Sudan University of Science and Technology

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Detection of Salmonella in yoghurt and cheese in Khartoum State – Sudan

الكشف عن السالمونيلا في الزبادي والجبن بولاية الخرطوم – السودان

A thesis Submitted in partial for the Requirements of Degree of Master of Science in Preventive Veterinary Medicine (MPVM)

BY…

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DEDICATION

To my beloved ones in my life,

My parents

~ My husband ~

And

~my sons ~

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Abstract

The study was conducted to isolate and identify Salmonella spp and to determine Total bacterial count in 60 yoghurt and cheese during 2017 in Khartoum state .A total of 60 samples of local yoghurt and cheese chosen randomly from retail points (local shopes and super markets) in Khartoum state (Bahri ,Khartoum, Omdurman localities).For isolation of bacteria the samples were cultured and for identification of these bacteria the biochemical tests were used .Total bacterial count was also used .The results revealed that there was no statistical difference between 3 localities ($P > 0.05$) after using total bacterial count of cheese and yoghurt samples .High total bacterial count of cheese was recorded in Omdurman ($P=0.8801$),while in Yoghurt was $P=0.9491$ recorded in Bahri .The contamination of cheese higher in local markets (137.08+ 2.84) than super markets(132.08 + 2.56),whereas in Yoghurt were higher in super markets(644.07+ 631.07) than local markets (141.08 + 247.07). The bacteria isolated from these samples were E.coli , Salmonella spp, Klebsiella spp and Proteus spp .In conclusion ,the contamination of milk by microorganisms due to handling ,equipment and machines which are normal flora, but can cause of the disease in non hygienic environment .

المستخلص

اجريت الدراسة لعزل وتحديد السالمونيلا والعد الباكتيري الكلي في 60 عينة زبادي وجبن خلال الفتره 2017 في ولاية الخرطوم . تم اختيار 60 عينه من الزبادي والجبن المحلي بشكل عشوائي من نقاط البيع بالتجزئه (الاسواق المحليه والاسواق الكبيرة) في ولاية الخرطوم (محليات بحري,الخرطوم , أمدرمان).ولعزل البكتريا التي تم استزراعها وللتعرف علي هزه البكتريا تم استخدام الاختبارات البيوكيميائيه ,كما تم استخدام العد الكلي للبكتريا , وأظهرت النتائج عدم وجود فرق احصائي بين الثلاثة محليات ($P<0.05$) بعد استخدام العد البكتيري الكلي لعينات الجبن والزبادي ,سجلت أعلى حموله باكتيريا للجبن في أمدرمان ($P=0.8801$) بينما في الزبادي $P=9491$ سجلت في بحري .تلوث الجبن أعلى في الاسواق المحليه (2.84+ 137.08) من الاسواق الكبرى (132.08+2.56),بينما في الزبادي كانت اعلى في الاسواق الكبرى (644.07+631.07) من الاسواق المحليه (141.08+247.07) .كانت البكتريا المعزوله من هزه العينات هي الايكولاي, السالمونيلا , الكليبسيلا , البروتيس .في الختام , تلوث الحليب بالكائنات الدقيقه الحيه بسبب المناولة , المعدات والالات التي تعتبر نبت جرثومي طبيعي ,ولكن يمكن ان تسبب المرض في بيئه غير صحيه .

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Chapter One

1. Introduction

During the last 25 years, there has been a continued increase in milk production and consumption of fermented dairy products (Magda , 2010).

Fermented dairy products are cheese, yogurt and fermented milk. They are fermented by the addition of a starter culture such as lactic acid bacteria which are responsible for the production of lactic acid from lactose that gives the product its characteristic flavor. Cheese is one of the most consumed milk products (McGee, 2004).

Cheese is an excellent source of protein fat and minerals such as calcium, iron and phosphorus, vitamins and essential amino acids and therefore is an important food in the diet of both young and old people (Tripathi , 1999) (O'Connor, 1993).

Family of *Enterobacteria ceae* contains many species which cause hazard to the consumer, other species are important from economic point of view as milk products (El-Kouly, 1995). Salmonellosis may cause an outbreak of gastroenteritis (Ahmed, 2000).

Such infection not only has economic importance in agriculture and animal husbandry but also is believed to be major factor in the transmission of *Salmonella* to humans via food chain (Morris, 1997).

Cheese like other types of food is exposed to contamination with microorganisms during the production process. Fresh cheese such as cottage and white cheese may be subjected to spoilage by coliforms, yeast and molds that enter as post-pasteurization contaminants(Sadek, 2009).

During cheese processing stages microbes can be introduced by cross contamination to raw milk as the raw material or from infected humans during handling and transmission (Temelli, 2006).

Yogurt and other fermented milk products are a very unfit food matrix for allowing growth and even the survival of *Salmonella*. The Cheese is among the foods which are less likely to cause salmonellosis in humans due to their production process (Little *et al*., 2008).

Several food borne pathogens have usually been detected in raw milk, including *Salmonella* spp., *Campylobacter* spp., *E. coli, Listeria monocytogenes*, and *S. aureus* (Oliver *et al*., 2005).

Fecal contamination during milking constitutes a primary route for pathogen transmission. The consumption of raw milk represents a well issue for human health (Headrick *et al.*, 1998; LeJeune and Rajala-Schultz, 2009).

However, increasing number of consumers demand raw milk and/or products made from raw milk and other unprocessed products. According to epidemiological data, it is necessary to consider documented not only the risk of raw milk consumption, but also the likely episodes of recontamination and cross-contamination that can take place at the industry or home kitchens if milk is not pasteurized or heat-treated. However, pasteurized milk has also been involved in salmonellosis infections and outbreaks, as reported by (Olsen *et al*. 2004).

Cross-contamination of cheese may be originated from starter culture, brine, floor, packaging material, cheese vat, cheese cloth, curd cutting knife, cold room, and production room air (Temelli *et al*., 2006), (Domínguez *et al.*, 2009) The main transmission routes of this pathogen are foods of animal origin contaminated with fecal matter (Swartz, 2002., Haeghebaert *et al*., 2003). However, a wide range of other foodstuffs such as milk, dairy products, fruits, vegetables, and fishery products can be sources of Salmonella infection (Todd, 1997).

The Objectives:

- Detection of *Salmonella* in local yoghurt and cheese were collected from shops super market in Khartoum state.
- To determine the bacterial count in local cheese and local yoghurt.

