The impact of food preparation practices on food borne diseases

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Abstract: Food preparation practices were found to be an important aspect that affect the food safety. In this study, a total of One hundred twenty two (122) food samples were used for the isolation of food poisoning, food spoilage bacteria, yeasts and molds. All the samples were collected from different hotels and resorts kitchens in Egypt. All the samples were subjected to aerobic plate count and total mold / yeast count as the results indicated that the count vary from satisfactory to unsatisfactory according to the standards as ISO (International standards organization) 4833/2003 for Ready to Eat Foods, (Egyptian standards of frozen minced meat) ES 1694/2005 for frozen minced meat, (Egyptian standards of frozen meat) ES 1522/2005 for frozen meat and (Egyptian standards of frozen poultry) ES 1090/2005 for frozen chicken and ISO 4833/2003. The most important food borne pathogens, *Staph. aureus*, *E. Coli, Salmonella* Sp. and *B. Cereus* were isolated by using different specific media. Different preparation practices were found that affect the aerobic plate count or proliferation of food pathogens as unhygienic handling; cross contamination; inadequate cleaning and sanitation of all contact surfaces; improper cooling, cooking and holding temperature. *Salmonella typhimurium* was selected in our study for the rapid detection by molecular techniques as Polymerase Chain Reaction (PCR) which indicated that PCR test combined with RV selective enrichment is more sensitive in detecting *Salmonella* serovars than bacteriological methods.
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1. Introduction

Food safety is a matter that affects anyone who eats food. Whether or not a person consciously thinks about food safety before eating a meal, a host of other people has thought about the safety of that food, from farmers to scientists to company presidents to federal government officials and sanitarians (Roberts, 2001).

Food-borne illness is a major international problem and an important cause of reduced economic growth. The contamination of the food supply with the pathogens and its persistence, growth, multiplication and/or toxin production has emerged as an important public health concern. Most of these problems could be controlled with the efforts on the part of the food handlers, whether in a processing plant, a restaurant, and others (Mensah *et al.*, 2002).

Many high-risk pathogens that cause disease in humans are transmitted through various food items. Due to increased morbidity and mortality leading to time lost in the work place and reduced productivity, food-borne disease across the world costs billions of dollars annually (Todd, 1989). There are main microorganisms related to the manipulation practices such as *Staphylococcus* spp*., Escherichia coli and Salmonella* spp. and different factors regarding the food-borne illness related to the impact of good manipulation practices on the food safety and food quality (Lukinmaa *et al.,* 2004).

As urbanization increases worldwide, eating meals outside the home is becoming more frequent. Despite the growth of this sector, there is no effective education or training of the food handlers or hygienic control of the food sold on the streets.

Over two hundred different diseases are known to be transmitted by the food (Bryan, 1982). Despite this, only a fraction of all food-borne infections are ever diagnosed and officially reported, or can be traced to a definite Vehicle and a specific causative agent (Lukinmaa *et al.,* 2004). In this context, the manipulation practices represent an important factor. The epidemiological data indicate that the cross contamination during the food preparation contributes notably to the occurrence of the foodborne diseases (Forsythe and Hayes, 1988).

To ensure that the food is microbiologically safe, both the manipulators (WHO, 2002) and the food need to be continually monitored (Gilling *et al.*, 2001). Bacteria need certain elements to survive and grow as water, food, proper temperature, time, oxygen and proper pH or acidity (Potter and Morris, 1996). The hygienic standard of food can be assessed by the analysis of the indicator microorganisms (Forsythe and Hayes, 1988).

Besides this, there are major microorganisms groups that are used alone or together in order to verify the microbiological characteristics and the hygienic condition of the food as fecal coliforms, e.g., *Escherichia coli,* which are presently used as an indicator of the sanitary conditions. *Staphylococcus aureus* is a major human pathogen capable of causing a wide range of infections. Staphylococcal food poisoning caused by the enterotoxigenic producing *S. aureus* is an important foodborne disease throughout the world (Bergdoll, 2000). *Salmonella* spp. is one of the most commonly reported causes of the food borne disease in UK and in USA and in many other countries around the world (Varma, 2005).

There are various factors contribute to the outbreaks of the food borne illness. The main ones are:

i) Inadequate food manipulation;

ii) Improper holding temperatures (failing to properly refrigerate food),

iii) Inadequate cooking,

iv) Contaminated equipment (failure to clean and disinfect kitchen or processing plant equipment),

v) Poor personal hygiene.

Other factors that may contribute to the food borne illness include:

i) Preparing food a day or more before serving with improper holding and reheating,

ii) Cross contamination (from raw to cooked products),

iii) Adding contaminated ingredients to the previously cooked food.

After foods are contaminated, the main factor is letting them remain at a temperature that allows the growth of the potentially hazardous microorganisms or its toxin production in the food.

The conventional microbiological methods for detection of these bacteria, however, usually include multiple subcultures and biotype or serotype-identification steps, which are laborious and time-consuming (Swaminathan & Feng, 1994; Feng, 1993). Rapid and easy detection of pathogenic organisms will facilitate precautionary measures to maintain healthy food (Feng, 1993). The advent of gene probe techniques has allowed the development of powerful tests by which particular bacterial strains can be rapidly identified without the need for isolating pure cultures (Rasmussen *et al.,* 1994; Cohen *et al*., 1993).

The aim of this work: Isolation and identification of food poisoning bacteria, food spoilage bacteria, molds, yeasts, and implementing rapid detection of *Salmonella typhimurium* using polymerase chain reaction.

2. Material and Methods

Collection of samples

A total of One hundred twenty two (122) food samples were used for the isolation of food poisoning, food spoilage bacteria, yeasts and molds. All the samples were collected from different hotels and resorts kitchens in Egypt.

All the samples were taken from the different stages of food manufacturing or preparation (prior, during and after preparation) to determine the impact of food manufacturing or preparation practices on growth of food poisoning microorganisms, the collected samples included ready to eat (RTE) foods, fresh vegetables, raw foods, improper cooked foods, cooked foods and food contact surfaces and employees swabs.

Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay and examined as quickly as possible. The collected samples were subjected to the microbiological examination to evaluate their quality.

Preparation of samples

Preparation of sample homogenate (ICMSF, 1996)

To each 25 grams of the sample, 225 ml of sterile peptone water were added and thoroughly mixed using sterile homogenizer for 1 – 1.5 minutes, from which tenfold serial dilutions were prepared.

Surface swabs

Swabs were sterile cotton screw capped plastic tubes ready for use. A template made of metal having an exposed inner area of 10 cm² (2×5 cm) was used to delineate area of sampling. The template were wrapped in aluminum foil and sterilized in hot air oven at 180ºC for 20 minutes. Buffered peptone water 1% was used as rinsing and diluents fluid. The solution was distributed to small heat resistant screw capped tubes, each containing 10 ml of rinsing fluid, and then sterilized in the autoclave at 121ºC for 20 minutes. For use, the sterilized template placed firmly against the surface of the food serving establishments and food handlers to limit the examined area.

