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# BACTERIAL HAZARDS OF CHICKEN SHAWERMA SANDWICHES

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## ABSTRACT

One hundred random samples of chicken Shawerma sandwiches were collected separately from different restaurants with variant localities at Mansoura city and subjected to bacteriological examination for determination of aerobic plate count; *Staph. aureus* count and its enterotoxins; *E. coli* count and its toxins; *Bacillus cereus count* and incidence of Salmonella. The log mean counts were 5.5±3.2, 2.2±1.6, 3.3±1.8 and 2.4±1.9 cfu/g for aerobic plate count; *Staph. aureus; E. coli* and *Bacillus cereus counts* respectively, with an incidence percent of 18%, 22%, 32% and 10% for *Staph. aureus; E. coli*; *Bacillus cereus* and Salmonella. Meanwhile, 3 out of 18 isolated *Staph. aureus* strains were enterotoxigenic which produce a SEE and SED enterotoxins and one isolate of *E. coli* produced Stx1. *Salmonella typhimurium could not be detected* in the examined chicken shawerma sandwiches. This study confirms that chicken Shawerma sandwiches may serve as a source of potential public health hazard. Thus, corrective action needs to be employed to minimize the risk of consuming this type of fast food during its cooking and serving, in addition handling and personal hygiene of the food vendors are the major factors of foodborne pathogens.

***Key words:*** Chicken Shawerma sandwiches, *Staph. aureus*, *E. coli* and their toxins, Bacillus cereus, Salmonella

## INTRODUCTION

Chicken and chicken products provide animal protein of high biological value for consumers at all ages, where they contain all the essential amino acids required for growth with high proportion of unsaturated fatty acids and low cholesterol value moreover, poultry meat is a good source of different types of vitamins and minerals (Zaki and Shehata, 2008).

Meanwhile, Braun *et al.* (2005) mentioned that these foods are manipulated extensively during processing and therefore have a potential for high bacterial contamination levels on the meat surface, as well as the inside. As a result, there is an increased risk of pathogens surviving and transferring not only by cross-contamination, but also by undercooking as observed in this kind of fast-food industry.

Ghafir *et al.* (2008) mentioned that several bacterial indicators are used to evaluate the hygiene during meat slaughtering process including the counts of *E.coli* and other Enterobacteriaceae and aerobic bacteria while, (Ahmad *et al.,* 2013) analyzed fresh shawerma for *E.coli*, Salmonella, aerobes and coliforms which were found in order of aerobes >*E. coli*> Salmonella, there is variation among the number of bacteria in every part aerobes were greater in number as compared to *E. coli* and Salmonella. (Logue and Nde 2007) mentioned that symptoms of food poisoning such as vomiting, nausea, cramps, diarrhea with or without blood, abdominal pain, or fever might appear after hours or even few days after consuming contaminated food. They are often mild and a person can recover alone at home but some people need to refer to the hospital. Risks of getting an infection are higher in infants or children since they don't have a well-developed immune system and in old people as the response of their immune system becomes weaker.

Bennett *et al.* (2008) reported that 1229 food borne outbreaks caused by *Bacillus cereus* and *Staph. aureus* from 1998 to 2008 in U.S., 39% were with a confirmed etiology. Vomiting was commonly reported in *Bacillus* cereus (75%) of cases and (87%) of *Staph. aureus* outbreaks in addition to Berdgoll (1989) concluded that a very small amount of *Staph. aureus* enterotoxins ranging from 20 ng to < 1 µg is

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needed to cause a typical symptoms of staphylococcal food poisoning. Furthermore, Asao *et al.* (2003) mentioned that an outbreak in Japan caused by low-fat milk contaminated with SEA showed that the total intake of SEA per individual was estimated to be 20–100 ng. More recently, Ostyn *et al.* (2010) in French found an outbreak caused by contaminated cheese, doses of SEE ingested by symptomatic persons were estimated to be about 90 ng. Presence of Salmonella in frozen meat products cause serious infection if not cooked properly (Dominguez and Schaffner, 2009).