Chapter one

Literature Review

1.1.Salmonella through History:

Salmonella was isolated for the first time from pig's intestine in 1885 by Theobald Smith, in research laboratory assistant at the Veterinary Division of the United States Department of agriculture. The research was performed under the guidance of Daniel Elmer Salmon, an American veterinary pathologist, responsible for the origin of the genus name. However, the history of *Salmonella* did not begin at the 19 th century. In ancient times, some important historical celebrities are believed to have died due to infections caused by this bacterium. In 2001, a group of researchers at the University of Maryland in the United States of America (USA) suggested that an infection by *Salmonella typhi* was the cause of death of Alexander the Great in 323 BC, based on a description of Alexander's symptoms written by the Greek author Arrian of Nicomedia (Moulopoulos, 1998).

In more recent times, Prince Albert, the husband of Queen Victoria, died in 1861 of typhoid fever. In fact, during the Victorian era, 50.000 cases of typhoid fever per year were estimated to occur in England (Morser 2005). Even at important wars, *Salmonella* caused more deaths than battle wounds. During the South African War (1899-1902), a typhoid outbreak in British camps mainly attributed to unsanitary conditions (1899-1902) killed 13.000 soldiers, as compared to 8.000 battle deaths (Cirillo, 2006).

In the early 1900's, Mary Mallon, also known as "Typhoid Mary", was responsible for several. Typhoid outbreaks, becoming the first famous carrier of typhoid fever in USA (Soper, 1907).

Mary Mallon was hired as a cook at several private houses, working in the New York area for wealthy families. She caused several Typhoid outbreaks, by moving from house to house and always disappearing before an epidemic could be traced back to her. In the end, she had worked for eight families, with 22 cases of .typhoid and at least 3 deaths directly attributed to her cooking job (Soper, 1907, Marineli, *et al*., 2013).

She was finally overtaken by the authorities in 1907 and committed to an isolation center on North Brother Island in New York. Mary Mallon was released in 1910, on the condition that she never again accepted employment involving food handling. She changed her name and was found working as a cook, after causing further typhoid outbreaks. She was admitted back to North Brother Island, where she lived until her death in 1938 (Marineli *et al*., 2013). These events showed that some

individuals have a natural immunity to *Salmonella* and Mary Mallon was the first asymptomatic typhoid carrier to be identified (Soper, 1907).

Only recently, medical science was able to clarify that *Salmonella* enterica serovar typhi can recently, medical science was able to clarify that *Salmonella* enterica serovar typhi can due to biofilm formation (Gonzalez-Escobedo and Gunn 2013).

1.2.*Salmonella* in Sudan

In Sudan isolation of salmonella was reported by different investigators .for example,(Mamoun *et al*., 1992) isolated 21 *salmonella* strains from several poultry farms in three different States in the Sudan, all were found to be *S.eneritidis* . (Yagoub *et al.*, 2005) isolated *Salmonella* sp.from 1.43% (1/70) of raw milk samples collected from Butana dairy factory ,farms and milk collection points located inHillat kuku, Khartoum North . (Yagoub *et al* ., 2006) isolated *S. paratyphi* A and *S.paratyphi* B from 6% of the white cheese sampes collected from retailer shops and restaurants in Khartoum and Omdurman cities during the period from February to November 2001.(

Yagoub ,2009)reported the isolation of *Salmonella* from 6.2% (28 /565) of the fish samples (gills, intestine skin and muscles) collected from fish markets in khartoum State .

(HagElsafi *et al* ., 2009) reported the isolation of four *salmonella* isolates from 119 fecal samples (3.4%) collected in Khartoum state.Two isolates were serotyped as *S.droganna* and the other two as *S.umbadah* . concerted efforts are needed to reduce the risk of *salmonella* contamination throughout the food supply chain .on- going laboratory –based *Salmonella* surveillance is an essential step to support these efforts .the purpose of this investigation was to determine the prevalence of *salmonella* from humans ,animals water and different food items in Khartoum State, Sudan .

1.3.Definition of the disease

Salmonellosis is a symptomatic infection caused by bacteria of the *salmonella* type. (CDC , 2015) The most common symptoms are diarrhea, fever, abdominal cramps, and vomiting.(CDC ,2015) Symptoms typically occur between 12 hours and 36 hours after exposure, and last form two to seven days.(WHO , 2016) Occasionally more significant disease can result in dehydration.(WHO , 2016) The old, young, and others with a weakened immune system are more likely to develop severe disease.(CDC ,2015) Specific types of *salmonella* can result in typhoid fever or paratyphoid fever.(CDC ,2015and Medlineplus 2017).

1.4. Characterization of *Salmonella.*

Bacteria classification is of major importance, creating order in the complex world of microbiology. Below the subspecies level, *Salmonella* isolates are discriminated using the White-Kauffmann-Le Minor serotyping scheme, established in the middle of the last century and still recognized as the reference method to discriminate between

Salmonella varieties The *Salmonella* genus includes two species, *S. enterica* and *S. bongori. S. enterica* is divided into 6 subspecies, I (enterica), II (salamae), IIIa (arizonae), IIIb (diarizonae), IV (houtenae) and VI (indica). The White-Kauffmann-Le Minor scheme, published by the WHO Collaborating Centre for Reference and Research on *Salmonella* at the Pasteur Institute France, uses serotyping to distinguish between Salmonella serovars, llowing the identification of more than 2.600 different serovars belonging (Grimont and Weill., 2007).

1.5. **Microbiological characterization**

Salmonellae are facultative anaerobic, Gram-negative rod shaped bacteria belonging to the Enterobacteriaceae family. Although most members of this genus are motile by peritrichous flagella, a few non-flagellated variants, such as *Salmonella* gallinarum and *Salmonella* pullorum are non-motile. Salmonellae are chemoorganotrophic, with the ability to metabolize nutrients by both respiratory and fermentative pathways (Gyles,*et al.,* 2010).

Salmonella is oxidase negative, catalase positive, indole and Voges Proskauer negative, methyl red and Simmons citrate positive, hydrogen sulfide producing and urea negative. Some of these characteristics are used for biochemical identification of *Salmonella* isolates (Grimont and Weill., 2007).

1.6. **Antigenic characterization**

Most human and food-producing animal serovars (more than 1.500) belong to the first subspecies, *Salmonella enterica* subspecies *enterica* (EFSA, 2010, Ranieri 2013).

While *S. enterica* normally includes all major serovars that are pathogenic to humans and animals, *S. bongori* is considered a bacterium of

coldblooded animals, most frequently associated with reptiles (Gyles *et al*., 2010). Salmonella enterica subsp. enterica serovars are usually designated by a name, frequently related to the geographical place where the serovar was first isolated (for example, *Salmonella havana*) or to the animal from which it was isolated for example, *Salmonella gallinarum* (Backer *et al*., 2000, Shah *et al*., 2005). Serovars belonging to other *S. enterica* subspecies and to *S. bongori* are designated by their antigenic formula (EFSA, 2010).