The sterile cotton swab drawn from the tubes, moistened in rinsing fluid solutions (buffered peptone water 1%), then rolled over the limited area inside the template rolled in one direction and perpendicular to this direction to represent all area. Finally, cotton swab was aseptically retained into the rinsing fluid screw capped tubes containing 10 ml buffered peptone water (1%).

Bacterial isolation, counting, purification and identification

The prepared samples and swabs were subjected to the following examinations:

Aerobic Plate Count (ICMSF, 1996)

One ml from each of the previously prepared dilutions was transferred into two separate sterile Petri-dishes to which approximately 15 ml of sterile melted and tempered plate count agar (45°C) were added. After thorough mixing, the inoculated plates were allowed to solidify before being incubated at 37°C for 24 hours. The count per gram was calculated on plates containing 30-300 colonies and each count was recorded separately.

Total Enterobacteriaceae count (Gork, 1976)

The same technique of the previous pour plate method was carried out using Violet Red Bile Glucose agar medium (VRBG). The plates were incubated at 37°C for 24 hours.

All purple colonies were then counted and the average number of colonies was determined. Hence, the Enterobacteriaceae count/g was calculated.

Total coliform count

The procedures recommended by ICMSF (1996) using Violet Red Bile agar medium were done. The same technique of the previous pour plate method was applied using Violet Red Bile agar medium. The plates were incubated at 37°C for 24 hours. All dark red colonies measuring 0.5 mm or more in diameter on non-crowded plates were then counted and the average number of colonies was determined.

Determination of total *Staphylococci* count (ICMSF, 1996)

Accurately, 0.1 ml from each of previously prepared serial dilutions was spread over duplicated plates of Baired Parker agar using a sterile glass spreader. The inoculated and control plates were incubated at 37°C for 48 hours. The developed colonies were enumerated and the total *Staphylococci* count /g was calculated. Also, the colonies were picked up and purified on nutrient agar slopes for further identification.

Screening for Enteropathogenic *Escherichia coli*

Pre-enrichment (ICMSF, 1996)

From the original dilution, one ml was inoculated into MacConkey's broth tubes supplemented with inverted Durham's tubes. Inoculated tubes were incubated at 37°C for 24 hours.

Enrichment broth

One ml from positive MacConkey's tube was inoculated into another MacConkey's broth tubes and incubated at 44°C for 24 hours.

Plating media

Loopfulls from positive MacConkey's broth tubes were separately streaked onto Eosin Methylene Blue agar medium (EMB), which was then incubated at 37°C for24 hours. Suspected colonies were metallic green in color.

Accordingly, the suspected colonies were purified and inoculated into slope nutrient agar tubes for further identification.

Serodiagnosis of *E. coli*

The isolates were serologically identified by using diagnostic sera, "Welcome *E. coli*" agglutinating sera, for diagnosis of the Enteropathogenic types.

Screening for Salmonellae

Pre-enrichment broth

Twenty five grams of examined samples were homogenized in 225 ml of sterile peptone water and incubated at 37°C for 18 hours.

Enrichment broth

One ml of the original dilution was inoculated into 9 ml Rappaport Vassilidis (RV) broth tube, then the tube was incubated at 43°C for 24 hours (Rappaport *et al.,* 1956 and Harvey and Price, 1981).

Selective Plating

Xylose lysine desoxychocolate agar (XLD) was used. Loopfulls from the inoculated tubes were separately streaked onto XLD agar medium and incubated at 37°C for 24 hours. Suspected colonies were red with or without black centers.

The suspected colonies were sub-cultured onto nutrient agar plate and incubated at 37°C for 24 hours. However, the purified suspected colonies were selected and streaked onto slope nutrient agar for further identification. The purified isolates were identified morphologically, biochemically and serologically.

Serological identification of Salmonellae

Isolates proved biochemically to be *Salmonella* were subjected to serological identification according to Kauffman white scheme as follows:

Isolates were sub cultured on nutrient slope for 24 hours at 37°C for application of slide agglutination technique, two homogenous suspensions were made on a slide by suspending a piece of suspected colony in a drop of sterile physiological saline. A drop of each of separate O and H *Salmonella* factors were added separately to each of the suspensions with standard loop thoroughly mixed to bring the microorganisms in close contact with antisera. Positive agglutination occurred within a minute and could be easily seen with the naked eye. A delayed or partial agglutination was considered as negative or false result.

Determination of O (somatic) antigens

Separate O antisera were applied to determine the group of the *Salmonella* isolates.

Determination of H (flagella) antigens

Polyvalent H antisera for both phase I and phase 2 were tried in order to determine the complete antigenic formula of the isolates. For this purpose, rapid diagnostic H antisera sets were used (Welcome

Diagnostic, a Division of the Welcome Foundation Limited, Dartford England DA15 AH).

Determination of *B. cereus* count (Harrigan, 1998)

From each previously prepared dilution, 0.1 ml was seeded onto the surface of Polymyxin Pyruvate Egg yolk Mannitol Bromothymol Blue Agar (PEMBA) by bent glass rod. The plates were then inverted and incubated at 37°C for 24 hours. The typical colonies of *B. cereus* were blue, turquoise to peacock blue surrounded by zone of egg yolk precipitation. The plates were re-incubated for further 24 hours to detect all *B. cereus* colonies which were enumerated and recorded as total *B. cereus* count. Moreover, the purified suspected colonies were selected and streaked onto slope nutrient agar for further identification.

Determination of total mold and yeast count

Duplicated Petri dishes of Sabouraud's Dextrose agar media were inoculated each with 1 ml of each dilution, left to solidify at room temperature then incubated at 25°C for 7 days. During the incubation period, inoculated plates were examined daily for the suspected colonies. Total mold and yeast count was then calculated and recorded.

Identification of the bacterial isolates by analytical profile index (API) strips

The identification of all bacterial isolates was performed using API strips API 20 E, and API 20 *Staph*. The strips were inoculated and incubated as described by the manufacturer (bio Merieux Vitek System, France). Examination of the strips was conducted after 18-24 hr incubation at 37°C. The results were read and analyzed using API instrument.

