 0.8%), Salmonella *typhi* (n=2/125;1.6%), and *Salmonella Paratyphi* (n=3/125; 2.4%).

Positive urine Salmonella culture result taken from food handlers (n=4/125; 3.2%) sub types into, *Salmonella species* (n=3/125; 2.4%), *Salmonella typhi* (n=1/125;0.8%), and *Salmonella Paratyphi* (n=0/125; 0.0%).

Part (III) : Patterns and Causes associated with Salmonella carriers among food handlers

1. Socio-demographic factors :

Table (3) : Socio-demographic factors among food handlers and Salmonella Culture Results.

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
1.	**Gender**				
	Male	**15(12.5%)**	**105(87.5%)**	**120 (100.0%)**	**Fisher's Exact Test P=0.502**
	Female	**1(20.0%)**	**4(80.0%)**	**5(100.0 %)**	
	Total	**16 (12.8%)**	**109(87.2%)**	**125(100.0%)**	
2.	**Nationality**				
	Sudanese	**14 (11.8%)**	**105 (88.2 %)**	**119 (100.0 %)**	**Fisher's Exact Test P=0.170**
	Non Sudanese	**2 (33.3 %)**	**4 (66.7%)**	**6 (100.0 %)**	
	Total	**16 (12.8 %)**	**109 (87.2 %)**	**125 (100.0 %)**	
3.	**Marital Status**				
	Yes	8 (8.2%)	90(91.8 %)	98 (100.0 %)	**Fisher's Exact Test P=0.007**
	No	8(29.6%)	19(70.4%)	27(100.0 %)	
	Total	16 (12.8%)	109(87.2%)	125(100.0%)	
4.	**Educational Level**				
	Secondary or beyond	0 (0.0%)	48(100.0 %)	48 (100.0 %)	**Pearson Chi-Square 14.085 P=0.001**
	Primary level	7 (15.6%)	38 (84.4 %)	45 (100.0 %)	
	Illiterate	9 (28.1 %)	23 (71.9 %)	32 (100.0 %)	
	Total	16 (12.8%)	109(87.2%)	125(100.0%)	

There is statistical a significant between (unmarried status, education level) and Salmonella positive culture based on Fisher's exact test and Chi-Square test (P=0.007 and P=0.001).

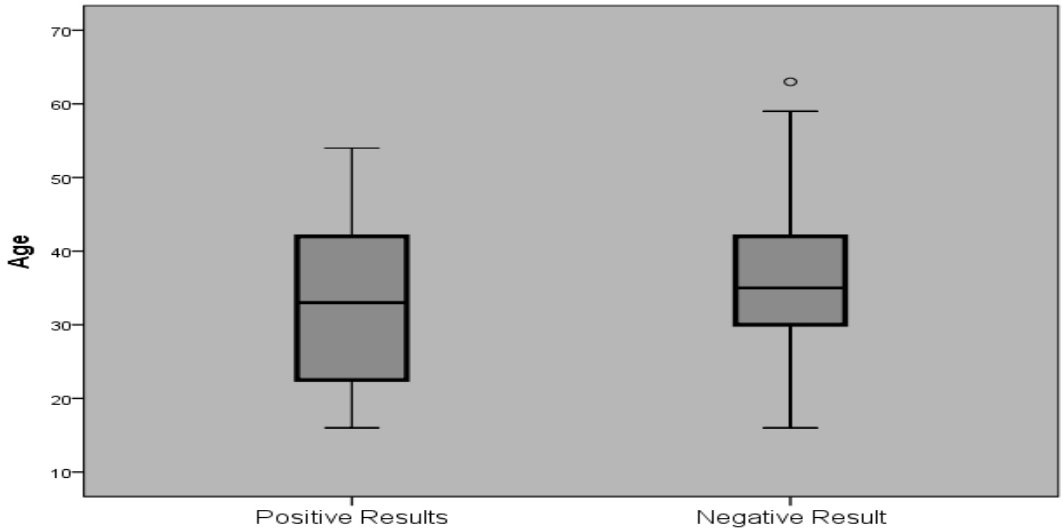

Figure 1: Parameters of age according to results of cultures

The analysis age according to culture results, there was a positive culture: Range; 16 -54, Mean; 33.63 ±SD; 12.159, Median; 33. On the other hand, the negative culture: 16 -63, Mean; 35.69 ±SD; 9.365, Median; 35.0. The results indicate no significant difference between positive and negative Salmonella culture results in relate to workers age (P = 0.432).

2. Work characteristics of the study population :

2.1. Type of work

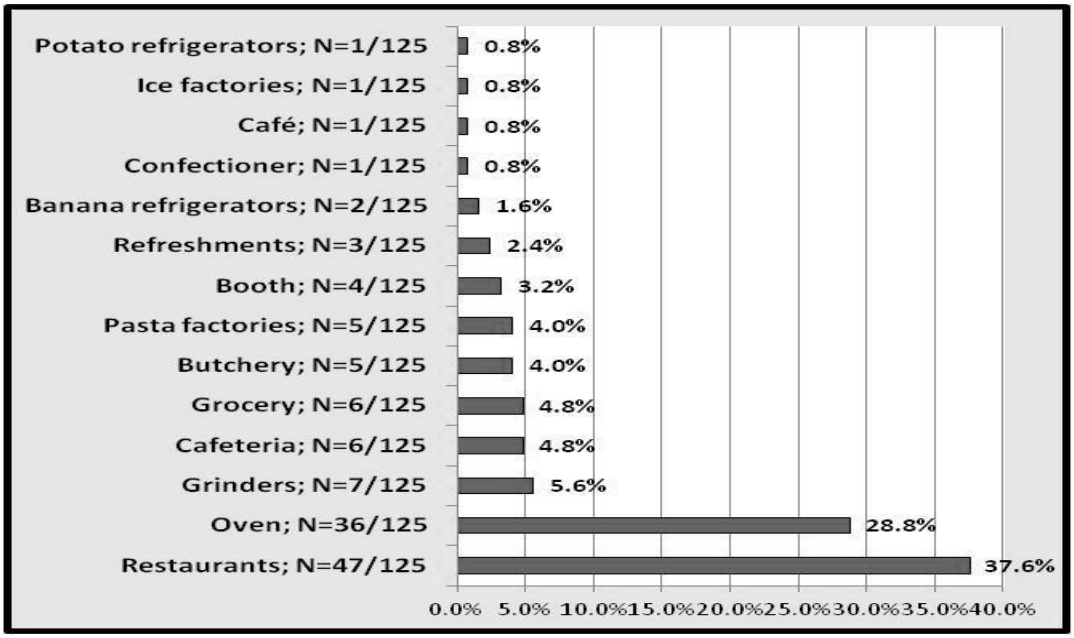

Figure 2: Distribution of the study population according to type of work .

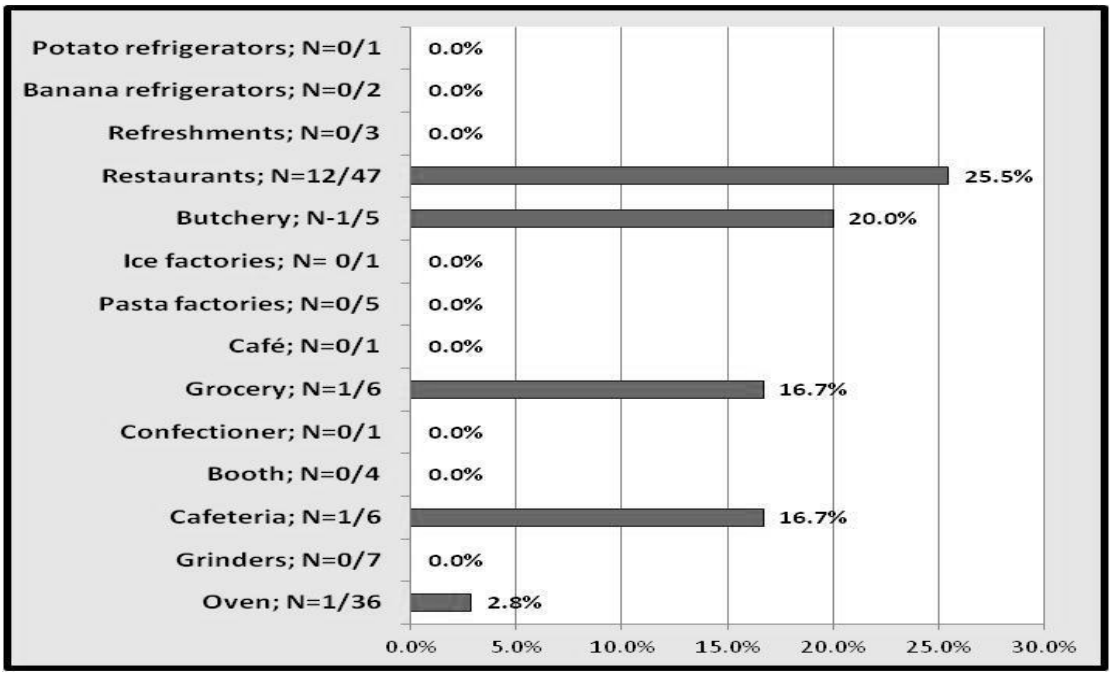

Figure 3: Comparison of rates of positive culture according to type of work.

Pearson Chi-Square 14.128 $P = 0.365$ (Non-significant)

2.2. Work experience:

Work experience of the participants ranged between 1 and 29 years. With mean of 7.5 years(SD=6.8 years) and median of 5 years.

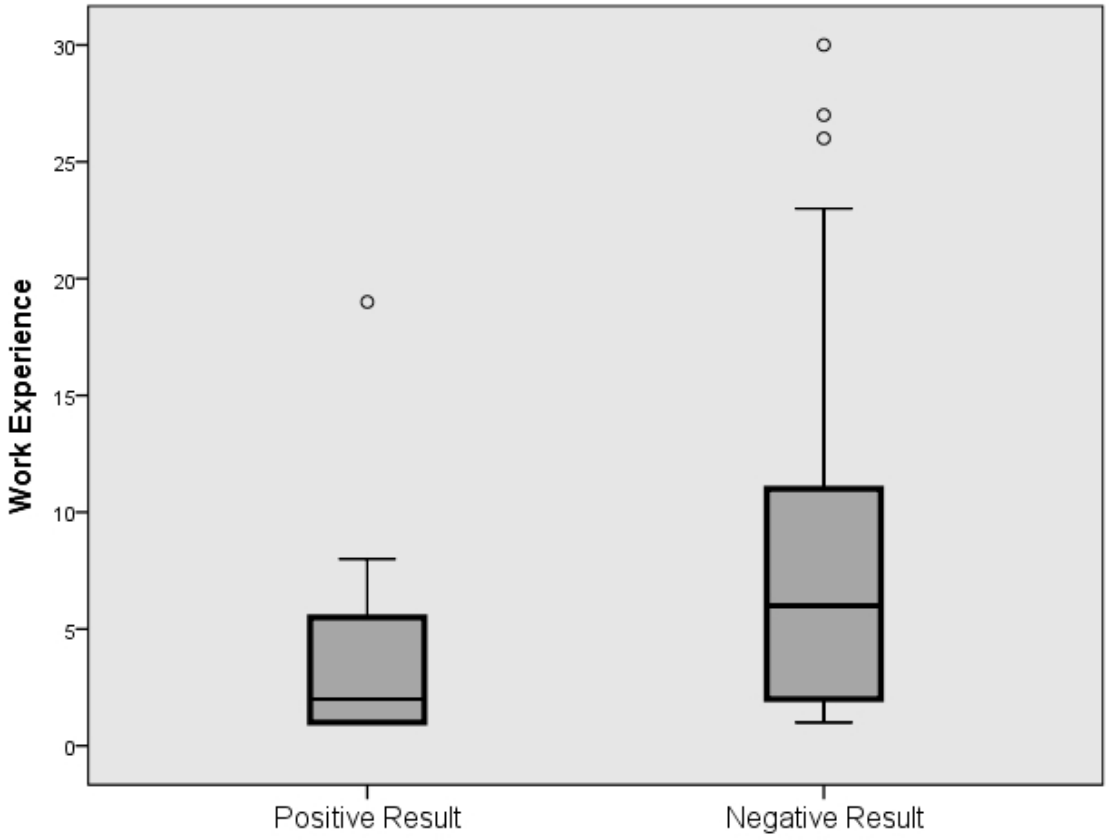

Figure 4: Comparison of years of work experience according to culture result

The analysis of duration in years of working experience and culture results, there was a positive culture: Range 1-19, Mean 4.00±SD 4.619, Median 2.00. On the other hand, the negative culture: Range 1-30 , Mean 7.95±SD 6.939, Median 6.00. The results indicate a significant difference between positive and negative Salmonella culture results in favor for workers with more work experiences (P = 0.008).