Serotyping is based on the antigenic characterization of surface structures, such as lipopolysaccharides (O-antigen), flagellar proteins (H-antigen), and capsular polysaccharides (Vi-antigen), through agglutination with polyvalent and monovalent antisera (Grimond and Weill, 2007, Switt *et al*., 2009).

The O-antigen is the external component of the lipopolysaccharide located on the cell wall, consisting of along linear polysaccharide containing 50 to 100 repeating saccharide units, with four to seven sugars per unit (EFSA, 2010). Different sugars and different linkages between sugars give origin to different antigens (Reeves and Wang, 2013).

The H-antigen corresponds to flagellin, the major component of flagella (Switt *et al*., 2009).

The Vi-antigen is a surface polysaccharide, which only occurs in three *Salmonella* serovars, namely *Salmonella typhi, Salmonella paratyphi* C and *Salmonella dublin* (Johnson, 1965, Snellings, 1977).

The full antigenic formula system determined by the White-Kauffmann-Le Minor scheme is represented as follows: first, O-antigens, followed by H-antigens of first phase and Hantigens of second phase; the three antigen designations are separated by colons (EFSA,2010).

According to this scheme, *Salmonella typhimurium* would be described as I 1,4,[5],12:i:1,2, indicating that this serotype belongs to subspecies I and carries the‘‘1,4,[5],12’’ O antigens, the ‘‘i’’ phase 1 H-antigen, and the ‘‘1,2’’ phase 2 H-. antigens. The underlined O factor 1 means that this factor is determined by phage conversion, bein g present only if the culture is lysogenized by a particular converting phage. The factor5 between square brackets means that the presence of the antigen is not related to phage conversion. *Salmonella* 1,4,[5],12:i:– thus shares all O antigens and phase 1 H-antigens with *Salmonella typhimurium*. Antigenic variants like the monophasic *Salmonella typhimurium* 1,4,[5],12:i:-that lack the second phase H-antigen have been described in the literature as “*Salmonella typhimurium*-like” strains (EFSA 2010b, Bugarel et al. 2012).

1.7. Laboratory methods for identification and characterization of *Salmonella*

Several laboratory techniques are used for identification and typing *Salmonella* In epidemiological order to track changes in epidemiology and to trace sources of foodborne outbreaks (Wattiau, 2011, Achtman *et al.* 2012).

Isolation of *Salmonella* from faeces and food samples may be difficult due to several factors, Additionally, *Salmonella* populations in food samples may be stressed due to unfavourable storage and processing conditions, including high or low temperatures, pH, or salt content. In order to insure the isolation of *Salmonella* and avoiding false negative results, several steps must be performed, including the use of a large sample volume, inoculation in a non-selective pre-enrichment medium, such as buffered peptone water, followed by the (sea) of a combination of two selective

enrichments broths such as Müller-Kauffmann Tetrathionate broth and Rappaport Vassiliadis Soy and plating on two selective media, such as Xylose Lysine Desoxycholate agar and Brilliant Green agar, or another selective agar media like Hektoen or Salmonella-Shigella agar. Subsequently, colonies with a typical *Salmonella* morphology are confirmed by further culturing in Triple Sugar Iron agar and Urea agar and using biochemical tests, including L-lysine decarboxylase, β-galactosidase, Voges Proskauer and Indole tests. Then, *Salmonella* colonies are serotyped classified at the subspecies level and eventually serotyped (WHO, 2010).

Serotyping is a relatively easy technique to perform, however it requires skilled technicians to interpret the results, being an expensive and prolonged procedure since hundreds of antisera are required (Grimont and Weill, 2007, Guibourdenche *et al.*, 2010).

In fact, the replacement of serotyping by molecular techniques is being tested. Through the years, several molecular techniques have been used to identify *Salmonella* serotypes based on microarrays (Fang *etal.,*2010*).*

Real-time polymerase chain reaction (PCR) (Maurischat *et al.*, 2015), repetitive sequence-based PCR (Wise *et al.*, 2009, Ranieri *et al.*, 2013). and multilocus sequence typing (Ranieri *et al.*, 2013).

Each of these methods has advantages and drawbacks in terms of costs, speed and sensitivity (Ranieri *et al.*, 2013).

Although considered improvements in several aspects, none has been considered as the ideal method to be conducted as routine in microbiology laboratories (Wattiau *et al.*,2011) .

and serotyping still remains an indispensable tool to discriminate *Salmonella* serovars. This technique provide valuable information regarding potential sources, as several serovars are correlated with specific hosts (subspecies IIIb serovars are common in reptiles) or geographical regions (Backer *et al.* , 2000). type of disease and severity (EFSA, 2010)

and potential multidrug resistance profile, since some serovars may be linked to certain antimicrobial resistance profiles (Clemente *et al.*, 2014). Additionally, serotyping information is also essential to achieve its main purpose, which is to allow for an internationally accepted nomenclature (Grimont and Weill ,2007).

Bacteriophages, or more commonly phages, are viruses that can only replicate within specific bacteria. Phage typing can differ between strains of the same serovar. This technique is based on the principle that specific phages will only lyse particular strains of a specific serovar, currently allowing the identification of over 200 definitive types (DT) (EFSA,2010).

The lysis pattern can be compared to a standard scheme to identify the strain phage type (De Lappe *et al.*, 2009).

Typing schemes were conceived for particular *Salmonella* serovars and showed to be valuable epidemiological tools to investigate outbreaks. Information regarding the typing schemes in combination with antimicrobial susceptibility testing has led to identification of many large international outbreaks (O'Mahony *et al.*, 1990, Mahon *et al.*, 1997, Backer *et al.*, 2000, Mossong *et al.*, 2007). Nowadays, molecular methods are critical for outbreak detection, investigation and control. Pulsed Field Gel Electrophoresis (PFGE) is a molecular typing method, considered to be the “Gold Standard” for supporting the identification of epidemiological links between isolates(Wattiau *et al.*, 2011) .

However, multilocus sequence typing (MLST) and whole genome sequencing are becoming more favoured options (Achtman *et al.*, 2012) .

1.8.Host susceptibility

Host factors predisposing to severe NTS infection include reduced gastric acidity, pernicious anemia, antacids and H2 blockers administration (Hohmann, 2001) .

Impaired cell mediated and humoral immunity and decreased phagocytic function were also described as related with severe disease (Gondwe *et al*., 2010, Dougan *et al*., 2011).

Salmonellae are unable to survive at a gastric pH inferior to 2.5 and patients with anatomical or functional achlorhydria are at increased risk of developing infection (Tennant *et al.*, 2008).

Other risk factors for salmonellosis include extremes of age, intestinal microbiota variation, diabetes, malignancy, rheumatic disorders, reticuloendothelial blockade as a result of malaria, Human Immunodeficiency Virus (HIV) infection, and therapeutic immunosuppression (Hohmann,2001, Crum-Cianflone , 2008).