DNA-based techniques

Bacterial DNA extraction using thermo scientific kit

The bacteriologically positive strains for *Salmonella* were grown in10 ml tryptic soya broth (TSB) at 37°C for 24 h. The overnight cultures were centrifuged at 3000 rpm for 5 min and the supernatant were decanted carefully. The bacterial pellets were washed three times with phosphate buffer saline pH 7.2, resuspended in 400 µl tris-EDTA buffer (pH 8.0) and heated in water bath at 100°C for 20 min. There were left to cool at room temperature and centrifuged at 14,000 rpm for 10 min. An aliquot of 5 µl of the supernatant was used as template DNA in the PCR. While the extraction of DNA from the field samples enriched in RV broth was carried out by the same method reported by (Oliveira *et al*., 2003).

Polymerase chain reaction

Oligonucleotide primers

The used Primers were designed for *S. typhimurium* based on the fimA gene sequence (Naravaneni and Jamil 2005; Moussa *et al.,* 2011). It was found that The fimA gene in *S. typhimurium* encodes the major fimbrial subunits (Clegg and Gerlach, 1987; Nichols *et al.,* 1990; Moussa *et al.,* 2011). This gene has been cloned and sequenced from *S. typhimurium* (Swenson *et al.,* 1991), and a particular region was found to be specific for *Salmonella*.

The nucleotide sequence of the fimA gene is available from GenBank (accession no. M18283) and the sequence was retrieved for designing the used primers according to Kisiela *et al.* (2013). The sequence of the primers designed is: Forward primer, 5'- CCT TTC TCC ATC GTC CTG AA -3'; Reverse primer, 5'- TGG TGT TAT CTG CCT GAC CA -3'.

This set of primers was used for PCR amplification and also for the subsequent sequencing of the PCR product which all carried out in accordance with the protocols available at the Sigma Egypt sequence service using ABI 3730xl DNA sequencer. The sequence was then compared with similar sequences retrieved from DNA databases by using the NCBI n-BLAST search program in the National Center for Biotechnology Information (NCBI).

DNA amplification and agarose gel electrophoresis

PCR amplifications were performed in a final volume of 50 µl in micro-amplification tubes (PCR tubes).

The reaction mixtures consisted of 5 µl of the DNA template, 5 µl 10× PCR buffer (75 mM Tris-HCl, pH 9.0, 2 mM MgCl2, 50 mM KCl, 20 mM (NH4)2SO4), 1 µl dNTPs (40 µl), 1 µl (1U Ampli Taq DNA polymerase), 1 µl (25 pmol) from the forward and reverse primers of both primer pairs and the volume of the reaction mixture was completed to 50 µl using distilled-deionized water (DDW).

The thermal cycler was adjusted as follows: Initial denaturation at 94°C for 5 min., followed by 35 cycles of (denaturation at 94°C for 1 min., annealing at 56°C for 1 min. and extension at 72°C for 1 min). Final extension was carried out at 72°C for 10 min and the PCR products were stored in the thermal cycler at 4°C until they were collected.

The PCR products were tested for positive amplification by agarose gel electrophoresis described by (Sambrook *et al.,* 1989) with Gene Ruler (50bp) DNA ladder. The gel was electrophoresed to the desired level of voltage and the DNA was visualized and imaged.

The used PCR kits were: 1- Gene Jet genomic DNA purification kit (thermo Scientific) for extraction. 2- Maxima Hot Start PCR masters mix (thermo Scientific) for PCR. 3- Gene Jet PCR purification Kit (thermo Scientific) for PCR cleanup.

3. Results and Discussion

Table (1) revealed the percentage of food samples collected from different stages of food preparation or manufacturing to determine the impact of food manufacturing or preparation practices on the growth of food poisoning microorganisms.

Table (2) showed the first group of food samples (Ready to eat foods) which were subjected to Aerobic Plate Count, Total Molds and Yeast Count to determine the safety of foods the safety of ready to eat food samples according to ISO 4833/2003 as the standard of aerobic plate count for RTE foods $\leq 10^5$ cfu/g, so all samples are accepted as are not exceed the standard of aerobic plate count limit, this is correlated to the implementation of good manufacturing/preparation practices during the different stages of food preparation as following:

Proper cleaning and sanitation of all food contact surfaces and raw vegetables prior to preparation using chlorine tablets within the contact time according to material safety data sheet (MSDS) which leads to minimize the aerobic plate count to the acceptable limit.

Cooling or chilling of all food items after preparation till to serving within the proper temperature which is far from Temperature Dangerous Zone (TDZ). All those food samples free from the most common food pathogens except the sample no.14 (C/SE/07) which is contaminated with *E. Coli* O86: K61 which is pathogenic, However the APC of the sample 8.2× 10^3 which was conformed to the standard.

The contamination of this sample refers to bad manufacturing or preparation practices which may be cross contamination from other contaminated foods, bad personal hygiene or improper cleaning and sanitation.

It was found that good preparation practices as good cleaning and proper sanitation of fresh vegetables by using food grade chlorine tablets leads to minimize the microbial load to the acceptable limits which conformed to standards as revealed in table (3) of the samples of fresh vegetables after and during sanitation.

Table 1. The percentage of food samples collected from different stages of food preparation or manufacturing

Stages of the collected foods	No. of samples	Percentage %
Ready To Eat (RTE) foods	21	17.2%
Fresh Vegetables	7	5.7%
Raw Foods	30	24.6%
Improper cooked foods	4	3.3%
Cooked foods	18	14.8%
Food contact surfaces and employees swabs	42	34.4%
Total	**122**	**100.00%**

Table 2. Bacterial, mold and yeast count in ready to eat foods (RTE) samples

NO.	Code	APC	TMC	TYC	NO.	Code	APC	TMC	TYC
1	C/B/01 green salad	10^3×7.5	10^3×2.7	10^2×8.5	2	C/B/02 green salad	10^3×4.9	10^2×5.6	----
3	C/B/07 Tahina (sesame paste) salad previously prepared	10^3×2.8	10^2×8.0	10^2×2.0	4	C/B/09 improper chilled salad	10^3×8.5	10^3×3.3	10^2×7.4
5	C/B/26 tart (dressing + gelatine+ tart)	10^3×6.3	10×7.0	---------	6	C/I/01 green salad	10^3×3.4	10^3×4.0	10^2×8.3
7	C/I/02 tahina salad	10^3×9.1	10^2×6.9	10^2×2.0	8	C/I/07 salad previously prepared	10^3×7.2	--------	--------
9	C/I/09 improper chilled salad	10^4×1.2	10^3×4.9	10^3×1.2	10	C/LM/01 mixed salad	10^3×9.2	10^2×8.3	10^2×2.9
11	C/LM/07 salad prev. prepared	10^3×3.6	10^3×4.1	10^3×1.0	12	C/se/01 green salad	10^3×1.9	10^2×5.0	10^2×1.9
13	C/se/02 mixed salad	10^3×5.6	10^2×2.0	10^2×1.0	14	C/se/07 salad previously prepared	10^3×8.2	10^3×1.1	10^2×6.0
15	C/se/09 improper chilled salad	10^3×9.4	10^2×9.0	10^2×4.0	16	C/se/26 fruit salad	10^3×3.6	10^2×1.6	10^2×3.0
17	C/MR/01 green salad	10^3×2.3	10^2×3.0	10^2×7.1	18	C/ MR /02 green salad	10^2×8.9	10^2×1.2	10^2×9.0
19	C/ MR /09 improper chilled salad	10^3×6.5	10^2×1.3	10×7.0	20	SH/AP/01 green salad	10^3×1.0	10×9.0	10^2×4.5
21	SH/AP/01 green salad	10^3×1.0	10×9.0	10^2×4.5					