2.3. Work place :

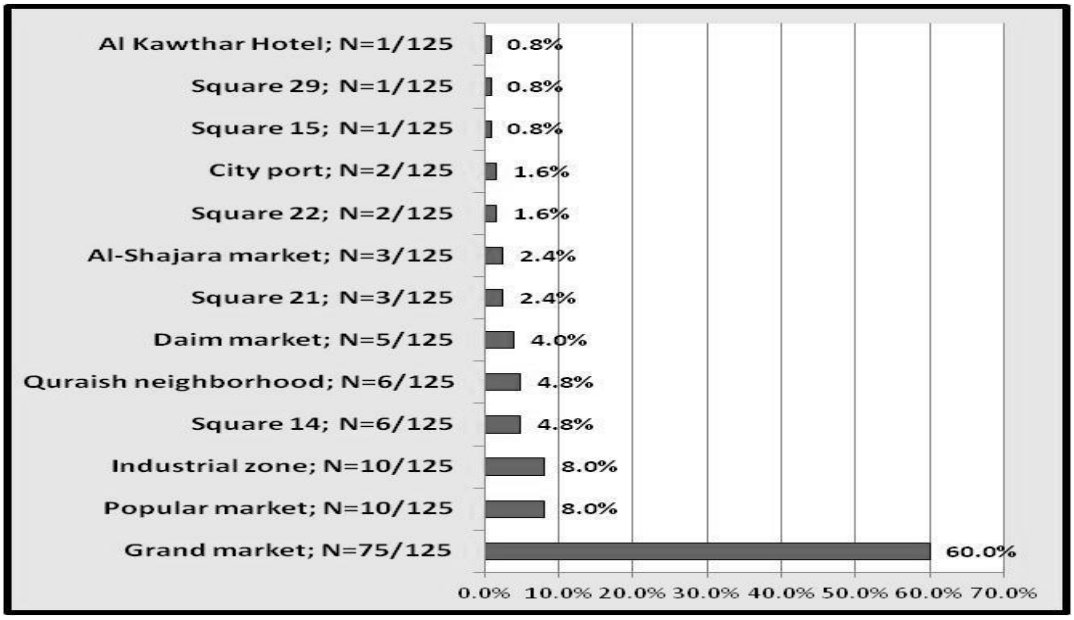

Figure 5: Distribution of the study population according to place of work .

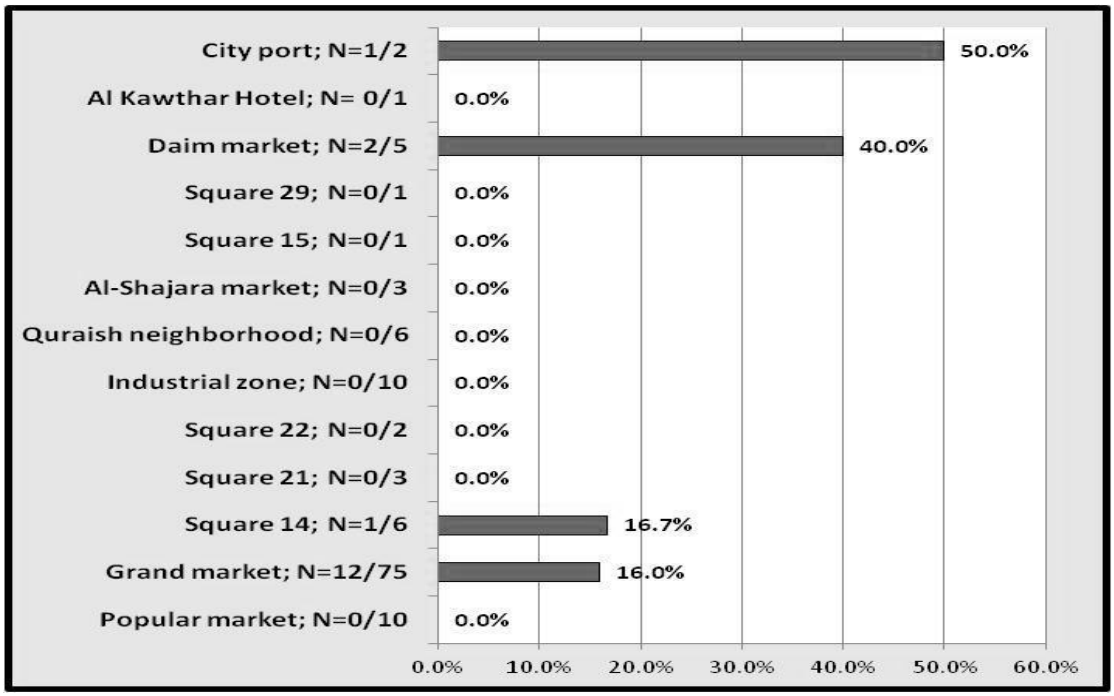

Figure 6: Comparison of rates of positive culture according to place of work

The Chi-Square test indicated there was no significant difference between positive Salmonella culture and place of work (Chi-square=11.994, P=0.446).

2.4. Monthly income:

Table (4) : Monthly Income associated with Salmonella Culture Results of food handlers :

Monthly Income	Sample Culture Results		
	Positive culture	**Negative culture**	**Total**
Satisfactory	12 (11.1%)	96(88.9 %)	108 (100.0 %)
Unsatisfactory	4 (23.5%)	13(76.5%)	17(100.0 %)
Total	16 (12.8%)	109(87.2%)	125(100.0%)

Fisher's Exact Test P = 0.231(Non-significant)

The Fisher's Exact test indicated there was no significant difference between participants monthly income and positive Salmonella culture results (P=0.23).

3. Health Training and knowledge of food handlers:

Table (5) : Training and knowledge of food handlers associated with Salmonella Culture Results

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
1.	**Food safety training before**				
	Yes	0 (0.0%)	15 (100.0 %)	15 (100.0 %)	**Likelihood Ratio X2 4.398 P = 0.036, Fisher's Exact Test; P = 0.214 (Equivocal result)**
	No	16 (14.5%)	94 (85.5%)	110 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Knowledge about food borne infection.**				
	Yes	2 (2.9 %)	67 (97.1 %)	69 (100.0 %)	**Pearson Chi-Square 13.528**
	No	14 (25.0 %)	42 (75.0 %)	56 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	***P* <0.001**
3.	**Knowledge that man is the most important source of food contamination.**				
	Yes	2 (2.9 %)	67 (97.1 %)	69 (100.0 %)	Pearson Chi-Square
	No	14 (25.0 %)	42 (75.0 %)	56 (100.0 %)	13.528
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	*P* <0.001
4.	**Knowledge about law and certificate.**				
	Yes	9 (8.2 %)	101 (91.8 %)	110 (100.0 %)	Fisher's Exact Test
	No	7 (46.7 %)	8 (53.3 %)	15 (100.0 %)	*P* = 0.001
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The Fisher's and Pearson Chi-Square test indicated that there is significant association between lack of training and knowledge with positive Salmonella culture results.

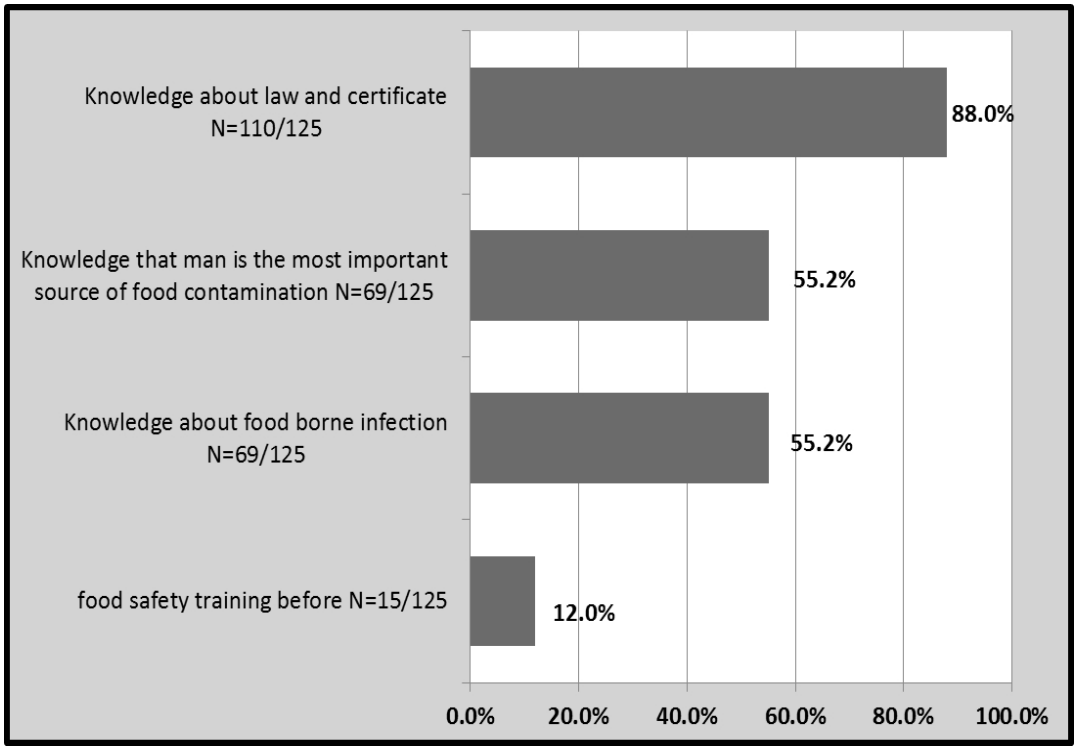

Figure 7: Distribution of the study population according to food safety training and knowledge .

