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DETERMINATION of PREVALENCE and INCIDENCE of *Salmonella* spp. and *Shigella* spp. in SOME FOODS in IRAQ/SULAYMANIYAH/QALADZE

MASTER DEGREE THESIS

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THESIS ACCEPTANCE AND APPROVAL

DETERMINATION of PREVALENCE and INCIDENCE of *Salmonella* spp. and *Shigella* spp. in SOME FOODS in IRAQ/SULAYMANIYAH/QALADZE prepared by Rahman Khdir IBRAHIM, has been accepted thesis study on the date 24/11/2017, was accepted as a Master's degree in the Department of Food Engineering at Siirt University, with majority votes by the jury below.

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I confirm the above results.

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
SS agar	Salmonella Shigella agar
XLD agar	Xylose Lysine Deoxycholate agar
TSA	Tryptic Soy Agar
HE agar	Hectoen Enteric agar
RV	Rappaport-Vassiliadis enrichment broth
SIM	Sulfide Indol Motility
BPW	Buffered peptone water
g	Gram
mg	Milligram
h	Hour
L	Liter
ml	Milliliter
µl	Microliter
a_w	Water activity
cfu	Colony forming unit
µ	Mikron
µm	Micrometer
n	Number
MR	Methyl Red
VP	Voges-Proskauer
O/R	Oxidation/Reduction

ÖZET

YÜKSEK LİSANS TEZİ

Irak Sülaymaniyah/Qaladze bölgesinde bazı gıdalarda *Salmonella* spp. ve *Shigella* spp. türlerinin varlığı ve yaygınlığının belirlenmesi.

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Bu araştırmada; Irak/Sülaymaniye/Qaladze bölgesinde tüketime sunulan 10'ar adet (fabrika üretimi çiğ tavuk eti, köy üretimi çiğ tavuk eti, tavuk et döner, kırmızı et döner, çiğ köy yumurtası, pişirilmiş köy yumurtası, ev yapımı ayran, ev yapımı yoğurt, içme suyu ve yıkamada kullanılan su) olmak üzere toplam 100 adet örnek *Salmonella* ve *Shigella* türleri yönünden üç farklı besiyerinde incelenmiştir.

Araştırmada *Salmonella* türlerinin tanımlanmasında ISO 6579, *Shigella* türlerinin tanımlanmasında EN ISO 21567 metodu referans olarak kullanılmıştır. Numunelerin % 58'i *Salmonella* ve *Shigella* türleri yönünden pozitif olarak belirlenmiştir. Kontamine olan 58 adet örneğin 45'i (% 77.60) *Salmonella* spp. ve 32'si (% 55.20) de *Shigella* spp. olarak tanımlanmıştır.

Salmonella türleri içinde 17'si (% 37.70) *S. enteritidis*, 11'i (% 24.40) *S. bongori*, 8'i (% 17.70) *S. typhimurium*, 8'i (% 17.70) *S. paratyphi* ve 1'i (% 2.20) *S. typhi* olarak tespit edilirken; *Shigella* türleri içinde 16'sı (% 50.00) *S. dysanteria*, 6'sı (% 18.75) *S. sonnei*, 6'sı (% 18.75) *S. flexneri* ve 4'ü (% 12.50) *S. boydii* olarak belirlenmiştir.

İncelenen gıda gruplarında *Salmonella* enfeksiyonu en sık olarak fabrika üretimi çiğ tavuk etleri, köy üretimi çiğ tavuk etleri ve çiğ köy yumurtalarında (% 80.00), *Shigella* enfeksiyonuna da en sık olarak yıkamada kullanılan sular (% 80.00) ile tavuk et dönerleri ile çiğ köy yumutalarında (% 50.00) rastlanılmıştır. *Salmonella* enfeksiyonu açısından ev yapımı yoğurtlar ve içme sularının güvenli olduğu tespit edilirken, *Shigella* enfeksiyonu açısından tüm gıda gruplarının potansiyel risk taşıdığı tespit edilmiştir.

İstatistiksel olarak tavuk et döner örneklerindeki *Salmonella* spp. ile pH ve a_w arasında pozitif yönde ($p<0.01$), kırmızı et döner örneklerinde pH ve a_w arasında negatif yönde ($p<0.05$), ev yapımı ayran örneklerinde ise pozitif yönde ($p<0.05$) korelasyon tespit edilmiştir. Ev yapımı yoğurtlarda da *Shigella* spp. ile pH ve a_w arasında pozitif yönde ($p<0.01$) bir korelasyon belirlenmiştir.

Sonuç olarak bölgede tüketime sunulan ve incelenen örneklerde *Salmonella* ve *Shigella* türlerine rastlanma oranları oldukça yüksek bulunmuş ve bu ürünlerin halk sağlığı açısından potansiyel bir risk oluşturabileceği kanaatine varılmıştır.

Anahtar Kelimeler: *Salmonella* spp., *Shigella* spp., Irak Halk Sağlığı, Gıda Güvenliği

ABSTRACT

MSc THESIS

DETERMINATION of PREVALENCE and INCIDENCE of *Salmonella* spp. and *Shigella* spp. in SOME FOODS in IRAQ/SULAYMANIYAH/QALADZE

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In this study was conducted to determine the prevalence and incidence of *Salmonella* spp. and *Shigella* spp. in some foods from Iraq/Sulaymaniyah/Qaladze City. An about 100 samples were analyzed of 10 different groups (10 samples of each: factory raw chicken meat, village raw chicken meat, chicken meat shawarma, red meat shawarma, raw village egg, cooked village egg, homemade ayran, homemade yogurt, drinking water and washing water) were collected from different sources.

A total of 100 samples were examined in three different media for *Salmonella* and *Shigella* spp. and study based on ISO 6579 for *Salmonella* isolation and EN-ISO 21567 for *Shigella* isolation as a method.

The results showed that 58% of the samples were positive for *Salmonella* spp. and *Shigella* spp. Out of 58 contaminants, the percentage of isolated *Salmonella* spp. were 77.60% and *Shigella* spp., were 55.20%, at the same time, the highest incidence among *Salmonella* spp. was *S. enteritidis* (37.70%), *S. bongori* (24.40%), *S. typhimurium* (17.70%), *S. paratyphi* (17.70%), and *S. typhi* (2.20%); the highest frequency among the *Shigella* spp. was determined as *S. dysanteriae* (50.00%), *S. sonnei* (18.75%), *S. flexneri* (15.62%) and *S. boydii* (12.50%), while the frequency of infection among the food groups is highest in village raw chicken meat and raw village eggs, the lower infection is found in homemade yogurts.

Salmonella infection was most frequently observed in raw village eggs and village raw chicken meats at 80.00%, also *Shigella* infection was found to be the most common at 80.00% in the washing water.

Statistically, *Salmonella* spp. a significant correlation was found $p<0.01$ between pH and a_w in the positive direction in chicken meat shawarma, while in red meat shawarma $p<0.05$ between the pH and a_W in the negative direction, whereas in homemade ayran significance was found $p<0.05$ in the positive direction. *Shigella* spp. in homemade yogurt was positive correlation between pH and a_w $p<0.01$.

As a result, infection rates of salmonellosis and shigellosis were found to be very high in the region and it was determined that they pose a potential health risk for public health.

Key words: *Salmonella* spp., *Shigella* spp., Iraqi Public Health, Food Safety

1. INTRODUCTION

Food microbiology is science that deals with the study of the general biology of the microorganisms that are found in foods including: their growth features, identification, and pathogenesis. Specifically, areas of interest which concern food microbiology are food spoilage, food poisoning, food preservation, and food legislation. Pathogens in product or harmful microorganisms, result in major public health problems in the worldwide and are the leading causes of diseases and death (Hueston and Bryant, 2005).

Bacteria, yeasts, molds, and viruses are important in food for their ability to cause foodborne diseases and food spoilage and to produce food and food ingredients. Many bacterial species and some molds and viruses, but not yeasts, are able to cause foodborne diseases. Most bacteria, molds, and yeasts, because of their ability to grow in foods, can potentially cause food spoilage. However, microbes can play an important role in food; advantageous microorganisms are used in foods in many ways. These include actively growing microbial cells, non-growing microbial cells, and metabolic by-products and a cellular component of microorganisms, for example of the use of growing microbial cells is the conversion of milk to yogurt by bacteria (Ray, 2003).

In this research, were study two enteric pathogens: *Salmonella* spp. and *Shigella* spp. These important members of *Enterobacteriaceae* are enteric pathogens that cause typhoid/paratyphoid and bacillary dysentery, respectively. These organisms are highly infectious and are responsible for many thousands of morbidity and mortality each year particularly in the undeveloping regions of the world with poor environmental sanitation. Studies have shown *Salmonella* and *Shigella* species predominance as major causes of diarrhoeal disease in various countries of the world (Holt et al, 1994).

Both pathogens have been the cause of morbidity and mortality in children and the elderly especially in developing countries, members of the family *Enterobacteriaceae* are Gram (-), non spore forming rods. Some of them are human and animal pathogens producing intestinal infection and food poisoning. *Salmonella* and *Shigella* are among the most important bacterial causes of diarrhea (Koehler and Fein, 1996).

The aims of this study to determination of prevalence and incidence of *Salmonella* spp. and *Shigella* spp. in some foods from Qaladze city, isolation and

identification of this two pathogenic bacteria with qualitative and quantitative detection of microorganisms in some food and water, detemine those types of foods that are a good host for the two pathogenic bacteria, and transfer mechanism of the disease to human beings, determination of salmonellosis and shigellosis how much included potential risks, determine the role of oxidation-reduction (O/R), water activity (a_w) and pH on the growth of *Salmonella* and *Shigella*, and aimed to using the result and techniqes to prevent and controle of salmonellosis and shigellosis.

Ten types of food selected, that provide good flora for the growth of these bacteria. Many studies in the past have used Salmonella Shigella (SS) agar which is a selective media to grow *Salmonella* spp. and *Shigella* spp. In this study three different media chose, to grow the bacteria and find out which one of this media is more selective to the other. Founded out that Hecton Enteric (HE) agar media is more selective and the number of colonies of bacteria grew on it were lesser in comparison to the other two media. Despite the fact that, the SS agar has been used for a long time for isolation of *Salmonella* spp. and *Shigella* spp., but appeared in this study that SS agar is not strictly selective and other Gram (-) bacteria can grow on it.

Founded out in the study that the major cause of food contamination by *Salmonella* spp. and *Shigella* spp. are food preparation techniques, these contaminations result from raw food preparation techniques, then transfer the disease to consumers at home and food services, while thermal processing such as cooking, roasting, and boiling for different types of foods killed the bacteria.

Generally, Iraq one of the in developing countries, has poor quality control over food safety, monitoring food services and restaurants, there are poor or no training of those who work in food services places, and finally the tv's and radio station is not playing a role in informing person citizens to be careful about food safety, and lack of electricity in Qaladze as well is a big problem to preserve food, dairy, and meat products in refrigerators, and feeding for animals and poor quality.

Founded out that each year in this city hundreds of people are infected with foodborne disease such as salmonellosis and shigellosis. Therefore; chose this study to work on *Salmonella* spp. and *Shigella* spp. and send the result of this investigation to the relevant parties to work on it and do something about it.

2. LITERATURE REVIEW

2.1. Bacteriology of *Salmonella* and *Shigella* Organisms

Basically, *Salmonella* and *Shigella* belong to the family of *Enterobacteriaceae*. This family is the largest and most heterogeneous collection of medically important, Gram (-) bacilli. It is totally consist of thirty genera and more than one hundred and twenty species which have been described and they have been classified based on biochemical properties including: antigenic structure, nucleic acid hybridization and sequencing. Despite the complexity of this family, more than 95% of the medically important isolated and it is belong to only ten genera and constitute fewer than twenty five species (Murray et al, 1998).

Generally this family consists of *Arsenophonus*, *Budvicia*, *Buttiauxella*, *Cedecea*, *Citrobacter*, *Edwardsiella*, *Enterobacter*, *Erwinia*, *Escherichia*, *Ewingella*, *Hafnia*, *Klebsiella*, *Kluyvera*, *Leclercia*, *Leminorella*, *Moellerella*, *Morganella*, *Obesumbacterium*, *Pantoea*, *Pragia*, *Proteus*, *Providencia*, *Rahnella*, *Salmonella*, *Serratia*, *Shigella*, *Tatumella*, *Xenorhabdus*, *Yersinia* and *Yokenella* (Holt and Williams, 1994). *Enterobacteriaceae* are ubiquitous organisms that are found worldwide in soil, food, water, and vegetation, which are parts of the normal intestinal flora, in most animal and humans (Chessbrough, 2002).

Historically, Salmon and Smith were the first to isolate *Salmonella* from pigs in 1885 (Ryan and Ray, 2004). As explained before, *Salmonella* is an important genus of the family *Enterobacteriaceae*. Family members of the genus are Gram (-), facultative anaerobes and inhabit, which are the intestinal tract of man and animals. They may be recovered from a wide range of hosts such as; poultry, swine, human, foods and from the environment. Consequently; *Salmonella* may be pathogenic to wild or domestic of animals and humans (Holt et al, 1994).

Finally; *Salmonella* it is an important pathogen to the food industry and it has been frequently identified as the etiological agent of foodborne outbreaks in human. The pathogenic conditions of *Salmonella* include enteric fever, gastroenteritis and septicemia (Siqueira et al, 2003).

Shigella spp. is the causative agents of shigellosis, or “bacillary dysentery”. This is firstly discovered by Kiyoshi Shiga, as a Japanese scientist (Pelczar, 1981).

The genus *Shigella* belongs to family *Enterobacteriaceae*. It comprises four species, such as; *S. dysenteriae*, *S. flexneri*, *S. sonnei*, and *S. boydii*, which are classified into serotypes based on biochemical differences and variations in their O-antigen groups. Thus, *S. dysenteriae* (group A) has 17 serotypes, *S. flexneri* (group B) has 14 classical serotypes and subserotypes, *S. sonnei* (group C) have a single serotype and *S. boydii* (group D) has 20 serotypes (Johnson et al, 1975).