Also, co-infection with *Schistosoma* has been reported to cause prolonged and severe illness due to altered macrophage function and *Salmonella* replication and survival ability of Salmonella within the parasite (Abruzzi and Fried ,2011).

1.9. Infectious Dose

Salmonella infective dose 50 (ID50), which is the number of viable cells required to cause infection in 50% of the experimental subjects, was determined in human volunteers. Those experiments pointed to an ID50 of 10^3 to 10^6 cells, which can be considered a relatively large dose when

compared to other foodborne pathogens like *Campylobacter jejuni* (500 microorganisms) or *Shigella* spp. (10 microorganisms) (Hara-Kudo and Takatori , 2011).

However, other reports regarding salmonellosis outbreaks investigations suggest that the infective dose is often lower, being calculated to be inferior to 10^3 bacilli (Blaser and Newman,1982).

Higher doses are associated with higher rates of illness and shorter incubation periods (Hara-Kudo and Takatori , 2011).

Lower infective doses may promote and cause infection, if *Salmonella* is co-ingested with foods that rapidly pass the stomach, such as liquids, or that includes a high content of fat and protein, like ice cream, cheese and chocolate,which may play a role in protecting *Salmonella* from gastric acidity (Kothary and Babu , 2001).

Antacids administration or defective immune systems are conditions that may require lower ID to cause infection. Even within the genus, the infective dose is variable among different serovars. For example, it was reported that *Salmonella pullorum* infective dose is 10^9 to 10^{10} bacilli, while *Salmonella newport* infective dose is estimated to be 10^5 to 10^6 microorganisms (Kothary and Babu, 2001).

1.10.Virulence and infectivity

Once ingested, *Salmonella* spp. must survive the low pH of the stomach, adhere to the small intestine epithelial cells and overcome host defence mechanisms to enable infection (Jay *et al*., 2003).

Salmonella spp. possess a number of structural and physiological virulence factors, enabling them to cause acute and chronic disease in humans. The virulence of *Salmonella* spp. varies with the length and structure of the O

side chains of lipopolysaccharide molecules at the surface of the bacterial cell. Resistance of *Salmonella* spp. to the lytic action of complement (part of the immune response) is directly related to the length of the O side chain (Jay *et al*., 2003).

Other important virulence factors include the presence and type of fimbriae, which is related to the ability of *Salmonella* spp. to attach to host epithelium cells, as well as the expression of genes responsible for invasion into cells (Jones , 2005).

Some of these virulence genes are encoded on *Salmonella* pathogenicity islands (SPI). SPI-1 is required for bacterial invasion into intestinal epithelial cells, while systemic infections and intracellular accumulation of *Salmonella* spp. are dependent on the function of SPI-2 (Valle and Guiney 2005).

Salmonella spp. produce a heat labile enterotoxin, resulting in the loss of intestinal fluids (causing diarrhoea). This enterotoxin is closely related functionally, immunologically and genetically to the toxin of Vibrio choleraeand the heat labile toxin of pathogenic Escherichia coli(Jay *et al.,* 2003).

Most *Salmonella* strains also produce heat labile cytotoxin which may cause damage

to the intestinal mucosal surface and results in general enteric symptoms and inflammation. Infection with non-typhoidal *Salmonella*is generally limited to a localised intestinal event. However, the presence of virulence plasmids has been associated with non-typhoidal *Salmonella* spp. surviving in phagocytes and spreading from the small intestine to the spleen and liver (Jay *et al*., 2003; Hanes, 2003).

Multiple antibiotic resistant strains of *Salmonella* have emerged, an example being *S. Typhimurium* definitive phage type 104 (DT104). Multi-resistant *S. Typhimurium* DT104 infects both humans and animals, such as cattle and sheep. To date, this organism is not 4 endemic in Australia, although it is a significant health problem in European countries, North America, the Middle East, South Africa and South-East Asia (Jay *et al*. 2003).

1.11.*Salmonella* infection in human being

1.11.1Transmission

Salmonella spp. are transmitted by the faecal-oral route by either consumption of contaminated food or water, person-to-person contact, or from direct contact with infected animals (Jay *et al*., 2003).

Hand-to-mouth and object-to-mouth behaviors are common among young children and can increase their risk for contracting salmonellosis. (US-CFR,2010).

1.11.2.Clinical signs

Gastroenteritis symptoms are generally mild and may include abdominal cramps, nausea, diarrhea, mild fever, vomiting, dehydration, headache and/or prostration. The incubation period is 8–72 hours (usually 24–48 hours) and symptoms last for 2–7 days (WHO/FAO 2002; Darby and Sheorey 2008).

Severe disease such as septicaemia sometimes develops, predominantly in immunocompromised individuals. This occurs when *Salmonella* spp. enter the bloodstream, leading to symptoms such as high fever, lethargy, abdominal and chest pain, chills and anorexia; and can be fatal. A small number of individuals develop a chronic condition or sequelae such as

arthritis,appendicitis, meningitis or pneumonia as a consequence of infection (Hohmann 2001; WHO/FAO 2002; FDA 2012).

Salmonella spp. are shed in large numbers in the faeces of infected individuals at the onset of illness. (Jay *et al*. 2003; Crum-Cianflone 2008).

1.11.3. Prevention

Food must be cooked to 145 -165 ^{o}F(63-74 ^{o}C),and liquids such as soups or gravies should be when reheating .Freezing kills some *salmonella* ,but it is notsufficient to reliably reduce them below infectious levels. While *salmonella* is usually heat-sensitive ,it acquires heat-resistance in high –fat environments sush as panut butter(FDA , 2009) .

Antibodies against nontyphoidal salmonella were first found in Malawi children in research published in 2008 . The Malawian researchers identified an antibody that protects children against bacterial infections of the blood caused by nontyphoidal *salmonella* .Astudy at queen Elizabeth Hospital in Blantyre found that children up to tow years old develop antibodies that aid in killing the bacteria .this could lead to a possible *salmonella* vaccine for humans. (Maclennan *et al* .,2008).

Eradicated *salmonella* without vaccines and antibiotics by focusing on eliminating the infection from "breeder stocks", implementing various measures to prevent infection , and taking azero-tolerance policy towards salmonella in chickens .(Contaminated chicken , 2014).

1.11.4.Treatment

Although *Salmonella spp.* gastroenteritis is generally a self-limiting illness that may requirefluid and electrolyte replacement, antibiotics are needed in severe cases. Fluoroquinolones are the antimicrobials most widely regarded as optimal for the treatment of salmonellosis in adults. Third-generation cephalosporins are widely used in children with serious infections. The earlier drugs chloramphenicol, ampicillin and amoxicillin and

trimethoprim-sulfamethoxazole could be used as alternatives for salmonellosis treatment (Hohmann, 2001; WHO, 2005b).