Therefore, this work was planned to evaluate some of the bacteriological dangers in chicken shawerma sandwiches sold in Mansoura city.

## MATERIALS AND METHODS

**A- Collection of samples:** A total of 100 random samples of chicken shawerma sandwiches were collected separately from different restaurants with variant localities at Mansoura city and aseptically transferred without delay in an insulated ice box to the laboratory, then subjected to the following.

**B - Bacteriological examination:**

**1**- Preparation of food homogenate: according to the technique recommended by ISO, 6887-2 (2003) 25 g of each sample were taken by a sterile knife and stomached using Seward stomacher 80 biomaster England with 225 ml sterile buffered peptone water (0.1%) to give a homogenate of 1/10 dilution from which six fold serial dilutions were prepared and subjected to the following bacteriological examination.

**2- Aerobic Plate Count (APC):** It was carried out according to APHA (2001)

**3- *Staph. aureus* count: FDA (2002)** using Baird-Parker agar plates, incubated at 35$^{o}$C for 48 hr and the suspected *Staph. aureu*s colonies were isolated, and confirmed bycatalase, coagulase, thermos table nuclease and Voges-Proskauer tests.

**4- *Bacillus cereus* count:** according to the technique recommended by ISO, (2004) using Mannitol egg yolk polymexinphenol red agar and confirmed biochemically by catalase, nitrate reduction test, Voges-Proskauer test and detection of haemolysis.

**5- Counting and Identification of Beta-glucuronidase-positive *E. coli*** according to the technique recommended by ISO, 16649/2(2001) *E. coli* was detected by using sorbitol Mac Conkey agar medium (Oxoid, England). Pure suspected colonies were biochemicaly identified by using urease, Indol, methyl red, $H_2S$ production test and citrate utilization test according to (Koneman *et al*., 1997). And Salmonella according to the technique recommended by ISO, 6579 (2002) on enrichment Rappaport vassiliades broth at 42$^{0}$C for 24-48h, platting on XLD agar at 35$^{0}$C for 24h. The presumptive colonies were confirmed biochemically and by multiplex PCR.

**C- Detection of virulence genes:** The isolated *Staph. aureus*, *E. coli* and Salmonella were examined by using PCR for detection of *Staph. aureus* enterotoxins; *E. coli* (stx1 and stx2) and *Salmonella typhimurium* (invA, and stn). Reference Lab. for Quality Control on Poultry Production, Animal Health Research Institute, Dokki -Egypt.

**1- DNA extraction for isolated *Staph. aureus*:**

**a-** DNA extraction from samples was performed using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56$^{O}$C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**b-** Oligonucleotide Primers used were supplied from Metabion (Germany) are listed in Table 1.

**c-** For multiplex PCR of enterotoxins, Primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmolconcentration, 8 µl of water, and 7 µl of DNA template. The reaction was performed in an Appliedbiosystem 2720 thermal cycler.

**d-** Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 30 µl of the multiplex PCR products were loaded in each gel slot. Gelpilot 100 bp DNA ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was

photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**2- DNA extraction for isolated *E. coli*:**

**a-** DNA extraction from samples was performed using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at $56^{O}$C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

**b-** Oligonucleotide Primers used were supplied from Metabion (Germany) are listed in Table 1.

**c-** PCR amplification For stx1, stx2 duplex PCR, primers were utilized in a 50- µl reaction containing 25 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 13 µl of water, and 8 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

**d-** Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the uniplex PCR products and 30 µl of the duplex PCR products were loaded in each gel slot. Generuler 100 bp DNA ladder (Fermentas, sigma) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**3- DNA extraction for isolated *Salmonella typhimurium:***

**a-** DNA extraction from samples was performed using the QIA amp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at $56^{O}$C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer.