2.2. Classification of *Salmonella* spp. and *Shigella* spp.

The genus *Salmonella* has been divided into two species: *S. enterica* (comprising six subspecies) and *S. bongori*. *Salmonella enterica* is an important agent of foodborne illness, over 99% of human *Salmonella* spp. Infections are caused by *S. enterica* subsp. e*nterica.* This species is sub-classified into 6 subspecies namely; *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica* (Reeves et al, 1989).

Genus *Shigella* includes four species, *S. dysenteriae* (subgroup A), *S. flexneri* (subgroup B), *S. boydii* (subgroup C) and *S. sonnei* (subgroup D). It is believed that the manifestation of *S. dysenteriae* type 1 infection is more severe because of its exclusive property to produce shiga toxin, a potent enterotoxin (Wei et al, 2003).

The scientific classification of *Salmonella* and *Shigella* (Reeves et al, 1989).

Domain:	*Bacteria*
Kindom:	*Monera*
Phylum:	*Proteobacteria*
Class:	*Gamma Protobacteria*
Order:	*Enterobacteriales*
Family:	*Enterobacteriaceae*
Genus:	*Salmonella* and *Shigella*

In the present study isolated 9 spicies.

Salmonella spp.	*Shigella* spp.
S. enteritidis	*S. dysenteriae*
S. typhimurium	*S. flexneri*
S. bongori	*S. boydii*
S. paratyphi	*S. sonnei*
S. typhi	

2.3. Growth Condition Characteristics of *Salmonella* spp. and *Shigella* spp.

Salmonella spp. growth in food or other hosts, because they are affected by a varios of factors such as pH, temperature, a_w and the presence of preservatives. *Salmonella* spp. will can growth in the temperature range around 5.20-46.20°C, with the optimal temperature being 35-43°C. It is not particularly heat resistant, because most serotypes will be killed by normal cooking conditions. Freezing can be detrimental to *Salmonella* survival, although it does not guarantee breaking up of the organism (Doyle and Beuchat, 2007).

There is a beginning rapid decrease in the number of viable organisms at temperatures close to the freezing point due to the freezing damage. However, at lower temperatures (from -17 to -20°C), there is also a notably decline in the number of viable organisms. *Salmonella* have the capacity to survive in long periods of time at storage temperatures more than <-20°C. Heat resistance of *Salmonella* in foods is depending on composition, nature of solutes, pH, and a_w of the food. *Salmonella* spp. may survive in feed for 16 months at 25°C (Hafez, 2005).

Generally, heat resistance increases as the a_w of the food decreases, any reduction in pH results in reduction of heat (Banwart and Ayres, 1956).

Foods, which are high in lipid and low in moisture such as chocolate and peanut butter, may have a preservative effect against heat. In low pH conditions, heat resistance of *Salmonella* spp. will decrease. *Salmonella* spp. will grow in a broad pH range of 3.80-9.50 with an optimum pH range around 7.00-7.50. Beside of pH range for growth, cells may become deactivate. Although this is not immediate but cells have been exposed to survive for long phase of time in acidic products (Gast, 1997).

Salmonella spp. is classified as facultative anaerobic organisms and they do not require oxygen to growth. Water activity, has an important effect on the growth of *Salmonella* spp. with the optimum a_w around 0.99 and the lower point for growth is 0.93. *Salmonella* spp. can survive for months or for years in foods with a low a_w such as black pepper, chocolate, peanut, butter and gelfoof (Podolak et al., 2010).

Growing and surviving of *Shigella* spp. in foods is affected by many factors including temperature, pH, salt content and the presence of preservatives. For example, survival of *S. flexneri* has been shown to increase with decreasing temperature,

increasing pH, and decreasing salt or sodium chloride (NaCl) concentration (Zaika and Phillips, 2005).

The temperature scale for growing of *Shigella* spp. is about 6-8 to 45-47°C. However, *Shigella* spp. will cause rapid inactivation at temperatures around 65°C, under frozen -20°C or refrigerated 4°C conditions *Shigella* spp. can remain alive for extended phase of time (Lightfoot, 2003).

Therefore; *Shigella* spp. grows in a pH range of 5.00-9.19, and minimum a_w which can survive 0.97 (Zaika, 2001). It is demonstrated that *S. flexneri* is tolerant to acid and it can remain alive at pH 4 for 5 days in soup when it incubated at 28°C, *Shigella* spp. is be can to survive lower pH conditions at decrease temperatures, with *S. flexneri* and *S. sonnei* are be able to survive for 14 days in tomato juice (pH 3.90-4.10) and apple juice (pH 3.30-3.40) when they are keeped at 7°C (Bagamboula and Debevere, 2002).

S. flexneri is salt tolerant and also able to grow in media containing 7% of NaCl at 28°C (Zaika, 2002a). It is sensitive to organic acids typically used to reserve food. For example, lactic acid has been verified to be effective at inhibiting *S. flexneri* growth, followed in order by acetic acid, citric acid, malic acid and tartaric acid (Zaika, 2002b).

Shigella spp. has been shown to survive on various surfaces and *S. sonnei* has been isolated and cultured from hands for several hours after hand contamination. A study by Nakamura (1962) explained that *S. sonnei* was able to survive on glass, cotton, wood, metal and paper, with survival times about 2 days on metal surface to 28 days on paper at 15°C. *S. dysenteriae* serotype 1 has also been shown to alive on surfaces such as plastic, aluminium, glass, cloth and wood (Islam et al, 2001).

2.4. Pathogenesis of *Salmonella* and *Shigella* Infections

Salmonellosis is an infectious disease of humans and animals caused by microorganisms of the two species of *Salmonella* (*S. enterica*, *S. bongori*). Salmonellosis is one of the main infectious causes of enteric disease in human being in the worldwide, and in most cases they are related to food products of animal origin (Williams et al, 2015). Approximately 95% of cases for human salmonellosis associated with the consumption of contaminated products such as, poultry meat or poultry meat production, eggs or egg production, milk or milk production, seafood, and fresh

produce. Although, 12 hours to 3 days are the incubation period for *S. enteritis*, enteric fever usually appears after 7-28 days (Mead et al, 1999).

Salmonellosis symptoms are usually gastrointestinal including, nausea, vomiting, abdominal cramps and bloody diarrhea with mucus. Headache, fatigue and rose spots are also possible. These symptoms can be severe, especially in young children and the elderly. In sickle-cell anemia, osteomyelitis due to *Salmonella* infections is more common than in the general population (WHO/FAO, 2002).

Symptoms will end generally up to a week, and can appear 12 to 72 h after ingesting the bacterium. After bacterial infections, reactive arthritis (Reiter's syndrome) will develop. In most cases, the illness will end four to seven days, and most people recover without treatment. Although, the diarrhea may be so severe, the patient becomes dangerously dehydrated and they must be hospitalized. At the hospital, the patient may receive intravenous fluids to treat the dehydration, and they may be given medications to provide symptomatic relief, such as fever reduction. In severe cases, the *Salmonella* infection may spread from the intestines to the blood stream; it will cause death unless the person is treated promptly with antibiotics (Jay et al, 2003).

Despite of the type of *Salmonella* usually associated with infections in humans non-typhoid *Salmonella*, is usually contracted from sources such as: poultry, pork and beef, if the meat is prepared incorrectly or infected with the bacteria after preparation. Infected eggs, egg products and milk when not prepared, handled or refrigerated properly reptiles, such as turtles, lizards and snakes, which may carry the bacteria in their intestines (Darby and Sheorey, 2008).

About the shigellosis and clinical symptoms of it, the most acute form of shigellosis is produce by the *S. dysenteriae* serotype 1. *S. sonnei* causes the mild form of sickness, while *S. flexneri* and *S. boydii* can cause either severe or mild illness (FDA, 2012). *S. dysenteriae* may also lead to dangerous complications such as persistent diarrhoea, severe anorexia, weight loss and malnutrition, dilation of the large intestine, seizures, kidney damage, and hemolytic-uremic syndrome (Sur et al, 2004). Bacteremia may be described in infants and immune compromised adults. Pneumonia associated with *S. sonnei*, and it has also been described in the following; malnourished children, in human immunodeficiency virus (HIV) infected patients, and in patients with chronic diseases (Miller et al, 2005).

A symptom, ranges from watery diarrhea to severe symptoms such as fever, abdominal pain, tenesmus, and bloody diarrhea. Severity of the disease varies by the infecting species, *S. dysenteriae* infections usually cause dysentery, which may also occur in infections caused by *S. flexneri*. Where as *S. boydii* and *S. sonnei* generally often is self-limited watery diarrhea. Acute complications such as; toxic megacolon, peritonitis and septicemia are mostly observed in severely malnourished children and although they may occur in absence of early antibiotic treatment (Von Seidlein et al, 2006).

2.5. Transmission Mode of Salmonellosis and Shigellosis

Salmonellosis is mainly transmitted by the fecal-oral route. They are carried asymptomatically in the intestines or gall bladder of many animals, in which they are continuously or intermittently shed in the feces. They can also be passed latently in the mesenteric lymph nodes or tonsils. Fomites and mechanical vectors (insects) can also spread *Salmonella*. Vertical transmission occurs in birds with contamination of albumen and possibly the yolk of eggs (Kozlica et al, 2010).

Animals may become infected from contaminated feed and drinking water or close contact with infected animals. Birds and rodents can spread *Salmonella* to the livestock, meat locations such as rumen; rectum, caecum and colon contain high concentration of *Salmonella*. People are often infected when they are eating contaminated foods of animal origin including meat, eggs, milk, vegetables and fruits. They may also be infected by ingesting organisms in animal feces either directly or in contaminated foods or water (Craun et al, 2010).

Transmission of *Salmonella* is cyclic between humans, animals, foods and environmental sources. Usually, non-typhoidal *Salmonella* spread along the food chain. In farm livestock animal feed and high levels of fecal shedding of infected animals has been recognized as an important entry site in the food chain. Another factor of contamination is the slaughtering of the animals (Figure 2.1.). In undeveloped countries, fecal contamination of water is a significant source for *S. typhi* and *S. paratyphi* for human infections (Liu and Yang, 2010).

Shigellosis will transfer by the fecal oral route by either person-to-person contact or use of contaminated food or drinking (Nygren et al, 2012). Contaminated water is another way for transmission of *Shigella* species. This can occur due to poorly treated

contaminated water when it is used for drinking and food preparation. Because of this, escape of sewage through the earth or fecal contaminant of recreational water is other conduct of transportation *Shigella* spp. Shigellosis is endemic in many developing and none developing countries and also it occurs in epidemics causing considerable morbidity and mortality (Alsanius, 2010).

Among the four species of *Shigella* such as *Shigella dysenteriae* type 1 especially important, because it causes the most severe disease and may occur in large regional epidemics however, the bacteria can infect person by contaminate water or food (Lightfoot, 2003).

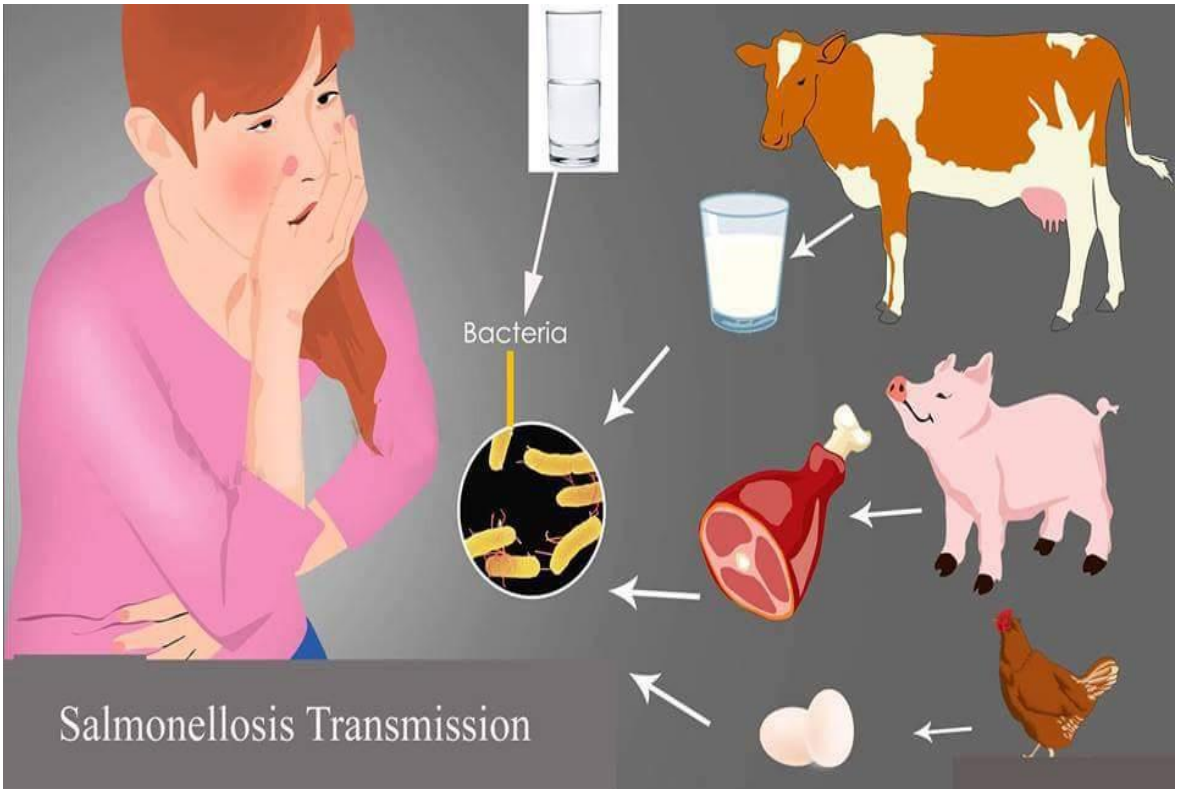

Figure 2.1. Transmission mode of salmonellosis

2.6. Infectious Dose of *Salmonella* spp. and *Shigella* spp.

The infectious dose of *Salmonella* spp. varies with the serotype. For non-typhoid salmonellosis the infectious dose is approximately 10^3 bacilli, but for enteric fever the infectious dose is about 10^3 bacilli by ingestion. Patients with achlorhydria, depressed cell-mediated immunity, or who are elderly may become infected with at a lower infectious dose. The infectious dose may also be dependent on the level of acidity in the patient's stomach (Bronze and Greenfield, 2005).