1.12.Salmonella infection in animals

1.12.1Transmission

The most common mode of *Salmonella* transmission is through the ingestion of the bacterium in food derived from an infected animal or contaminated by feces of an infected animal or person (Mead et al., 1999).

1.12.2.Clinical signs

Salmonella infection can be either symptomatic or asymptomatic. For example, in cattle, and particularly calves, infection with *Salmonella Typhimurium* can cause serious systemic disease in addition to severe diarrhea, often resulting in death if not treated. Infecti on with *Salmonella Dublin* can cause abortions in cattle. In infected calves, *Salmonella Dublin* causes severe diarrhea whereas in adult cattle it is responsible for septic abortion associated with a 70% mortality and prolonged carriage by surviving animals. Similarly, in poultry *Salmonella Pullorum* or *Gallinarum* can produce severe symptoms characteristic of ‘fowl typhoid.’ In contrast, *Salmonella Enteritidis* in poultry is mostly asymptomatic, as is *Salmonella Derby* in pigs(Maskey *et al*., 2006).

1.12.3. Prevention

Control of *Salmonella* disease can be exerted at three levels – the individual , the community (the herd), and the environment. Such control may be exerted by vaccination, control of the source, or by interruption of transmission. Factors exacerbating the emergence and spread of particular strains, such as the indiscriminate use of antimicrobials, may also be important, and the control of anti microbials, particularly in animal husbandry, has been highlighted as

an important factor in combating the emergence of strains with resistance to key antimicrobial classes. Vaccines are available for some nontyphoidal *Salmonella* in animal husbandry (Fisher,2005).

1.12.4. Treatment

The rehydration therapy is the mainstay of management. However, Multiple drug resistance, defined as resistance to ampicillin, chloramphenicol, and trimethoprim-sulfamethoxazole is common, but resistance to fluoroquinolones, extended-spectrum cephalosporins, and azithromycin is increasing in some Salmonella serovars (Crump *et al*., 1996 -2007) .

1.13. Epedimulogy of *salmonella:*

The incidence of salmonellosis has markedly increased in many countries; however, a paucity of good surveillance datd exists .in2000, approximately 21.6 million worldwide cases of typhoid fever caused 216,500 deaths (moulopoulos ,1998).

Salmonella is an important cause of diarrheal disease, that is responsible for substantial illness and death worldwide (GBD , 2013). Typhoid fever was estimated to cause 11.9 million illnesses and 129,000 deaths in 2010 (Mogasale V *et al*.,2014), while invasive nontyphoidal *Salmonella* was estimated to cause 3.4 million illnesses and 680,000 deaths the same year (Ao TT ,2010).

Typhoid and paratyphoid fever is an important cause of morbidity and mortality among children and adults in developing countries. The disease remains endemic in developing countries in Africa, and South and Southeast Asia. Enteric fever is also commonly reported from the Middle East, South and Central America, the Pacific Islands, and some countries in Southern and Eastern Europe. In contrast, in developed countries such as the United Kingdom or the United States,*Salmonella*

Typhi is uncommon with the majority of cases occurring among travelers returning from endemic areas. For example, in the United States approximately 240 *Salmonella Typhi* infections are reported annually, of which 79% were recorded in patients with a history of recent travel to endemic areas (Lynch MF et al.,1999-2006).

The epidemiology of paratyphoid fever is less well documented than that of typhoid. Nevertheless, approximately 25% (CDC, 2015) .

countries, such as those in South America, Africa, and parts of Asia (Heymann, 2008). in developed countries where there is active, coordinated foodborne disease surveillance, other serovars such as Typhimurium and Enteritidis are frequently reported. Sixty to eighty percent of all human salmonellosis cases in the United States occur intermittently and sporadically throughout the population. Clusters of large outbreaks in restaurants, institutions for children, hospitals, and nursing homes have occurred recently and remain major public health threats. These outbreaks are usually the product of contamination from a production source, such as chicken farms, feed blending mills, and slaughterhouses. One of the more well-known *Salmonella* outbreaks in the United States occurred in 2010. This outbreak resulted from contamination in the food production chain, leading to a massive egg recall of over half a billion eggs and more than 2,000 reported cases of*Salmonella*-related illness (Hutchison, 2010).

Although less common, outbreaks from food handling by an ill person or carrier have been reported in recent years (Cruickshank *et al*., 1987; Khuri-Bulos *et al.,* 1994).

For instance, in 2000 an ill food handler in a bakery that supplied hamburger buns to restaurants was found responsible for an outbreak among several burger restaurants across Southern California and Arizona. This outbreak was atypical in that it resulted from consumption of

commercially distributed bread, which is a highly unusual vehicle for most foodborne infectious agents (Kimura *et al.,* 2005).

Outbreaks from person-to-person transmission can also be of particular concern, especially among hospital workers who have the potential to spread the bacterium with their hands or through contaminated instruments. Outbreaks of *Salmonella* infection have occurred in places like maternity wards where staff members with contaminated hands and/or the use of contaminated medical instruments result in the transmission of *Salmonella* to abies and mothers (Rowe *et al.,* 1969).

In 2008, an outbreak strain of *Salmonella* serotype Tennessee occurred in a neonatal intensive care unit in the United States, where limited access to sinks for hand washing likely facilitated the transmission to infants (Boehmer, 2009).

Chapter Two
Materials and Methods
2.1 Study area;
The study was conducted in Khartoum State, which is located in North Eastern part of the centre of Sudan. The state is located between 21o, 25-24o, 45 East and15o, 9-16o, and 45 norths. The state covers 20, 736km2. Khartoum State is divided into three administration governorates: Khartoum, Omdurman and Khartoum North. The governorates are bounded by North Kordofan in the west and in the north by Nile River State and in the North West by the Northern State and by the White Nile State in the South and Gazeera State in the east (Abdelgadir 2011) .
2.2 Collection of Samples:
A total of 60 cheese and yoghurt ssmples (35 cheese ,25 yoghurt) were collected during the year 2017 from retail points (shops and super markets) in Bahri ,Khartoum and Omdurman localities –Khartoum State ,All samples were inoculated in nutrient broth and inocubated over night .Small portion was straked with sterile loop on Macconkey agar and incubated at 37°c for 24h **.**
2.3. Sterilization:
2.3.1. Sterilization by red heat
The method was used for sterilization wire loops, straight wire and tissue forceps it was done by holding the object over the flame as near and vertical as possible until it becomes red-hot(Cruickshank et al., 1975).
2.3.2. Sterilization by autoclaving
This method was used for sterilizing of culture media and for materials that could not with stand the dry heat .The temperature was 115°-121° C under 10-15 pounds pressure for 15-20 minutes (Barrow and Feltham, 1993).
2.4.Bacteriology:
All media were prepared according to (Barrow and Feltham 1993)**.**
2.4.1. solid media
2.4.1.1. Nutrient agar
The medium was prepared by adelition of 25grams of the powder were added to one liter of distilled water and brought to boiling for dissolving the powder completely .It was sterilized by autoclaving for 15 minutes at

121°c and 15 pounds per square inch. Then distributed septically as 18-20 ml in Petri-dishes.