4. Health Status of Food handler :

Table (6) : Health Status associated of food handler with Salmonella Culture Results

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
1.	**Fever in last 30 days**				
	Yes	3 (42.9 %)	4 (57.1 %)	7 (100.0 %)	**Fisher's Exact Test P=0.044**
	No	13 (11.0 %)	105 (89.0 %)	118 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Diarrhea in the last 30 days**				
	Yes	8 (47.1 %)	9 (52.9 %)	17 (100.0 %)	**Fisher's Exact Test P <0.001**
	No	8 (7.4 %)	100 (92.6 %)	108 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
3.	**Vomiting in the last 30 days**				
	Yes	2 (50.0 %)	2 (50.0 %)	4 (100.0 %)	**Fisher's Exact Test P=0.079**
	No	14 (11.6 %)	107 (88.4 %)	121 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
4.	**Stomach ache in the last 30 days**				
	Yes	8 (47.1 %)	9 (52.9 %)	17 (100.0 %)	**Fisher's Exact Test P <0.001**
	No	8 (7.4 %)	100 (92.6 %)	108 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
5.	**headache in the last 30 days**				
	Yes	3 (42.9 %)	4 (57.1 %)	7 (100.0 %)	**Fisher's Exact Test P=0.044**
	No	13 (11.0 %)	105 (89.0 %)	118 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
6.	**Blood in stool**				
	Yes	3 (50.0 %)	3 (50.0 %)	6 (100.0 %)	**Fisher's Exact Test P=0.027**
	No	13 (10.9 %)	106 (89.1 %)	119 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
7.	**Medical Examinations during the recent period**				
	Yes	2 (28.6 %)	5 (71.4 %)	7 (100.0 %)	**Fisher's Exact Test P=0.220**
	No	14 (11.9 %)	104 (88.1 %)	118 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
8.	**Received treatment recently**				
	Yes	2 (50.0 %)	2 (50.0 %)	4 (100.0 %)	**Fisher's Exact Test P=0.079**
	No	14 (11.6 %)	107 (88.4 %)	121 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The fisher’s test indicated there is significant association between health status. (Fever, Diarrhea, stomach ache, headache and Blood in stool in the last 30 days) and positive Salmonella culture results (P=0.044, P <0.001, P<0.001, P=0.044 and P=0.027, respectively).

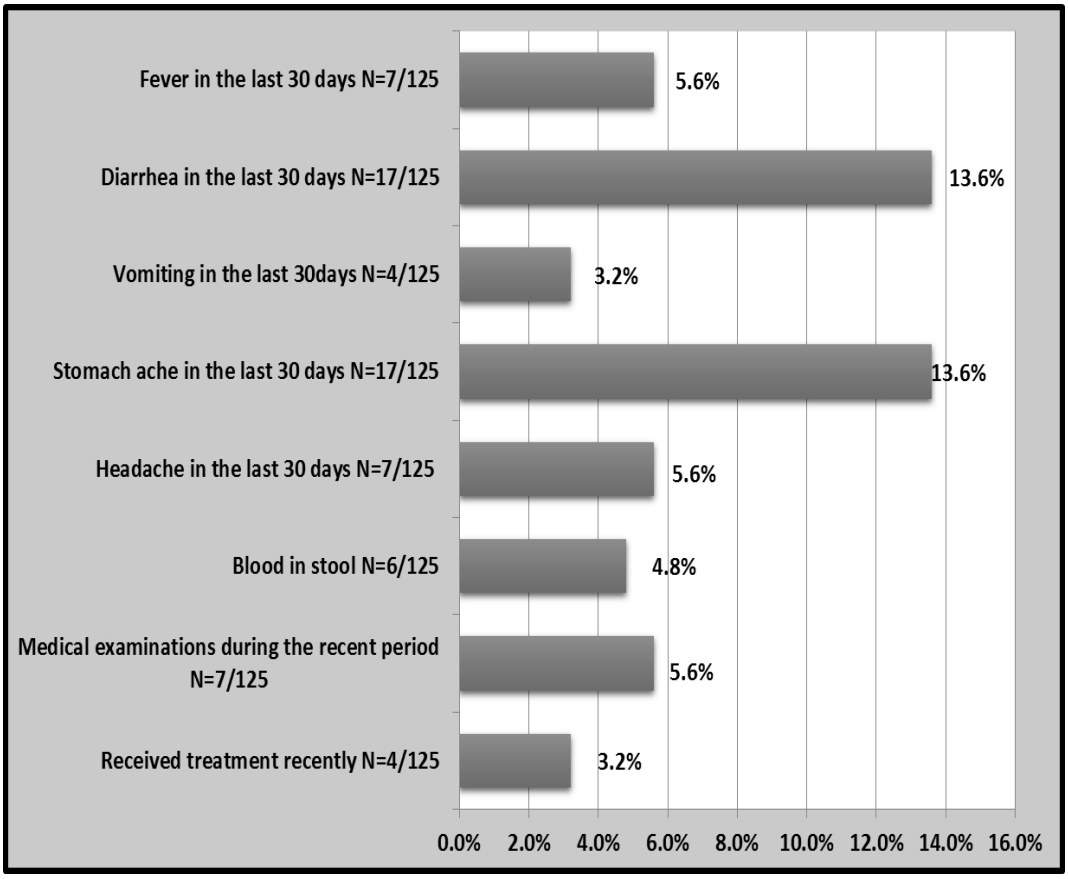

Figure 8: Distribution of the study population according to health status

5. Hygienic practices of the food handlers

5.1. Hygienic Practice Related to Personal Hygiene:

Table (7): Personal Hygiene of food handler associated with Salmonella Culture Results.

No.	Factors	Salmonella Culture Results			Statistical index
		Positive culture	Negative culture	Total	
1.	**Wearing of special clothes for the work**				
	Yes	1 (3.0 %)	32 (97.0 %)	33 (100.0 %)	**Fisher's Exact Test P=0.067**
	No	15 (16.3 %)	77 (83.7 %)	92 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Wearing of clean clothes and unspecial for the work**				
	Yes	15 (16.5 %)	76 (83.5 %)	91 (100.0 %)	**Fisher's Exact Test P=0.067**
	No	1 (2.9 %)	33 (97.1 %)	34 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
3.	**Covering of the hair**				
	Yes	2 (4.0 %)	48 (96.0 %)	50 (100.0 %)	**Pearson Chi-Square 5.782 P=0.016**
	No	14 (18.7 %)	61 (81.3 %)	75 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
4.	**Cut the fingernails**				
	Yes	5 (4.7 %)	101 (95.3 %)	106 (100.0 %)	**Fisher's Exact Test P <0.001**
	No	11 (57.9 %)	8 (42.1 %)	19 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
5.	**General body cleanliness**				
	Yes	5 (5.1 %)	93 (94.9 %)	98 (100.0 %)	**Fisher's Exact Test P <0.001**
	No	11 (40.7 %)	16 (59.3 %)	27 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
6.	**The presence of hand wounds uncovered**				
	Yes	6 (75.0 %)	2 (25.0 %)	8 (100.0 %)	**Fisher's Exact Test P <0.001**
	No	10 (8.5 %)	107 (91.5 %)	117 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
7.	**The presence of hand wounds covered**				
	Yes	1 (11.1 %)	8 (88.9 %)	9 (100.0 %)	**Fisher's Exact Test P=1.000**
	No	15 (12.9 %)	101 (87.1 %)	116 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
8.	**The presence of nose secretions**				
	Yes	3 (100.0 %)	0 (0.0 %)	3 (100.0 %)	**Fisher's Exact Test P=0.002**
	No	13 (10.7 %)	109 (89.3 %)	122 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
9.	**Health certificate**				
	Yes	14 (11.7 %)	106 (88.3 %)	120 (100.0 %)	**Fisher's Exact Test P=0.122**
	No	2 (40.0 %)	3 (60.0 %)	5 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The Fisher's and Pearson Chi-Square test indicated that there is significant association between most items of personal hygiene practices (uncovering of the hair, non-Cutting fingernails, unsatisfactory general body cleanliness, The presence of hand wounds uncovered and The presence of nose secretions) with positive Salmonella culture results (P=0.016, P<0.001, P<0.001, P<0.001 and P=0.02 respectively).

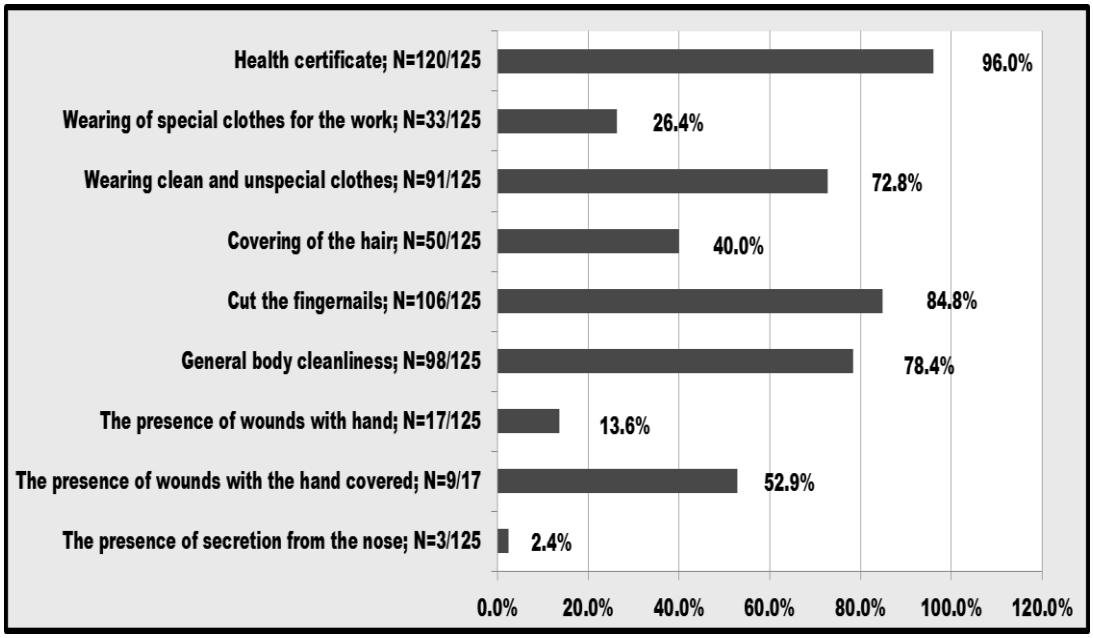

Figure 9: Distribution of the study population according to personal hygiene

5.2. Concern with Health Practices

5.2.1. Hand washing practices:

Table (8): Hand washing practice of food handler associated with Salmonella Culture Results

No.	Factors	Salmonella Culture Results			Statistical value
		Positive culture	Negative culture	Total	
1.	**Washing hands with water only**				
	Yes	16 (20.5 %)	62 (79.5 %)	78 (100.0 %)	**Pearson Chi-Square 11.056 P=0.001**
	No	0 (0.0 %)	47 (100.0 %)	47 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Washing hands with water and soap**				
	Yes	0 (0.0 %)	46 (100.0 %)	46 (100.0 %)	**Pearson Chi-Square 10.684 P=0.001**
	No	16 (20.3 %)	63 (79.7 %)	79 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
3.	**Washing hands before starting work**				
	Yes	0 (0.0 %)	99 (100.0 %)	99 (100.0 %)	**Fisher's Exact Test P < 0.001**
	No	16 (61.5 %)	10 (38.5 %)	26 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
4.	**Washing hands after going to toilet**				
	Yes	15 (12.1 %)	109 (87.9 %)	124 (100.0 %)	**Fisher's Exact Test P=0.128**
	No	1 (100.0 %)	0 (0.0 %)	1 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
5.	**Washing hands after blowing nose**				
	Yes	0 (0.0 %)	57 (100.0 %)	57 (100.0 %)	**Pearson Chi-Square 15.380 P< 0.001**
	No	16 (23.5 %)	52 (76.5 %)	68 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
6.	**Washing hands after touching body parts**				
	Yes	0 (0.0 %)	17 (100.0 %)	17 (100.0 %)	**Fisher's Exact Test P=0.125**
	No	16 (14.8 %)	92 (85.2 %)	108 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
7.	**Washing hands after touching any objects**				
	Yes	0 (0.0 %)	28 (100.0 %)	28 (100.0 %)	**Fisher's Exact Test P=0.022**
	No	16 (16.5 %)	81 (83.5 %)	97 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The Fisher's and Pearson Chi-Square test indicated that there is significant association between hand washing practice (washing hands with water only, Washing hands with water and soap, washing hands before starting work, Washing hands after blowing nose and washing hands after touching any objects) with positive Salmonella culture results.($P=0.001$,$P=0.001$, $P<0.001$,$P<0.001$ and $P=0.022$, respectively).