Shigella infectious dose very low, because a few little data is obtainable on the dose-response relation for *Shigella* spp. during the 1960s and 1970s, human feeding trials were using strains of *S. dysenteriae* serotype 1, *S. flexneri., S. sonnei* were performed to determine the dose which is need to cause shigellosis. The dose response may be varied between strains. Illness will cause by *S. dysenteriae* serotype 1, *S. flexneri*, or *S. sonnei* with ingestion of 10, 100 and 500 organisms, respectively (DuPont, 1989).

2.7. Incidence of *Salmonella* and *Shigella* in Some Food Samples

2.7.1. Poultry and poultry meat products

Poultry products are considered to be most crucial nutritious sources for human being, because it contains high protein which is essential for growth and development of human, further processing of poultry meat involves conversion of raw poultry carcasses into value added products such as reconstructed products, cold cuts or breaded products. Processing of poultry is really important in terms of improving juiciness, taste, shelf life and holding capacity (Sahoo et al, 1996).

Raw poultry is one of the important sources of major foodborne bacterial pathogens such as *Salmonella* spp., *Shigella* spp. and *Listeria monocytogenes*. *Enterobacteriaceae* considered the major indicator in poultry carcasses to determine the healthy status and storage conditions. Otherwise this leads to colonies some toxin produced bacterial including *Salmonella*. *Salmonella* species are responsible for a variety of acute and chronic diseases in both poultry and humans. Infected poultry products are among the most important sources for foodborne disease in humans. *Salmonella* are more common in poultry products that in incidence among animals. Contamination control during slaughter and processing has been identified as an ultimate requirement in order to detect the prevalence of pathogenic microorganisms in poultry products. Microbiological status of broiler carcasses depends on several factors, such as: infection level of living birds, cross contamination, amount and variety of pathogens among others (Abu-Ruwaida et al, 1994).

2.7.2. Milk and milk products

Milk is a suitable substrate for microbial growth and development. The fluid or semi-fluid nature of milk and its chemical composition renders it one of the good culture media for microbial growth and multiplication (Fekadu, 1994).

Milk could be contaminated in many phased including procurement, processing and distribution, one of the causes of milk contamination is using non-portable water. It is known that tropical conditions which have a hot, humid climate for much of the year are ideal for quick milk deterioration so pose particular problems because the temperature is ideal for growth and multiplication of many bacteria (Godefay and Molla, 2000).

Milkborne disease has been frequently recorded and unpasteurized milk appears to be commonly implicated in such outbreaks. Milk may become infected by contamination with infected materials like utensils, water and flies. Milk handlers may be carriers of infectious agents and also cause contamination (O'Connor, 1995).

The main sources of raw milk contamination are air, milking equipment, feed, soil, feces and grass. Many key factors directly influence the quality of raw milk including environment where the cows are kept and milked, sanitation of milk and storage tools. All these factors affect the total bacterial count test and species of bacteria (Coorevits, 2008).

In raw or unpasteurized milk can affect the safety, quality, and consumer acceptance of dairy products. Several human microbial pathogens such as *Salmonella* spp., *Staphylococcus aureus*, *Shigella* spp., *Campylobacter jejune*, *Listeria monocytogenes* and *Mycobacterium tuberculosis* have been found to be associated with milk and milk products (Murphy and Boor, 2000).

Standard pasteurization methods are very important and effective in destroying *Salmonella*. A cheese made from pasteurized milk could be considered safe. Raw milk cheeses have been contaminated in several *Salmonella* this would suggest that raw milk cheeses could pose health risks, for the population especially for people with compromised immune systems (Donnelly, 2001).

Contamination of milk and its products with microorganism are significant indicator for safety, quality, regulations and public health stated that milk from the farm can become contaminated with Gram (-) bacteria present on teats, the teat ends, teat canal, udder surfaces, mastitis udders and contaminated water used to clean the milking systems and those that are resident in the milking system. For example, high microbial counts in raw milk are responsible for quality defects in pasteurized milk, processed milk, dried skim milk, butter and cheese. *Salmonella* could be found in raw milk in farm

bulk tanks. Many studies have been reported that raw milk or raw milk products are a well-contaminate with *Salmonella* species. Raw milk products including nonfat dry milk and ice-cream have also been the vehicle for outbreaks of *Salmonella* (McManus and Lanier, 1987).

The safety of milk products with respect to foodborne diseases is a great concern around the world. This is especially true in developing countries where production of milk and various dairy products take place under rather unsanitary conditions and poor production practices (Zelalem and Faye, 2006).

2.7.3. Water

Waterborne disease mostly caused by *Shigella*. Most of the enteric agents could be transferred by water. However, the rate of inactivation in the water environment and infectious dose are the critical characteristics of an organism that defines the risk of a waterborne outbreak of disease. The most common water pathogenes are *Vibrio cholera, Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Giardia lamblia* and *Cryptosporidium parvum* (other routes of infection are food, soil, person to person); however, they are all enteric pathogens that may remain live but cannot multiply in treated water because of the killing agents in the water (Edberg et al, 2000).

Salmonella is a ubiquitous intestinal pathogen with a worldwide distribution that comprises a large number of serovars characterized by different host specificity and distribution. This organism is one of the leading causes of intestinal illness all over the world as well as the etiological agent of more severe systemic diseases such as typhoid and paratyphoid fevers (Pond, 2005).

Shigella is commonly living in the gastrointestinal tract of humans and other primates and it is released in very large amount in the feces of suffered individuals. It's basically transmitted through contaminated water, sewage water, and food or by direct contact with an infected person. Its presence in the population is maintained by a few asymptomatic carriers. In water, *Shigella* can last for at least six months at room temperature and this high survival make it transmissible by water. The total number of *Shigella* outbreaks that occur each year throughout the world is estimated to be 164.7 million, including 163.2 million cases in developing countries, 1.1 million of which result in death. Children under 5 account for 61% of all deaths attributable to shigellosis in poor countries the fecal contamination with water is very common because of poor

education and sanitation which is considered to be the most serious source of microbial contamination these water bodies ultimately serve as municipal raw supplies, which indicate possibilities for the transmission of these pathogens to the end-point users (Emch et al, 2008).

2.7.4. Fruits and vegetables

Raw leafy vegetables normally have nonpathogenic epiphytic microorganism (which live non-parasitically on the surface of a plant on various organs such as the leaves, roots, flowers, buds, seeds and fruit). However, during the process of harvesting and further handling the products could be contaminated with pathogenic agents from animal and human sources. As most of these produce are eating without further processing, their microbial content may represent a risk factor for the consumer's health. Microbiological contamination of fruits and vegetables can occur directly or indirectly from animals or insects, soil, manures, water and equipment used to grow the horticultural commodities as well as human handling along the food chain. The microbiological contaminants may have an adverse health effect (Aycicek, 2006).

Pathogen exposure directly associated with the raise of foodborne disease in many developing countries. Foodborne disease could be caused mainly by microorganisms and/or their exotoxins and enterotoxins. Practicing of vegetables may have related to pathogenic contamination. Manures (organic matter, mostly derived from animal feces) used to promote the growth of crops and vegetables contain a large number of pathogenic microorganisms including *Salmonella* spp., *E. coli*, *B. anthracis*, *Mycobacterium* spp., *Brucella* spp., *L. monocytogenes*, *Y. enterocolitica*, *C. perfringens*, *Klebsiella* spp. *and M. paratuberculosis*. Using fertilizers may pose a serious health risk to the local consumers (Rahman and Noor, 2012).

The number of documented outbreaks of human infections associated with the consumption of raw fruits, vegetables and unpasteurized fruit juices has increased in recent years (Buck et al, 2003).

More recently, salmonellosis has been linked to tomatoes, seed sprouts, cantaloupe (muskmelons), mamey sapote, apple juice and orange juice there are also documented associations of shigellosis with lettuce, scallions and parsley (Martin et al, 2003).

2.7.5. Egg

Eggs are an inexpensive and highly nutritious food, providing 18 vitamins and minerals, the composition of which can be affected by several factors such as hen diet, age, strain as well as environmental factors (Samman et al, 2009).

There is a general consensus that eggs contain other biological agents that may have play important role in the therapy and prevention of chronic and infectious. The presence such compounds with antimicrobial, immunomodulation, antioxidant, anti-cancer or anti-hypertensive properties have been reported in eggs (Abeyrathne and Lee, 2013).

At the same time, the many nutrient substances present in eggs create an excellent environment for the development of bacterial microflora, including pathogenic bacteria (Stępień, 2010).

Bad quality of egg shell possibly means injuring of egg shell cause contamination of egg with microorganisms which may lead to spoilage consequently economic losses or perhaps transmission of pathogens inducing cases of foodborne infection or intoxication to consumers (Kaneko et al, 2009).

Freshly laid eggs are generally devoid of organisms. However, following exposure to environmental conditions for example, soil, feces and dirty nesting materials, eggs become contaminated with different types of microorganisms (Ellen, 2000).

Microorganisms may contaminate the egg in two ways firstly by penetration or withdrawal through pores of the shells and secondly by the trans ovarian route. Growth and penetration of *S. enteritidis, S. heidelberg and S. typhimurium* in eggs. Predisposing factors such as environmental temperature and humidity influence the bacterial penetration thus enhancing infection and spoilage (Theron et al, 2003).

Wide rages of poultry product have been reported to be contaminated with *Salmonella* spp., consequently cause outbreaks of salmonellosis. Different species of *Salmonella* including *S. choleraesuis, S. enterica, S. bongori, S. typhi, S. paratyphi* and *S. typhimurium* causes gastro intestinal tract infection and typhoid fever (Bhunia, 2008).

Eggs contaminated with *Salmonella* spin or move during the farms and outlets my increase with both horizontal and vertical transmissions. Vertical transmission

means contamination of egg yolk, albumin, membranes or egg shells. While in horizontal transmission disease is penetrated during or after ovipositor through the egg shell from the gut or fecal contamination (Aoust et al, 2000).

2.7.6. Meat and meat products

Meat and meat products are unsafe to a variety of infectious diseases that can visible in food processing areas due mainly to poor personal hygiene, processing and cleanliness practices which cause develop the growth of microorganisms. Meat and meat products are important transport of foodborne illnesses in the world especially developing countries. There are two routes by which diseases may be transmitted through meat and it's products to humans. The first route is direct contact which includes anthrax, streptococcal skin infections, fungal and viral diseases. The second route of contamination by ingestion of half or uncooked meat or meat products (Aoust et al, 2000).

Meat processing and marketting the main causes higher levels of contamination meat carcasses. The presence of little numbers of microorganism in carcass meat and edible offal may lead to high level contamination of meat when it is cut into pieces increased more microorganisms to the surfaces of meat (Ejeta et al, 2004).

There are four main pathogens that have usually been associated with meat and meat products contamination including *Salmonella* spp., *Campylobacter* spp., *L. monocytogenes* and *E. coli.* These organisms have been associated to a number of cases of human illness (Mershal et al, 2010).

Salmonella infection of the red meat is the most reported cause of foodborne illness. Foodborne salmonellosis often follows consumption of contaminated animal products, which usually results from infected animals used in food production or from contamination of the carcasses (Alemayehu et al, 2002). *Salmonella* infection in meat animals arises from intensive rearing preparation and the use of contaminated feeds, cross-contamination of carcasses with *Salmonella* can also occur during slaughtering processes (Baird, 1990).

3. MATERIAL AND METHODS

3.1. Materials

Food samples were collected from different sources in Sulaymaniyah/Qaladze city from Iraq and then transferred immediately in 1-2 h, to laboratory by sterile ice box. A total of 100 samples (10 different samples of each factory raw chicken meat, village raw chicken meat, chicken meat shawarma, red meat shawarma, raw village egg, cooked village egg, homemade ayran, homemade yogurt, drinking water and washing water) were used in this study. The samples were kept cold at 4°C until analysis.

3.1.1. Solid media preparation

These media were prepared according to the manufacturers instructions; different media were used in this study (Figure 3.1.).

Salmonella shigella (SS) agar (Lab 052)

Prepare by dissolving 63 g in one liter of distilled water, mix well and bring to the boil (do not autoclave), cool to near 50°C, mix and distribute in to sterile petri dishes.

Xylose lysine desoxycholate (XLD) agar (Merck KGaA VM718887603)

Prepare by dissolving 53 g in one liter of distilled water, heat with frequent mixed until the medium boils (do not autoclave), transfer immediately to a water bath at 50°C and pour into sterile petri dishes.

Hectoen enteric (HE) agar (Merck KGaAVM742381625)

Prepare by dissolving 76 g of the powder in one liter of distilled water, heat with frequent mixed until the medium boils (do not autoclave), and pour into sterile petri dishes.

Sulfide indole motility (SIM) agar (HMEDIA M181)

Prepare by dissolving 30 g of the media to one liter of distilled water, heat to boiling and mix to dissolve completely then dispense medium into tubes to an approximate depth of 3 inches, sterilize in the autoclave at 121ºC, for 15 minutes.

Simmons citrate agar (CM O155)

Prepare by dissolving 28 g in one liter distilled water, boiled to dissolve the medium completely, mix well and distribute in tubes, sterilize by autoclaving (121°C for 15 minutes), cool in slanted position (long slant, for tubes dispense 4.0 to 5.0 ml into 16 mm tubes).