APC=Aerobic Plate Count, TMC=Total Mold Count, TYC=Total Yeast Count

Table 3. Bacterial, mold and yeast count in fresh vegetables samples after and during sanitation

NO.	Code	APC	TMC	TYC	Contact Time of chlorine tablets
1	C/B/10.1 Vegetable during sanitation	$10^3\times2.8$	-----	10×6.0	5 minutes
2	C/B/10 Vegetables after sanitation	$10^2\times9.7$	-----	-----	10 minutes
3	C/I/10 Vegetables after sanitation	$10^3\times1.6$	-----	-----	10 minutes
4	C/Se/10 Vegetables after sanitation	$10^3\times5.1$	10×2.0	------	7-10 minutes
5	C/MR/13 Vegetables after sanitation	$10^2\times8.8$	10×1.0	------	7-10 minutes
6	SH/AP/10.1 Vegetables during sanitation	$10^2\times9.8$	-----	10×2.0	5 minutes
7	SH/AP/10 Vegetables after sanitation	$10^2\times7.3$	-----	-----	10 minutes

Table 4. Bacterial, mold, yeast count and pathogenic isolates in raw food samples

NO.	Code	APC	Pathogens	TMC	TYC	NO.	Code	APC	Pathogens	TMC	TYC
1	C/B/11 Raw meat	$10^5\times1.2$	*Staph.* 7×10^2 *E.coli* O111:K58	$10^3\times1.2$	$10^2\times9.0$	16	C/se/11 Raw meat	$10^4\times7.6$	*S.enteritidies* *E.coli* O26:K60	$10^2\times4.0$	10×8.0
2	C/B/12 Prepared meat	$10^3\times8.7$		10×7.0	----	17	C/se/12 Prepared meat	$10^3\times4.8$	*Staph.* 2×10^2	$10^2\times1.2$	-----
3	C/B/14 Raw chicken	$10^5\times3.8$	*Staph.* 2×10^3	$10^3\times3.7$	$10^3\times1.5$	18	C/se/14 Raw chicken	$10^5\times2.0$	*Staph.* 1.1×10^4 *S.munester*	$10^2\times7.0$	$10^2\times1.5$
4	C/B/15 prepared chicken	$10^3\times9.9$		$10^3\times1.0$	$10^2\times6.2$	19	C/se/15 prepared chicken	$10^3\times7.2$	*Staph.* 4×10^2	$10^2\times2.0$	10×9.0
5	C/B/22 Prep.minced meat	$10^4\times7.3$	*B.cereus* 6×10^2	$10^3\times1.3$	$10^2\times6.8$	20	C/se/22 Prep.minced meat	$10^4\times8.2$	*E.coli* O128:K67	$10^2\times6.0$	$10^2\times1.7$
6	C/LM/11 Raw meat	$10^5\times6.8$	*E.coli* O55:K59	$10^3\times3.6$	$10^3\times2.0$	21	SH/AP /11 Raw meat	$10^4\times2.2$		$10^2\times3.0$	------
7	C/LM/12 Prepared meat	$10^4\times1.7$		$10^2\times5.0$	$10^2\times2.1$	22	SH/AP /12 Prepared meat	$10^3\times3.1$	*Staph.* 1.0×10^2	10×8.0	------
8	C/LM/14 Raw chicken	$10^5\times4.2$		$10^3\times2.5$	$10^3\times1.7$	23	SH/AP /14 Raw chicken	$10^4\times5.7$		$10^2\times4.0$	$10^2\times2.3$
9	C/LM/15 prepared chicken	$10^4\times2.5$		$10^3\times2.1$	$10^2\times8.8$	24	SH/AP /15 prepared chicken	$10^3\times3.8$		$10^2\times1.0$	10×3.0
10	C/LM/22 prep.minced meat	$10^4\times8.9$	*B.cereus* 3×10^2	$10^3\times1.7$	$10^3\times1.0$	25	SH/AP /22 prep.minced meat	$10^3\times9.2$	*Staph.* 3×10^2 *B.cereus* 1.0×10^2	$10^2\times5.0$	10×8.0
11	C/I/11 Raw meat	$10^4\times9.5$	*Staph.* 1×10^3	$10^2\times8.0$	$10^2\times5.3$	26	C/MR /11 Raw meat	$10^4\times4.7$		$10^2\times5.0$	$10^2\times2.8$
12	C/I/12 Prepared meat	$10^3\times8.8$		$10^2\times1.0$	------	27	C/MR /12 Prepared meat	$10^3\times6.0$	*Staph.* 9×10^2	10×9.0	10×4.0
13	C/I/14 Raw chicken	$10^5\times9.0$	*Staph.* 5×10^3 *S.typhimurium*	$10^3\times7.0$	$10^3\times3.1$	28	C/MR /14 Raw chicken	$10^4\times7.1$	*S.enteritidies*	$10^2\times7.0$	$10^2\times3.0$
14	C/I/15 prepared chicken	$10^4\times4.6$		$10^3\times1.4$	$10^2\times7.9$	29	C/MR /15 prepared chicken	$10^3\times5.5$	*Staph.* 1.0×10^3	$10^2\times2.2$	10×5.0
15	C/I/22 prep.minced meat	$10^5\times1.1$	*Staph.* 9×10^2 *B.cereus* 1.0×10^3 *E.coli* O124:K72	$10^3\times2.4$	$10^3\times1.1$	30	C/MR /22 prep.minced meat	$10^3\times6.4$	*E.coli* O111:K58	$10^2\times3.1$	10×9.0

APC=Aerobic Plate Count, TMC=Total Mold Count, TYC=Total Yeast Count, S.=*salmonella*, Staph. =*Staphylococcus aureus*, B.cereus= *Bacillus cereus*, E.coli= *Escherichia coli*

One of the good manufacturing or preparation practices is the addition of different spices and herbs to the raw meat and chicken which lead to minimize of APC and the prevention of food pathogens growth which enhance both of food safety and Quality. Table (4) revealed that the addition of different spices and natural herbs has a great impact on the growth of different food pathogens, molds and yeast in addition to APC. It was observed that the addition of different spices and natural herbs to the raw foods (meat or chicken) leads to minimize of APC and the prevention the growth of food pathogens as *Salmonella* Sp. *or E. Coli* as samples C/B/11, C/B/12, C/B/14, C/B/15, C/LM/11 and C/LM/12 However, it leads to the contamination of the sample (cross contamination) with *Staph. aureus* which related to bad preparation practices as mixing the raw foods with herbs or spices with improper cleaned or sanitized hands directly, using tools or utensils which is improper cleaned and sanitized then the preservation or cooling of raw foods after the preparation (additives) at improper temperature within Temperature Dangerous Zone (TDZ) which leads to increase the growth of *Staph. aureus* as samples C/SE/11, C/SE/12, SH/AP/11, SH/AP/12, C/MR/11, C/MR/12 and C/MR/14. The results of these bad preparation practices were observed in different samples which isolated from three different hotels but the same method of preparation is used.