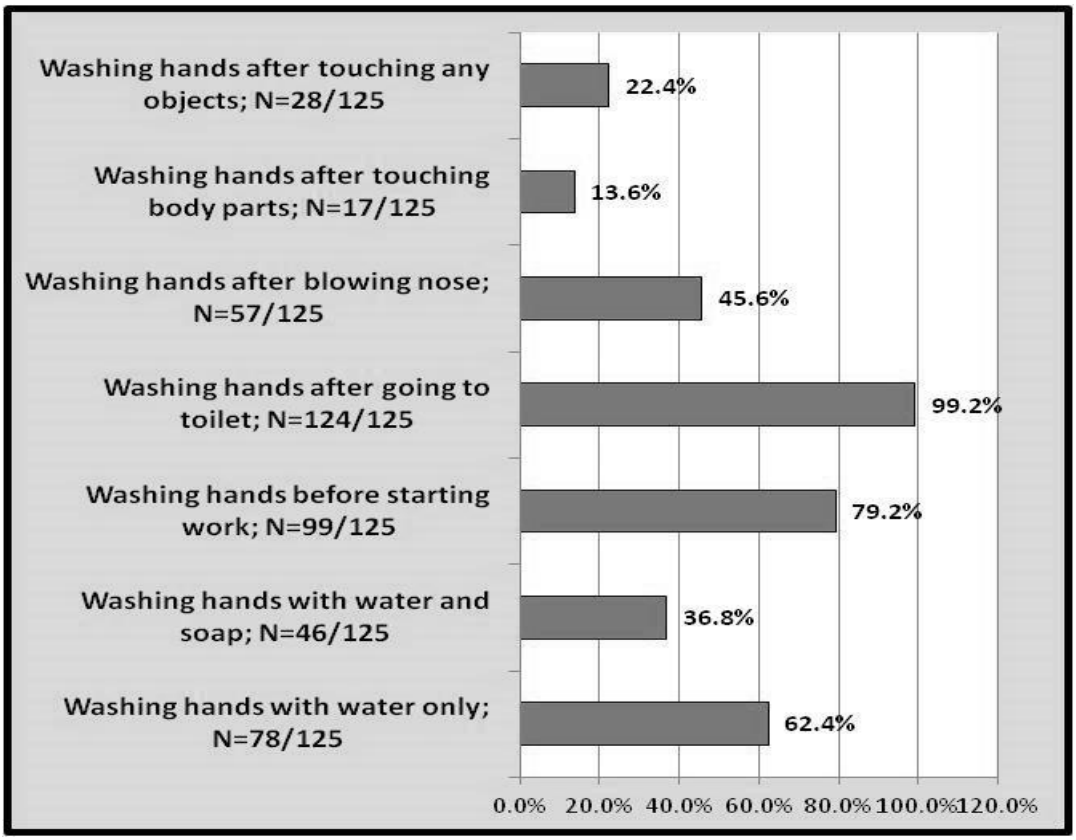

Figure 10: Distribution of the study population according to hand washing practice

5.2.2. Other Hygienic practices: Analysis of other hygienic practice during Work:

Table (9) : Other Hygienic practice during Work factor associated with Salmonella Culture Results of food handler

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Statistical test	Total	
1.	**Shaking hands while working**				
	Yes	13 (17.8 %)	60 (82.2 %)	73 (100.0 %)	**Pearson Chi-Square 3.943 P=0.047**
	No	3 (5.8 %)	49 (94.2 %)	52 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Wear nail polish when handling food**				
	Yes	0 (0.0 %)	2 (100.0 %)	2 (100.0 %)	**Fisher's Exact Test P=1.000**
	No	16 (13.0 %)	107 (87.0 %)	123 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
3.	**Wearing rings and watch or other similar items**				
	Yes	12 (15.4 %)	66 (84.6 %)	78 (100.0 %)	**Pearson Chi-Square 1.242 P=0.265**
	No	4 (8.5 %)	43 (91.5 %)	47 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
4.	**Biting nail with your teeth**				
	Yes	8 (50.0 %)	8 (50.0 %)	16 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	8 (7.3 %)	101 (92.7 %)	109 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Negative culture	Total	
5.	**Keeping food in the place designated for it away from contamination**				
	Yes	6 (5.4 %)	106 (94.6 %)	112 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	10 (76.9 %)	3 (23.1 %)	13 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
6.	**Avoiding Sneezing and coughing on food**				
	Yes	1 (1.0 %)	100 (99.0 %)	101 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	15 (62.5 %)	9 (37.5 %)	24 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
7.	**Smoker?**				
	Yes	9 (14.8 %)	52 (85.2 %)	61 (100.0 %)	**Pearson Chi-Square 0.408 P=0.523**
	No	7 (10.9 %)	57 (89.1 %)	64 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
8.	**Avoiding Smoking on food or near food**				
	Yes	7 (7.4 %)	88 (92.6 %)	95 (100.0 %)	**Fisher's Exact Test P=0.003**
	No	9 (30.0 %)	21 (70.0 %)	30 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The Fisher's and Pearson Chi-Square test indicated that there is significant association between other hygienic practice during work factor with positive Salmonella culture results of food handler. The following elements showed significant; Shaking hands while working, Biting nail with your teeth, Keeping food in the place designated for it away from contamination, Avoiding Sneezing and coughing on food and Avoiding Smoking on food or near food showed significant association with sample culture results (P=0.047, P<0.001, P<0.001, P<0.001 and P=0.003, respectively).

However, certain elements did not show significant association; Wear nail polish when handling food, Wearing rings and watch or other similar items, Smoker? (P=1.000, P=0.265 and P=0.523 respectively).

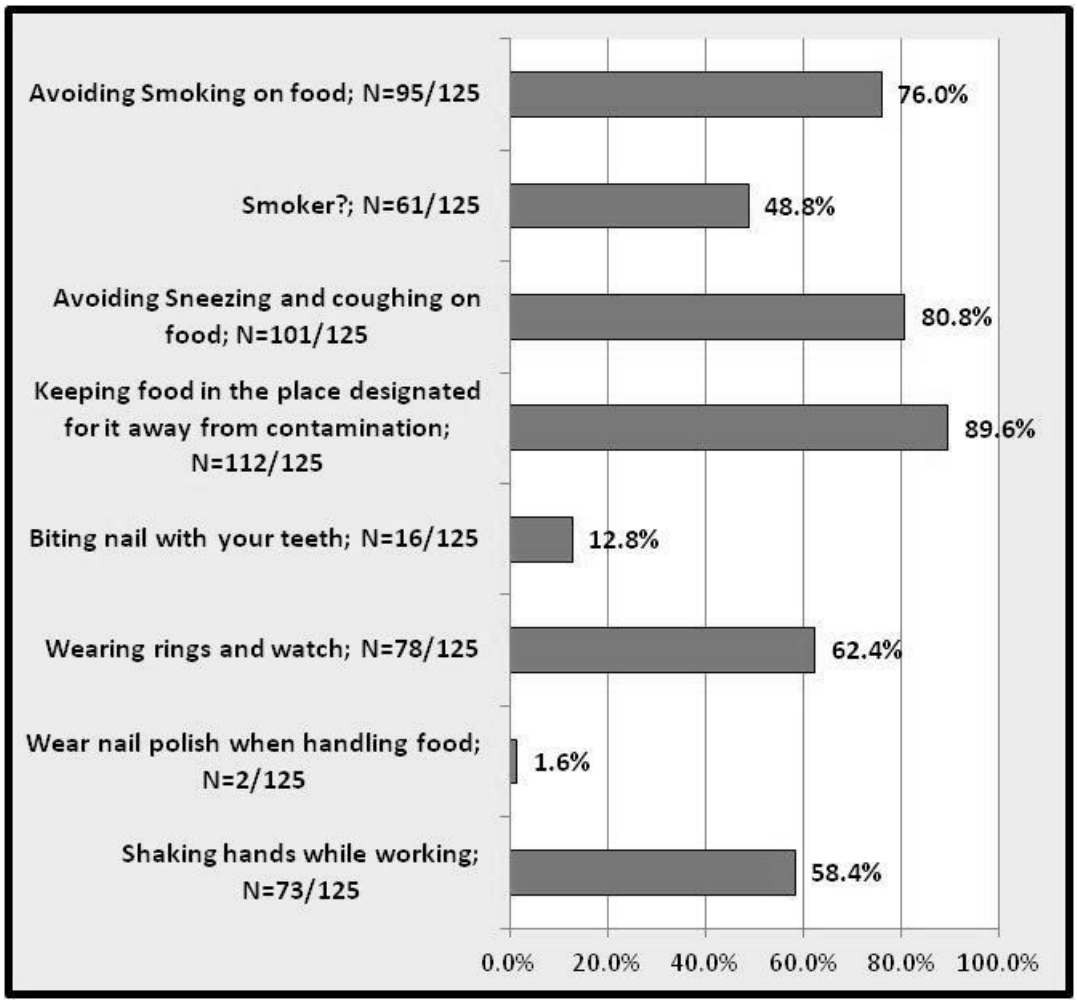

Figure 11: Distribution of the study population according to hygienic practice

6. Workplace Health Conditions:

Table (10) Workplace Health Conditions of food handler associated with Salmonella Culture Results

No.	Factors	Salmonella Culture Results			Statistical test
		Positive culture	Statistical test	Total	
1.	**The Building is in good condition**				
	Yes	15 (12.2 %)	108 (87.8 %)	123 (100.0 %)	**Fisher's Exact Test P=0.241**
	No	1 (50.0 %)	1 (50.0 %)	2 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
2.	**Sanitation of Work Surfaces**				
	Yes	0 (0.0 %)	43 (100.0 %)	43 (100.0 %)	**Pearson Chi-Square 9.622 P=0.002**
	No	16 (19.5 %)	66 (80.5 %)	82 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
3.	**Cleaning of Instrument and Equipment**				
	Yes	6 (5.6 %)	101 (94.4 %)	107 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	10 (55.6 %)	8 (44.4 %)	18 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
4.	**Cleaning of Working Place**				
	Yes	3 (2.9 %)	101 (97.1 %)	104 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	13 (61.9 %)	8 (38.1 %)	21 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
5.	**Daily dispose of Wastes**				
	Yes	3 (2.9 %)	100 (97.1 %)	103 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	13 (59.1 %)	9 (40.9 %)	22 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

No.	Factors	Sample Culture Results			Statistical test
		Positive culture	Statistical test	Total	
6.	**Using the sanitizer when washing utensils**				
	Yes	1 (1.5 %)	65 (98.5 %)	66 (100.0 %)	**Pearson Chi-Square 15.954 P<0.001**
	No	15 (25.4 %)	44 (74.6 %)	59 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
7.	**Use of safe water**				
	Yes	13 (10.7 %)	108 (89.3 %)	121 (100.0 %)	**Fisher's Exact Test P=0.006**
	No	3 (75.0 %)	1 (25.0 %)	4 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
8.	**Presence of a wash basin**				
	Yes	12 (11.9 %)	89 (88.1 %)	101 (100.0 %)	**Fisher's Exact Test P=0.508**
	No	4 (16.7 %)	20 (83.3 %)	24 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
9.	**Insect infestation**				
	Yes	8 (57.1 %)	6 (42.9 %)	14 (100.0 %)	**Fisher's Exact Test P<0.001**
	No	8 (7.2 %)	103 (92.8 %)	111 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	
10	**Availability of refrigerator**				
	Yes	11 (15.9 %)	58 (84.1 %)	69 (100.0 %)	**Pearson Chi-Square 1.362 P=0.243**
	No	5 (8.9 %)	51 (91.1 %)	56 (100.0 %)	
	Total	16 (12.8%)	109 (87.2%)	125 (100.0%)	

The Fisher's and Pearson Chi-Square test indicated that there is significant association between workplace health conditions of food handler with sample culture results. The following elements showed significant;

Sanitation of Work Surfaces, Cleaning of Instrument and Equipment, Cleaning of Working Place, Daily dispose of Wastes, Using the sanitizer when washing utensils, Use of safe water and Insect infestation showed significant association with sample culture results (P=0.002, P <0.001, P <0.001, P<0.001, P<0.001, P=0.006 and P<0.001, respectively). However, certain elements did not show significant association; The Building is in good condition; P=0.241, presence of a wash basin; P=0.508 and availability of refrigerator; P=0.243 .