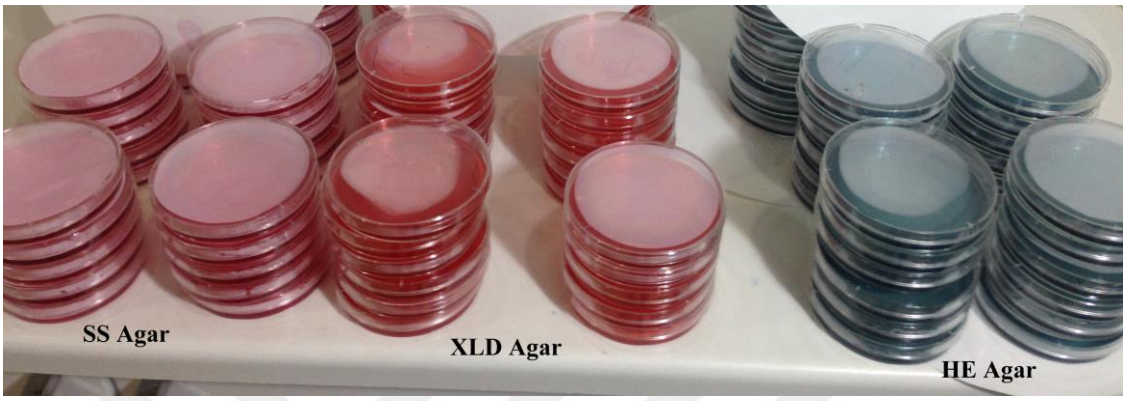

Figure 3.1. Solid media

3.1.2. Liquid media preparation

Buffered peptone water (BPW)

It was prepared by dissolving 50 g of powder in one liter distilled water, mixed well and distributed into test tubes and sterilized by autoclaving at 121ºC for 15 minutes, then stored at 4ºC until used (Figure 3.2.).

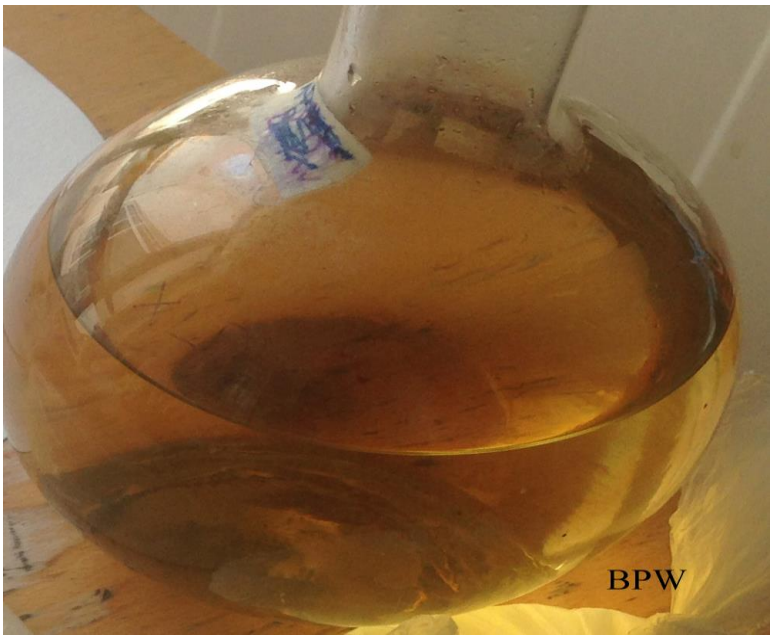

Figure 3.2. Buffered peptone water (BPW)

Peptone water sugars for carbohydrate fermentation

The media composed of peptone water and different sugars, distributed amounts 2 ml into sterile test tubes containing inverted Durhaim's tube then sterilized by steaming for 30 minutes and stored at 4ºC until used.

Shigella broth (BAM Media M136)

Dissolve 31.5 g of the powder in one liter of distilled water, heating if necessary. Distribute in suitable containers and sterilize in the autoclave at 121ºC for 15 minutes. Cool to 45ºC and aseptically add novobiocin to reach a final concentration of 0.5 mcg/ml. The complete medium must be used the day of preparation. The basal broth without antibiotic can be stored in refrigeration for 4 weeks.

Rappaport-vassiliadis (RV) enrichment broth (Merck KGaAVM711400603)

Prepare by dissolving 26.6 g of the medium in one liter of distilled water, mix thoroughly and autoclave at 116°C for 15 minutes (Figure 3.3.).

Figure 3.3. Rappaport-vassiliadis (RV) enrichment broth

3.1.3. Solutions and reagents preparation

Kovac's reagent

This reagent was prepared for indole test. 5 g of p-dimethyl amino benzaldehyde was dissolved in 75 ml of amyl alcohol by warming in a water bath (50-55ºC), then cooled and 25 ml of HCl was added. It was protected from light and stored at 4ºC (Cowan and Steel, 1985).

Oxidase test reagent

It was prepared by adding a loop full of tetramethyl-phenylenediamine hydrochloride solution to 3 ml of distilled water; one procedure of this test place a piece of filter paper in petri dish and add 3 drops of freshly prepared oxidase reagent, using a sterile glass rod, remove a colony of test organisms from a culture plate and smear it on the filter paper (Cruickshank, 1972).

Normal saline solution

This was prepared by dissolving 8.5 g of sodium chloride in one liter of distilled water (Cowan and Steel, 1985).

Methyl red (MR) solution

This solution was prepared by dissolving 0.04 g of methyl red in 10 ml ethanol and diluted with water to 100 ml (Cowan and Steel, 1985).

Voges-proskauer (VP) reagent

With alpha-naphthol in the presence of 40% potassium hydroxide (KOH), some bacteria produce stable acid as end products when growths in some specific media, after glucose fermentation, particular enteric bacteria metabolize pyruvic acid to acetylmethyl carbinol, when positive this product reacts with alpha-naphthol (α-naphthol) in the presence of 40% KOH to produce a red color complex (Cowan and Steel, 1985).

3.2. Methods

3.2.1. Isolation

In the present study for isolation of *Salmonella* spp. used ISO 6579 as a method (Anonymous, 2002) and for isolation of *Shigella* ssp. used EN-ISO 21567 as a method (Anonymous, 2004). The steps of two methods cleared in Figure 3.4.

Homogenizing and parametering food samples

After collection the food samples and transferred to laboratory by sterile ice box, and homogenized by stomacher (Sjia-04c), then determined the some parameters for each samples such as pH, O/R (Pro 2013, Fat Technical Lab) and a_w (a_w-meter, series 3, Aqua Lab), two other importance machine used in the current study firstly for colony counting called acolyte 3 (8000/syn) and secondly for automated identification called VITEK 2 Compact (VK2 C9753).

Pre-enrichment medium

Buffered peptone water (BPW), is used to help recovery or activate bacteria before transfer to a selective media, this media is free from inhibitors and is well buffered and provides conditions for resuscitation of the cells that have been injured in the time of food preservation. In this step, 25 g of all homogenized food samples added to 225 ml of BPW and incubated at 37ºC for 16-22 h.

Selective broth medium

For all food samples 1 ml of the pre-enrichment was taken, by using a sterile pipette transferred into the test-tube containing 10 ml of the rappaport-vassiliadis (RV) broth, are placed into sterile tubes containing 10 ml RV broth (with serial dilution) and the culture was incubated at 41.50±1°C aerobically for 24 h.

But for *Shigella* detection used Shigella broth not used RV broth, because inhibit the growth of *Shigella*.

Plating (inoculation of plates)

10 µl or a loop full of the RV broth was streaked on a plate of three different selective media (SS agar, Hectoen agar, and XLD agar) and incubated aerobically at 37ºC for 24 h.

Colony counting

For counting isolated bacterial colonies, were used advanced computerized machine called acolyte 3 (8000/syn), this device can take photos of the plates and colonies at the same time classical colony counting used.

Storage of isolated bacteria and purification

Colonies were purified by repeated subculture on Tryptic Soy Agar (TSA) at least 5 suspected colony selected; pure isolates were stored on TSA slopes in the refrigerator at 4ºC.

3.2.2. Identification of isolates

Performed identification according to Cowan and Steel (1985), by classical biochemical tests and automated identification (Figure 3.4.).

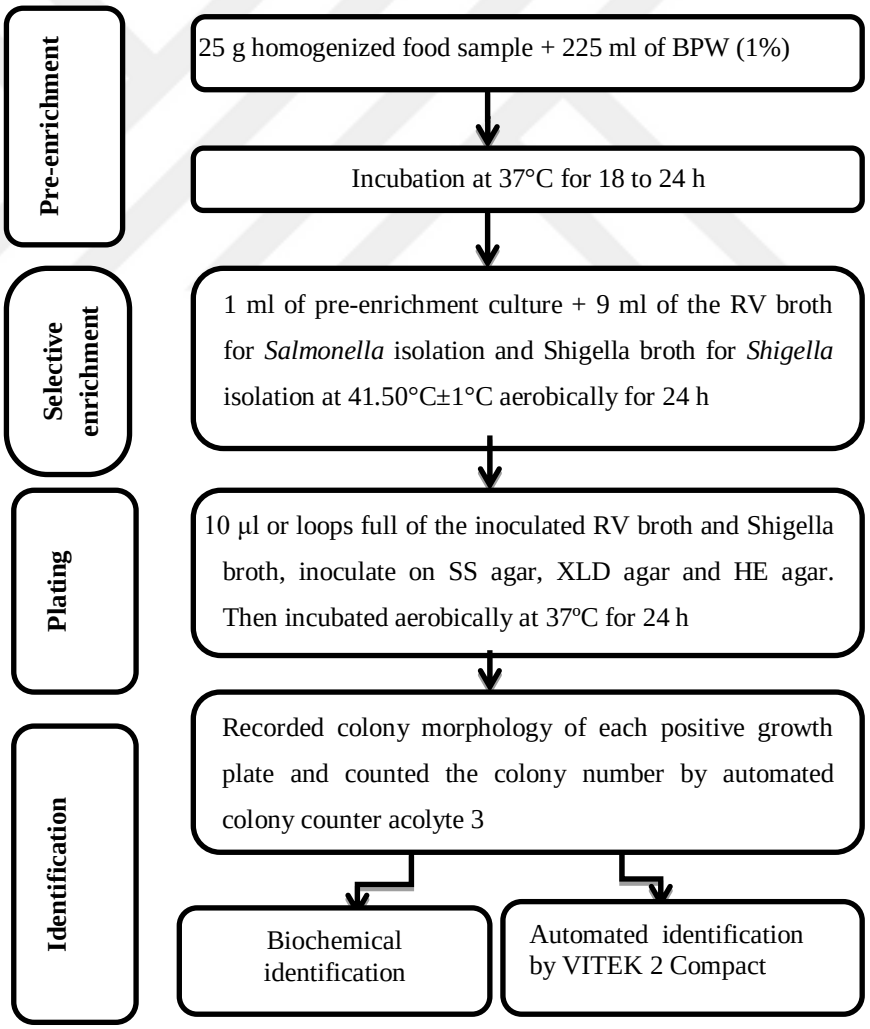

Figure 3.4. Isolation and identification of *Salmonella* spp. and *Shigella* spp.

3.2.2.1. Classical identification of isolates

Microscopic examination

For this purpose by a sterelized loop make a thin layer of bacterial cells on a clean glass slide from a fresh 18-24 h of growth culture. After staining with a gram stain, the cultures examined in the immersion objective, were apeared blue-violet as Gram (+) and pink-red as Gram (-) (Figure 3.5.).

Microscopic properties of *Salmonella* Gram (-) bacteria, shape bacilli, usually motile and *Shigella* also Gram (-), non motile and shape bacilli or rods, non motile (Bartholomew, 1962).

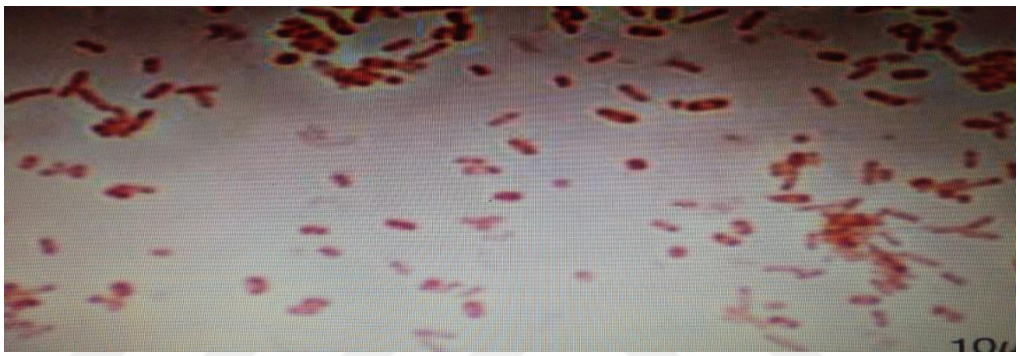

Figure 3.5. Microscope appearance of Gram (-) bacteria

Oxidase test

A pices of filter paper were soaked in 10% solution of tetramethyl-p-phenylene diamine hydrochloride (oxidase test reagent) in and then left to dry, then a new young test culture, on nutrient agar, was picked up with a sterile glass rod and streaked on that filter paper dark purple color that developed for five to ten seconds was considered positive reaction, oxidase test for *Salmonella* spp. and *Shigella* spp. are negative (Cruickshank, 1972) (Figure3.6.).