Table (5) revealed that when the results of APC of cooked samples are compared with the standards ISO 4833/2003 which stipulated that it must be $\leq 10^4$ cfu/g , in addition to ISO 16649- part 2 for *E. Coli* detection , ISO 6589/2005 for *Salmonella* detection , and ISO 6888-1 for *Staph . aureus* detection it was found that cooked meat and cooked chicken samples were treated with the proper temperature during the cooking step (80°C/2minutes) then preserved at cooling temperature which is beyond the temperature dangerous zone, so APC was conformed to the standard. On the other hand, the samples of meat previously prepared as after cooking at proper temperature it was preserved at improper cooling temperature, so APC start to increase over the standard as shown 1.1×10^4 cfu/g.

Table 5. Bacterial, mold, yeast count and pathogenic isolates in cooked food samples

NO.	Code	APC	Pathogens	TMC	TYC	NO.	Code	APC	Pathogens	TMC	TYC
1	C/B/13 cooked meat	2.4×10^3		-----	-----	10	C/SE/20 Meat with beans prev. prepared	$10^5\times4.5$	*S.enteritidies* *E.coli* O124:K72 $10^3\times2.0$	$10^3\times1.2$	$10^2\times5.8$
2	C/B/16 cooked chicken	4.5×10^3		$10^2\times7.0$	----	11	C/SE/21 cooked rice	$10^4\times2.7$	*B.cereus* 8.0×10^2	$10^2\times1.0$	10×3.0
3	C/B/20 Meat previously prepared	$10^4\times1.1$		$10^2\times9.0$	$10^2\times4.3$	12	SH/AP/13 Cooked meat	$10^2\times6.9$		10×6.0	------
4	C/B/25 cooked Rice	$10^3\times8.0$	*B.cereus* 2×10^2	10×3.0	10×1.0	13	SH/AP/25 Cooked rice	$10^3\times5.7$		10×6.0	10×2.0
5	C/I/13 Cooked meat	$10^3\times3.9$		10×5.0	-----	14	H/M/01 Cooked meat	$10^2\times8.5$		10×1.0	------
6	C/I/16 Cooked chicken	$10^3\times5.9$		$10^2\times8.0$	10×1.0	15	C/B/21 mixed soup	$10^3\times4.9$		$10^3\times1.8$	$10^2\times7.9$
7	C/I/25 Cooked rice	$10^4\times1.4$	*B.cereus* 5×10^2	$10^2\times1.6$	10×2.0	16	C/I/21 Improper holding for cooked fish	$10^4\times5.3$		$10^4\times1.0$	$10^3\times3.0$
8	C/SE/13 Cooked meat	$10^3\times1.1$		$10^2\times1.0$	-----	17	C/se/25 Improper holding for cooked rice at 30^{0}C	$10^4\times5.1$	*B.cereus* 3.0×10^3	$10^4\times1.0$	$10^3\times3.0$
9	C/SE/16 cooked chicken	$10^3\times3.9$		$10^2\times1.4$	-----	18	C/MR/25 Improper holding for cooked rice at 45-50^{0}C	$10^3\times9.2$	*B.cereus* 8.0×10^2	$10^2\times1.7$	$10^2\times5.3$

APC=Aerobic Plate Count, TMC=Total Mold Count, TYC=Total Yeast Count, S.=*salmonella*, *Staph*. =*staphylococcus aureus*, *B. cereus*= *Bacillus cereus* , *E. coli*= *Echrichia Coli*

The same results of the implementation of good preparation practices were shown for samples of cooked rice as APC was 8.0×10^3 cfu/g, however there is growth detected for *B. cereus* but still within the standard 2.0×10^2 cfu/g.

Another example for bad preparation practices for other cooked rice sample C/SE/21 was observed as after preparation and cooking, it was preserved at temperature dangerous zone but for short time, so APC showed increasing beyond the standard 2.7×10^4 cfu/g and *B. cereus* growth with count 8×10^2 cfu/g which is marginally satisfactory.

Cooked meat with beans sample C/SE/20 was a clear example for bad preparation practices as shown that it was cooked at improper temperature, cooled at improper temperature within TDZ at 15°C and cross contaminated due to bad preservation, so it is observed that APC was very high as 4.5×10^5 cfu/g and there was detection for the growth of food pathogens as *S. enteritidies, E. coli* (O124:K72*)* and *B. cereus* with count 2.0×10^3 cfu/g which is unsatisfactory. The cooked fish sample revealed another example of bad preparation practices as the holding /preservation of cooked fish at improper temperature within TDZ (45°C) that lead to increasing of APC to 5.3×10^4 cfu/g in addition to increasing the total mold count to 1.0×10^4 and total yeast count to 3.0×10^3.

The other two samples of cooked rice, refers to bad preparation practices, the first one C/SE/25 which was preserved at low temperature 30°c within TDZ so APC was increased to 5.1×10^4 cfu /g in addition to the high count of *B. cereus* which is detected 3.0×10^3 cfu/g which is unsatisfactory, while the second sample C/MR/25 was preserved at low temperature 45°C for short time , shows APC within acceptable limit 9.2×10^3 cfu/g, however the growth of *B. cereus* was detected with count 8×10^2 cfu/g which is marginally satisfactory.

Table (6) showed the samples of improper cooked foods to determine the impact of food preparation practices on the growth of food poisoning microorganisms, especially the impact of core temperature of cooked foods which must be within standard (80°C/2 minutes). The first sample (C/B/24) of cooked meat as the core temperature was 80°C/2 minutes, so APC within the standard which is 6.2×10^3 cfu/g, no growth was detected for food pathogens and both of TMC and TYC was conformed to the standard.

Sample no. 2 (C/SE/24) which is improper cooked meat as the core temperature of the sample was 78°C/1 minute, so it is observed that APC was 2.9×10^4 cfu/g which is beyond the standard APC while both of TMC and TYC was conformed to the standard that refers to the negative impact of bad preparation practices.

