Original Research Article

Bacteriology Screening of roasted and raw Chicken sold in Tripoli

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ABSTRACT

Aims: This work was carried out to screen for the presence of bacteria in roasted chicken sold in market, poultries shop and restaurants in Tripoli.
Study design:. A total of 20 roasted chicken and 20 raw chicken parts randomly collected from different selling points in Tripoli
Place and Duration of Study: microbiology laboratory in microbiology and immunology department in faculty of pharmacy in university of Tripoli, January 2013 to September 2013.
Methodology: bacteriologically examined using standard microbiological method according to Based on the colonial morphological and biochemical test, the following bacteria species were isolated.
Results: Prevalence of *Salmonella* was higher in raw chicken samples (100%) compared to the roasted one (28%), *E. coli* was detected in both raw and roasted chicken (32%), whereas *Shigella* and *E. coli O157:H7* were detected only in roasted chicken [(8%) and (24%)] respectively.
Conclusion: the study found that the raw chicken samples were more susceptible to bacterial contamination than the roasted chicken samples, therefore a special strategies are needed to decrease the prevalence of bacterial pathogens in chicken samples present in Tripoli area. Therefore good handling/hygiene in processing and preheating of roasted chicken before consumption is recommended.

Keywords*: Raw Chicken, Roasted Chicken, Shigella, E. coli O157:H7, Bacteria, Screening.*

1. INTRODUCTION

Food-borne illnesses in human beings due to bacterial pathogens and their toxins are well documented worldwide[1], Food-borne illness imposes a substantial economic and quality of life burden on society by way of acute morbidity[2]. Foods are one the main sources of food borne pathogens due to high contents of proteins and carbohydrate which represent an enriched media for growth and multiplication of pathogens. Several pathogenic bacteria such as *Staphylococcus aureus*, *E. coli*, *Salmonella spp*. have been isolated from different foods. The most important are those transmitted by the faecal-oral route, which includes bacteria, viruses, and parasites[3].

The common ways in which bacteria and other microorganisms spread are by the air, contact, insect and other creatures, cross contamination is a cause of food poisoning that is often overlooked. This occurs when harmful bacteria are spread between food surfaces and equipment[4].

Meat contamination could constitute human health hazard due to production of toxin by some bacteria[5]. Data on food borne diseases are well documented worldwide. In United States, it has been estimated that seven pathogens found in animal products such as *Eschericiah coli* 057:7, *Listeria monocytogenes*, *Campylobacter jejuni*, *Clostridium perfringe*s, *Salmonella spp*., *Toxoplasma gondi*i and *Staphylococcus aureus* account for

approximately 303.12.3 million cases of food borne illness and a record of 39,000 each year[6]. Chicken is often contaminated with *Camylobacter* bacteria and sometimes with *Salmonella* and *Clostridium* bacteria[7]**.**

The presence of bacteria in roasted and raw meat at times may be as a result of slaughtering of animals that are previously infected with a particular disease without proper treatment or as a result of surface contamination by the meat vendors, wind or by ingredient used in meat treatment such a barbecue, knife, sharp pointed sticks, charcoal, roasted trays, spoon, water[8]. Roasted meat being displayed uncovered by the meat vendors exposed the meat to bacteria contamination[9].

This study aimed to bacteriological contamination in raw and roasted chicken samples collected from different areas in Tripoli, to Collect of raw and roasted chicken sample from markets, poultries shop and different restaurants in Tripoli and Isolation and identification of collected samples using routine microbiological technique, and then propose possible protection measures for problems developed from bacterial contamination in Tripoli markets and restaurants**.**

2. MATERIAL AND METHODS

The identification and control of food contaminations relies on careful investigation using biochemical and microbiological techniques and the implementation of appropriate legal and management strategies. Bacteriological method for detecting pathogens typically involved in culturing the organism in selective media and identifying isolates according to their morphological, biochemical and immunological characteristics. This method is sensitive and permits the specific detection of microorganism of interest[10].

To perform this step, culture media of broth and agar media were prepared as indicated by the manufacturer. Prepared plates were left to dry before performing work. All preparation and drying process were performed using strict aseptic technique.