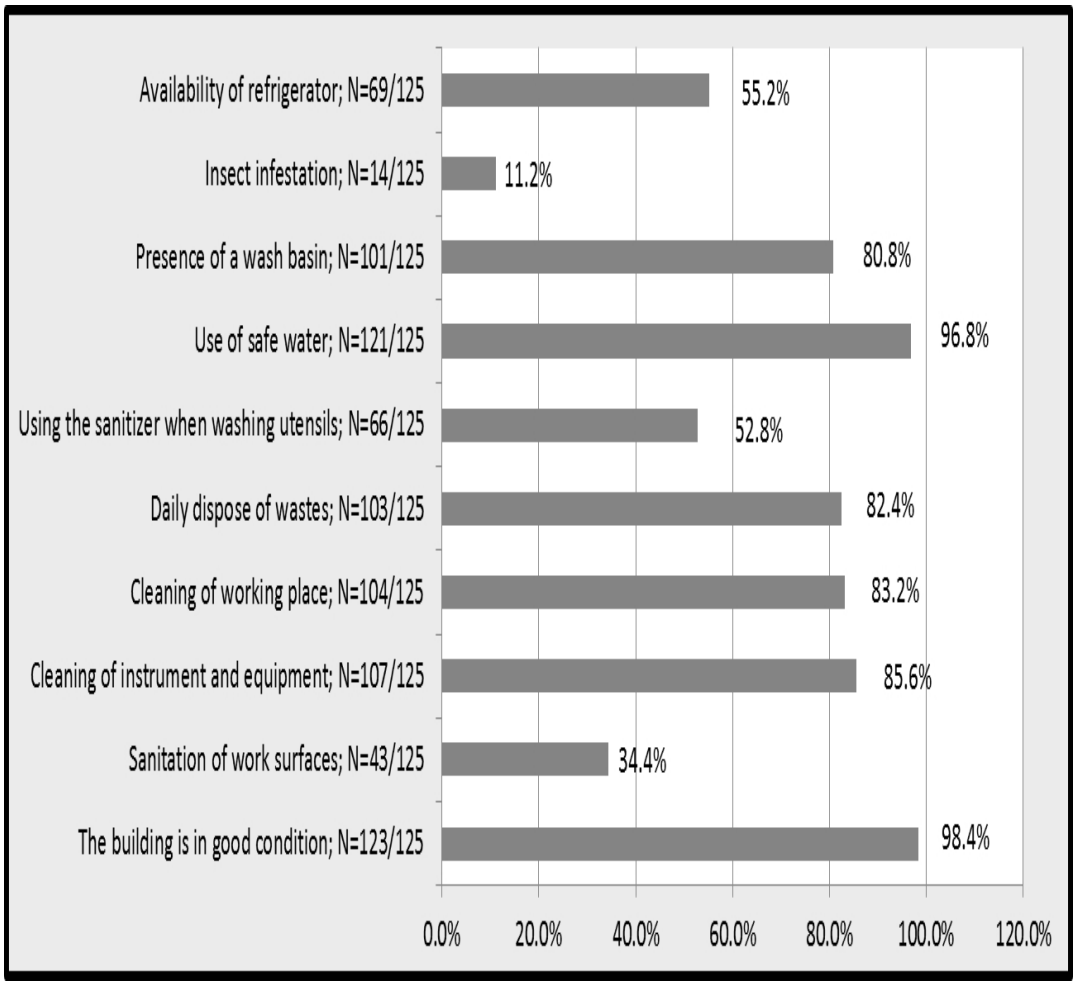

Figure 12: Distribution of the study population according to workplace conditions

Multivariate analysis of the investigated factors:

Table (11) : Multivariate analysis of factors related to positive culture

Factor	B	Wald X^2	*P*
Being Married	4.052	0.000	1.000
Educated Secondary and Beyond	5.181	0.000	1.000
Work Experience	0.184	0.000	1.000
Knowledge about FBI	-21.024	0.000	0.999
Knowledge certificate	1.321	0.000	1.000
Diarrhea last30 Days	-2.584	0.000	1.000
Headache last30 Days	-26.062	0.000	1.000
Blood in stool	-37.724	0.000	1.000
Hair cover	-3.095	0.000	1.000
Nail cut	-4.879	0.000	1.000
Body cleanliness	-3.578	0.000	1.000
Wounds uncovered	4.065	0.000	1.000
Eye secretions	59.345	0.000	1.000
Hand Wash Water only	-0.479	0.000	1.000
Hand Wash Water and Soap	-3.757	0.000	1.000
Hand Wash Before work	-20.434	0.000	1.000
Hand Wash After toilet	-21.248	0.000	1.000
Hand Wash After nose blow	2.816	0.000	1.000
Hand Wash After touching body	-0.221	0.000	1.000
Hand Wash After touching objects	-13.738	0.000	1.000
Shaking hands	2.923	0.000	1.000
Biting nails	-4.909	0.000	1.000
Safe food keeping	-34.744	0.000	1.000
Avoid sneeze and cough	-7.872	0.000	1.000
Avoid smoking	-4.792	0.000	1.000
Surfaces sanitizing	-0.775	0.000	1.000
Equipment cleaning	30.974	0.000	1.000
Place cleaning	-16.072	0.000	1.000
Waste disposal daily	-15.443	0.000	1.000
Utensils sanitizing	19.352	0.000	0.999
Safe water	-11.057	0.000	1.000
Infestation	-21.482	0.000	1.000
Refrigerator available	-3.236	0.000	1.000
Constant	34.390	0.000	1.000

No significant independent predictor found.

Patterns of Antimicrobials:

The antimicrobial test of the isolates of Salmonella from the three different sites of carrier showed variation in pattern as susceptible, resistance and intermediate and with different rates according to identification of Salmonella isolates.

Table (12): Frequency and distribution of antimicrobial pattern to *Salmonella typhi*. N=4

Antimicrobial Patterns	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible	**2**	**3**	**0**	**0**	**2**	**1**	**2**
Resistance	**1**	**1**	**4**	**4**	**2**	**3**	**1**
Intermediate	**1**	**0**	**0**	**0**	**0**	**0**	**1**

Salmonella typhi showed susceptible pattern to cotrimoxazole (3/4;75%), ciprofloxacin and ceftriaxone (2/4; 50%), meanwhile all *Salmonella typhi* (4/4; 100%) showed high level of resistance patterns to tetracycline and ampicillin.

Table (13): Frequency and distribution of antimicrobial pattern to *Salmonella Paratyphi* A. N= 5

Antimicrobial Patterns	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible	**2**	**3**	**0**	**3**	**5**	**4**	**0**
Resistance	**2**	**1**	**5**	**0**	**0**	**0**	**0**
Intermediate	**1**	**1**	**0**	**2**	**0**	**1**	**5**

Salmonella Paratyphi A isolates showed susceptible pattern to gentamycin (5/5; 100%) and chloramphenicol (4/5; 80%) meanwhile all Sa*lmonella Paratyphi A* (5/5; 100%) showed high level of resistance patterns to

ampicillin. In addition all *Salmonella Paratyphi A* (5/5; 100%) showed Intermediate pattern level to ciprofloxacin.

Table (14): Frequency and distribution of antimicrobial pattern to *Salmonella species*. N=10

Antimicrobial Patterns	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible	**7**	**7**	**0**	**6**	**8**	**8**	**5**
Resistance	**3**	**3**	**10**	**4**	**1**	**2**	**1**
Intermediate	**0**	**0**	**0**	**0**	**1**	**0**	**4**

Salmonella species other than *Salmonella typhi* and *Salmonella Paratyphi A* showed susceptible patterns with variation in rates for the different antimicrobials as Gentamicin and Chloramphenicol (8/10;80%), cotrimoxazole and ceftriaxone (7/10; 70%), tetracycline (6/10; 60%) and ciprofloxacin (5/10;50%). On the other side all other *Salmonella species* showed high level of resistance pattern to ampicillin (10/10; 100%). Sixteen participants of the food handlers showed positive culture in one site of meanwhile three participants of the food handlers showed positive cultures in two sites (urine +fingernail swabs),(urine +stool),(Fingernail swabs+ stool). (Appendix VII)

5.1. Discussion:

The interpretation of the results revealed high prevalence (n=16/125; 12.8%) of Salmonella carriers among food handlers in Shendi city. Positive culture were detected in (n=19/375; 5.7%) of the collective samples. Pattern sites of frequency and distribution of Salmonella carriers involved stool (n=9/125; 7.2%), urine (n= 4/125; 3.2%) and finger nails (n=3/125; 2.4%).

Patterns of positive stool culture for Salmonella were identified and counted as [*Salmonella spp* (n= 6/125; 4.8%), *Salmonella typhi* (n= 1/125; 0.8%) and *Salmonella Paratyphi A* (n= 2/125; 1.6%) .

The prevalence of Salmonella carriers among food handlers in Shendi city (12.8%) was high than other studies carried in some areas of other countries. The prevalence in Arba Minch University students' cafeteria was (6.9%) [54]. and at Al Neelain University cafeterias in Khartoum was (6.25%) [52]. In Lagos, Nigeria [58] the prevalence was (5.7% for *S. typhi*) among healthy food handlers. In Jirapa, Ghana [53] the prevalence was (4.1%) with most isolates belonged to *Salmonella typhi* (71.4%). In North west Ethiopia [48] the prevalence was (3.6%) meanwhile In Addis Ababa, Ethiopia [56] the prevalence was (3.4%). In Malaysia [51] the prevalence was (2.8%) among total of 317 Asian migrant food handlers. and in India [49]. the prevalence of *Salmonella typhi* was 0.7% among 300 asymptomatic food handlers.

Patterns of positive fingernails swab culture for Salmonella were identified and counted as *Salmonella spp* (n=1/125; 0.8%). *Salmonella typhi* (n=2/125; 1.6%) and *Salmonella Paratyphi* (n= 3/125 ; 2.4%).These rates were significant in relation to the study at Debre Markos University, Northwest Ethiopia [48] as none of the food handlers fingernail specimens

had positive cultures for Salmonella. However, it would be very low when compared to results reported in a study [50] at Kuala Lumpur as Salmonella was detected on 48% of the food handlers hands. The differences in techniques might be of importance in those results. Nevertheless, it is worth emphasis that the good level of hand hygiene practices in the present study may be also worthy.

Patterns of positive urine culture for Salmonella were identified and counted as *Salmonella spp* in (3/125 ; 2.4%) and *Salmonella typhi* in (1/125;0.8%).

Collectively, positive culture results were found in (n=16/125 ; 12.8%) participants, which confers a relatively high prevalence among food handlers in the city.

Patterns of antimicrobials:

In the present study, the antimicrobial patterns of Salmonella revealed variation in percentage of susceptibility and resistance. The isolated Salmonella show susceptible to gentamycin, chloramphenicol, cotrimoxazole, tetracycline, ceftriaxone. All isolates were resistant to ampicillin and all isolates were resistant at least to one of the antimicrobials tested. (ceftriaxone, cotrimoxazole, tetracycline, gentamycin, chloramphenicol, ciprofloxacin).