**b-** Oligonucleotide Primers used were supplied from Metabion (Germany) are listed in Table 1.

**c-** PCR amplification Primers were utilized in a 25- µl reaction containing 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

**d-** Analysis of the PCR Product: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 20 µl of the products was loaded in each gel slot. Gelpilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) and generuler 100 bp ladder (Fermentas, Germany) were used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

**Table 1:** Primers sequences, target genes, amplicon sizes and cycling conditions for *Staph.aureus*, *E. coli* and *Salmonella typhimurium* used in multiplex PCR.

| Organism | Target gene | Primers sequences | Amplified segment (bp) | Primary denaturation | Amplification (35 cycles) | | | Final extension | Reference |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Secondary denaturation | Annealing | Extension | | |
| *Staph. aureus* | *Sea* | GGTTATCA ATGTGCGG GTGG<br>CGGCACTT TTTTCTCTT CGG | 102 | 94˚C 5 min. | 94˚C 30 sec. | 50˚C 40 sec. | 72˚C 40 sec. | 72˚C 10 min. | Mehrotra *et al*., 2000 |
| | *Seb* | GTATGGTG GTGTAACT GAGC<br>CCAAATAG TGACGAGT TAGG | 164 | | | | | | |
| | *Sec* | AGATGAAG TAGTTGAT GTGTATGG<br>CACACTTT TAGAATCA ACCG | 451 | | | | | | |
| | *Sed* | CCAATAAT AGGAGAAA ATAAAAG<br>ATTGGTAT TTTTTTTCG TTC | 278 | | | | | | |
| | *See* | AGGTTTTT TCACAGGT CATCC<br>CTTTTTTTT CTTCGGTC AATC | 209 | | | | | | |
| *E. coli* | stx1 | ACACTGGA TGATCTCA GTGG<br>CTGAATCC CCCTCCAT TATG | 614 | 94˚C 5 min. | 94˚C 30 sec. | 58˚C 40 sec. | 72˚C 45 sec. | 72˚C 10 min | Dipineto *et al.*, 2006 |
| | stx2 | CCATGACA ACGGACAG CAGTT<br>CCTGTCAA CTGAG CAGCACTT TG | 779 | | | | | | |
| Salmonella typhimurium | stn | TTG TGT CGC TAT CAC TGG CAA CC<br>ATT CGT AAC CCG CTC TCG TCC | 617 | 94˚C 5 min. | 94˚C 30 sec. | 59˚C 45 sec. | 72˚C 45 sec. | 72˚C 10 min | Murugkar et al., 2003 |
| | invA | GTGAAATT ATCGCCAC GTTCGGGC AA<br>TCATCGCA CCGTCAAA GGAACC | 284 | 94˚C 5 min. | 94˚C 30 sec. | 55˚C 30 sec. | 72˚C 30 sec. | 72˚C 7 min. | Oliveira et al., 2003 |

**Statistical analysis:**
The results are expressed as log mean ± Standard Error (SE). Data were statistically analyzed using statistical analysis systems.

**Table 2:** Statistical Analytical Results of Bacterial Incidence inthe examined Chicken Shawerma Sandwich esexpressed as log mean±SE. (n=100)**.**

| Sample type \ Isolated organisms | | APC | *Staph. aureus* | *E. coli* | *Bacillus cereus* | Salmonella |
|---|---|---|---|---|---|---|
| Chicken shawerma sandwiches | bacterial count | 5.5±3.2 | 2.2±1.6 | 3.3±1.8 | 2.4±1.9 | - |
| | incidence % | 100 | 18 | 22 | 32 | 10 |