2.1 Sample collection:

Samples of raw chicken meat were collected from chicken slaughtered at poultries shop and markets, whereas each samples of roasted chicken meat were collected from different restaurant in Tripoli. A total of 50 samples were examined. The samples were immediately transported to the laboratories in a cool thermos and were processed for culture.

2.2 Cultivation and isolation of *Salmonella* and *Shigella* from collected samples:

Salmonella and Shigella was isolated according to standard methods. 25g sample of chicken was added to 225ml of buffered peptone water, and incubated for 24hr at 37°C. one ml pre-enriched carcass culture was then transferred to selenite F broth and incubated for 24hr at 37°C. after 24hr of incubation, one loopful from each of enriched broths was streaked into plates of *Salmonella Shigella* (S.S) ager and xylose lysine deoxycholate (XLD) ager and incubated at 37°C for 24hr.

The plates were examined for the presence of typical colonies of *Salmonella*, i.e transparent colonies with black center on S.S ager and pink colonies and black center one XLD ager. Suspected colonies were confirmed by conventional biochemical methods TSI, API 20E, *Salmonella* latex kit [11].

2.3 Identification of *Salmonella* and *Shigella*:

After cultivation and isolation of *Salmonella* and *Shigella* from collected samples, identification was confirmed by the following biochemical tests:

Triple Sugar Iron agar (TSI) test for H2S production:

This medium was originally designed as a multi-test medium. It is often required when differentiating members of the *enterobacteriaceae*. The medium is used principally as a standard test for H2S.

Medium is prepared by dissolving a measured amount of dry powder in dissolving water as indicated by the manufacturer, solution was heated in water bath, 10ml of dissolved medium was transferred to tubes before sterilization, placed into autoclave for an 1hr, tubes were left to solidify after sterilization to create the slant at 45 angle.

Slant tubes were inoculated with pure culture by streaking over the entire surface of the slant (zig-zag to cover surface) and the stabbing deep into the butt, and then incubated at 37°C for 24hr to allow H2S production.

ii) API 20E (Analytical Profile Index):

These are now widely used by laboratories across the world for the definitive identification of many groups of organisms. The rapid 20E system allows the prompt identification of *Enterobacteria* by detection of preformed enzymes in suspension of the test organism and gives a result in 4hr. they may be used manually, but automated technology allows standardization of inoculum, reads the results, analyses the date and provides a print –out.

A plastic strip holding twenty mini-test tubes is inoculated with a saline suspension of a pure culture (as per manufacturer's directions). This process also rehydrates the desiccated medium in each tube. A few tubes are completely filled (CIT, VP and GEL) and some tubes are overlaid with mineral oil such that anaerobic reactions can be carried out (ADH, LDC, ODC, H_2S, URE).

After incubation in a humidity chamber for 4 hours at 37°C, the color reactions are read (some with the aid of added reagents), and the reactions (plus the oxidase reaction done separately) are converted to a seven-digit code which is called the Analytical Profile Index, from which name the initials "API" are derived. The code can be fed into the manufacturer's database via touch-tone telephone, and the computerized voice gives back the identification, usually as genus and species. An on-line database can also be accessed for the identification.

***Salmonella* latex kit:**

Is an agglutination test for the presumptive identification of *Salmonella spp*. additional investigation have shown it can be used to screen presumptive *Salmonella* colonies isolated on selective ager plates, from both food and clinical samples. The test allows the user to presumptively identify and confirm the presence of *Salmonella spp.*

Place 1 drop of saline on the surface of the reaction card, remove a typical looking colony from the plate using a loop and emulsify in the drop of saline, rock the card gently for 2 mins to check for agglutination, add 1 drop of test latex to the suspension, mix using a mixing stick, rock the card for up to 2min and examine for agglutination.

Agglutination within 2min is indicative for the presence of *Salmonella spp.* in the sample, whereas absence of agglutination is an indicative for the absence of *Salmonella spp*.

2.4 Cultivation and isolation of *E. coli and E. coli O157:H7* from collected samples:

To perform this step, culture media of broth and agar media was prepared as indicated by the manufacturer. Prepared plated was left to dry before performing work. All preparation and drying process were performed using strict aseptic technique. 25g sample of chicken was added to 225ml of buffered peptone water, and incubated for 24hr at 37°C. after 24hr of incubation of streaked onto plates of *MacConkey* ager (Mc) and sorbitol MacConker ager (S.Mc) and incubated at 37°C for 24hr. The plates were examined for the presence of typical colonies of *E. coli* and *E. coli O157:H7* respectively.

3.2.5 Identification of *E. coli* and *E. coli O157:H7:*

After cultivation and isolation of E*. coli* from collected samples, identification was confirmed by TSI as previously mentioned.

Indole test:

This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole, which accumulates in the medium. Indole is then tested by colorimetric reaction with p-dimethyl aminobenzaldehyde giving red ring that indicates the presence of *E. coli* and giving yellow ring that indicates the presence of *klebsiell*a. The test is positive for *E. coli* and negative for *Klebsiella.*

Pure bacterial culture must be grown in sterile tryptophan or peptone broth for 24-48hr before performing the test. Following incubation, add 5 drops kovac's reagent (isoamyl alcohol, para-dimethylaminobenzaldhyde, concentrated HCL) to the culture and observed for the ring produced

***E. coli O157:H7* latex kit:**

The value of a latex agglutination test (*E. coli O157:H7* latex kit) for rapid presumptive detection of *E. coli* serotype *0157:H7* was determined by laboratory trials and during an outbreak of hemorrhagic colitis. The latex kit was found to be a simple, highly efficlent and reliable test in detecting *E. coli O157:H7* with 100% sensitivity and specificity.

Place 1 drop of saline on the surface of the reaction card, remove a typical looking colony from the plate using a loop and emulsify in the drop of saline, rock the card gently for 2mins to check for agglutination, add 1 drop of test latex to the suspension, mix using a mixing stick, rock the card for up to 2min and examine for agglutination.

Agglutination within 2min is indicative for the presence of *E. coli O157:H7* in the sample, absence of agglutination is an indicative for the absence of *E. coli O157:H7*

3. RESULTS AND DISCUSSION

Roasted chicken is a popular meat product, which is prepared with fresh chicken that is garnished with hot spices and then roasted over fire. Roasted chickens as sources of food are frequently involved in food illnesses because they provide an ideal medium for the growth of disease causing microorganisms[12].

In this study we collected 50 chicken samples (25 Raw and 25 Roasted) from markets, poultries shop and restaurants from different areas in Tripoli. Samples were investigated for the bacteriological contamination using routine microbiological technique.

From 25 samples collected from the different areas in Tripoli restaurants the results shows that the presence of *Salmonella* and *Shigella spp.* in roasted chicken collected from

From 7 areas showed positive results for *Salmonella*, where they showed positive results for *Shigella* only in 2 other different areas. The presence and absence of *E. coli* and *E. coli O157:H7* in roasted chicken collected from restaurants in different areas in Tripoli, samples collected from 5 areas showed positive results of both *E . coli* and *E. coli O157:H7,* whereas other samples collected from another 2 area showed no growth of *E. coli O157:H7,* **Table 1**.

The presence and absence of *Salmonella* and *Shigella spp* in raw chicken samples collected from different poultries shops and markets in Tripoli. All samples collected showed positive results for *Salmonella spp.* and *Shigella* isolated from raw chicken **Table 2**.

There was high prevalence of these bacteria in roasted chicken sold in Tripoli as show in this study. The highest percentage was *E. coli* 32%, then *Salmonella* percentage 28% and *E. coli O157:H7* (24%) where the *Shigella* was 8%. **Table 3** This finding agrees with the earlier publications of FAO/WHO, (2003) which stated that salmonellosis, shigellosis is prevalent due to people's feeding habit as well as unhygienic way of preparing and roasting of the meat. The presence of contamination in our study may be due to unhygienic and improper handling of the chicken during processing or selling. [13].

In this study *E. coli* 32% was the highest percentage, *E coli* may also come from the water used in washing hands by the chicken sellers during processing and after roasting and these may include spoilage, Coliforms and pathogenic species[14]. *E. coli* O157: H7 can survive and even multiply in meat, poultry and vegetables[15]. *E. coli* 0157:H7 was isolated from a frozen raw beef patty of the kind implicated in outbreaks in 1982 the United State[16].

The illness caused by *Salmonella* is called salmonellosis, which is one of the most frequently reported foodborne pathologies worldwide[17]. In this study the *Salmonella* percentage 28%, *Salmonella* is the most significant pathogen transmitted by raw poultry to the kitchen[18]. In this study the percentage of *salmonella 100%,* and *E. coli* were 32% and no any *E. coli* 0157:H7 and *Shigella* in raw chicken **table 3.**

E. coli was detected in both raw and roasted chicken samples (32% for each) indicating that this bacterial can resist both freezing and heating. Roasted chicken samples showed the presence of both *E. coli O157:H* (24%) and *Shigella* (8%) that could be attributed to the poor personal and restaurant hygiene.

Table 1 biochemical and microbiological tests used to identify *E. coli* and *E. coli O157:H7* and *Salmonella* and *Shigella* isolated from roasted chicken, isolated from roasted chicken.

	Detection of *Salmonella* and *Shigella* in roasted chicken:					*E. coli* and *E. coli O157:H7* isolated from roasted chicken.			
N.O	Isolation media		Identification test			Isolation media	Identification test		
	S.S	XLD	TSI	API 20E	*Salmonella* latex kit	Mc	TSI	Indole test	*E. coli O157:H7* latex kit
1	-ve	-ve	/		/	-ve	-ve	/	/
2	-ve	-ve	/		/	-ve	-ve	/	/
3	-ve	-ve	/		/	-ve	-ve	/	/
4	-ve	-ve	/		/	+ve	+ve	+ve	-ve
5	-ve	-ve	/		/	+ve	+ve	+ve	+ve
6	+ve	+ve	+ve		+ve	+ve	+ve	+ve	-ve
7	+ve	+ve	+ve	*s. arizona*	+ve	+ve	+ve	+ve	+ve
8	-ve	-ve	/		/	-ve	-ve	/	/
9	+ve	+ve	+ve		+ve	+ve	+ve	+ve	+ve
10	-ve	-ve	/		/	-ve	-ve	/	/
11	+ve	+ve	+ve	*s. arizona*	+ve	-ve	-ve	/	/
12	-ve	-ve	/		/	+ve	+ve	-ve	-ve
13	-ve	-ve	/		/	-ve	-ve	/	/
14	+ve	+ve	+ve		+ve	+ve	+ve	+ve	+ve
15	+ve	+ve	+ve		-ve	-ve	-ve	/	/
16	-ve	-ve	/		/	-ve	-ve	/	/
17	-ve	-ve	/		/	-ve	-ve	/	/
18	-ve	-ve	/		/	-ve	-ve	/	/
19	-ve	-ve	/		/	+ve	+ve	+ve	+ve
20	-ve	-ve	/		/	+ve	+ve	+ve	+ve
21	-ve	-ve	/		/	-ve	-ve	/	/
22	+ve	+ve	+ve	*S. arizona*	+ve	+ve	+ve	-ve	-ve
23	-ve	-ve	/		/	-ve	-ve	/	/
24	+ve	+ve	+ve		-ve	-ve	-ve	/	/
25	+ve	+ve	+ve		+ve	+ve	+ve	-ve	-ve

Table 2 biochemical and microbiological tests used to identify *E. coli* and *E. coli O157:H7* and *Salmonella* and *Shigella* isolated from raw chicken.

	Detection of *Salmonella and Shigella*				E. coli and E. coli O157:H7 isolated from				
No of sample	Isolation media		Identification		Isolation media		Identification Test		
	S.S	XLD	TSI	*Salmonell* a latex kit	Mc	S. Mc	TSI	Indole test	*E. coli O157:H7* latex kit
1	+ve	+ve	+ve	+ve	+ve	/	+ve	+ve	-ve
2	+ve	+ve	+ve	+ve	+ve	/	+ve	+ve	-ve
3	+ve	+ve	+ve	+ve	+ve	/	+ve	+ve	-ve
4	+ve	+ve	+ve	+ve	-ve	/	/	/	/
5	+ve	+ve	+ve	+ve	+ve	/	+ve	-ve	-ve
6	+ve	+ve	+ve	+ve	-ve	/	/	/	/
7	+ve	+ve	+ve	+ve	-ve	/	/	/	/
8	+ve	+ve	+ve	+ve	+ve	/	+ve	-ve	-ve
9	+ve	+ve	+ve	-ve	-ve	/	/	/	/
10	+ve	+ve	+ve	+ve	-ve	/	/	/	/
11	+ve	+ve	+ve	+ve	+ve	/	+ve	-ve	-ve
12	+ve	+ve	+ve	+ve	-ve	/	/	/	/
13	+ve	+ve	+ve	+ve	-ve	/	/	/	/
14	+ve	+ve	+ve	+ve	-ve	/	/	/	/
15	+ve	+ve	+ve	+ve	-ve		/	/	/
16	+ve	+ve	+ve	+ve	-ve	-ve	/	/	/
17	+ve	+ve	+ve	+ve	-ve	-ve	/	/	/
18	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
19	+ve	+ve	+ve	+ve	-ve	-ve	/	/	/
20	+ve	+ve	+ve	-ve	+ve	-ve	+ve	+ve	-ve
21	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
22	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
23	+ve	+ve	+ve	+ve	-ve	-ve	/	/	/
24	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve
25	+ve	+ve	+ve	+ve	-ve	-ve	/	/	/

Table 3: Microbial contamination in roasted and Raw chicken samples in percentages.

Bacteria	The percentage %	
	Roasted chicken	Raw chicken
Salmonella	**28**	**100**
Shigella	**8**	**0**
E. coli	**32**	**32**
E. coli O157:H7	**24**	**0**

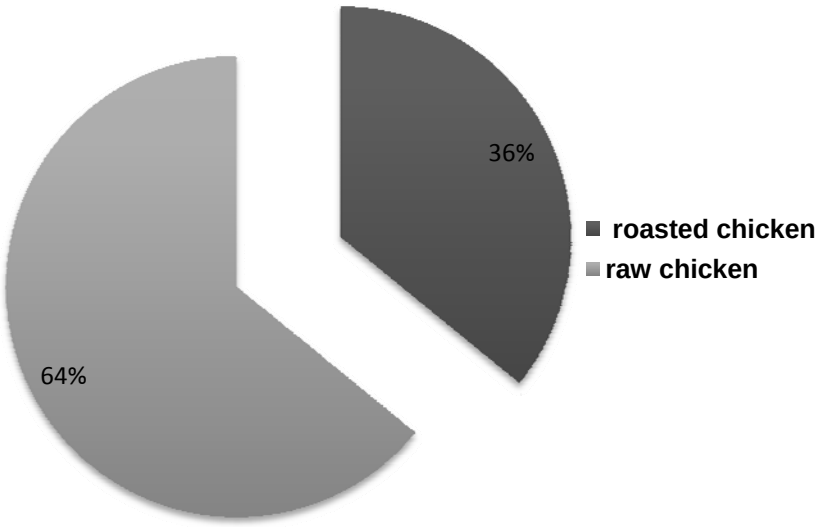

Figure 1 : Microbial contamination in roasted chicken and raw chicken samples in percentage

In this study the bacterial contamination in roasted chicken samples was detected in percentage of (36%%), whereas raw chicken samples showed a bacterial contamination of 64% **Figure 1** indicating that heating may be sufficient to kill any possible organism that could contaminated the chicken samples.

4. CONCLUSION

In conclusion, the presence of bacteria shown in the result may be that the organism were present in the raw chicken that were roasted or due to cross-infection during preparation, insufficient application of heat to the deep tissues and perhaps because of contamination from potential buyers, meat handlers, hands, trays and the open air environment.

The above bacteria organisms isolated in this study could be pathogenic or opportunistic pathogens and pose a health risk especially in infants or immune-compromised individual. Special strategies should be considered in order to avoid spread of bacterial contamination such as hand washing, proper heating of food, holding food under appropriate condition disinfecting of equipment and food contacting surfaces. This may indicate poor hygienic practice and suggest the risk of infection and health hazard to consumers. We therefore

recommend good handling/hygiene in processing. More so, preheating of roasted chicken before consumption is recommended.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

CONSENT

All authors declare that verbal informed consent was obtained from the paraticbate for publication of this case report.

ETHICAL APPROVAL

The study protocol was reviewed and approved by the Ethical Committees of University of Tripoli of Libya.

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