According to identification of the isolates of Salmonella antimicrobial patterns, *Salmonella typhi* is susceptible to cotrimoxazole (3/4 ;75%), ciprofloxacin, gentamicin and ceftriaxone (2/4; 50%), and resistance to ampicillin and tetracycline (4/4,100%). Multidrug-resistant and fluoroquinolone-resistant strains of *Salmonella typhi* have been reported from various parts of India and created a major therapeutic problem [20].

Salmonella Paratyphi A is susceptible to gentamicin (5/5; 100%), chloramphenicol (4/5; 80%), and resistance to ampicillin (5/5 ;100%).

Other *Salmonella species* is susceptible to chloramphenicol and gentamicin (8/10; 80%), cotrimoxazole and ceftriaxone (7/10 ;70%), tetracycline (6/10; 60%) and ciprofloxacin(5/10 ;50%), meanwhile resistance to ampicillin (10/10 ;100%) Such findings comply with the studies conducted in Ghana[53] , In Malaysia [51] and In Addis Ababa [56].

Emergences of new Salmonella strains or strains that have anti-microbial drug resistance contribute to the concern regarding imported food. Anti-microbial drug resistant Salmonella infections are associated with an increased hospitalization rate, morbidity, and mortality.

In review of patterns of socio-demographic data, the gender showed no significant difference in the rate of positive Salmonella culture which was not concordant with the study of Salmonella carrier states among food handlers of Addis Ababa university students cafeterias [56], where all Salmonella positive food handlers were females, this might be due to the better education of Sudanese females. In regarding marital status, the rate of positive culture was higher among the unmarried participants (n=8/27; 29.6%) than married ones (n= 8/98; 8.2%). The result was statistically significant (P = 0.007) and this may reflect the role of marital support in keeping the personal hygiene.

Additionally, regarding the educational level, (n=32/125; 25.6%) participants were illiterate, (n= 45/125; 36.0%) were educated at primary level, (n= 35/125; 28.0%) were secondary level educated and the rest (n=13/125; 10.4%) were university graduates. No positive results were reported among those with secondary education or beyond. On the other hand, the positive culture rates (n= 7/45; 15.6%) were primary school

graduates and (n= 9/32; 28.1%) were among illiterates. The difference was statistically significant (*P*= 0.001). The current study outcomes indicated that the higher the education the stronger awareness and adherence to food hygiene protocols.

Patterns of positive Salmonella culture among food handlers was distributed according to the work characteristics (Type of work, work place, work experience), and were presented in 5 types of food services establishments; restaurant workers (n=12/47; 25.5%), butchery worker (n=1/5; 20.0%), grocery worker (n=1/6; 16.7%), cafeteria worker (n=1/16; 16.7%) and oven worker (n=1/36; 2.8%). Anyhow, this difference was not statistically significant. In addition, pattern of positive Salmonella culture among food handlers for the work place were confirmed only in the four locations areas; City port (n=1/2; 50%), Daim market (n=2/5; 40%), Square 14 (n=1/6; 16.7%) and Grand market (n=12/75; 16%). The distribution was not found statistically significant. Nevertheless, it should be more investigation to find out the factors might be behind this. Nonetheless, those with positive culture in the work experience ranged between 1 and 19 years with Mean = 4.00±SD 4.6 years, and Median = 2.0 years. While those with negative salmonella Culture, the ranged between 1 to 30 years and Mean= 7.95±SD 6.9 years and Median= 6.0 years. The longer experience among participants with negative culture was statistically significant (*P* = 0.008). This is consistent with the study at Debre Markos University, Northwest Ethiopia [48] where the isolation of Salmonella had a significant inverse relationship with the number of service years (*P* = 0.017).

The outcomes in this current study document that income satisfaction was not found to be statistically associated with different rate of positive culture.

Furthermore, when observation the training and knowledge in the field of food safety, (n=110/125; 88.0%) of the participants reported that they had knowledge about law and certificate in food hygiene, (n= 69/125 ; 55.2%) reported that they had knowledge that man is the most important source of food contamination and similar proportion reported that they had knowledge about food borne infection. Only (n=15/125; 12%) received a structured training about food safety. All of these factors were found to be significantly associated with positive culture results (P <0.001 for the first three and = 0.036 with likelihood ratio for the last). This is not concordant with the study conducted in Lagos Southwest Nigeria [55] Regression analysis concluded no significant association between food handlers knowledge and their previous infection. This may reflect the cultural difference or the difference in training program contents and conduction[55].

For the present study Additionally, when assessing the health status of the participants, (n=17/125; 13.6%) reported abdominal pains and similar proportion reported diarrhea, while less proportions reported fever (n=7/125; 5.6%), headache (n=7/125; 5.6%) and blood in the stool (n=6/125 ; 4.8%). Moreover, the rates of positive culture were higher among those reported symptoms or sought medical advice. Nonetheless, the differences were statistically significant where following symptoms and signs were reported; Fever in last 30 days, (n =3/7; 42.9%) versus (n=13/118 ; 11.0%) (P =0.044), Diarrhea in the last 30 days,(n =8/17; 47.1%) versus (n =8/108; 7.4%) (P<0.001), Stomach ache in the last 30 days, (n=8/17; 47.1%) versus (n= 8/108 ; 7.4%) (P<0.001), Headache in the last 30 days, (n = 3/7; 42.9%) versus (n =13/118; 11.0%) (P=0.044) and Blood in stool (n= 3/6 ; 50.0%) versus (n= 13/119; 10.9%) (P=0.027). On the other hand, other reported findings did not exhibit statistical significance such as, vomiting in the last 30 days, (n=2/4; 50.0%) versus

(n=8/108; 11.6%) (*P*=0.079). Food handlers had routine medical examinations, (n=2/7; 28.6%) versus (n=14/118; 11.9%) (*P*=0.220) and received treatment recently, (n=2/4; 50.0%) versus (n=14/121; 11.6%) (*P*=0.079). Those findings were concordant with the study of Salmonella carriage among food handlers and patients attending Joseph Hospital in Jirapa, Upper West Region where abdominal pains, headache and diarrhea were the common complaints presented by patients [53].

In regarding practice related to personal hygiene, the majority have health certificate (n=120/125 ; 96.0%), while adherence to general cleanliness (n=98/125 ; 78.4%), wearing clean and un-special clothes (n= 91/125; 72.8%), cutting fingernails (n=106/125 ; 84.8%) and covering hair (n=50/125; 40.0%) were less practiced. A small proportion (n= 17/125; 13.6%) had uncovered wounds in their hands, were (n= 3/15 ; 2.4%) had eye secretions. Furthermore, there was a statistically significant higher rates of positive culture related to uncovering of the hair,(n=2/50; 4.0%) versus(n= 14/75; 18.7%) (*P*=0.016), having fingernails cut,(n=5/106 ;4.7%) versus (n=11/19; 57.9%) (*P* <0.001), unsatisfactory general body cleanliness, (n=5/98; 5.1%) versus (n=11/27; 40.7%) (*P* <0.001), the presence of hand wounds uncovered (n= 6/8 ; 75.0%) , (n=10/117 ; 8.5%) (*P* <0.001), the presence of nose secretions, (n=3/3 ; 100.0%) versus (13/ 122 ; 10.7%) (*P*=0.002).

The current study looked at practices related to hand washing**,** near all reported that they wash hands after toilet use (n= 124/125 ; 99.2%). However, the practice of hand washing otherwise seems to be unsatisfactory. The study reported washing hands before starting work accounted for (n= 99/125; 79.2%). When looking at whom practices washing hands after blowing nose was (n=57/125; 45.6%), touching body

parts (n= 17/125 ; 13.6%) and touching any objects was only reported by (n=28/125 ; 22.4%) . also, use of soap with water in hand washing was practiced only by (n=46/125; 36.8%). Furthermore, when looking at the practices of washing hands with water only, (n= 16/78 ; 20.5%) versus 0.0%; (P=0.001), washing hands with water and soap, 0.0% versus (n=16/79 ; 20.3%) (P=0.001). Furthermore, washing hands before starting work, 0.0% versus (n=16/26; 61.5%) (P<0.001), washing hands after going to toilet, (n=15/124; 12.1%) versus (n=1/1; 100.0%); (P=0.128), washing hands after blowing nose, 0.0% versus (n=16/68; 23.5%) ; (P< 0.001), washing hands after touching body parts 0.0% versus (n=16/108 ; 14.8%) P=0.125 and washing hands after touching any objects, 0.0% versus (n=16/97;16.5%); (P=0.022). Those practices reflect adherence and respect to food hygiene roles as general and they are strongly related to positive culture. Multivariate analysis shows no independent predictor for positive culture, this reflects the integration of those factors in the causation of the Salmonella carrier state. The unsatisfactory rates of those practices may contribute to the relatively high rate of carrier state.

Additionally, the current study reported other hygienic practices, and showed that most of participants (n=112/125; 89.6%) reported proper storage of food in safe place. Moreover, a large but still unsatisfactory proportion (n=101/125; 80.8%) reported avoiding sneezing and coughing on food and other (n=95/125; 76.0%) reported avoiding smoking on food. Some bad practices were unfortunately present in a large rates while working like wearing accessories (rings and watch) (n=78/125; 62.4%), shaking hands (n=73/125; 58.4%) and even biting nails (n=16/125;12.8%).

shaking hands while working, (n=13/73; 17.8%) versus (n=3/52; 5.8%; (P=0.047) , biting nail with teeth,(n=8/16; 50.0%) versus (n=8/109; 7.3%;

(P<0.001), keeping food in the place designated for it away from contamination,(n=6/112; 5.4%) versus (n=10/13; 76.9%) (P<0.001), avoiding Sneezing and coughing on food, (n=1/101; 1.0%) versus (n=15/24; 62.5%) (P<0.001) and avoiding Smoking on food,(n=7/95; 7.4%) versus (n=9/30; 30.0%; (P=0.003).

The study at Debre Markos University, Northwest Ethiopia [48], the author suggested that screening of food handlers, training for food handling and hand hygiene practices, and regular monitoring of the food handling practices should be done in order to avoid diseases that can be acquired through improper food handling, like bacterial infections [48] . Furthermore, a study conducted at Kuala Lumpur, Malaysia [50] concluded that the food handlers had adequate food safety knowledge, but perceived knowledge failed to be translated into practices at work. The study in Arba Minch University, South Ethiopia [54] reported that finger nail status, hand washing practice after toilet and touching food with bare hands were independent predictors of infectious enteric diseases among the food handlers. The authors strongly recommended that training on personal and food hygiene should be provided to all food handlers with regular sanitary inspection to improve adherence of food handlers to personal hygiene and food safety practices [54] .

In a study at Addis Ababa University Students' Cafeteria, Ethiopia [56] documented that, hand washing habit after toilet with and without soap had a statistically significant protective association with carrier status of salmonellae (P=0.003) and odds ratio of 0.07 with a 95% confidence interval (0.008, 0.58) [56]. Moreover, in a study conducted in the Federal Capital Territory of Nigeria [57] , the study recommended compulsory and proper treatment of staff with active illness. They also recommended the

practices of disposable rubber gloves, plasters and other first-aid items for management of minor cuts/injuries should be provided for use by the food handlers when necessary [43]. This recommendation was also supported by another study conducted in Nigeria [58] that stated that an inadequate personal hygiene can facilitate the transmission of these pathogens via food to humans [58].

The current study looked at workplace health conditions, were all of the studied workplaces were suitable and licensed. most were in good condition (n=123/125; 98.4%), have safe water supply (n=121/125; 96.8%), cleaning of instrument and equipment (n=107/125; 85.6%), cleaning of working place(n=104/125;83.2%), daily dispose of wastes (n=103/125; 82.4%), using the sanitizer when washing utensils (n=66/125; 52.8%). insect infestation were reported by (n=14/125; 11.2%) and sanitation of work surfaces was practiced in (n=43/125; 34.4).