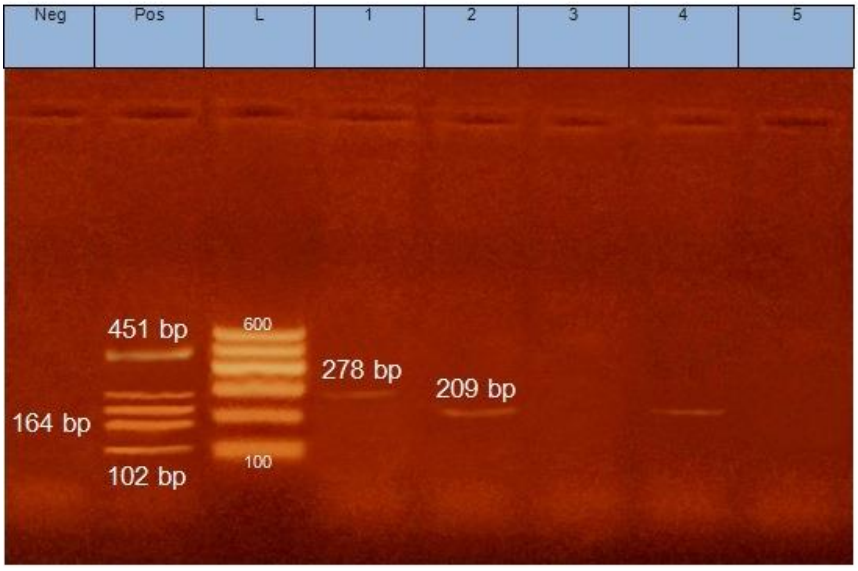


**Fig (1):** Agarose gel electrophoresis of *Staph. aureus* PCR products using enterotoxins Staphylococcus primer. Pos=positive control, Neg=negative control, L=100 bp DNA ladder
**Lane "1":** Positive amplification of 278bp for enterotoxin D
**Lane "2" and Lane "4":** positive amplification of 209 bp for enterotoxin E
**Lane "3" Lane "5"** were negative

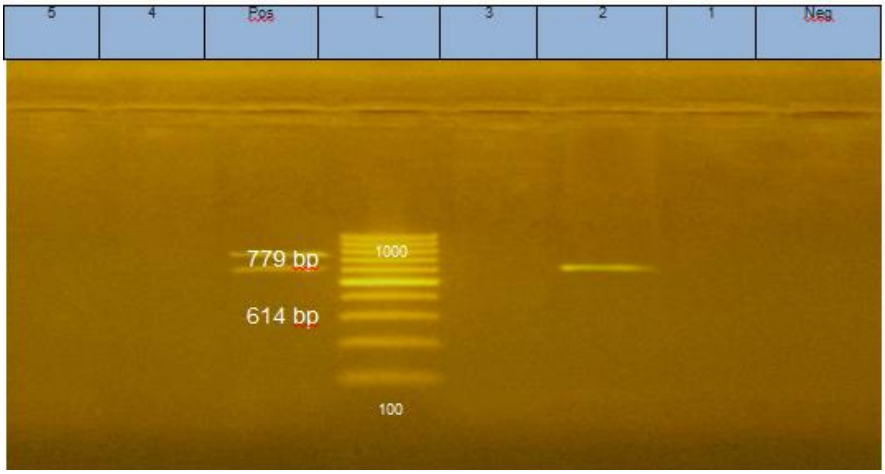


**Fig (2):** Agarose gel electrophoresis of *E. coli* PCR products using *stx1 and stx2* primers.
**Lane "1"; Lane "3 "; Lane "4 "and Lane "5"** were negative
**Lane "2":** Positive amplification of 614 bp for stx1 gene

## DISCUSSION

Poultry are known to be a reservoir of large number of bacteria which may be pathogenic to human being. Typically, these occur in low sanitation levels and may pose a threat to the consumer if the product is not handled in a safe manner. Therefore, the production, transportation and sale of meat products must be performed with the almost care and preferably be subjected to HACCP evaluation, to prevent the presentation of any undue hazard (Madden, 1994). Hence, the importance of food as a vehicle in transmission of several diseases has been documented especially in developing countries where hygienic standards are not strictly followed or enforced (Harakeh *et al.,* 2005). Foodborne diseases do not always result in acute gastroenteritis but also, food represents an important vehicle for pathogens (Flint *et al.,* 2005).