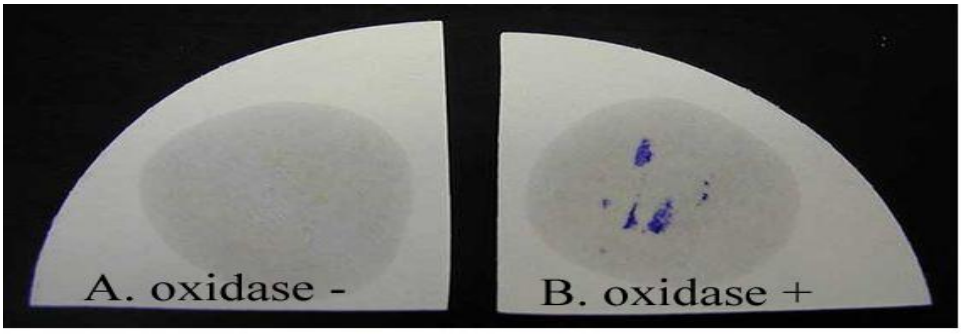

Figure 3.6. Oxidase test

Catalase test

A drop of 3% solution of hydrogen peroxide (H_2O_2) was placed on a clean glass slide. A colony of test culture was then placed on the H_2O_2, when gas bubbles appeared on the surface of the culture material the test was considered positive. Catalase test for *Salmonella* spp. always positive but *Shigella* species are variable (Temiz, 2010) (Figure 3.7.).

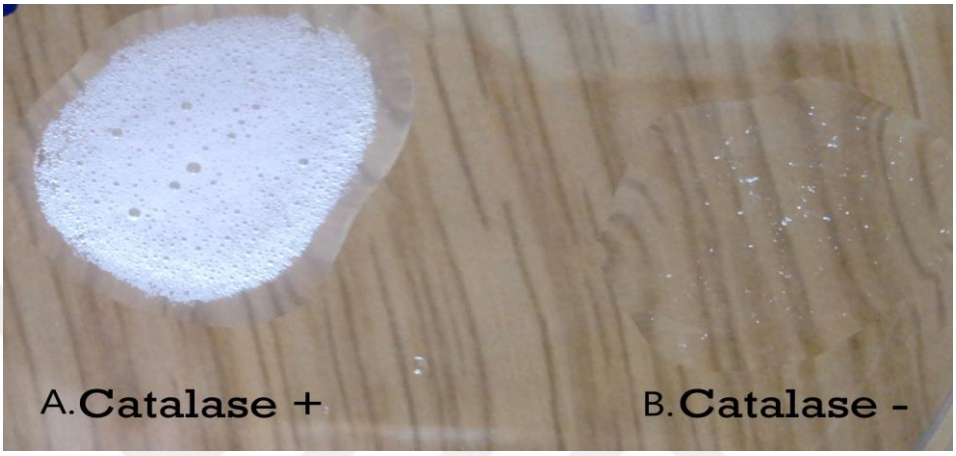

Figure 3.7. Catalase test

Gas production test

The ability of organisms to ferment different sugars has long been used in differentiation of the *Enterobacteriaceae*, when peptone water sugar was inoculated with bacterial culture; the tube was then incubated at 37ºC and examined for up to 2 days, where as gas production was indicated by development of an empty space in the Durham's tube (Temiz, 2010) (Figure 3.8.).

Figure 3.8. Gas production test

Sulfide, indole and motility (SIM) test

Sulfide, indole and motility (SIM) medium was used for determination each, motility, H_2S production and indole tests, were incubated at 37ºC for 24 or 48 h, in the case of positive motility was shown by turbidity away from the line of inoculation, while growth confined at the point of inoculation was a negative result (Darland, 1978) (Figure 3.9.).

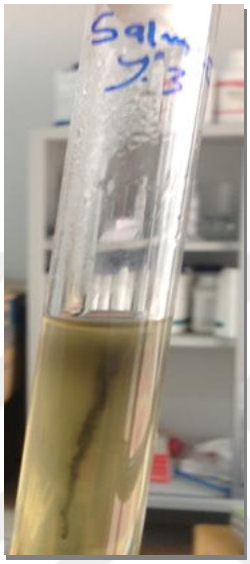

Figure 3.9. Sulfide, indole and motility test

Methyl red (MR) test

A positive reaction was indicated by appearance of a red color, methyl red for *Salmonella* spp. are positive, while methyl red for *Shigella* spp. is negative (Murray, 1998) (Figure 3.10.).

Figure 3.10. Methyl red test

Voges proskauer (VP) test

A positive reaction was indicated by development of bright pink color within 30 minutes. The result of VP test for *Salmonella* spp. and *Shigella* spp. are negative (Cowan and Steel, 1985) (Figure 3.11.).

Figure 3.11. Voges proskauer test

Citrate test

A positive test was indicated by change of color from green to blue, the citrate test result of *Salmonella* spp. between strong and weak positive in some serotype are negative, but citrate test for *Shigella* spp. are negative (Murray, 1998) (Figure 3.12.)

Figure 3.12. Citrate test

3.2.2.2 Automated identification of isolates

To further identification of *Salmonella* and *Shigella*, despite the traditional tests, used VITEK 2 Compact (VK2 C9753) which is advanced machine and it is used in culture field throughout the world. It compact has every thing healthcare laboratories need for fast, accurate microbial identification, and antibiotic susceptibility testing.

The innovative microbial identification system includes an expanded identification database, the most automated platform available, rapid results, improved confidence, with minimal training time. The system next-generation platform provides greater automation while increasing safety and eliminating repetitive manual operations. The rapid response time means results can be provided more quickly than with manual microbial identification techniques (David, 2016).

3.2.3. Statistical analyses

Statistical analysis is the science of collecting data and uncovering patterns and trends. It's really just another way of saying statistics. After collecting data you can analyze it to summarize the data.

In the current study used SPSS (Version 18.0), enumerated and analyzed for founding relationship between *Salmonella* and *Shigella* isolation and pH, a_w, oxidation-reduction (O/R) and three different media wich used in the study.

Correlation analysis is used to indicate the association or relationship between two or more quantitative variables. This analysis is fundamentally based on the assumption of a straight line, relationship between the quantitative variables similar to the measures of association for binary variables; it measures the strength or extent of an association between the variables and also its direction.

The end result of a correlation analysis is a correlation coefficient whose values range from -1 to +1. A correlation coefficient of +1 indicates that two variables are perfectly related in a positive (linear) manner, a correlation coefficient of -1 indicates that two variables are perfectly related in a negative (linear) manner, while a correlation coefficient of zero indicates that there is no linear relationship between the two variables being studied (Gogtay, 2017).

4. RESULTS

4.1. Isolated Bacteria

In the current study was analyzed 100 food samples (10 different groups), 58 samples of all 100 samples are positive growth. The number of the isolated *Salmonella* spp. of the all food samples was 45 (77.60%); and the number of isolated *Shigella* spp. was 32 (55.20%).

The highest incidence among *Salmonella* spp. was *S. enteritidis* (37.70%), *S. bongori* (24.40%), *S. typhimurium* (17.70%), *S. paratyphi* (17.70%), *S. typhi* (2.20%). The highest frequency among the *Shigella* species was determined as *S. dysanteria* (50.00 %), *S. sonnei* (18.75%), *S. flexneri* (18.75%) and *S. boydii* (12.50%) (Figure 4.1. and Table 4.1.).

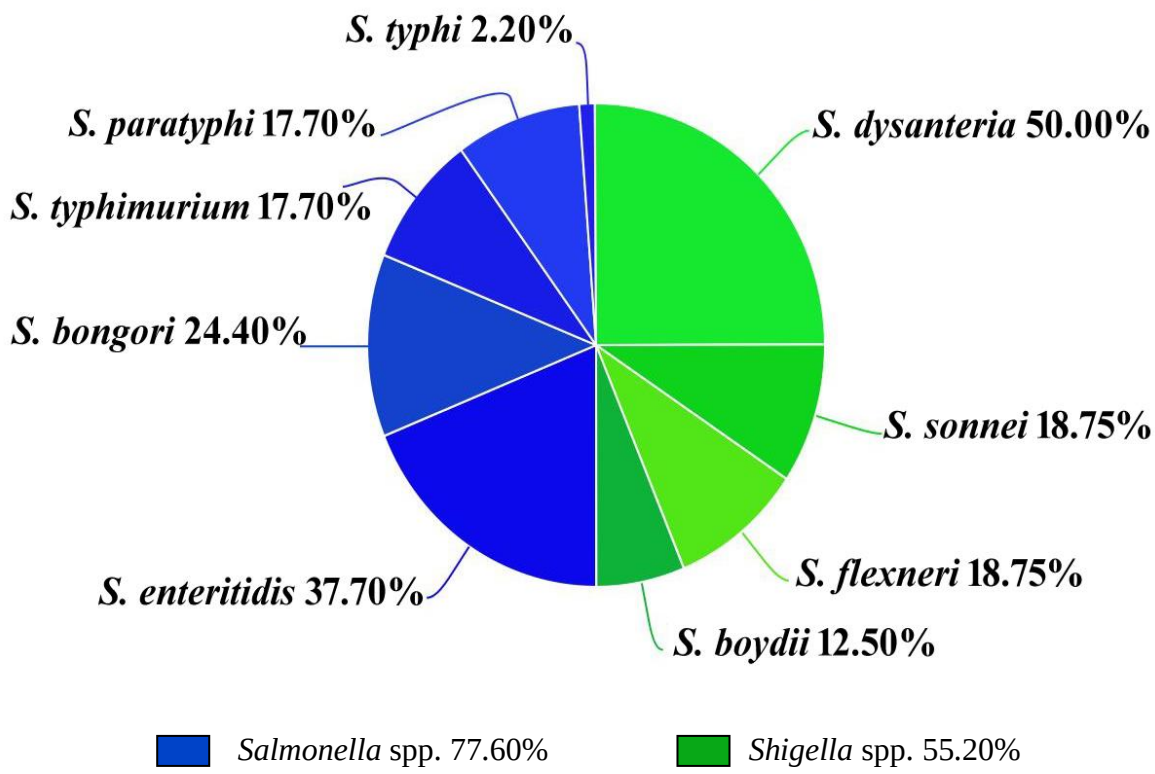

Figure 4.1. Percentages of isolated *Salmonella* spp. and *Shigella* spp.

Table 4.1. *Salmonella* spp. and *Shigella* spp. isolated from food samples

Food groups		Number of isolated		*Salmonella* spp.	n	*Shigella* spp.	n
		Salmonella spp.	*Shigella* spp.				
A	**Factory raw chicken meat**	8	3	*S. enteritidis* *S. bongori* *S. paratyphi* *S. typhi*	4 2 1 1	*S. dysenteriae* *S. sonnei*	2 1
B	**Village raw chicken meat**	8	2	*S. enteritidis* *S. bongori* *S. typhimurium* *S. paratyphi*	3 3 1 1	*S. dysenteriae*	2
C	**Chicken meat shawarma**	7	5	*S. enteritidis* *S. typhimurium* *S. bongori* *S. paratyphi*	4 1 1 1	*S. dysenteriae* *S. flexneri* *S. boydii*	2 1 2
D	**Red meat shawarma**	5	4	*S. enteritidis* *S. typhimurium* *S. bongori*	1 2 2	*S. dysenteriae* *S. flexneri* *S. sonnei*	2 1 1
E	**Raw village egg**	8	5	*S. enteritidis* *S. typhimurium* *S. paratyphi* *S. bongori*	3 2 2 1	*S. dysenteriae* *S. flexneri* *S. boydii* *S. sonnei*	2 1 1 1
F	**Cooked village egg**	1	1	*S. paratyphi*	1	*S. sonnei*	1
G	**Homemade ayran**	1	1	*S. paratyphi*	1	*S. flexneri*	1
H	**Homemade yogurt**	-	1	-	-	*S. sonnei*	1
I	**Drinking water**	-	2	-	-	*S. dysenteriae*	2
J	**Washing water**	7	8	*S. enteritidis* *S. typhimurium* *S. bongori* *S. paratyphi*	2 2 2 1	*S. dysenteriae* *S. flexneri* *S. sonnei* *S. boydii*	4 2 1 1
Total		**45**	**32**		**45**		**32**

Table 4.2. Minimum, maximum and mean of pH, a_w and O/R of food groups

Food groups		pH			a_w			Oxidation/Reduction		
		Min	**Max**	**Mean**	**Min**	**Max**	**Mean**	**Min**	**Max**	**Mean**
A	**Factory raw chicken meat**	6.4	7.5	7.09	0.88	0.95	0.916	-200	-76	-141.40
B	**Village raw chicken meat**	6.6	7.1	6.90	0.92	0.98	0.937	-190	-59	-136.90
C	**Chicken meat shawarma**	4.8	7.7	6.72	0.84	0.99	0.947	229	315	291.78
D	**Red meat shawarma**	4.3	7.8	6.21	0.93	0.99	0.953	292	318	305.20
E	**Raw village egg**	6.1	7.2	6.90	0.83	0.94	0.937	493	504	-136.90
F	**Cooked village egg**	6.4	7.8	6.95	0.48	0.6	0.534	488	501	497.10
G	**Homemade ayran**	4.2	6.7	4.32	0.85	0.91	0.88	165	300	232.10
H	**Homemade yogurt**	4	6.5	4.79	0.79	0.95	0.835	100	190	158.30
I	**Drinking water**	6.5	7.2	6.87	0.98	1.1	1.007	477	501	497.10
J	**Washing water**	6.6	7.2	6.94	0.88	1.1	0.991	444	501	485.70

4.2. Cultural Properties of *Salmonella* and *Shigella*

4.2.1. Growth properties in liquid media

Growth in peptone water was indicated by the formation of turbidity and slight white sediment after 24 h of incubation at 37ºC.

Growth in RV enrichment broth was detected by change color of medium from blue to yellow after 24 h of incubation at 37ºC (Figure 4.2.).