Additionally, sanitation of work surfaces, 0.0% versus (n=16/82; 19.5%) (p=0.002), cleaning of instrument and equipment,(n=6/107; 5.6%) versus (n=10/18; 55.6%) (p<0.001), cleaning of working place,(n=3/104; 2.9%) versus (n= 13/21; 61.9%; (p<0.001), daily dispose of wastes (n=3/103; 2.9%) versus (n=13/22; 59.1%) (p<0.001), using the sanitizer when washing utensils (n=1/66; 1.5%) versus (n=15/59; 25.4%); (p<0.001), use of safe water (n=13/121; 10.7%) versus (n=3/4; 75.0%; (p=0.006) and insect infestation (n=8/14; 57.1%) versus (n=8/111; 7.2%) ; (p<0.001) were significant risk factors contributed to hygiene status.

ž 5.2. Conclusions;

Salmonella Carriers exist among food handlers and there were significant association of causes and Salmonella carriers. Food handlers who are carriers of Salmonella are considering being one of the main sources of transmission of salmonella infection and food poisoning.

Salmonella infection continues to be a major public health problem in shendi city. as In the current study (n=16/125 ; 12.8%) of food handlers were positive for salmonella.

The food handler's knowledge and practice of personal hygiene standards are vital in prevention of transmission of salmonella.

Being unmarried, with lower education, less work years' experience, no previous training or lack of knowledge on food safety, presence of recent gastrointestinal symptoms, poor hand washing practice, negligence of hygiene at work place were all associated with higher percentage of positive Salmonella culture. The study outcomes ensure the significant association of the causes and Salmonella positive food handlers and indicated the presence of antimicrobial resistance among food handler's carriers. Such outcomes influence the occurrence of epidemic infection and food poisoning with Salmonella.

5.3. Recommendations

Food control authorities at the population and health administration of Shendi locality are recommended to:

- To aware food handlers to pay attention to their health and behavior when handling and processing food.
- To train food handlers on basic principles of food safety and hygiene as part of conferring the license of the work.
- To use a check list that contains health measures and standard for monitoring properly all food handlers with license.
- To apply legislations and laws of food control on food handlers without license.
- To implement screening of salmonella carriers among food handlers and co-operate with other health authorities to follow changes of antimicrobial patterns.
- To incite an electronic register for the health status of the food handlers.
- To hold Health education and training to food handlers about personal hygiene, environmental sanitation regularly.

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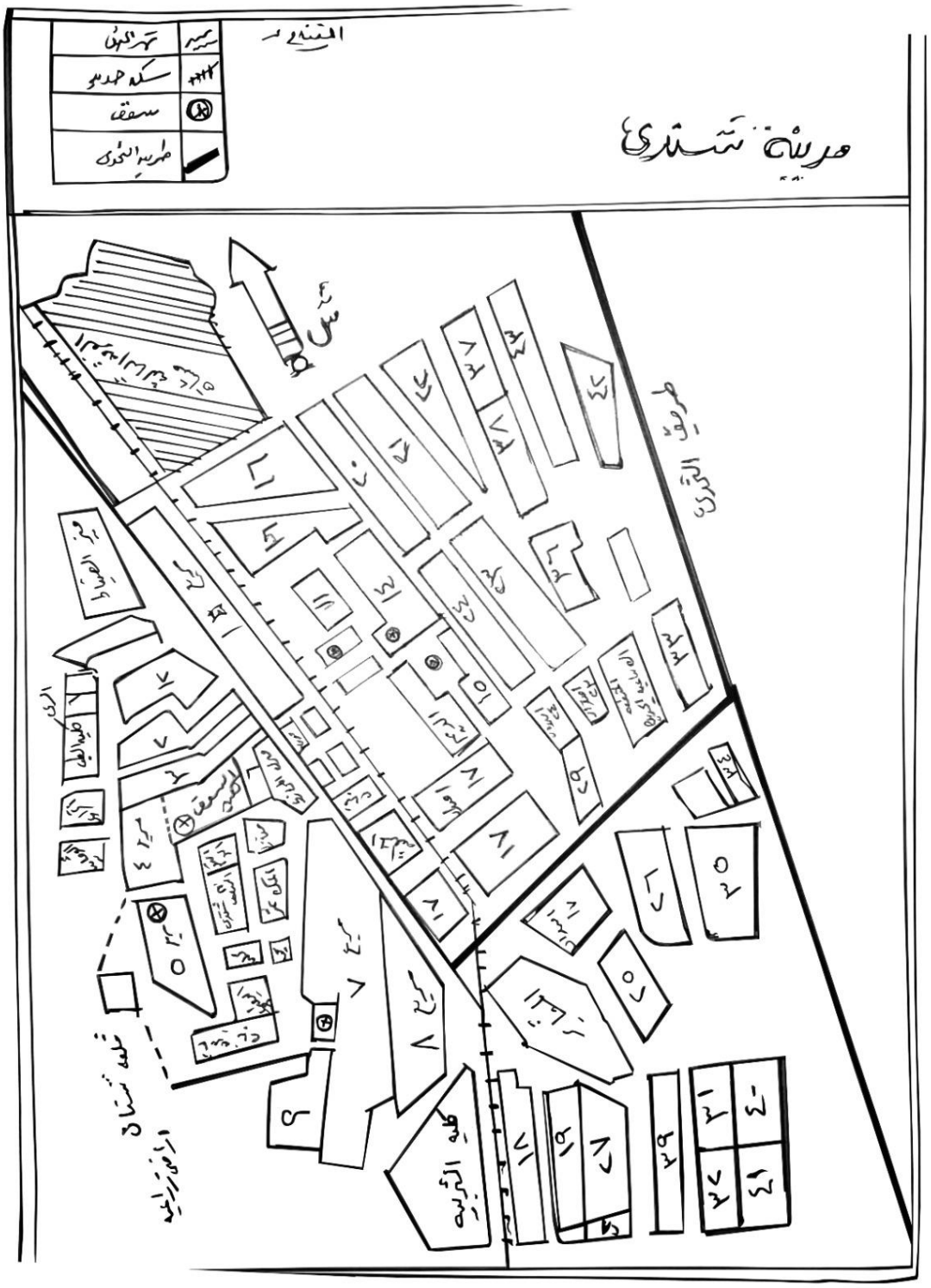
Appendix (I): Study Area.



Appendix (II): Distribution of population of the study and sample size according to food services establishments.

Type of food services establishments	Number of food services establishments	Number of food handlers	Sample Size
Ovens	**51**	**204**	**36**
Grinders	**41**	**41**	**7**
Cafeteria	**9**	**31**	**6**
Booths	**24**	**24**	**4**
Confectioner	**8**	**8**	**1**
Grocers	**33**	**33**	**6**
Cafes	**8**	**8**	**1**
Pasta factories	**3**	**30**	**5**
Ice factories	**2**	**8**	**1**
Butchery	**25**	**25**	**5**
Restaurant	**72**	**268**	**47**
Refreshment shop	**18**	**18**	**3**
Warehouse for Banana refrigerators	**5**	**10**	**2**
Warehouse for potato refrigerators	**3**	**6**	**1**
Total	**302**	**714**	**125**

Appendix (III): Distribution of the sample of the food handlers

Appendix (III-1):
Distribution of oven workers sample:

الموقع	عدد العينة
السوق الكبير	8
سوق الشجرة	3
السوق الشعبي	7
المنطقة الصناعية	1
المربع 14	6
المربع 21	3
المربع 22	2
حي قريش	6
الاجمـــــــالي	36

Appendix (III-2):
Distribution of grinder workers sample:

الموقع	عدد العينة
السوق الكبير	5
السوق الشعبي	1
المربع 15	1
الاجمـــــــالي	7

Appendix (III-3):
Distribution of cafeteria workers sample:

الموقع	عدد العينة
السوق الكبير	3
مدخل المدينة	2
فندق الكوثر	1
الاجمـــــــالي	6

Appendix (III-4):
Distribution of booth workers sample:

الموقع	عدد العينة
سوق الديم	1
المنطقة الصناعية	3
الاجمـــــــالي	4

Appendix (III-5):
Distribution of restaurant workers sample:

الموقع	عدد العينة
السوق الكبير	44
سوق الديم	3
الاجمـــــــالي	47

Appendix (III-6):
Distribution of grocery workers sample:

الموقع	عدد العينة
السوق الكبير	5
سوق الديم	1
الاجمـــــــالي	6

Appendix (III-7):
Distribution of Café workers sample:

الموقع	عدد العينة
السوق الكبير	1
الاجمـــــــالي	1

Appendix (III-8):
Distribution of patisserie workers sample:

الموقع	عدد العينة
المنطقة الصناعية	5
الاجمـــــــالي	5

Appendix (III-9):
Distribution of ice factories workers sample:

الموقع	عدد العينة
المربع 29	1
الاجمـــــــالي	1

Appendix (III-10):
Distribution of butchery workers sample:

الموقع	عدد العينة
السوق الكبير	5
الاجمـــــــالي	5

Appendix (III-11):
Distribution of confectioner workers sample:

الموقع	عدد العينة
السوق الكبير	1
الاجمـــــــالي	1

Appendix (III-12):
Distribution of refreshment workers sample:

الموقع	عدد العينة
السوق الكبير	3
الاجمـــــــالي	3

Appendix (III-13):
Distribution of banana refrigerators workers sample:

الموقع	عدد العينة
السوق الشعبي	2
الاجمـــــــالي	2

Appendix (III-14):
Distribution of potato refrigerators workers sample

الموقع	عدد العينة
المنطقة الصناعية	1
الاجمـــــــالي	1

Appendix (IV): Consent form of the food handlers

وزارة التعليم العالي والبحث العلمي **التاريخ : .. /.. / .. 20 م**

جامعة شندي ولاية نهر النيل

كلية الدراسات العليا

الموافقة للمشاركة في الدراسة

السيد / السيدة : العامل بـ .....

السلام عليكم ورحمة الله وبركاته وبعد ،،،

أضع أنا الباحث/ عبدالرحمن هدية سعود .. بين أيديكم هذه الاستبانة وهي احدي المتطلبات الدراسية للحصول على درجة الدكتوراه في مجال الصحة العامة بعنوان :

الأنماط الوبائية لحملة السالمونيلا بين متداولي الأغذية في مدينة شندي ولاية نهر النيل - السودان

آملا منكم الموافقة على الانخراط في الدراسة وذلك بالإجابة على أسئلة استبانة الدراسة ، واعطى عينات من (البراز – البول – مسحة من الأظافر) .مع العلم بأن المعلومات ستكون تحت السرية التامة .

أوفق أنا /..... علي الانخراط في الدراسة، بعد أن أطلعت على أهداف والغرض من الدراسة .

التوقيع/.....

التاريخ/.....