2.4.1.2. Mac Conkey's agar medium

Fifty-two grams of Mac Conkey's agar powder were added to one liter of distilled water and brought to boiling until dissolved completely. The PH was+ or- adjusted to 7.4 then sterilized by autoclaving at 121°C for 15 minutes. Then it was aseptically distributed in sterile Petri –dished as 15-20ml portion and left to solidify.

2.4.1.3. Simmon's citrate agar

Twenty three grams of Simmon's citrate agar powder were suspended into 1000 ml of distilled water and boiled to dissolving completely. The medium was distributed in 2 ml volumes into test tubes and sterilized by autoclaving at 121° C for 15 minutes.

2.4.1.4. Kilgler iron agar

Kilgler iron agar powder (5.5 grams) were suspended into 100 ml of distilled water and boiled to dissolving completely.

Then the medium was distributed in 6 ml amounts in large size test tubes sterilized by autoclaving at 121°C for 15 minutes. The medium was allowed to solid in a sloped position to give a butt about 25 mm deep and a slope about 25 mm long.

2.4.1.5.*Salmonella Shiglla* agar

Suspend 60 g of the medium in one liter of deionized or distilled water. Mix well. Heat with frequent agitation and boil for one minute. Do not autoclave the media. Pour into plates. Let the agar solidify and store in the refrigerator (avoid freezing). Prepared culture media can be kept for at least a week in refrigeration.

2.4.2. Semi solid media

2.4.2.1. Motility medium

The medium was described by(Cruickshank *et al*.,1975). 0.2% was dissolved in nutrient broth and distributed in sterile test tubes containing Craigie tubes and then the media was autoclaved at 121oc and 15 pounds per square inch.

2.4.3.Liquid media

2.4.3.1. Peptone water

Peotone water was prepared according to(Cruikshank *et al* ., 1975) ten grams peptone and 5 grams NaCL were dissolved by heating in 1000 ml distilled water. The PH was adjusted to 7.2 and the medium was distributed in test tubes (5ml) and serialized by autoclaving at 115 ^{o}C for 15minutes under pressure 15Ib per square inch. The stock was preserved in the refrigerator utill used **.**

2.4.3.2. Nutrient broth

Nutrient broth contained lab-lemco powder (1g) yeast extract (2g), peptone (5g) and sodium chloride (5g). PH was adjusted to 7.4 approximately. An amount of 13g of the dehydrated medium was added to one liter of distilled water .the reconstituted medium was mixed well then distributed in 5ml amounts and sterilized by autoclaving at 121 ^{o}c for 15 minutes under pressure of 15Ib per square inch (Oxoid,2006).

2.4.3.3. MR-VP medium

MR-VP medium (Oxoid lab) contained peptone (5g), dextrose (5g) and phosphate buffer (5g). The PH was adjusted to 7. One liter distill water was mixed well with 15 gram of the medium .Then distributed in test tubes with cotton plugs and sterilized by autoclaving at 121°c for 15 minutes under pressure 15Ib per square inch.

2.4.4. Examination of cultures

Examination of all cultures on solid media was performed for detection of growth, pigmentation, colonial morphology as well as changes in the media. Plates that showed visible growth were subjected to further bacteriological tests while those that did not show visible growth were incubated for further 48hours and discarded if no growth was detected.

2.4.5. Purification of cultures :

The primary isolates were sub cultured on nutrient agar. The subculture was repeated several times until pure colonies were obtained.

2.4.6. Gram stain technque :

Using a sterile wire loop apart of isolates colonies four primary pure plots were taken and spread on microscopes slides to make thin smears. They were fixed with heat and placed in 2.6 staining rack. They were covered by crystal violet for two minutes and washed off by tap water, then decolorized

with acetone for few seconds and washed off by tap water, then covered with carbol fuchsine for thirty seconds. Finally, the stained smears were washed and air-dried. Then they were examined under oil immersion lens (100°). The gram positive and negative organisms shape and arrangement of organisms were identified according to (Barrow and Feltham ,1993).

2.4.7. Biochemical Tests:

2.4.7.1.Catalase test

Using sterile glass rod apart of isolated colony was emulsified in one drop of hydrogen peroxide on a clean slide. Gsas bubbles indicated positive reaction(Barrow and Feltham 1993)**.**

2.4.7.2. Motility test

The isolates were studied for motility by Craigie technique(Cruicshank *et a l*., 1975) in which the bacteria was inoculated into a central tube containing semi solid agar placed in test tube using straight wire. After incubation at 37°c for 24 hours, the tubes were examined for migrating of bacteria outside the tube.

2.4.7.3.Oxidase Test

Strips of Whatman's No. I filter papers were soaked in a freshly prepared 1% solution of tetramethy-p-phenylenediaminedi hydrochloride. After draining from about 30 seconds, the papers were freeze dried and stored in dark sterile screw capped bottles. For use, a strip was laid with a sterile forceps on a clean Petri-dish and moistened with sterile distilled water. Colonies from a fresh young culture of the test organism (18-24 hrs old) were picked with a sterile bent glass-rod and rubbed on the filter paper. A dark purple color that developed within 5 to 10 seconds was considered a positive reaction.

2.4.7.4. Methyl-red Test

The tested cultures were inoculated in test tubes containing 1.5 ml glucose phosphate medium each. The tubes were then incubated at 37 ^{o}C for 48 h. About five drops of methyl red reagent were added to each test culture, mixed well and the results were read immediately. Abright red color indicated positive reaction and a yellow color indicated negative reaction.

2.4.7.5. Indole production test

Indole production test was carried out as described by Barrow and Feltham (1993). The tested organism was inoculated into tryptone water and

incubated at 37 ^{0}Cfor 48 h. One milliliter of the Kovac's reagent was run down along side of the test tube. Appearance of pink color within a minute indicated positive reaction.

2.4.7.6. Simmon's citrate test

Tested organisms were inoculated on the slop, of Simmon's citrate medium , incubated for 24-to 48 hours at 37 ^{o}C the positive color of medium turnned to the blue color .