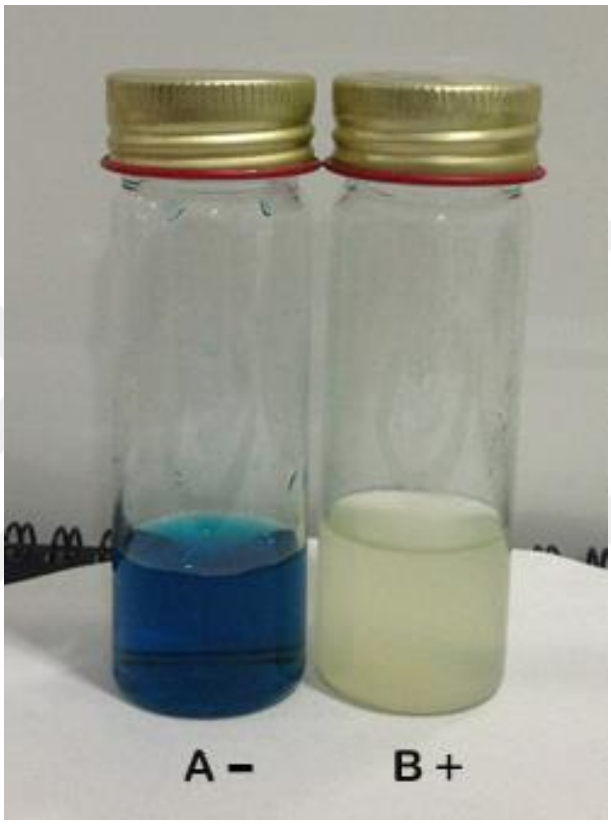

Figure 4.2. Rappaport-vassiliadis (RV) enrichment broth and change color

4.2.2. Growth properties on solid media

Salmonella and *Shigella* grow on different media in present study used three different media such as SS agar, HE agar and XLD agar. The *Salmonella* and *Shigella* colonies appear with different shapes and colors according to the used media.

Salmonella and *Shigella* colony on SS agar are colorless colonies because they are non-lactose fermenters, with smooth and circular surface. Hydrogen sulfide (H_2S) positive for *Salmonella* spp., there for produce black center colonies (Figure 4.3).

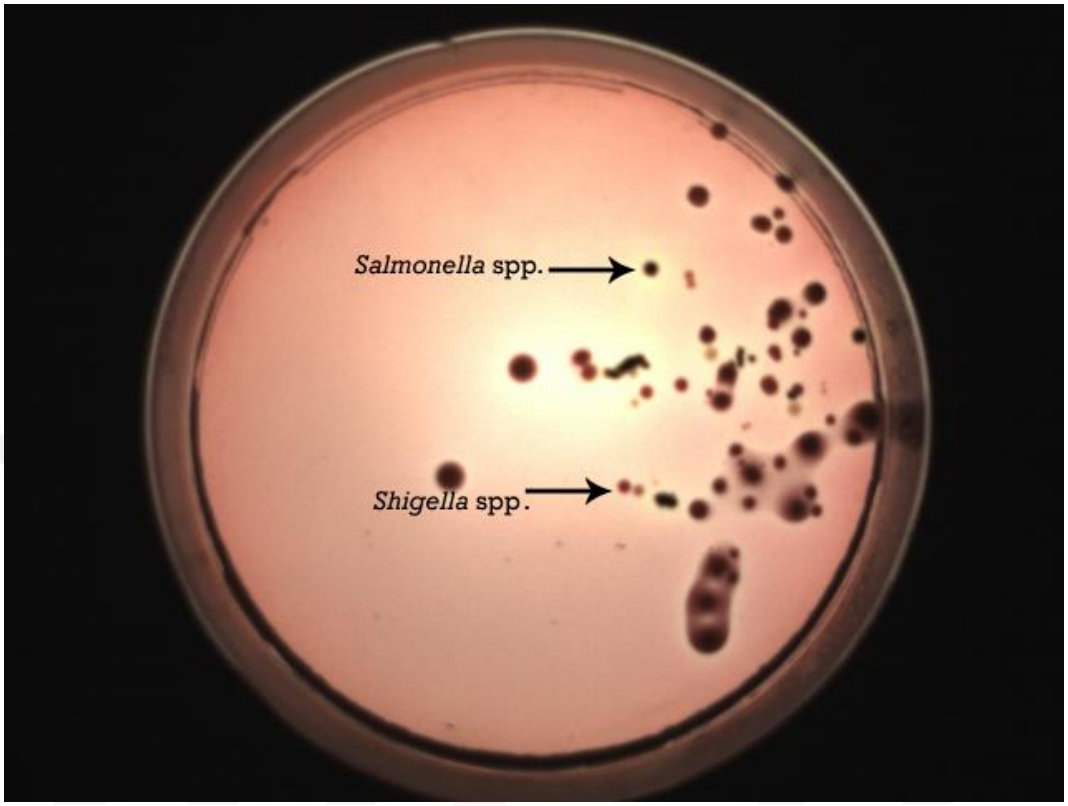

Figure 4.3. Colony morphology on Salmonella Shigella (SS) agar

Colonies of *Salmonella* and *Shigella* on HE agar are green to bluish-green in color, but *Salmonella* spp. because produce H_2S appear as blue-green colonies with black centers (Figure 4.4.).

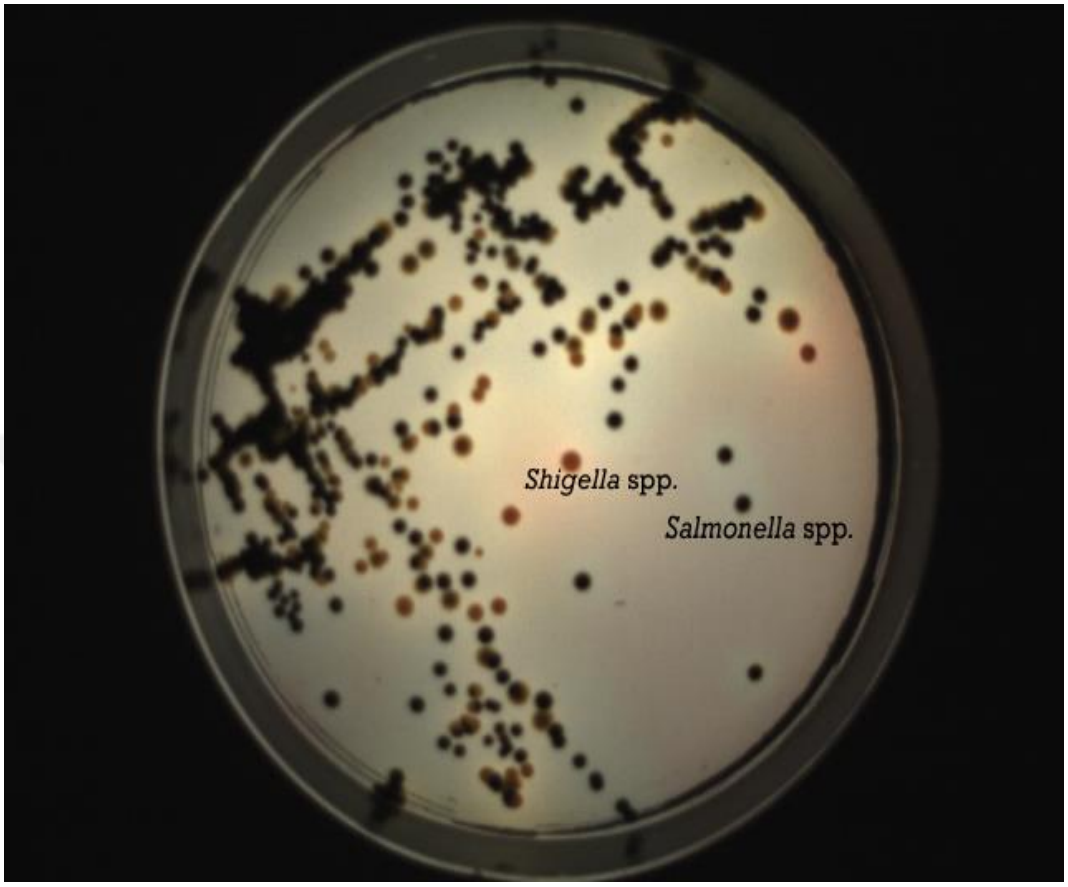

Figure 4.4. Colony morphology on Hectoen Enteric (HE) agar

Salmonella and *Shigella* colony on XLD agar appears red colony, *Salmonella* also metabolise thiosulfate to produce H_2S, which leads to the formation of colonies with black centers and allows them to be differentiated from the colourless *Shigella* colonies (Figure 4.5).

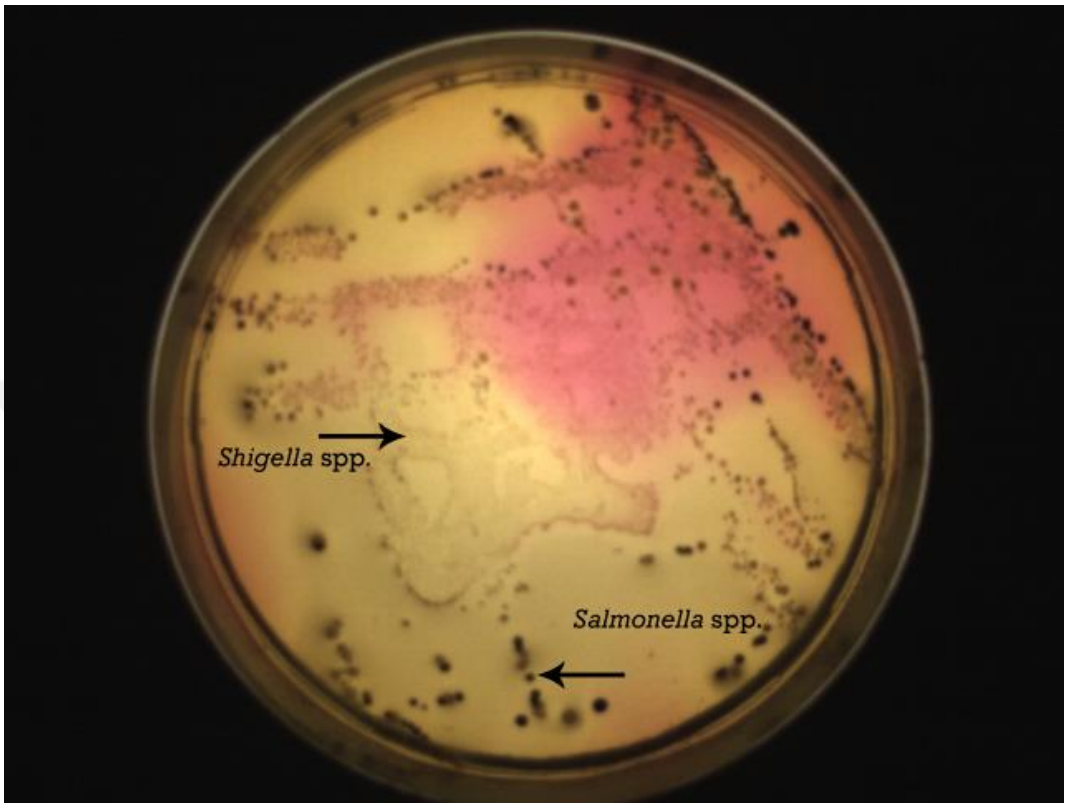

Figure 4.5. Colony morphology on Xylose Lysine Deoxycholate (XLD) agar

On sulfide indole motility (SIM) medium, were determined the motility and H_2S production. *Salmonella* colonies was indicated by black discoloration because produced H_2S, and found motile zone by a diffuse of growth flaring from the line of inoculation. But were not H_2S production and motility zone for inoculated *Shigella* colonies (Figure 4.6.).

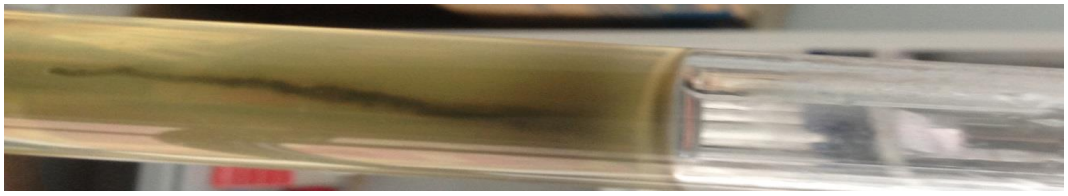

Figure 4.6. Sulfide indole motility (SIM) medium growth result

4.2.3. Microscopic properties

All *Salmonella* isolates were Gram (-), short rods, single or groups (Figure 4.7.) and all *Shigella* isolated are Gram (-) and rod-shaped bacilli (Figure 4.8.).

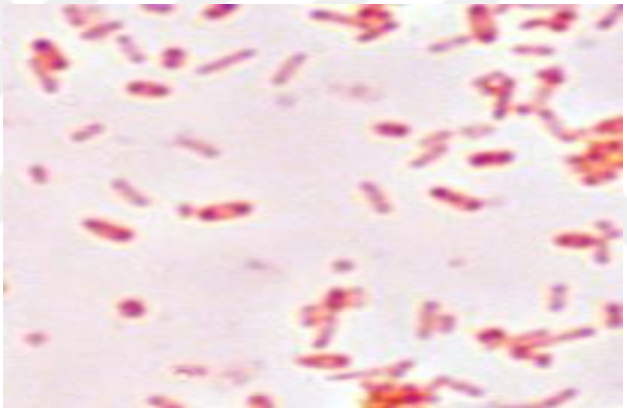

Figure 4.7. Microscopic properties of *Salmonella* spp.

Figure 4.8. Microscopic properties of *Shigella* spp.

4.2.4. Biochemical reactions results

Biochemical reactions results of *Salmonella* spp. and *Shigella* spp. were isolated in the current study cleared in the Table 4.3.