The obtained results of aerobic platecount in Table 2 of chicken shawerma sandwiches were 5.5±3.2 $log_{10}$cfu/g these results were nearly in accordance with those achieved by (Sharaf and Sabra, 2012) in cooked chicken shawerma which were $1.2x10^5$cfu/g and lower than the results recorded by (Salem *et al.,* 2016) in chicken shawerma sandwiches which were $2.8x10^6 \pm 1.0x10^6$cfu/g. this difference may be due to contamination of raw food, food handlers, utensils, unefficient cooking process and post cooking contamination.

Obviously, for the safety of shawerma sandwiches, it is necessary to cut a not more than 2-cm piece of fillet every time to be sure that these pieces are efficiently cooked and the latter is ready-made. Microbiological quality problems of Shawerma sandwiches depends greatly on the quality of raw meat and other ingredients, efficiency of cooking process, proper sanitary practices for personnel and for cooking/processing utensils (Kayaardi *et al.,* 2006). The achieved resultsof *Staph. aureus* in Table 2 declared that the mean count of *Staph. aureus* in chicken shawerma sandwiches were 2.2± $1.6log_{10}$cfu/g with incidence percent of 18% and the results of Fig (1) showed that 3 out of 18 isolated strains were enterotoxigenic produced sed and see virulent genes.

These results were nearly similar to Hatakka (1998) who found that hot foods have been the source of outbreaks of *Staph*. *aureus* food poisoning and Nimri *et al.* (2014) examined chicken shawerma sandwiches for *Staph*. *aureus* which were 8.3% and some strains are capable of producing a heat-stable enterotoxin that causes food poisoning in humans also, Salem *et al.* (2016) found *Staph*. *aureus* in 26% of the examined samples. (Todd 2017) isolate *Staph. aureus* from shawerma and Chen *et al*. (2015) mentioned that *Staph. aureus* in cooked meat products were 4.84% in addition to (Hu ShouKui *et al.,* 2013) Surveyed that the positive rates of *Staph. aureus* in cooked meat were 10.0% and 43.7% of the isolated strains produced enterotoxins while, Abou Hussein (2007) and Sharaf and Sabra (2012) can't detect *Staph*. *aureus* in cooked chicken shawerma.

The main sources of pathogenic bacteria in food are contaminated raw food, food handlers, dust, water, utensils & insects (Ray, 1996). The results of examined chicken shawerma sandwiches in Table2 indicated that the mean count of *E. coli* were 3.3±1.8 $log_{10}$cfu/g with incidence percent of 22% and the results of Fig (2) showed that presence of stx1 in one isolate and the other isolates were not toxigenic. these results were higher than that obtained by Abou Hussein (2007) who mentioned that the incidence of *E*. *coli* in cooked chicken shawerma was 13.33% while nearly similar results obtained by Sharaf and Sabra (2012) who examined cooked chicken shawerma samples for *E. coli* count which were $3.9x10^2$cfu/g with an incidence percent 20%, while higher percentage were recently reported by (Nimri *et al.,* 2014) examined shawerma (donair kebab) where the predominant species was *E. coli* (28.3%), with six isolates of serotype O157:H7 and Banna and Nawas (2016) tested chicken shawerma sandwiches for incidence of *E. coli* which were 20%.

Shinagawa (1990) and Sousa and Stamford (2013) stated that *Bacillus cereus* is able to form spores, founded in ready meals and it is responsible for causing two distinct types of food poisoning: an emetic toxin pre-formed in food where cooked rice is the most common vehicle, the diarrhoeal type is caused by an enterotoxin. Its virulence factors include the production of haemolysins and phospholipases toxins and the symptoms are similar to those of *Staph. aureus* intoxication. The heat resistance of *Bacillus cereus* spores and the non fastidious nature of the organism facilitate its survival and/or growth in a wide variety of foods.

The achieved results in Table 2 declared that the mean count of *Bacillus cereus* were 2.4±1.9 $log_{10}$cfu/g with incidence percent of 32%, these results were higher than Chen *et al.* (2015) who detected *Bacillus cereus* in 12.66% of cooked meat products and lower than those recorded by Salem *et al.* (2016) who examined chicken shawerma sandwiches for the presence of *Bacillus cereus* incidence which were identified in 40% of examined samples.