2.4.7.7. Kliglar Iron test

Kligler iron agar medium was heavily inoculated with the test cultures by straight wire, first stabbed the butt and then streaked the slope in a zigzag, then incubated at 37°C for 24-48 hours black coloration due to formation of iron sulphide (H_2s)in the positive cases**.**

2.4. 7.8. Total Count

total plate count agar as described by (Bell, 1997). The medium was autoclaved and maintained at 46ºC. Samples were serially diluted and an aliquot of 1 ml of each of serial dilution was transferred to the petri dishes (4inchdiameter) and molten agar (15-20 ml) was poured on it. Plates were gently swirled to uniformly mix the sample and incubated at 37ºC for 48 hours. After incubation APC was determined from appropriate plates.

2.4. 7.9. Statistical Analysis

The symmetrical data in all phases of the study were compiled in excel database, and organized for statistical analysis. The analysis was done using IBM SPSS version 21(Coakes, 2005), a computer-based statistical software package. Different statistical approaches were used for comparing between means which include One Way ANOVA Paired T test as well as linear logistic regression to estimate the coefficients of the linear equation. Data represented by mean (standard deviation "SD") of (60) samples collected per each For comparing counts between per each .

Data with different superscripts are significantly difference at P value < 0.05 (using One Way ANOVA). For comparing between successive per each Data with different superscripts are significantly difference at P value < 0.05 (using paired samples T test.

Chapter Three

Results

3.1. Total Bactria Count of chesses samples collected from Bahre, Khartoum and Omdurman:

The results of one way analysis of variance for bacterial count revealed non-significant difference (P =0.8801) among the three places. The result in table (3-1) showed that the total bacterial count of cheese was 117.08, 137.08 and 142.08 colony forming unit CFU/ ml at Bahri, Khartoum and Omdurman, respectively.

3.2 Total Bactria Count of Yoghurt samples collected from Bahri, Khartoum and Omdurman:

The result of one way analysis of variance for bacterial count revealed non-significant difference (P=0.9491) among the three Places and the result in table (3-2) showed that the total bacterial Count in yoghurt samples was135.08, 121.08 and141.08 colony forming unit CFU/ ml per ml at the Bahri, Khartoum and Omdurman respectively.

3.3 comparison between cheese samples collected from Bahri, Khartoum and Omdurman According to Sources of samples:

T-test for comparing the mean of total bacterial count in cheese collected from the two sources, indicated non-significant differences (P =0.9491)

Shown in table (3-3) the total bacterial count in cheese samples was 137.08 and 132.08 CFU/ ml at the local and super point respectively.

3.4 comparison between yoghurt samples collected from Bahri, Khartoum and Omdurman according to Sources of samples:

T- test showed comparison the mean of the total bacterial count in yoghurt

samples collected from the two sources indicated significant difference (0.5752) as shown in table (3-4). The total bacterial count in yoghurt samples was141.08 and 699.07 CFU/ ml respectively at the local shops and super markets respectively.

Table 3.1 Total Count of Bactria in Cheese samples collected from Bahri, Khartoum and Omdurman Localities –Khartoum State

Place of samples	Number of samples	Means-SE
Bahri	9	117.08±3.8607[a]
Khartoum	13	137.08±3.2207[a]
Omdurman	13	142.08±3.2207[a]

Table 3.2 Total Count of Bactria in Yoghurt samples collected from Bahri, Khartoum and Omdurman Localities –Khartoum State

Place of samples	Number of samples	Means±SE
Bahre	11	135.08±3.621[a]
Khartoum	7	121.08±4.541[a]
Omdurman	7	141.08±4.541[a]

Table 3.3 comparison between the sources of Cheese samples (n=35) in Khartoum state

Source of samples	Number of samples	T.Value	P- Vale	Means$\pm$ SE	SD
Local	13	0.11	0.9110	$137.08^{a} \pm 2.8907$	1.0408
Super	22	0.12	0.9077	$132.08^{a} \pm 2.5607$	1.2008

.

Table (3.4) comparison between the sources of yoghurt samples (n=25) in Khartoum State

Sample Source	Sample Number	T.Value	P- Vale	Means$\pm$SE	SD
Local	22	1.01	0.3244	$141.08^{b} \pm 247.07$	115.08
Super	3	1.05	0.3785	$699.07^{a} \pm 631.07$	115.08

3.5 Frequencies and percentage of type of bacteria in local cheese were collected from Bahri, Khartoum and Omdurman 2017.

Three samples out of 13 local cheese samples that collected from shops were positive to *E.Coli, protus spp* and *salmonella spp* table (3.5) . The organism *E.Coli* was represented16.7% in Khartoum locality, while *protus spp* and *salmonella spp* (14.3% and 14.3% respectively) were found in Omdurman locality.

3.6 Frequencies and percentage of type of bacteria in super market cheese were collected from Bahri, Khartoum and Omdurman localities.

In table(3-6) demonstrated that 8 out of 22 local cheese samples were positive (36.4%) in Bahri the organism *Klebsila* spp(22.2%) and *E.coli* (11.1%) were isolated . Whereas ,*proteus spp* (28.6%) was isolated in Khartoum localty.While both *E.coli* and *proteus spp* were fawnd in Omdurman locality .

Table (3.5) Frequencies and percentage of type of bacteria in local cheese were collected from Bahri, Khartoum and Omdurman localities.

Place	Samples Number	Type of Bactria	Frequencies	Percentage %
Bahre	-	-	-	-
Khartoum	6	*E, coli*	1	16.7
Omdurman	7	*Protaus*	1	14.3
		Salmonella	1	14.3
Samples negative	10	-	-	76.9
Samples positive	3	-	-	23.1
Total samples	13	-	-	100.0%

Table (3.6) Frequencies and percentage of type of bacteria in super market cheese were collected from Bahre, Khartoum and Omdurman localities.

Place	samples Number	Type of Bactria	Frequencies	Percentage%
Bahre	9	*klebsiella*	2	22.2
		E.coli	1	11.1
Khartoum	7	*Protaus*	2	28.6
Omdurman	6	*E.coli*	1	16.7
		Protaus	2	33.3
Samples negative	14	-	-	63.6
Samples positive	8	-	-	36.3
Total samples	22	-	-	100.0%

3.7 Frequencies and percentage of type of bacteria in local yoghurt were collected from Bahri, Khartoum and Omdurman localities.

Out of 22 local yoghurt samples are collected from local shop 6 were positive to bacterial identification resulting in 27.3%table (3.7).

In yoghurt6 samples were positive to bacteria in local shops (3.7) .But the 3 samples (100.0%) collected from super markets were negative to bactria (3.8).

Table (3.7) Frequencies and percentage of type of bacteria in local yoghurt were collected from Bahri, Khartoum and Omdurman localities.