Table 4.3. Biochemical and automated identification test for isolated bacteria

	Tests	***Salmonella* spp.**	***Shigella* spp.**
Classical identification	**Microskopic examination**	Bacil	Bacil or rod
	Gram stain	Gram (-)	Gram (-)
	Oxidase	-	-
	Catalase	+	(-/wk+)*
	Gas production	-	-/+
	H_2S	+/-	-
	Indole	-	-/+
	Motility	+	-
	MR	+	-
	VP	-	-
	Citrate	+/wk+/	-
Automated identification	Note: VITEK 2 Compact an automated identified microbiology system utilizing-growth-based technology	**5 different species**	**4 different species**
		S. enteritidis 17 *S. bongori* 11 *S. typhimurium* 8 *S. paratyphi* 8 *S. typhi* 1	*S. dysenteriae* 16 *S. flexneri* 6 *S. sonnei* 6 *S.boydii* 4
	Toplam	**45**	**32**

*wk+: Means weak positive reaction of biochemical tests

4.2.5. Statistical analyses in foods

Group A. Factory raw chicken meat

Table 4.4. Physical condition and number of bacterial colony of group A

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigell a* spp.**
7.1	0.91	-76	2.200	-	3.600	-	1.000	-
7.5	0.92	-89	3.500	6.820	3.940	5.987	1.100	1.900
6.4	0.94	-159	-	7.000	-	5.400	-	4.100
7.4	0.90	-200	4.500	-	4.100	-	3.00	-
6.6	0.95	-156	5.600	-	5.020	-	5.100	-
7.5	0.94	-178	3.950	-	3.520	-	2.830	-
7.0	0.89	-100	4.000	-	4.400	-	4.040	-
6.9	0.93	-192	8.000	-	7.610	-	4.630	-
7.2	0.90	-165	7.260	3.400	1.650	2.470	1.820	0.402
7.3	0.88	-99	4.960	-	2.600	-	2.000	-

Table 4.5. Statistical analysis of group A

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigela* spp.**
pH	**Pearson Correlation**									
a_w		-.678*								
O/R		-.453	.546							
***Salmonella* spp.**		.594	-.211	-.063						
***Shigella* spp.**		-.630	.086	.011	-.606					
***Salmonella* spp.**		.616	-.129	-.037	.957**	-.636*				
***Shigella* spp.**		-.625	.079	.004	-.615	1.000**	-.645*			
***Salmonella* spp.**		.579	-.078	.035	.969**	-.696*	.959**	-.706*		
***Shigella* spp.**		-.577	.028	-.046	-.662*	.974**	-.695*	.980**	-.760*	

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)

Group B. Village raw chicken meat

Table 4.6. Physical condition and number of bacterial colony of group B

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigell*a spp.**	***Salmonella* spp.**	***Shigella* spp.**
7.1	0.93	-180	11.98	-	8.7	-	5.8	-
7.0	0.93	-59	14.1	-	9.84	-	7	-
6.8	0.98	-100	3.24	-	2.42	-	3.4	-
6.9	0.92	-166	-	5.91	-	5.1	0	4.9
6.9	0.94	-172	15.4	-	9.2	-	6.9	-
7.1	0.91	-190	8.6	-	7.8	-	4.83	-
6.7	0.97	-98	5.45	-	4.8	-	3.34	-
6.6	0.95	-125	-	16.3	-	12.95	0	7.845
7.0	0.89	-156	6.37	-	2.8	-	3	-
6.9	0.95	-123	5.36	-	5	-	2.8	-

Table 4.7. Statistical analysis of group B

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		-.678*								
O/R		-.453	.546							
***Salmonella* spp.**		.594	-.211	-.063						
***Shigella* spp.**		-.630	.086	.011	-.606					
***Salmonella* spp.**		.616	-.129	-.037	.957**	-.636*				
***Shigella* spp.**		-.625	.079	.004	-.615	1.000**	-.645*			
***Salmonella* spp.**		.579	-.078	.035	.969**	-.696*	.959**	-.706*		
***Shigella* spp.**		-.577	.028	-.046	-.662*	.974**	-.695*	.980**	-.760*	

*: Correlation is significant at the 0.05 level (2-tailed)

**: Correlation is significant at the 0.01 level (2-tailed)

Group C. Chicken meat shawarma

Table 4.8. Physical condition and number of bacterial colony of group C

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
7.7	0.98	295	7.83	5.91	4.11	2	3.72	2.4
7.1	0.96	298	8.3	7.1	5.6	3.4	3.84	2.13
6.9	0.96	315	5.2	4.86	6.9	3.33	4.55	3.9
6.7	0.99	310	2.8	1.3	3.1	1.5	1.2	0.9
7.0	0.97	275	7.55	-	4.722	-	15.4	-
6.8	0.98	298	5.62	4.02	2.2	4.456	2.5	1.001
7.3	0.96	300	3.3	-	10.36	-	13.6	-
6.2	0.84	235	-	-	-	-	-	-
4.8	0.88	300	-	-	-	-	-	-
6.0	0.86	229	-	-	-	-	-	-

Table 4.9. Statistical analysis of group C

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		.726*								
O/R		.292	.773**							
***Salmonella* spp.**		.779**	.794**	.487						
***Shigella* spp.**		.540	.525	.480	.733*					
***Salmonella* spp.**		.702*	.639*	.564	.545	.284				
***Shigilla* spp.**		.408	.572	.536	.592	.852**	.196			
***Salmonella* spp.**		.525	.468	.208	.488	-.147	.739*	-.198		
***Shigigella* spp.**		.466	.474	.540	.563	.846**	.374	.736*	-.115	

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)

Group D. Red meat shawarma

Table 4.10. Physical condition and number of bacterial colony of group D

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
6.5	0.98	300	1.82	2.3	2.7	2.567	1.2	1.002
7.8	0.97	312	1.4	0.8	1.3	1.321	1.002	0.99
6.7	0.99	305	4.5	-	3.1	-	2	-
7.7	0.96	300	2.706	3.4	2.9	1.1	1.98	0.96
5.2	0.95	318	3.38	1.5	4	3.4	1.7	1.2
4.3	0.93	312	-	-	-	-	-	-
7.3	0.86	310	-	-	-	-	-	-
4.4	0.97	305	-	-	-	-	-	-
6.7	0.98	298	-	-	-	-	-	-
5.5	0.94	292	-	-	-	-	-	-

Table 4.11. Statistical analysis of group D

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		.002								
O/R		-.142	-.275							
***Salmonella* spp.**		.287	.437	.239						
***Shigella* spp.**		.389	.230	-.058	.465					
***Salmonella* spp.**		.249	.398	.265	.936**	.674*				
***Shigella* spp.**		.072	.210	.377	.500	.664*	.755*			
***Salmonella* spp.**		.405	.440	.185	.961**	.675*	.954**	.578		
***Shigella* spp.**		.333	.256	.310	.508	.818**	.736*	.911**	.666*	

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)

Group E. Raw village egg

Table 4.12. Physical condition and number of bacterial colony of group E

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
7.1	0.93	-180	11.98	-	8.7	-	5.8	-
7.0	0.93	-59	14.1	-	9.84	-	7	-
6.8	0.98	-100	3.24	-	2.42	-	3.4	-
6.9	0.92	-166	-	5.91	-	5.1	-	4.9
6.9	0.94	-172	15.4	-	9.2	-	6.9	-
7.1	0.91	-190	8.6	-	7.8	-	4.83	-
6.7	0.97	-98	5.45	-	4.8	-	3.34	-
6.6	0.95	-125	-	16.3	-	12.95	-	7.845
7.0	0.89	-156	6.37	-	2.8	-	3	-
6.9	0.95	-123	5.36	-	5	-	2.8	-

Table 4.13. Statistical analysis of group E

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		-.105								
O/R		.515	.180							
***Salmonella* spp.**		.489	.463	.105						
***Shigella* spp.**		-.184	.484	.229	.083					
***Salmonella* spp.**		.446	.447	-.041	.841**	.102				
***Shigella* spp.**		-.233	.485	.221	.077	.991**	.102			
***Salmonela* spp.**		.312	.571	.328	.752*	.187	.820**	.200		
***Shigella* spp.**		-.286	.533	.287	.141	.927**	.048	.926**	.291	

*: Correlation is significant at the 0.05 level (2-tailed)
**: Correlation is significant at the 0.01 level (2-tailed)

Group F. Cooked village egg

Table 4.14. Physical condition and number of bacterial colony of group F

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
6.7	0.60	501	-	-	-	-	-	-
7.8	0.55	499	-	-	-	-	-	-
7.4	0.51	496	-		-		-	-
6.6	0.49	499	-	0.9	-	1	-	0.02
6.7	0.57	500	-	-	-	-	-	-
6.9	0.52	488	-	-	-	-	-	-
6.4	0.48	490	-	-	-	-	-	-
7.0	0.50	500	3.23	-	1.1	-	0.977	-
7.1	0.55	498	-	-	-	-	-	-
6.9	0.57	500	-	-	-	-	-	-

Table 4.15. Statistical analysis of group F

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigela* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		.150								
O/R		.196	.537							
***Salmonella* spp.**		.043	-.300	.226						
***Shigella* spp.**		-.301	-.389	.148	-.111					
***Salmonella* spp.**		.043	-.300	.226	1.000**	-.111				
***Shigella* spp.**		-.301	-.389	.148	-.111	1.000**	-.111			
***Salmonella* spp.**		.043	-.300	.226	1.000**	-.111	1.000**	-.111		
***Shigella* spp.**		-.301	-.389	.148	-.111	1.000**	-.111	1.000**	-.111	

**: Correlation is significant at the 0.01 level (2-tailed).

Group G. Homemade ayran

Table 4.16. Physical condition and number of bacterial colony of group G

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonell*a spp.**	***Shigella* spp.**	***Salmonell*a spp.**	***Shigella* spp.**
4.0	0.89	180		-	-	-	-	-
6.7	0.90	222	2.6	-	1.89	-	0.345	-
3.5	0.89	298	-	-	-	-	-	-
3.7	0.85	167	-	-	-	-	-	-
4.2	0.87	260	-	-	-	-	-	-
3.8	0.88	165	-	-	-	-	-	-
4.3	0.85	300	-	-	-	-	-	-
3.4	0.86	266	-	-	-	-	-	-
6.1	0.91	298	-	2	-	2.98	-	1.8
3.5	0.90	165	-	-	-	-	-	-

Table 4.17. Statistical analysis of group G

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmnella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		.503								
O/R		.247	-.041							
***Salmonell a* spp.**		.730*	.325	-.060						
***Shigella* spp.**		.546	.488	.393	-.111					
***Salmnella* spp.**		.730*	.325	-.060	1.000**	-.111				
***Shigella* spp.**		.546	.488	.393	-.111	1.000**	-.111			
***Salmonell a* spp.**		.730*	.325	-.060	1.000**	-.111	1.000**	-.111		
***Shigella* spp.**		.546	.488	.393	-.111	1.000**	-.111	1.000**	-.111	

*: Correlation is significant at the 0.05 level (2-tailed)

**: Correlation is significant at the 0.01 level (2-tailed)

Group H. Homemade yogurt

Table 4.18. Physical condition and number of bacterial colony of group H

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
4.7	0.87	100	-	-	-	-	-	-
4.2	0.8	167	-	-	-	-	-	-
4.8	0.84	173	-	-	-	-	-	-
6.5	0.95	165	-	4.56	-	5.9	-	1.003
4.1	0.84	190	-	-	-	-	-	-
5.2	0.8	160	-	-	-	-	-	-
4.4	0.82	155	-	--	-	-	-	-
4.0	0.81	158	-	-	-	-	-	-
4.7	0.83	163	-	-	-	-	-	-
5.3	0.79	152	-	-	-	-		-

Table 4.19. Statistical analysis of group H

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**	.635*								
a_w										
O/R		-.075	-.111							
***Salmonella* spp.**		.b	.b	.b	.b					
***Shigella* spp.**		.809**	.860**	.102	.b					
***Salmonella* spp.**		.b	.b	.b	.b	.b	.b			
***Shigella* spp.**		.809**	.860**	.102	.b	1.000**	.b			
***Salmonella* spp.**		.b	.b	.b	.b	.b	.b	.b	.b	
***Shigella* spp.**		.809**	.860**	.102	.b	1.000**	.b	1.000**	.b	

*: Correlation is significant at the 0.05 level (2-tailed)

**: Correlation is significant at the 0.01 level (2-tailed)

. b: Cannot be computed because at least one of the variables is constant (SPSS will not perform Spearman's Rank Correlation for the two Construct)

Group I. Drinking water

Table 4.20. Physical condition and number of bacterial colony of group I

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
6.5	1.00	500	-	-	-	-	-	-
7.2	1.00	494	-	-	-	-	-	-
6.7	1.00	501	-	-	-	-	-	-
7.0	1.00	477	-	-	-		-	-
7.2	0.99	487	-	-	-	-	-	-
6.7	1.00	497	-	-	-	2.67	-	1.8
7.1	1.00	500	-	-	-	-	-	-
6.6	0.98	499	-		-	-	-	-
6.7	1.10	496	-	1.2	-	0.4	-	0.22
7.0	1.00	500	-	-	-	-	-	-

Table 4.21. Statistical analysis of group I

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***salmonella* spp.**	***Shigella* spp.**	***salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**				.[a]		.[a]		.[a]	
a_w		-.192			.[a]		.[a]		.[a]	
O/R		-.450	.041		.[a]		.[a]		.[a]	
***Salmonella* spp.**		.[a]	.[a]	.[a]	.[a]		.[a]		.[a]	
***Shigella* spp.**		-.313	.307	.101	.[a]		.[a]		.[a]	
***Salmonella* spp.**		.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	
***Shigella* spp.**		-.267	.073	.094	.[a]	.970**	.[a]		.[a]	
***Salmonella* spp.**		.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]	.[a]
***Shigella* spp.**		-.261	.046	.093	.[a]	.963**	.[a]	1.000**	.[a]	

**. Correlation is significant at the 0.01 level (2-tailed)

.[a]: Cannot be computed because at least one of the variables is constant (It means that there is no variance in one of the drinking water variables, if the variable are the same for every case, values for the variables are constant)

Group J. Washing water

Table 4.22. Physical condition and number of bacterial colony of group J

Physical condition			cfu on SS Agar*10^2		cfu on XLD Agar*10^2		cfu on HE Agar*10^2	
pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
6.9	1.00	495	6	12.6	6.3	6.41	4.4	4.5
7.1	0.99	497	10.566	12.6	8.21	14.4	7.2	9.9
6.8	1.1	501	5.3	2.7	3.8	1.6	3.2	2.34
6.6	1.00	444	6.2	5.209	5.34	4.7	3.56	3.48
7.0	0.99	487	4.3	8.6	3	7.5	1.3	2.87
7.1	0.88	490	-	-	-	-	-	-
6.8	0.98	500	6.34	-	5.55	-	4.2	-
7.2	1.00	488	-	11.9	-	9.93	-	7.9
6.9	1.00	455	-	16.5	-	14.6	-	10.456
7.0	0.97	500	-	2.23	-	1.68	-	1

Table 4.23. Statistical analysis of group J

Correlations										
					SS Agar		XLD Agar		HE Agar	
		pH	**a_w**	**O/R**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**	***Salmonella* spp.**	***Shigella* spp.**
pH	**Pearson Correlation**									
a_w		-.408								
O/R		.469	.018							
***Salmonella* spp.**		-.343	.343	.142						
***Shigella* spp.**		.256	.226	-.329	.062					
***Salmonella* spp.**		-.381	.304	.126	.983**	.083				
***Shigella* spp.**		.314	.167	-.345	.137	.938**	.107			
***Salmonella* spp.**		-.298	.307	.188	.976**	.088	.980**	.148		
***Shigella* spp.**		.283	.248	-.344	.105	.917**	.083	.970**	.153	

**: Correlation is significant at the 0.01 level (2-tailed)

5. DISCUSSION AND CONCLUSION

Foodborne outbreaks occur in developing countries and its main concern public health which do not meet health requirements. Producers and consumers are unconscious, in undeveloped countries, ready-to-eat food is half of the increase in consumption and new treatment technologies are among the main causes of the occurrence and incidence of poisoning occurs (Mansfield and Forsythe, 2000).