Sample no. 3 (C/MR/13) of improper cooked chicken sausage with steam represented a clear example of bad preparation practices as during this cooking method the core temperature cannot reach to the standard, however the APC still within the standard of cooked foods, but on the other hand this improper temperature represented negative impact as it leads to the growth of food pathogens as *Staph. aureus* with count 4×10^2 cfu/g which is not conformed to the standard (ES 2911/2005 of frozen poultry sausage) in addition to the growth of *Bacillus cereus* with count 7×10^2 cfu/g which is marginal satisfactory, both of *E. coli* O26:K602 and *Salmonella typhimurium* were detected which are not conformed to the standard.

Table 6. Bacterial, mold, yeast count and pathogenic isolates in improper cooked food samples

NO.	Code	APC	Pathogens	TMC	TYC
1	C/B/24 Improper cooked meat	$10^3\times6.2$	No pathogens detected	$10^2\times6.5$	10×9.0
2	C/SE/24 Improper cooked meat "medium rare"	$10^4\times2.9$	No pathogens detected	$10^2\times2.0$	10×5.0
3	C/MR/13 Improper cooked sausage	$10^3\times4.6$	*Staph.* 4×10^2 *B.cereus* 7×10^2 *S.typhi* *E.coli* O26:K60	$10^2\times1.3$	10×6.0
4	C/SE/27 improper cooked chicken	$10^4\times6.7$	No pathogens detected	$10^2\times4.0$	10×7.0

Table 7. Bacterial, mold and yeast count in food contact surfaces / food handler's swabs

No.	Code	APC	TMC	TYC	Staph.	No.	Code	APC	TMC	TYC	Staph.
1	C/B/03 Slicer swab	3.3×10^2	-----	-----	-----	22	C/SE/03 Slicer swab	6.0×10^2	-----	-----	-----
2	C/B/04 Surface swab1	8.4×10^2	1.4×10^2	3.0×10	-----	23	C/SE/04 Surface swab1	3.3×10^3	3.0×10	-----	-----
3	C/B/05 Surface swab2	1.6×10^3	5.3×10^2	1.6×10^2	-----	24	C/SE/05 Surface swab2	9.7×10^2	-----	-----	-----
4	C/B/06 F.H.swab	9.8×10^2	-----	-----	-----	25	C/SE/06 F.H.swab	7.5×10^4	-----	-----	9.0×10^3 Hand Injury
5	C/B/17 Surface swab .But.1	1.9×10^3	-----	-----	-----	26	C/SE/17 knife swab .But.	8.6×10^2	3.0×10	-----	-----
6	C/B/18 Surface swab .But.2	6.7×10^2	-----	-----	-----	27	C/SE/18 Surface swab .But.	1.1×10^3	-----	-----	-----
7	C/B/19 F.H.swab	2.5×10^3	-----	-----	1.0×10^2	28	C/SE/19 F.H.swab .But.	6.9×10^3	-----	-----	7×10^2
8	C/LM/03 Slicer swab	8.1×10^2	-----	-----	-----	29	C/MR/03 Slicer swab	2.6×10^2	-----	-----	-----
9	C/LM/04 Surface swab1	5.2×10^2	-----	-----	-----	30	C/MR/04 Surface swab1	5.8×10^2	-----	-----	-----
10	C/LM/05 Surface swab2	2.9×10^3	4.6×10^2	1.0×10^2	-----	31	C/MR/05 Surface swab2	1.1×10^3	-----	-----	-----
11	C/LM/06 F.H.swab	1.4×10^3	-----	-----	-----	32	C/MR/06 F.H.swab	7.3×10^2	-----	-----	1.0×10^2
12	C/LM/17 Surface swab .But.1	1.0×10^3	3.2×10^2	-----	-----	33	C/MR/17 Surface swab .But.1	5.2×10^2	1.0×10	1.0×10	-----
13	C/LM/18 Surface swab .But.2	9.6×10^2	2.0×10^2	-----	-----	34	C/MR/18 Surface swab .But.2	8.7×10^2	4.0×10	1.0×10	-----
14	C/LM/19 F.H.swab .But.	6.0×10^3	-----	-----	-----	35	C/MR/19 F.H.swab	7.1×10^2	-----	-----	-----
15	C/I/03 Slicer swab	7.6×10^2	-----	-----	-----	36	SH/AP/03 Slicer swab	1.7×10^2	-----	-----	-----
16	C/I/04 Surface swab1	3.5×10^3	4.0×10^2	7.1×10^2	-----	37	SH/AP/04 Surface swab1	4.2×10^2	-----	-----	-----
17	C/I/05 Surface swab2	8.3×10^2	1.3×10^2	1.0×10^2	-----	38	SH/AP/05 Surface swab2	8.5×10^2	-----	-----	-----
18	C/I/06 F.H.swab	1.1×10^3	-----	-----	2.0×10^2	39	SH/AP/06 F.H.swab	5.1×10^2	-----	-----	-----
19	C/I/17 Surface swab .But.1	6.8×10^2	-----	2.7×10^2	-----	40	SH/AP/17 knife swab .But.	4.3×10^2	1.0×10	-----	-----
20	C/I/18 Surface swab .But.2	4.2×10^3	7.8×10^2	3.1×10^2	-----	41	SH/AP/18 Surface swab .But.	7.9×10^2	7.0×10	-----	-----
21	C/I/19 F.H.swab	4.8×10^3	-----	-----	4.0×10^2	42	SH/AP/19 F.H.swab H.S	4.0×10^2	-----	-----	-----

F.H=food Handler, But=Butcher section, H.s=Hot section

Tables (7 & 11) revealed that the swabs of C/B, the first two samples (C/B/03) for slicer swab and (C/B/04) for surface swab from Ready To Eat Section (RTE), it is observed that both of the samples are conformed to the standard (10 cfu/ cm^2) as an indicator for the implementation of good practices as proper cleaning and disinfection using food grade chlorine tablets taking in consideration the contact time which is stipulated in Material Safety Data Sheet (MSDS) of chlorine tablets.

Swab no. 4 (C/B/06) of food handler swab from the same section (RTE) represent bad practices as improper cleaning/washing and disinfection with sanitizer gel, so APC not conformed to the standard,

However, swab no. 5 (C/B/18) for raw meat contact surface act as indicator for good practices as proper cleaning and disinfection as APC conformed to the standard.

Swabs of C/LM shows results for both of good and bad practices, swab no. 8 (C/LM/03) of slicer and swab no. 9 (C/LM/04) of RTE contact surface conformed with the standard , on the other hand swab no. 14 (C/LM/19) of food handler of raw meat, after washing and disinfection of the hands, it was observed that APC was very high 6×10^3 cfu/g which is not conformed to the standard and represent an indicator for bad practices.