Place	samples Number	Type of Bactria	Frequencies	Percentage %
Bahre	10	*E.coli*	1	10.00
		Klebsiella	1	10.00
Khartoum	7	*E.coli*	1	14.3
		Klebsiella	1	14.3
Omdurman	5	*E.coli*	1	20.00
		Klebsiella	1	20.00
Samples negative	18	-	-	72.73 %
Samples positive	6	-	-	27.27 %
Total samples	22	-	-	100%

Table (3.8) Frequencies and percentage of type of bacteria in super market yoghurt were collected from Bahri, Khartoum and Omdurman localities.

Place	**samples Number**	**Type of Bactria**	**Frequencies**	**Percentage %**
Bahri	1	**-**	-	100.0%
Khartoum				
Omdurman	2	**-**	**-**	100.0
Samples negative	3	**-**	-	100.0
Samples positive	-	**-**	**-**	**-**
Total samples	3	**-**	**-**	**100.0%**

Chapter Four

Discussion

Cheese and yoghurt are obtained by subjecting raw milk to one or more technical processes that change the components of milk and its rheological properties to a greater or lesser extent. These “treatments” may be the addition of salt and the removal of water (seasoning) or the addition of natural enzymes and/or milk ferments that trigger these complex biochemical processes that ageing of cheese or fermented milks. *Salmonella*, as well as other pathogenic agents of foodborne disease, can contaminate raw milk by direct inside the mammary gland (very rare event), during milking, because the bacteria are often present in the faeces of milk animals and on their coat, after milking, because salmonellae can contaminate work surfaces with which the raw milk comes into contactor in subsequent phases, still due to the presence of *Salmonella* on work surfaces and/or cross contamination. The fate of salmonellae in milk and milk products widely depends on the antimicrobial effects the different transformation processes may have on the bacteria, as bactericidal effect or more simply bacteriostatic effect. (Jayarao and Henning, 2001).

The study was showed that one out of the total 60 (35cheese -25yoghurt) samples were found to be contaminated with *Salmonella*. thes samples from local shops and supermarkets from Khartoum state . Salmonella was present in alocal shop cheese sample, the data were collected thorough the microbiological analysis which include determination of Total bacteria Count per colonies forming unit/ml and determination Frequencies and percentage of each type of bacteria.

It was concluded that the higher Bacteria Count of cheese was 142 it was recorded in Omdurman while the lower was 117 colonies forming unit/ml in bahre.in table(3.1) , in case of yoghurt the higher Bacteria Count was 141 it was recorded in Omdurman while the lower was 121 colonies forming unit/ml /ml in Khartoum.in table(3.2).

Salmonella species such as *Salmonella typhi* is a bacterium that causes typhoid fever (enteric fever), an acute, life-threatening febrile illness (CDC, 2008). The disease is a cause for concern and a major public health problem in developing countries (Asia, Africa); especially in Nigeria due to poor sanitary conditions and lack of or inadequate potable water (Ibekwe *et al.,* 2008). It is mainlytransmitted through food or drink or water, contaminated with urine or faeces of infected people or a chronic carrier (CDC, 2008; Ibekwe *et al*., 2008).

There is no significant different between bacterial load of cheese when comparing with source of sample (132 and 137).

But there significant different between bacterial load of yoghurt when comparing with source of sample (141 and 699),in table. Through this result it can be concluded that the samples yoghurt obtained from super markets was best when comparing by samples yoghurt obtained from local market. The presence of high loads of lactic acid bacteria, coupled with low pH levels (4.0 to 4.1 on average) and Awmean that yogurt and other fermented milk products are a very unfit food matrix for allowing the growth and even the survival of *Salmonella* (Little *et al*., 2008).
The frequencies of bacteria in samples cheese obtained from local market was 3 out of 13 samples were positive result for *E.coli* , *Proteus* and *salmonella* (16.7%). While the frequencies of bacteria in cheese samples

obtained from super market was 8 out of 22 samples were positive (36.7%) for *E.coli, Proteus* and *Klebsiella* .

It was concluded that the frequencies of Bacteria in samples yoghurt obtained from local market was 6 out of 22 samples were positive (27.3%).for E.coli andKlebsila While the frequencies of Bacteria in yoghurt samples obtained from super market was not present out of 3 samples were negative (100%) in table (3.7),(3.8).

The presence of interobacteria in local cheese is due to several foodborne pathogens have usually been detected in raw milk, including *Salmonella* spp., *Campylobacter*spp. *E. coli*, *Listeria monocytogenes*,and S. *aureus* (Oliver, 2005.) *Klebsiella* spphave been implicated in cases of acute gastroenteritis due to consumption of contaminated raw foods (Lindsay 1997). *K. pneumoniae* has also been reported as a cause of superior venta cava syndrome (Kim *et al*. ,1997) and retropharyngeal abscess (Pontell et al, 1995) in humans. Klebsiella pneumonia is present in the respiratory tract and faeces of about 5% normal individuals (Brooks *et al*., 2001).

E. coli, which are normal flora of the human and animal intestine, have been identified as aleading cause of food borne illness all over the world (Hussein, 2007). However, diarrhea caused by enterotoxigenic *E. coli* (ETEC) is highly prevalent in young children in developing countries as well as in travelers.It spreads through contaminated water and food (Qadri *et al*., 2005).

The contamination with these organisms in this study may be due to condition of the handling, tables, equipment and machines, which had been used during
processing, preparing, and the hygienic practice. Although most of organisms found in this study are normal flora of different parts of man and

animal body, some of them have been associated with many disease problems. They might cause disease in their presence in the animal body or by contamination of food. Animal health, dressing skills, personnel hygiene, abattoir cleanliness, and adequate storage and holding temperature during distribution and retail influence the constitution and number of microorganisms present (Hudson 1996).

Conclusions and Recommendations

Conclusions

At the end of our research, contamination of the local product of cheese and yoghurt, which appears to be a result of poor hygiene practices, from production to consumption. Disease-causing bacteria, such as E.Coli, salmonella, klebsiella, protaus have been isolated, and under certain conditions they may be dangerous. To prevent them from being damaged, good hygiene practices must be followed, starting from production to consumption, and a lot of work and effort is required to regulate the most vulnerable points of contamination.

Recommendations

- The authorities must provid laboratory for the manufacture of local cheeses ,simple, small, mobile and high efficiency in production and owner ship of small producers at appropriate prices and subsidized and training employees to increase the quality and quantity of local cheeses and yoghurt to benefit economically.
- This study recommends the consumers to pasteurized raw milk and salted cheese
- The local cheeses and local yoghurt must be packagad in different packages of weights so as not to be exposed to pollution during the deportation ,storage and marketing .
- The hands should also be washed before handlingcheese and yoghurt.

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