This study investigated the isolation and identification of *Salmonella* and *Shigella* from 10 different food groups, samples were collected and analyzed in Iraq/Sulaymaniyah/Qaladze city. 100 different food samples were examined using different media, total bacterial colonies of all of the samples were counted on the three different agar.

The results showed in (Table 5.1.), the number of positive samples ranged from 1 to 10 per group with average 5 to 8 per groups. The maximum value of the positive sample was 10 per food group also the minimum value of the positive sample was 1 per food group.

58 out of 100 samples are detected positive. While the percentage of *Salmonella* spp. was 77.60%, the percentage of *Shigella* spp. of the positive growth food samples was detected 55.20%. Generally the incidence of *Salmonella* spp. was higher than that of *Shigella* spp. The highest number of *Salmonella* contamination was given from three groups (factory raw chicken meat, village raw chicken meat and raw village egg) and the highest number of *Shigella* spp. contamination was taken from washing water group.

The comparison of the groups showed that these three food groups (factory raw chicken meat, village raw chicken meat, and raw village egg) were more contaminated with *Salmonella* spp. and the number of isolated *Salmonella* of each of them were 8 *Salmonella* per group, which were more than other food groups. Food groups with least isolated number of *Salmonella* were cooked village chicken egg and homemade ayran that in each of this two group's only one food sample are contaminated with *Salmonella* spp., no *Salmonella* were detected in drinking water and homemade yogurt.

Table 5.1. Number of positive and negative growth of food samples per group

Food group		Number of positive growth	Number of negative growth
A	**Factory raw chicken meat**	10	-
B	**Village raw chicken meat**	10	-
C	**Chicken meat shawarma**	7	3
D	**Red meat shawarma**	5	5
E	**Raw village egg**	10	-
F	**Cooked village egg**	2	8
G	**Homemade ayran**	2	8
H	**Homemade yogurt**	1	9
I	**Drinking water**	2	8
J	**Washing water**	9	1

In general isolated *Shigella* spp. was less than *Salmonella* spp. with exception in washing water where higher number of *Shigella* spp. was isolated than *Salmonella* spp. The percentage of *Salmonella* and *Shigella* isolated per group of positive samples viewed in the table (Table 5.2.).

Other aims of this study to record important comparative data in three types of media for growth efficiency, and identification of two specific bacterial in food microbiology. In the present study were used three different media, because for all bacterial laboratories, assurance the media to get a specific problem out of certain bacteria, individual enrichment and color media have been investigated in many studies (Orji et al., 2007).

Different types of bacteria cannot be covered by a single medium; therefore, it is important to compare the effectiveness of media growth routinely used in food microbiology.

The growth comparison of *Salmonella* and *Shigella* on the three media used in this study showed that HE agar was more selective than other selective culture media

(SS agar and XLD agar), and the number of counted colony which grow less than two other media.

HE agar has the benefit that it only prevents a bit of growth *Salmonella* and *Shigella*, thus giving high yields of these microorganisms, but at the same time accompanied by ensures adequate inhibition of other microorganisms.

Table 5.2. Percentage of *Salmonella* spp. and *Shigella* spp. isolated per group

Food group		***Salmonella* spp. (%)**	***Shigella* spp. (%)**
A	**Factory raw chicken meat**	100.0%	71.43%
B	**Village raw chicken meat**	100.0%	80.00%
C	**Chicken meat shawarma**	80.00%	30.00%
D	**Red meat mhawarma**	80.00%	20.00%
E	**Raw village egg**	77.80%	88.90%
F	**Cooked village egg**	-	100.0%
G	**Homemade ayeran**	50.00%	50.00%
H	**Homemade yogurt**	80.00%	50.00%
I	**Drinking water**	50.00%	50.00%
J	**Washing water**	-	100.0%

The other objective of this study was to assess the effect of pH, a_w and O/R on the growth of *Salmonella* and *Shigella* because of the wide spread of this two microorganism into the food and it is important to control the growth of *Salmonella*, *Shigella* and microorganisms generally.

Statistical analysis of experimental responses was shown that among the three factors test, pH, a_w and O/R has a major effect on the growth of *Salmonella* and *Shigella*.

Statistically, *Salmonella* spp. a significant correlation was found $p<0.01$ between pH and a_w in the positive direction in chicken meat shawarma, while in red meat shawarma $p<0.05$ between the pH and a_w in the negatve direction, whereas in homemade ayran significance was found $p<0.05$ in the positive direction. *Shigella* spp. in homemade yogurt was positive correlation between pH and a_w $p<0.01$.

As a result, infection rates of salmonellosis and shigellosis were found to be very high in the region and it was determined that they pose a potential health risk for public health.

In the present study *Salmonella* spp. and *Shigella* spp. were founded in the poultry meat carcasses and poultry meat products, was supported by Bekar et al. (1993) in Ankara, reported the isolation of 116 (18.60%) of *Salmonella*, 68 were *S. enteritidis*, 10 *S. typhimurium*, 12 *S. bredeney* and 7 *S. gallinarum* from a total of 623 samples of skin, liver and intestinal contents of fowls or male chicken.

Mohamed (1987) isolated 43 (3.90%) *Salmonella* strains from 1104 samples collected from slaughtered chickens within 18 months in Khartoum.

According the study in Netherlands during 1984 to 1988 the proportionality of *S. enteritidis* isolates were about 12.00% of 3699 chicken samples (Edel and Visser, 1988).

From Sudan, Ezdihar (1996) examined 610 infected chickens' samples and reported the isolation of 14 bacterial genera, among them two genera are *Salmonella* and *Shigella*.

This results is not suported by other countries findings in term of isolation of *Salmonella* and *Shigella* in the poultry. The results showed a higher prevalence, due to the processing of slaughtered and prepared chicken carcasses in the home and chicken shops and reused water in this processes, in Iraq generally and the Qaladze city especially, because not or little slaughter house and factory.

Also the higher prevalence of *Salmonella* and *Shigella* in poultry, in this study in relation to other prevalence data in the other country, high point the presence of a high degree of contamination of the poultry in the factory and village area, this in turn suggested that the slaughter, retailer poultry meat markets, knife and utensils used for slaughtering process, slaughterhouse personal and could have been cause contamination. Also carcass can contaminated by bacteria when contact with ingesta or feces from eliminatory tract during grow and due to poor personal hygiene practices and environmental sanitation and ignorance of health-promotion practices.

About the water contamination with *Salmonella* spp. and *Shigella* spp., a study from Cameroon identified and isolated 1.242 of enteric bacteria from a variety of

drinking water sources, which 0.24% had *Shigella* and 1.30% had *Salmonella* appearance (Ihejirika et al, 2011).

From different drinking water sources of Madhyapur Thimi which included *S. dysenteriae* (2.80%), *S. typhi* (2.10%) and *S. paratyphi* (1.40%) (Jafari et al, 2006).

In the study from Korea isolated 10 *Salmonella* (66.70%) from the starting of river and 5 (33.30%) end of the river waters (Bae et al, 2013).

Also recovered *Shigella* spp. (71.00%) and *Salmonella* spp. (71.00%) from Imo River, Nigeria (Sila et al, 2001). River one of the main sources of drinking water in the world, Iraq one of the country used river as a drinking water, the greatest microbial risks are associated with ingestion of river water that is contaminated with human or animal feces. Acute water microbial diarrhea are a major public health problem in developing countries and developed countries.

In the present study the isolation of *Salmonella* and *Shigella* in drinking water is slightly, that only one bacterium (*S. dysenteriae*) was isolated, *Salmonella* not isolated. It is known that *Salmonella* do not develop in a wide range of pH such as *Shigella* species. The appearance of this difference suggests that disinfectants such as chlorination of waters can be used.

Many water sources in Qaladze city are natural and not contaminated with microorganisms. Water may contaminate during the water collection at home. At the same time the present study shows the level of isolation of *Salmonella* and *Shigella* from washing water high level, due to the high rate of contamination with animal and human fecal and in Iraq generally and Qaladze city sewage water treatment is almost non-existent.

A total of 120 random samples of a city (fresh, non-salted) cheese were collected from different markets of Sulaymaniyah city in Iraq during October 2009 to June 2010. The results showed that three (2.50%) out of the total 120 cheese samples were found contaminated with *Salmonella* species. *Salmonella enteritidis* was the only serotypes that have been found (Arif, 2010).

Dairy products considered one of the factors that lead to cause and spreading salmonellosis and shigellosis. In this study two groups of dairy products which are homemade yogurt and homemade ayran, were the rate of isolation of these two

pathogens not high level reported, in homemade ayran only one *Salmonela* isolated (*S. paratyphi*) and one *Shigella* isolated (*S. flexneri*).

The result also showed that in the homemade yogurt, only *Shigella sonnei* was isolated, while *Salmonella* not isolated. Because yogurt is a chemically acidic food, *Salmonella* spp. do not have the chance to live in yogurt, but *Shigella* spp. can also grow in acidic foods, and water activity is thought to significantly reduce the development of microorganisms.

During the process of homemade yogurt and homemade ayran milk boiling and pathogenic bacteria are eradicated or killed, but it causes the contamination during the process of preparation of homemade yogurt and homemade ayran.

About the egg contamination of *Salmonella*, it was reported that the shells and contents of 2.090 packs of 6 fresh eggs from markets in Northern Ireland were examined and 9 isolates of *Salmonella* were detected from separate packs of eggs (0.43%) (Wilson et al, 1998).

A study from Bangladesh a total 103 poultry chicken egg samples were analyzed, among them 89 (86.40%) isolates were examine positive as *Salmonella* spp. Among these 89 eggs, 86 eggs (97.00%) were positive for *Salmonella* spp. from egg shell and 3 eggs (3.00%) were found positive from internal of egg (Mohammad, 2015).

A study from Pakistan, reported that total prevalence of positive *Salmonella* ratio were egg shells 86 (34.12%), egg contents 32 (12.69%), and egg storing trays 48 (36.36%). Out of 34.12% occurrences of *Salmonella* in egg shells and 12.69% in egg contents, the incidence of the pathogen was 29.36 and 38.88% in egg shells and 10.31 and 15.07% in egg contents of eggs collected from chicken farms and market outlets (Adil, 2012).

In the current study the incidence of *Salmonella* and *Shigella* in egg high level when compared to other study, the main factors of this big different because in the present study collected from different village sources, not used package or factory eggs, village eggs are more contaminated between chickens. The lack of vaccination for eradication of salmonellosis and shigellosis, inadequate personnel hygiene and inadequate hazard analysis of critical conrol points (HACCP) in food production, and the spread of the technical infectious disease are threatening both animal health and public health.

A study conducted in North Jordan in 2014, to isolate and identify bacterial pathogens from meat and chicken shawarma sandwiches sold to the public, also revealed that 26.30% of shawarma sandwiches were also contaminated with *Salmonella* (Nimri et al, 2014).

If the results are compared in the previous studies with this study, we see that the prevalence of *Salmonella* spp. and *Shigella* spp. in shawarma higher were this difference in results due to the contamination after cooking shawarma or halve cooking, cross contamination and during food preparation, hands, utensils and equipment such as cutting boards can become contaminated with bacteria, and salads which used with shawarma may cause contamination during cutting or washing by contaminated water.

As a result; this study is useful for using the results and techniques for the prevention and control of salmonellosis and shigellosis observed in the study. Hygiene and sanitation rules must be observed to prevent these diseases.

The best prevention technique at home, restaurant, or anywhere is to void flourishing microorganisms to high levels and to inactivate the items using cooking or boiling.

For secure food preparation, hands must be washed and surfaces must be cleaned frequently. Washing hands with hot soapy water 20 seconds before and after preparing food will be suitable. Besides, after using the toilet, it is better to change toilet towel, and if lack soap and water, use an alcohol-based hand sanitizer with at least 60% alcohol.

Food preparation equipment, cutting boards, and countertops must be cleaned with warm soapy water before and after handling each food process, and it must be considered to use clean paper towels to clean kitchen work surfaces.

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