C/SE swabs shows clear examples for the impact of bad practices as swab no. 23 (C/SE/04) and swab no. 24 (C/SE/05) both of them for RTE contact surfaces as shown APC was 3.3×10^3 cfu/g and 9.7×10^2 cfu/g, respectively which refers to improper cleaning and disinfection that lead to high APC which not conformed to the standard.

Another example of bad practices can be represented in swab no.25 (C/SE/06) for RTE food handler with hand injury/wound which is exposed so the handler can implement the proper practices during washing and disinfection of the hands that leads to very high APC (7.5×10^4 cfu/g) in addition to the presence of *Staph. aureus* with count (9.0×10^3 cfu/g) which act as a major risk especially with Ready To Eat food handler.

SH/AP swabs results assure or emphasize with the previous results coded with SH/AP which refers to implementation of Food safety system and good practices as all the swabs of food contact surfaces or food handlers are conformed with the standard which refers to the positive impact of implementation of good preparation practices.

Table (8) revealed that raw, prepared and cooked meat and chicken samples are contaminated with different species of spoilage bacteria, then the preparation step as addition of spices and natural herbs represented as good practices which leads to the prevention of the growth of many species as *Enterobacter aerogenes, Enterobacter hafniae, Klebsiella pneumonia, Proteus morganii and Serratia marcescen.* An example of bad practices which lead to the cross contamination of prepared meat or chicken is the improper method used to prepare it by addition of natural herbs and spices as shown the detection of *Staphylococcus epidermidis* in both prepared meat and chicken. There was a clear example of good preparation practices which implemented that agree with the previous results which was the proper sanitation for fresh vegetables by using food grade chlorine tablets with the contact time which stipulated in Material Safety Data Sheet (MSDS) that has positive impact as the prevention of the growth of different species of spoilage bacteria as *Enterobacter agglomerans, Proteus mirabilis, Proteus vulgaris and Staphylococcus epidermidis.* However; other species are showed resistance to chlorine tablets as *Enterobacter cloacae, Klebsiella ozaenae* and Micrococci. *Proteus vulgaris* showed resistance to the spices and natural herbs during the preparation step of the raw meat and chicken; in addition to resistance to the high temperature of cooking but showed sensitivity to chlorine tablets during the sanitation step of fresh vegetables after the contact time which is stipulated by MSDS, Additionally, table (9) emphasize that the improper chilling of salads represented as bad practices as shown the detection of different species of spoilage bacteria due to the temperature of chilling or cooling within TDZ.

Table 8. Incidence of spoilage bacteria isolated from raw, prepared and cooked meat and chicken samples

Isolated Bacteria	Raw meat	Prepared meat	Cooked meat	Raw chicken	Prepared chicken	Cooked chicken
Citrobacter diversus	-	-	-	+	-	-
Citrobacter freundii	+	+	-	+	+	+
Enterobacter aerogenes	+	-	-	+	+	-
Enterobacter agglomerans	-	-	-	+	-	-
Enterobacter cloacae	+	+	+	+	+	+
Enterobacter hafniae	+	-	-	-	-	-
Klebsiella ozaenae	+	+	-	+	+	+
Klebsiella pneumoniae	+	-	-	+	+	-
Proteus mirabilis	+	+	-	+	+	-
Proteus morganii	+	-	-	-	-	-
Proteus rettgeri	+	+	-	+	+	+
Proteus vulgaris	+	+	+	+	+	+
Serratia liquefaciens	-	-	-	+	-	-
Serratia marcescens	+	-	-	+	-	-
Staphylococcus epidermidis	-	+	-	-	+	-
Micrococci	+	+	+	+	+	+

Table (10) revealed that the preservation of cooked rice at proper temperature which is beyond TDZ leads to the prevention of the growth of spoilage bacteria except some species which can grow and survive at high temperature as *Enterobacter cloacae , Proteus vulgaris* and *Micrococci*.

The improper holding/preservation of cooked rice at low temperature (45°C-50°C) within TDZ

bacteria which can grow and survive at this low temperature within TDZ as *Enterobacter hafniae, Klebsiella pneumonia, Proteus mirabilis, Proteus rettgeri* and *Serratia liquefaciens*, these results agree with the previous results for the sample C/SE/25 which showed high APC 5.1×10^4 cfu/g that not conformed to the standard and high count of *B.* allow the growth of different species of spoilage bacteria as *Citrobacter freundii* and *Enterobacter aerogenes* which agree with the previous results observed as increasing in the *B. cereus* count to 8.0×10^2 cfu/g which is marginal satisfactory. Additionally, the improper holding/preservation of cooked rice at low temperature (30°C) showed that there was growth for many species of spoilage

cereus 3.0×10^3 cfu/g which is unsatisfactory. The improper cooking for chicken sausage as allow the surviving of food pathogens in addition to the growth of different species of spoilage bacteria (Coliforms) as *Citrobacter freundii, Enterobacter aerogenes, Enterobacter agglomerans, Enterobacter cloacae, Klebsiella pneumoniae* and *Proteus vulgaris.*

Table 9. Incidence of spoilage bacteria isolated from salads and fresh vegetables after and during sanitation

Isolated Bacteria	Green salad	Previous prepared salad	Improper chilled salad	Vegetable during sanitation	Vegetable after sanitation	Fruit salad
Citrobacter diversus	-	+	+	-	-	-
Citrobacter freundii	-	-	+	-	-	+
Enterobacter aerogenes	+	+	+	-	-	-
Enterobacter agglomerans	-	+	+	+	-	+
Enterobacter cloacae	+	+	+	+	+	+
Enterobacter hafniae	-	-	+	-	-	-
Klebsiella ozaenae	-	+	+	+	+	+
Klebsiella pneumoniae	+	+	+	-	-	+
Proteus mirabilis	+	+	+	+-	-	+
Proteus morganii	-	-	+	-	-	-
Proteus rettgeri	+	+	+	-	-	+
Proteus vulgaris	+	+	+	+	-	-
Serratia liquefaciens	+	+	+	-	-	+
Serratia marcescens	-	-	+	-	-	+
Staphylococcus epidermidis	-	+	+	+	-	-
Micrococci	-	+	+	+	+	-

Table 10. Incidence of spoilage bacteria isolated from cooked food samples

Isolated Bacteria	Rice	Rice at 45°-50°C	Improper hold rice 35°C	Minced meat with additives	Cooked sausage
Citrobacter diversus	-	-	+	-	-
Citrobacter freundii	-	+	+	-	+
Enterobacter aerogenes	-	+	+	+	+
Enterobacter agglomerans	-	-	-	-	+
Enterobacter cloacae	+	+	+	+	+
Enterobacter hafniae	-	-	+	-	-
Klebsiella ozaenae	+	+	+	+	-
Klebsiella pneumoniae	-	-	+	+	+
Proteus mirabilis	-	-	+	-	+
Proteus morganii	-	-	-	-	-
Proteus rettgeri	-	-	+	+	-
Proteus vulgaris	+	+	+	+	+
Serratia liquefaciens	-	-	+	-	+
Serratia marcescens	-	-	-	+	+
Staphylococcus epidermidis	+	+	+	+	+
Micrococci	+	+	+	+	+