Appendix (v): A questionnaire English Version:

Epidemiological Patterns of Salmonella Carriers among Food Handlers in Shendi City- River Nile State- Sudan

Case No. : ……. **Date : …./…./2020**

Establishment : …… **Location: …….**

FIRST PART:

- **Socio-demographic data of participant:**

1.	**Age:**	< 16 y	16-30 y	31-45 y	46-60 y	>60 y
2.	**Gender:**	Male		Female		
3.	**Nationality:**	Sudanese		None- Sudanese		
4.	**Marital Status:**	Single	Married	Divorced		
5.	**Educational Level :**	Illiterate		Primary		
		Secondary		University		
		Post graduate				

- **Work characteristics of the food handler:**

1.	**Type of work:**		
2.	**Work Experience:**		
3.	**Work Place:**		
4.	**Monthly Income:**	Satisfactory	Unsatisfactory

- **Health Training and Knowledge of food handler:**

No.	Items	YES	NO
1.	**Have you previously received training in food safety?**		
2.	**Do you know that there are diseases transmitted through food?**		
3.	**Do you know that humans are one of the sources of food contamination?**		
4.	**Do you know that the work is governed by laws such as (license - health card - ..)**		

❖ **Health Status of food handler:**

No.	Items	YES	NO
1.	**Have you had a fever in the last month?**		
2.	**Have you had diarrhea in the past month?**		
3.	**Have you had vomiting within the past month?**		
4.	**Have you had stomach ach in the past month?**		
5.	**Have you had a headache in the last month?**		
6.	**Do you suffer from the presence of blood in the stool?**		
7.	**Have you had medical examinations done recently?**		
8.	**Have you received treatment during the recent period?**		

SECOND PART: Check List by Observation:

❖ **Personal Hygiene:**

No.	Items	YES	NO
1.	**Wearing of special clothes for the work .**		
2.	**Wearing clean and special clothes for the work**		
3.	**Covering of the hair.**		
4.	**Cut the fingernails .**		
5.	**general body cleanliness**		
6.	**The presence of wounds with hand uncovered**		
7.	**The presence of wounds with the hand covered**		
8.	**The presence of secretions from the nose**		
9.	**Health Certificate**		

❖ **Hand Washing practices:**

No.	Items	YES	NO
1.	**Washing hands with water only.**		
2.	**Washing hands with water and soap .**		
3.	**Washing hands before starting work .**		
4.	**Washing hands after going to toilet .**		
5.	**Washing hands after blowing nose .**		
6.	**Washing hands after touching body parts .**		
7.	**Washing hands after touching any objects .**		

❖ **Other hygienic practices during the work:**

No.	Items	YES	NO
1.	**Shaking hands while working .**		
2.	**Wear nail polish when handling food .**		
3.	**Wearing rings and watch**		
4.	**biting nail with your teeth**		
5.	**Keeping food in the place designated for it away from contamination .**		
6.	**Avoiding Sneezing and coughing on food**		
7.	**Smoker**		
8.	**Avoiding Smoking on food**		

❖ **Workplace health condition:**

No.	Items	YES	NO
1.	**The Building is in good condition**		
2.	**Sanitization of Work Surfaces .**		
3.	**Cleaning of Instrument and Equipment .**		
4.	**Cleaning of Working Place .**		
5.	**Daily dispose of Wastes .**		
6.	**Using the sanitizer when washing utensils .**		
7.	**Use of safe water .**		
8.	**Presence of a wash basin .**		
9.	**Insect infestation**		
10.	**Availability of refrigerator**		

THIRD PART: Laboratory request forms :

1. **Stool Examination:**

Case No. :…… **Date of sample collection :…/…/20..**

a. Results of Stool Examination:

Results Examination	☐	Positive Salmonella Culture Results	☐	Negative Salmonella Culture Results

b. Species of Salmonella:

Species	☐	Salmonella typhi	☐	Salmonella Paratyphi	☐	Other Salmonella

c. Antimicrobial Patterns:

Antimicrobial / **Patterns**	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible							
Resistance							
Intermediate							

2. <u>Urine Examination:</u>

Case No. :…… **Date of sample collection :…/…/20..**

a. Results of Urine Examination:

Results Examination	☐	Positive Salmonella Culture Results	☐	Negative Salmonella Culture Results

b. Species of Salmonella:

Species	☐	Salmonella typhi	☐	Salmonella Paratyphi	☐	Other Salmonella

c. Antimicrobial Patterns:

Antimicrobial Patterns	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible							
Resistance							
Intermediate							

3. Fingernail Swabs Examination:

Case No. :…… **Date of sample collection :…/…/20..**

d. Results of Fingernails swabs Examination:

Results Examination	☐	Positive Salmonella Culture Results	☐	Negative Salmonella Culture Results

e. Species of Salmonella:

Species	☐	Salmonella typhi	☐	Salmonella Paratyphi	☐	Other Salmonella

f. Antimicrobial Patterns:

Antimicrobial Patterns	Ceftriaxone	Cotrimoxazole	Ampicillin	Tetracycline	Gentamicin	Chloramphenicol	Ciprofloxacin
Susceptible							
Resistance							
Intermediate							

Appendix (vi): A questionnaire Arabic Version:

الأنماط الوبائية لحملة السالمونيلا بين متداولي الأغذية في مدينة شندي ولاية نهر النيل – السودان

رقم الحالة: ..... **التاريخ: .../ .../ .. 20**

اسم المنشأة :..... **الموقع:.....**

الجزء الأول:

❖ **البيانات الاجتماعية والديموغرافية للمشارك:**

.1	العمر	أقل من 16 سنة	16- 30 سنة	31-45 سنة	46-60 سنة	أكبر من 60 سنة
.2	النوع	ذكر			أنثى	
.3	الجنسية	سوداني			غير سوداني	
.4	الحالة الاجتماعية	أعزب		متزوج		مطلق
.5	المستوى التعليمي	أمي			اساسي	
		ثانوي			جامعي	
		دراسات عليا				

❖ **خصائص العمل :**

.1	نوع العمل		
.2	مدة الخبرة		
.3	موقع العمل		
.4	الدخل الشهري	مُرضي	غير مُرضي

❖ **التدريب والمعرفة :**

ت	المحور	نعم	لا
.1	هل تلقيت تدريب سابقاً في مجال سلامة الغذاء		
.2	هل تعرف أن هناك أمراض تنتقل بواسطة الغذاء		
.3	هل تعرف أن الأنسان أحد مصدر تلوث الغذاء		
.4	هل تعرف أن العمل تحكمه قوانين مثل (الترخيص – الكرت الصحي- ..)		

❖ **الحالة الصحية :**

ت	المحور	نعم	لا
.1	هل عانيت من حمى خلال الشهر الماضي؟		
.2	هل عانيت من اسهال خلال الشهر الماضي؟		
.3	هل كان لديك قيء خلال الشهر الماضي؟		
.4	هل عانيت من اضطرابات في المعدة خلال الشهر الماضي؟		
.5	هل عانيت من صداع خلال الشهر الماضي؟		
.6	هل تعاني من وجود دم في البراز؟		
.7	هل عُملت فحوصات طبية خلال الفترة القريبة الماضية؟		
.8	هل تلقيت علاج خلال الفترة القريبة الماضية ؟		

<u>الجزء الثاني</u>: بالمشاهدة:

❖ **النظافة الشخصية :**

ت	المحور	نعم	لا
.1	ارتداء ملابس خاصة بالعمل		
.2	ارتداء ملابس نظيفة وغير خاصة بالعمل		
.3	يقوم بتغطية الرأس		
.4	يقوم بقص أضافره		
.5	يهتم بالنظافة العامة للجسم		
.6	وجود جروح باليد غير مغطاة		
.7	وجود جروح باليد ولكنها مغطاة		
.8	وجود افرازات من الأنف		
.9	توجد الشهادة الصحية		

❖ **ممارسات غسل اليدين :**

ت	المحور	نعم	لا
.1	غسل اليدين بالماء فقط		
.2	غسل اليدين بالماء والصابون		
.3	غسل اليدين قبل بدء العمل		
.4	غسل اليدين بعد الذهاب إلى الحمام		
.5	غسل اليدين بعد العطس		
.6	غسل اليدين بعد لمس أجزاء الجسم		
.7	غسل اليدين بعد لمس أي شيء أخر		

❖ **الممارسات الصحية الأخرى أثناء العمل :**

ت	المحور	نعم	لا
.1	المصافحة إثناء العمل		
.2	وجود مواد ملونة على الأظافر أثناء التعامل مع الغذاء		
.3	ارتداء الخواتم والساعة		
.4	عض الأظافر بالأسنان		
.5	حفظ الطعام في المكان المخصص بعيداً عن التلوث		
.6	تجنب العطس والسعال على الغذاء		
.7	مدخن		
.8	تجنب التدخين على الغذاء		

❖ **الحالة الصحية للمنشأة :**

ت	المحور	نعم	لا
.1	المبنى بحالة جيدة		
.2	القيام بتطهير أسطح العمل		
.3	القيام بتنظيف الأدوات والمعدات		
.4	القيام بتنظيف مكان العمل		
.5	التخلص اليومي من المخلفات		
.6	استخدام المطهر عند غسل الأواني		
.7	استخدام المياه الصالحة		
.8	وجود حوض للغسيل		
.9	وجود حشرات		
.10	وجود مبردات لحفظ الغذاء		

الجزء الثالث: استمارة فحص العينات:

1. فحص عينات البراز:

رقم الحالة :..... **تاريخ أخذ العينة:...../...../.....20**

أ- نتائج فحص عينات البراز:

نتيجة مزرعة بكتيريا السالمونيلا	□	**موجبة**	□	**سالبة**	

ب- أنواع بكتيريا السالمونيلا:

أنواع البكتيريا	□	**السالمونيلا التيفية**	□	**السالمونيلا نظيرة التيفية**	□	**أنواع أخرى للسالمونيلا**	

ت- الأنماط المضادة للميكروبات:

الأنماط	**مضادات الميكروبات**						
	سيفترياكسون	**كوتريموكسازول**	**أمبيسلين**	**التتراسيكلين**	**الجنتاميسين**	**الكلورامفينيكول**	**سيبروفلوكساسين**
استجابة							
مقاومة							
متوسط							

2. فحص عينات البول:

رقم الحالة :..... **تاريخ أخذ العينة:...../...../.....20**

أ- نتائج فحص عينات البول:

نتيجة مزرعة بكتيريا السالمونيلا	□	**موجبة**	□	**سالبة**

ب- أنواع بكتيريا السالمونيلا:

أنواع البكتيريا	□	**السالمونيلا التيفية**	□	**السالمونيلا نظيرة التيفية**	□	**أنواع أخرى للسالمونيلا**	

ت- الأنماط المضادة للميكروبات:

الأنماط	**مضادات الميكروبات**							
	سيفترياكسون	**كوتريموكسازول**	**أمبيسلين**	**التتراسيكلين**	**الجنتاميسين**	**الكلورامفينيكول**	**سيبروفلوكساسين**
استجابة							
مقاومة							
متوسط							

3. فحص عينات مسحة الأظافر:

رقم الحالة :..... **تاريخ أخذ العينة:...../...../.....20**

أ- نتائج فحص عينات مسحة الأظافر:

نتيجة مزرعة بكتيريا السالمونيلا	□	**موجبة**	□	**سالبة**	

ب- أنواع بكتيريا السالمونيلا:

أنواع البكتيريا	□	**السالمونيلا التيفية**	□	**السالمونيلا نظيرة التيفية**	□	**أنواع أخرى للسالمونيلا**	

ت- الأنماط المضادة للميكروبات:

الأنماط	**مضادات الميكروبات**						
	سيفترياكسون	**كوتريموكسازول**	**أمبيسلين**	**التتراسيكلين**	**الجنتاميسين**	**الكلورامفينيكول**	**سيبروفلوكساسين**
استجابة							
مقاومة							
متوسط							

Appendix (vii): Ethical Approval

River Nile state

Ministry of Health and Population

Administration of Researches and Projects

26 / 3 / 2020

Certificate

It is to certify that the proposal of the research under the title:

Pattern and risk factors for Salmonella carriage among food handlers in Shandi City , River Nile State Sudan , 2019-2020 .

by

Abdul Rahman Hadia Saud

Was ethically approved

DR. Samia Mohammed A/Elrahman

Head of the Administration (REC)

Appendix (viii): Antimicrobials Patterns: