PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

ANSInet
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
Mob: +92 300 3008585, Fax: +92 41 8815544
E-mail: editorpjn@gmail.com

Pakistan Journal of Nutrition 12 (2): 130-134, 2013
ISSN 1680-5194

Bacterial Contamination in Processed Chicken Shawarma (Meat) Sold in Various Parts of Lahore, Pakistan

Farooq Ahmad[1], Saqlain Abbas[2], Zahid Ali Butt[3], Adnan Skhawat Ali[1], Rashid Mahmood[1], Maqsood Ahmad[1], Muhammad Farhan[1], Abdul Wahid[4], Muhammad Nawaz[4] and Aqsa Iftikhar[1]
[1]Sustainable Development Study Centre, GC University, Lahore, Pakistan
[2]Government Islamia College, Civil Lines, Lahore, Pakistan
[3]Department of Botany, Abdul Wali Khan University, Mardan, Pakistan
[4]Department of Environmental Science, B.Z. University, Multan, Pakistan

Abstract: In this study bacterial flora of meat in chicken Shawarma (meat) were investigated from five different regions of Lahore. Samples were taken from internal and external part of Shwarma. The contamination was present in both external and internal part of meat. But external part was found to be little more contaminated as compared to internal part. Analysis of microbes includes *E. coli, Salmonella, Aerobes* and *Coliforms*. Microbs were found in order of *Aerobes* > *E. coli* > *Salmonella* there is not too much variation of contamination in different regions but there is variation among the number of bacteria. In every part *Aerobes* were in greater number as compared to *E. coli* and *Salmonella*. Shawarma analyzed from the Site III was more contaminated as compared to other sites. The external part of the product showed more microbial load as compared to internal part.

Key words: Bacterial contamination, shwarma, meat, chicken, Lahore

INTRODUCTION

Processing of meat can be difficult due to its perishable nature. Meat poultry and their products are major dietary items in our food. The knowledge of microbiology of meat and its products is very important to control the growth of undesirable microorganisms and retarding the conditions favorable for their growth and activity. Meat is an excellent medium for bacteria. Factor affecting microbial growth in meat include both intrinsic and extrinsic factors. *Salmonella* when present in frozen meat products can cause serious infections if not cooked properly (Dominguez and Schaffner, 2009).

Intrinsic factors are predominantly chemicals including concentration and availability of nutrients. These extrinsic factors are concerned mainly with the storage and processing condition. It also includes storage temperature, composition and relative humidity of the gasses atmosphere surrounding the meat. The metabolism of large number of bacteria or a small number of Yeast and mold is needed to cause the significant changes in the characteristic of meat products. Processed and fresh meat often harbors the different groups of microorganisms. Gram-positive spore forming bacteria and *Micrococci* are not common in processed meat. Microbes respond to the environmental changes during processing and storage, sometimes the period between the slaughtering and chilling affects their growth. The caresses are unlikely to support the growth of these microorganisms at any subsequent stage of processing. The frequency and number of campylobacter species was assessed in freshly processed, contaminated broiler carcass (Stern *et al*., 1995; Stern *et al*., 2007).

Spoilage of meat under aerobic conditions, bacteria make the surface slime, changes in colour, odor and taste which mostly occur due to *Pseudmonas, Streptococcus, Lactobacillus* and *Micrococcus*. Under aerobic condition yeast may grow on the surface of meat, causing sliminess, lipolysis, change in odor, taste and discoloration. Facultative and anaerobic bacteria are able to grow under anaerobic conditions and cause spoilage. When meat is held near 0°C microbial growth becomes limited but mold, yeast and some bacteria able to grow. Aerobic microorganisms are *Flavobacterium, Lactobacillus, Streptococcus* and *Clostridium* which can produce sliminess, discoloration and spots. The normal temperature for Coliforms is 37°C. The contamination of fecal coliforms *and E. coli* can be either direct or indirect. Coliform bacteria are detectable by gas formation of characteristic colonies. Comparison of microbial count of different species of bacteria can be done in chicken carcasses with or without visible faecal contamination (Jimenez *et al*., 2003).

The normal temperature of *Staphylococcus aureus* growth is 37°C but it may grow at 6.5°C. However it can not grow easily under chilled storage conditions. *Salmonella* can grow on meat at temperature below 6°C in 10 hours. The minimum growth temperature for *Salmonella* is 6.7 but it can grow upto 5.3°C. Studies

Corresponding Author: Farooq Ahmad, GC University, Sustainable Development Study Centre, Lahore, Pakistan

showed that it was isolated from farmhouse, slaughterhouse and market meat to be 7, 50 and 20%, respectively (Padungtod and Kaneene, 2006).
In the current study research work was carried out on a very famous dish of meat chicken shwarma which is popular in all classes of society and is consumed on large scale. Processed chicken meat (shawarma) was collected from various sites of Lahore and microbial contamination in internal and external parts of the samples was studied.

MATERIALS AND METHODS

Sampling: Samples were collected from five different regions Site I (Model Town), Site II (Shadbagh), Site III (Gullburg), Site IV (Defence) and Site V (Ghari Shohu) of Lahore. From each site samples were collected in duplicate from internal and external parts using sterile scalpels so the 60 samples of Shawarma were examined in duplicate for the presence of bacteria (Eglezos *et al*., 2010).

Aerobic plate count (enumeration of mesophilic aerobic bacteria)

(i) Preparation of food sample homogenate: Twenty five (25) grams of sample homogenate was weighed aseptically in to sterile blender jar and 225 ml BPW was added which gave 1:10 dilution of each sample. The food was blended at a speed of 15000-20000 rpm for 20 seconds.

(ii) Dilution: Form food homogenate 1.0ml was added by pipette in to a tube containing 9ml of the BPW using technique given in APHA (1976). From the first dilution, 1ml was transferred to the second dilution, using the different pipette. The second dilution tube contained 9ml of the BPW. The procedure was repeated with a third, forth pipette or until the desired number of dilution was prepared. The sample was agitated in the diluent mixture by shaking vertically 25 times through 1 foot arch per minute.

(iii) Pour plating: 1ml of food homogenate was poured into duplicate plates from different dilutions and 15ml of PCA was poured in each petri dish. The sample dilution in agar was mixed carefully and uniformly. Finally the medium was allowed to solidify.

(iv) Incubation: The prepared dishes were inverted and incubated at 32±1°C for 48±3 hours.

(v) Counting the colonies: Following incubation, all the colonies were counted on dishes containing 30-300 colonies and the results per dilution were recorded.

Detection of *salmonella*

(I) Preparation of food homogenate: 25 gram of the sample homogenate was aseptically in to a sterile jar and 225 ml of BPW was added. The food was blended at speed of 15000-2000rpm for 20 seconds.

(ii) Pre-enrichment: The food homogenate already blended with BPW, was aseptically transferred to a sterilized 500 ml bottle. This was incubated at 37±1°C for 16 to 20 hours.

(iii) Enrichment: 10 ml of each pre-enrichment bottle was transferred to 100 ml tetrathionate broth and another 10 ml were transferred to 100 ml of selective medium that were previously warmed to 42-43°C. The media was incubated at 42-43°C for 48 hours.

(iv) Plating out: After 18 to 43 hours, from each enrichment flask a petri dish was streaked on *Salmonella/Shegilla agar*. The plates were incubated at 37±1°C for 20 to 24 hours. The plates were examined after 24 to 48 hours for typical colonies of *Salmonella*.

(v) Confirmation: In *salmonella /shegilla agar* the colony were pink, translucent to opaque with surrounding medium pink to red.

Enumeration of *coliform bacteria*

(I) Preparation of food sample homogenate: 25g of the sample homogenate was weighed aseptically into a sterile blender jar and 225 ml of BPW was added. The food was blended at a speed of 15000-20000 rpm for 20 seconds.

(ii) Dilution: By shaking the food homogenate was mixed and 1.0ml was added by pipette into a tube containing 9 ml of the BPW. Further dilutions were made by using the started technique. From the first dilution, 1ml was transferred to the second dilution by using technique given in APHA (1976).

(iii) Inoculation: Each of the three tubes of LST broth containing inverted Durham tubes with 1 ml of the food homogenate (1: 10) were inoculated. The same operation was carried out from the first (1 in 1000) and the second (1 in 1000) dilution tube, for each dilution a new sterile pipette was used.

(iv) Incubation: The LST tubes were incubated at 37±1°C for 24 hours and 48 hours.

(v) Reading of Enrichment tubes (Presumptive test): The tubes showing gas production after 24 hours, were recorded and negative tubes were re-incubated for further 24 hours. Then the tubes that showed gas production were recorded.

(vi) Confirmatory test for coliforms: A loopful from each gas positive tube of LST was transferred to a separate tube of BGLB broth. The BGLB tubes were incubated at

37±1°C for 48 hours. The formation of gas confirmed the presence of coliform bacteria. The number of the tubes that were confirmed as positive for coliform were recorded.

Test for fecal coliforms: With the confirmatory procedure using brilliant green lactose broth, transfer was made simultaneously to all the presumptive tubes to EC medium. The inoculated EC tubes were incubated at 45.5°C for 24 hours and gas formation was recorded. The bacterial density was estimated from the tubes by MPN method.

Test for *E. coli*: A loopful was transferred from each gas positive tube of LST to a separate tube of EC broth. The EC broth tubes were incubated for 48 hours at 44.5°C the tubes with the production of gas were positive. One plate of EMB agar was streaked from each positive tube in a way to obtain discrete colonies and were incubated a 35°C for 18-24 hours. At the same time gram stains for each culture was prepared.

RESULTS AND DISCUSSION

Microorganism entered in meat from different sources i.e., air, water, processing and handling etc. Growth of contaminants in large numbers can lead to the food poisoning through the spoilage bacteria. Mead (2010) stated that poultry products are an important part of the global food market and are consumed at a large scale. Analysis of external and internal parts of shawarma showed that higher number of *E. coli*, Salmonella and Aerobes were found in shawarma collected from site III (internal part), site III (external part), site I (external part) respectively while lower number of *E. coli,* Salmonella and Aerobes were found in shawarma collected from site I (internal part), site I (internal part), site II (internal part) respectively (Table 1).

Site I: In Site I sample Shawarma was taken from internal and external sides from various shops. Table 3 indicates that table value (2.015) for all sample is smaller than the calculated value for *E. coli* (7.88) and aerobes (7.33) while it was lower for external part (25.68) than internal part (30.21). Here the presence of *E. coli* indicated that the contamination was due to washing of meat due to fecal contaminated water. The interior of meat gets contaminated during mincing process however chilling room and storage also contribute in the contamination. *E. coli* was found to be present in high numbers in samples taken from external surfaces. So in this cause null hypothesis was rejected and alternate was accepted means there was significant difference among the number of bacteria taken from the external and internal part of the product. Number of *E. coli* in external sample was 8.1×10^2 cfu/ml higher than internal sample is 5.7×10^2 cfu/ml. Similarly *Salmonella* in external part was 6.3×10^2cfu/ml that were also higher than internal sample is 5.6×10^2cfu/ml however Aerobes value for external sample was 2.56×10^3cfu/ml and for internal sample it was 2.64×10^3cfu/ml (Olsen *et al*., 2003).

Site II: In Site II samples were collected from six different food shops. Table 3 indicates that table value (2.015) is greater than calculated value (1.703) for *E. coli* in external part so in this cause null hypothesis was accepted and alternate was rejected. Table 2 of ANOVA indicates that there was no variation between samples but variation was there between bacteria. In this site again contamination was more in external part as compared to internal part and Aerobes were found greater in number compared to other bacteria.

Site III: In Site III samples were collected from different shops. Table 3 indicates that calculated value for *E. coli, Salmonella* and aerobes for both external and internal sample was 8.361, 6.28, 4.49, 4.62, 36.91 and 29.28 respectively. This indicates that *aerobes* were greater in number than others. Table 3 shows difference in number of bacteria for external and internal samples. In Site III region number of *E.coli* for external sample were 9.5×10^2cfu/ml and for internal sample 1.0×10^3cfu/ml. For *Salmonella* number of bacteria for external sample were 9.9×10^2cfu/ml and for internal sample 9.0×10^2cfu/ml. For Aerobes number of bacteria for external sample was 2.61×10^3cfu/ml and for internal sample those were 2.53×10^3cfu/ml. Special thing about this region was that this region having more contamination of pathogenic bacteria like *E. coli* and *Salmonella* as compared to other regions.

Site IV: Samples of shawarma were also collected from six different shops of site IV. Table 3 indicates that *E. coli* external and internal having table value smaller than calculated value so null hypothesis was rejected and alternate was accepted. Similarly for Aerobes calculated value of external and internal (43.90) and (49.89) which was greater than table value so null hypothesis was rejected. Table 3 indicates that there was difference of number of bacteria between external and internal samples. Samples were positive for *Salmonella*. In this zone F-crit (6.388) of *E. coli* was higher than calculated value (6.259) so again there was not much significance difference for this zone. For *Aerobes* there was much variation for bacteria in external and internal sample and significant difference was there because F crit. was (7.908) less than F-calculated value (14.08) but for this zone F crit (6.388) was less than F-calculated value (6.577) so there was again not much significant difference within zone. A surrogate was considered a suitable replacement for ECO157:H7 if the intervention produced a reduction in surrogate levels that was not significantly greater (P = 0.05) than that observed for ECO157:H7 (Ingham *et al*., 2010).

Table 1: Number of bacteria in external and internal parts of chicken shawarma collected from selected sites

Bacteria	Parts	Site I	Site II	Site III	Site IV	Site V
E. coli (cfu/ml)	External	81.16±25.2	76.16 ± 15.94	95.66 ± 28.02	66.0 ± 20.0	74.16 ± 11.7
	Internal	57.0±19.02	62.16 ± 16.67	100.0 ± 39.0	62.83 ± 14.77	53.10 ± 6.08
Salmonella (cfu/ml)	External	63.0±7.01	75.0 ± 14.95	99.0 ± 53.96	61.33 ± 8.35	70.66 ± 16.4
	Internal	56.16±8.25	55.0 ± 13.78	90.83 ± 47.87	55.00 ± 4.64	53.66 ± 6.05
Aerobes (cfu/ml)	External	256.66±25.33	267.16 ± 14.9	261.3 ± 17.33	313.33 ± 37.98	2603.66±27.23
	Internal	264.88±21.47	246.66 ± 12.11	253.16 ± 21.17	270.33 ± 16.17	257.8 ± 14.35

Table 2: ANOVA: - Comparison of various zones (Site I, Site II, Site III, Site IV, Site V) and bacteria (E. *coli, Salmonella* and A*erobes* external and internal)

Source of variation		SS	df	MS	F	P-value	F crit
E. coli	Between External and Internal	329.94	1	329.94	6.138	0.0684	7.709*
	Between All five Zones	345.94	4	336.48	6.259	0.0517	6.388*
Salmonella	Between External and Internal	330.63	1	330.63	16.681	0.0151	7.709 **
	Between All five Zones	1873.0	4	468.25	23.625	0.0048	6.388***
Aerobes	Between External and Internal	535.82	1	535.82	14.084	0.0199	7.709**
	Between All five Zones	1000.94	4	250.23	6.577	0.0477	6.388**

*Least significant difference ** Moderately significant difference, *** Highly significant difference

Table 3: t-analysis for processed shwarma in all regions

		E. coli		*Salmonella*		*Aerobes*	
		External	Internal	External	Internal	External	Internal
Site I	I t I ≥	2.015	2.015	2.015	2.015	2.015	2.015
	S.d	25.20	19.026	7.01	8.25	25.33	21.47
	T	7.88	7.33	22.01	16.66	25.68	30.21
Site II	I t I ≥	2.015	2.015	2.015	2.015	2.015	2.015
	S.d	15.94	16.67	14.95	13.784	14.90	12.11
	t	1.703	9.130	12.28	9.77	43.905	14.89
Site III	I t I ≥	2.015	2.015	2.015	2.015	2.015	2.015
	S.d	28.02	39.007	53.96	47.87	17.339	21.17
	T	8.361	6.280	4.492	4.62	36.917	29.280
Site IV	I t I ≥	2.015	2.015	2.015	2.015	2.015	2.015
	S.d	15.94	16.678	14.953	13.78	14.90	12.110
	T	11.703	9.130	12.286	9.77	43.90	49.89
Site V	I t I ≥	2.015	2.015	2.015	2.015	2.015	2.015
	S.d	11.70	6.08	16.4277	6.05530	27.237	14.35
	T	15.523	21.420	10.537	21.709	23.71	43.985

Site V: In Site V different samples were taken from six different shops. In this area contamination of bacteria was once again more in external parts of sample. Table 3 indicates that table value for *E. coli* external (2.015) was less than calculated value (15.523) so null hypothesis was accepted. Similarly for *E. coli* internal table value (2.015) was less than calculated value (21.420) so null hypothesis was rejected and alternate was accepted. Similarly for Aerobes table value (2.015) was less than calculated value (23.71) and the calculated value for external (43.98) so null hypothesis was rejected and alternate was accepted. In site V region Aerobes in external sample were 2.63×10^3 cfu/ml and in internal samples were 2.57×10^3cfu/ml. Similarly *Salmonella* in external sample were 7.4×10^2 cfu/ml and in internal sample were 5.3×10^2cfu/ml. so in this region Aerobes in external and internal sample were greater as compared to other bacteria. Studies were conducted on 187 broilers after the completion of their slaughter processing and found *Compylobactor jejuni* strains originating from the intestines of the previously processed C. jejuni-positive flock (Miwa *et al*., 2003; Georgiev and Vushin, 1981). Mean *Salmonella sp*. counts from breast (3.62 log10 cfu/g) or neck (3.40 log10 cfu/g) skin samples were significantly higher ($p<0.05$) than counts from vent skin samples (2.84 log10 cfu/g) (Kotula and Davis, 1999).

Conclusions: The microbiological contamination of meat is one of the major problem of meat industry because of it perishable nature. In our country, refrigeration and freezing are the major procedures commonly employed for the short and long term storage of raw and processed meat, respectively. It was concluded from the study that there were different type of bacteria present in meat like *Salmonella*, *E. coli,* Aerobes and coliforms. It was also noted that overall Aerobes were greater in number in all areas as compared to other bacteria like *Salmonella, E. coli* and coliform. The present research work was undertaken to investigate the bacterial level of processed meat and meat product (Shawarma) which is a vended food. Samples in duplicate were collected from six different shops of five zones were analyzed. It was noted that contamination

was present in both external and internal parts but external part was found to be more contaminated as compared to internal part.

Recommendations: To ensure the consumer and product safety, proper monitoring of microbiological quality of Shawarma should be conducted regularly. Monitoring of microbiological quality of all the meat products should be important concern. Proper hygienic conditions should be maintained at slaughtering as well as on processing places. More work is needed to see the effect of temperature variation on external part of the product due to discontinuation of the process while waiting for the next customer.

ACKNOWLEDGEMENTS

The authors acknowledge Government College University Lahore for providing funding for the current study and Director General, Pakistan Council of Scientific and Industrial Research (PCSIR) Lahore for proving equipments and laboratories facilities to fulfill this task.

REFERENCES

APHA, 1976. Compendium of methods for the microbiological examination of foods. American Public Health Association. New York.

Dominguez, S.A. and D.W. Schaffner, 2009. Survival of salmonella in processed chicken products during frozen storage. J. Food Prot., 72: 2088-2092.

Eglezos, S., B. Huang, G.A. Dykes and N. Fegan, 2010. The prevalence and concentration of Bacillus cereus in retail food products in Brisbane, Australia. Foodborne Pathog. Dis., 7: 867-870.

Georgiev, L. and I. Vushin, 1981. Microbiological studies of slaughterhouse broilers. Vet. Med. Nauki., 18: 55-59.

Ingham, S.C., R.J. Algino, B.H. Ingham and R.F. Schell, 2010. Identification of *Escherichia coli* O157:H7 surrogate organisms to evaluate beef carcass intervention treatment efficacy. J Food Prot., 73: 1864-1874.

Jiménez, S.M., M.C. Tiburzi, M.S. Salsi, M.E. Pirovani and M.A. Moguilevsky, 2003. The role of visible faecal material as a vehicle for generic Escherichia coli, coliform and other enterobacteria contaminating poultry carcasses during slaughtering. J. Applied Microbiol., 95: 451-456.

Kotula, K. L and M.E. Davis, 1999. Broiler skin sampling for optimum recovery of *Salmonella* sp. J. Food Prot., 62: 284-286.

Mead, G., A.M. Lammerding, N. Cox, M.P. Doyle, F. Humbert, A. Kulikovskiy, A. Panin, V.P. do Nascimento and M. Wierup, 2010. Salmonella On Raw Poultry Writing Committee. Scientific and technical factors affecting the setting of Salmonella criteria for raw poultry: A global perspective. J. Food Prot., 73: 1566-1590.

Miwa, N., Y. Takegahara, K. Terai, H. Kato and T. Takeuchi, 2003. Campylobacter jejuni contamination on broiler carcasses of C. jejuni-negative flocks during processing in a Japanese slaughterhouse. Int. J. Food Microbiol., 84: 105-109.

Olsen, J.E., D.J. Brown, M. Madsen and M. Bisgaard, 2003. Cross-contamination with Salmonella on a broiler slaughterhouse line demonstrated by use of epidemiological markers. J. Appl. Microbiol., 94: 826-835.

Padungtod, P. and J.B. Kaneene, 2006. Salmonella in food animals and humans in northern Thailand. Int. J. Food Microbiol., 108: 346-354.

Stern, N.J., M.R. Clavero, J.S. Bailey, N.A. Cox and M.C. Robach, 1995. Campylobacter spp. in broilers on the farm and after transport. Poult. Sci., 74: 937-941.

Stern, N.J., F. Georgsson, R. Lowman, J.R. Bisaillon, J. Reiersen, K.A. Callicott, M. Geirsdóttir, R. Hrolfsdóttir and K.L. Hiett, 2007. Frequency and enumeration of Campylobacter species from processed broiler carcasses by weep and rinse samples. Poult. Sci., 86: 394-399.