Table 11. Incidence of spoilage bacteria isolated from the examined samples of different swabs

Isolated Bacteria	Slicer swabs	Surface swabs1	Surface swabs2	F. H. swabs	Knife swabs
Citrobacter diversus	-	-	+	-	-
Citrobacter freundii	-	+	-	+	-
Enterobacter aerogenes	+	+	-	+	-
Enterobacter agglomerans	-	-	+	-	+
Enterobacter cloacae	-	-	+	+	+
Enterobacter hafniae	-	+	-	+	-
Klebsiella ozaenae	-	-	+	+	-
Klebsiella pneumoniae	+	+	-	+	-
Proteus mirabilis	-	+	-	+	+
Proteus morganii	-	+	-	-	-
Proteus rettgeri	+	-	-	+	+
Proteus vulgaris	+	+	+	+	
Serratia liquefaciens	+	-	+	+	+
Serratia marcescens	-	+	-	-	+
Staphylococcus epidermidis	-	+	+	+	-
Micrococci	+	-	+	+	+

Rapid detection of *Salmonella typhimurium* using PCR

The suspected sample of the minced meat was examined with bacteriological examination that appears the contamination with *S. typhimurium*, and then the same sample was subjected to the rapid detection with PCR technique.

The whole genomic DNA, extracted from the bacterial isolate *S. typhimurium* was used as templates and then amplified by using the specific primers.

The results of PCR amplification which was performed on the DNA extracted were confirmed by electrophoresis analysis. The electrophoresis was also used to estimate DNA weight depending on DNA marker (50 bp DNA ladder) and the PCR amplification of the fim A gene produced different amplicon sizes of approximately 350, 140 and 90 bp respectively (Figure 1).

The partial nucleotide sequence of the obtained products (558 nucleotides) was aligned and compared with similar sequences retrieved from DNA databases by using the NCBI n-BLAST search program in the National Center for Biotechnology Information (NCBI). The nearest match for the gene in our study obtained from (NCBI) was recorded for *Salmonella enterica* subsp. enterica serovar typhi strain RKS2866 FimA (fimA) gene (gene bank accession number KC405528).

Similar results were obtained by Geiger, 2005 who reported that; considerable attention has been directed to the antimicrobial properties of those plants used as herbs and spices to flavor food. Analysis of their volatile flavor and odor fractions, known as essential oils, has frequently identified compounds such as Allicin in garlic.

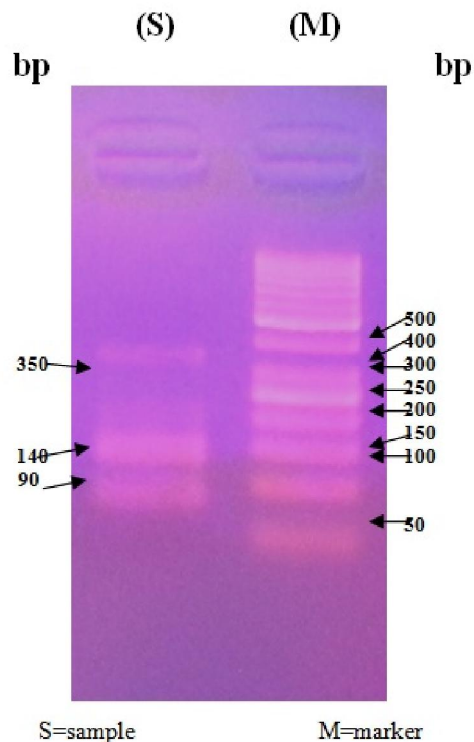

Figure 1. PCR amplification of genomic DNA of the selected *Salmonella* spp., showing three fragments at 90, 140, 350 bp approximately

Wanyenya *et al.* (2004) mentioned that cross-contamination during food preparation has been identified as an important factor associated with the food-borne illness. The food handlers play a major role in ensuring the food safety throughout the chain of the producing, processing, storage and preparation.

Mishandling and disregard for the hygiene measures on their part may result in food contamination and its attendant consequences.

Potter (1985) listed the elements that required for bacteria to survive and grow, the main ways of controlling bacterial growth is the temperature control which must be beyond TDZ (5^oC to 65^oC). The analysis of different swabs revealed the presence of *Enterobacter* sp., at surface, knives, slicers and food handler swabs after sanitation using chlorine tablets, which completely agree with Bergey's (2004) as *Enterobacter* species are found in the natural environment including water, sewage, vegetables, and soil. The increased prevalence of *Enterobacter* spp. as nosocomial pathogens may be due to a greater resistance to disinfectants and antimicrobial agents than that of other members of the Enterobacteriaceae.

Improper chilled salad samples which preserved at cooling temperature within TDZ (12^oC) contained different strains of food spoilage bacteria as *Citrobacter* sp., *Enterobacter* sp., *Serratia* sp., and *Proteus* sp., Potter and Morris (1996) reperted that the main ways of controlling bacterial growth is the temperature control which must be beyond TDZ (5^oC to 65^oC).

Oliveira *et al.* (2003) and Moussa *et al.* (2010) concluded that the PCR test combined with RV selective enrichment is more sensitive in detecting *Salmonella* serovars than traditional bacteriological methods.

Cohen *et al.* (1993) reported that all *Salmonella* strains amplified with the 85-bp fragment with the sets of primers under the PCR conditions. In this study, two primers which specifically amplify an 85-bp fragment in strains of *Salmonella* were selected and synthesized. The primers were selected completely internal to the fimA gene. By selecting primers completely internal to the fimA gene, all non-*Salmonella* strains responded negatively to the amplicon of the fimA gene. The size of the amplicon, 85 bp, made it a promising diagnostic tool for the sensitivity and the specificity. This agreed with Borowsky *et al*. (2009) who mentioned that the fimA gene was detected in 27 *salmonella* serovars with the same primer used resulting in 84 bp. Also, the same results were previously observed by Jawad *et al*. (2010).

Conclusion

- This paper studied the impact of food preparation practices on food borne diseases.
- This work recorded that food preparation practices were found to be an important aspect that affect the food safety.
- Molecular techniques as Polymerase Chain Reaction (PCR) were used for the rapid detection of food pathogens as *Salmonella Typhimurium* which will facilitate precautionary measures to maintain healthy food.

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