Cooking grilled chicken fillet dish shawerma has been found to ensure its freedom from Salmonella only in a piece less than 2 cm thickness where Deeper layers of chicken and its juice that accumulates in the grill tray may remain be Salmonella-contaminated throughout the heat treatment (Sergevnin *et al.,*

2012). The incidence results of Salmonellain the examined chicken shawerma sandwiches were 10% meanwhile, *Salmonella typhimurium* could not be detected. These results were lower than Nimri *et al.* (2014) who found Salmonella spp. In 26.3% from the examined Shawarma sandwiches and Banna and Nawas (2016) who tested chicken shawerma sandwiches for incidence of Salmonella which was 30%.

## CONCLUSION

This study confirms that chicken shawerma sandwiches may serve as a source of foodborne pathogens and accordingly a potential public health hazard. Corrective action needs to be employed to minimize the risk of consuming this type of fast food, knowing that even small doses of the organism may lead to food poisoning Such action must aim at minimizing the bacterial contamination during the production of chicken shawarma (cleaning, cutting, seasoning and stacking), its cooking and serving. More attention should be given to the cleanliness of utensils used in preparing the sandwiches (rod, knives, etc.) in addition to the personal hygiene of the workers preparing and stuffing the sandwiches. New regulations that will ensure the safety of the consumer.

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# الاخطار البكتيرية لسندوتشات شاورما الدجاج

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تعد منتجات الدواجن من الأغذية الضرورية للإنسان فى جميع بلدان العالم لما تحتويه من عناصر غذائيه ضروريه لبناء الجسم ولكنها تعتبر من أكثر المصادر المسببه للتسمم الغذائى إذا ما تم معاملتها بطرق خاطئه من الناحية الصحية أثناء إنتاجها وتصنيعها لذا أجريت هذه الدراسة للكشف عن الاخطار البكتيريه لسندوتشات الشاورما حيث تم جمع ١٠٠عينه من سندوتشات شاورما الدجاج من مطاعم زات مستويات واماكن مختلفة بمدينة المنصورة وعمل عد كلى للبكتيريا الهوائية، الاستافيلوكوكس اوريس الايجابى لاختبار تجمع البلازما، الايشيرشياكولاى، الباسيلس سيرس وكذا معرفة مدى تواجد ميكروب السالمونيلا حيث ان العد الكلى للبكتيريا الهوائية، الاستافيلوكوكس اوريس، الايشيرشياكولاى، الباسيلس سيرس كان ٥,٥±٣,٢، ٢,٢±١,٦، ٣,٣±١,٨و٢,٤±١,٩الوج./جم على الترتيب ونسب العزل كالتالي ١٨% و٢٢% و٣٢ % و١٠% على الترتيب لكل من الاستافيلوكوكساوريس، الايشيرشياكولاى، الباسيلس سيرس والسالمونيلا حيث تم فحص وجود جينات الضرواه لكل من الاستافيلوكوكس اوريس والايشيرشياكولاى والسالمونيلا باجراء اختبار تفاعل البلمرة المتسلسل لتحديد وجود جينات الضراوة حيث كانت النتائج سلبية الفحص لميكروب السالموونيلا تيفيميوريم بينما اثبتت النتائج وجود الانتيروتوكسين D&E في الاستافيلوكوكس اوريس والشيجا توكسين ١ فى الايشيرشياكولاى المعزولة وقد نوقشت قدرة الميكروبات فى احداث حالات مرضية عند تناول سندوتشات الشاورما الملوثة بهذه الميكروبات والتي تقوم بإثارة مراكز القئ في المخ وتشكل أحد الأسباب الرئيسية للتسمم الغذائي، والذي يحدث عادة بعد تناول الأطعمة المختلفة. وقد نوقشت الأهمية الصحية للمعزولات وكذلك كيفية الإقلال من تواجدها باتباع نظم إدارة سلامة الغذاء.