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

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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 Shawarma. 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. Microbes 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, shawarma, 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 Micrococcii 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 Pseudomonas,
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

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showed that it was isolated from farmhouses, 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 shawarma 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 (Gulburg), 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 80 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 of 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 15000-20000 rpm 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 tetrahionate 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/Shigella 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 /shigella 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 II (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 6.3×10^2 cfu/ml that were also higher than internal sample is 5.6×10^2 cfu/ml however Aerobes value for external sample was 2.56×10^2 cfu/ml and for internal sample it was 2.64×10^2 cfu/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, 38.91 and 28.28 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 9.5×10^2 cfu/ml and for internal sample 1.0×10^2 cfu/ml. For *Salmonella* number of bacteria for external sample were 9.9×10^2 cfu/ml and for internal sample 9.0×10^2 cfu/ml. For Aerobes number of bacteria for external sample was 2.61×10^2 cfu/ml and for internal sample those were 2.53×10^2 cfu/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 (14.06) but for this zone F crit (8.388) 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

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Parts</th>
<th>Site I</th>
<th>Site II</th>
<th>Site III</th>
<th>Site IV</th>
<th>Site V</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (cfu/ml)</td>
<td>External</td>
<td>81.16±25.2</td>
<td>75.16±15.94</td>
<td>95.96±20.02</td>
<td>66.0±20.0</td>
<td>74.16±11.7</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>57.0±19.02</td>
<td>62.16±16.67</td>
<td>100.0±39.00</td>
<td>62.83±14.77</td>
<td>53.10±6.08</td>
</tr>
<tr>
<td>Salmonella (cfu/ml)</td>
<td>External</td>
<td>63.0±7.01</td>
<td>75.0±14.95</td>
<td>99.0±33.95</td>
<td>61.33±8.35</td>
<td>70.66±6.4</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>56.1±8.26</td>
<td>25.9±17.83</td>
<td>90.83±47.87</td>
<td>55.0±6.44</td>
<td>53.66±6.05</td>
</tr>
<tr>
<td>Aerobes (cfu/ml)</td>
<td>External</td>
<td>259.6±23.33</td>
<td>29.16±14.9</td>
<td>261.3±17.33</td>
<td>313.3±37.98</td>
<td>2603.6±272.23</td>
</tr>
<tr>
<td></td>
<td>Internal</td>
<td>294.8±21.47</td>
<td>248.9±12.11</td>
<td>253.16±21.17</td>
<td>270.33±16.17</td>
<td>257.8±14.36</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>SS</th>
<th>df</th>
<th>MS</th>
<th>F</th>
<th>P-value</th>
<th>F crit</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>Between External and Internal</td>
<td>329.94</td>
<td>1</td>
<td>329.94</td>
<td>6.138</td>
<td>0.0845</td>
</tr>
<tr>
<td></td>
<td>Between All five Zones</td>
<td>345.94</td>
<td>4</td>
<td>336.48</td>
<td>6.250</td>
<td>0.0517</td>
</tr>
<tr>
<td>Salmonella</td>
<td>Between External and Internal</td>
<td>330.03</td>
<td>1</td>
<td>330.03</td>
<td>16.861</td>
<td>0.0151</td>
</tr>
<tr>
<td></td>
<td>Between All five Zones</td>
<td>1873.0</td>
<td>4</td>
<td>468.25</td>
<td>23.625</td>
<td>0.0048</td>
</tr>
<tr>
<td>Aerobes</td>
<td>Between External and Internal</td>
<td>535.82</td>
<td>1</td>
<td>535.82</td>
<td>14.084</td>
<td>0.0199</td>
</tr>
<tr>
<td></td>
<td>Between All five Zones</td>
<td>1003.94</td>
<td>4</td>
<td>250.23</td>
<td>6.577</td>
<td>0.0477</td>
</tr>
</tbody>
</table>

*Least significant difference  ** Moderate significant difference  *** Highly significant difference

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

<table>
<thead>
<tr>
<th></th>
<th>E. coli</th>
<th>Salmonella</th>
<th>Aerobes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>External</td>
<td>Internal</td>
<td>External</td>
</tr>
<tr>
<td>Site I</td>
<td>2.015</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>S.d</td>
<td>25.20</td>
<td>19.026</td>
<td>7.01</td>
</tr>
<tr>
<td>T</td>
<td>7.88</td>
<td>7.33</td>
<td>22.01</td>
</tr>
<tr>
<td>Site II</td>
<td>2.015</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>S.d</td>
<td>15.94</td>
<td>16.67</td>
<td>14.05</td>
</tr>
<tr>
<td>T</td>
<td>1.703</td>
<td>9.330</td>
<td>12.28</td>
</tr>
<tr>
<td>Site III</td>
<td>2.015</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>S.d</td>
<td>28.02</td>
<td>39.007</td>
<td>53.96</td>
</tr>
<tr>
<td>Site IV</td>
<td>2.015</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>T</td>
<td>11.703</td>
<td>9.130</td>
<td>12.286</td>
</tr>
<tr>
<td>Site V</td>
<td>2.015</td>
<td>2.015</td>
<td>2.015</td>
</tr>
<tr>
<td>S.d</td>
<td>11.70</td>
<td>6.08</td>
<td>18.4277</td>
</tr>
<tr>
<td>T</td>
<td>15.523</td>
<td>21.420</td>
<td>10.537</td>
</tr>
</tbody>
</table>

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 2.63×10^2 cfu/ml and in internal samples were 2.57×10^2 cfu/ml. Similarly Salmonella in external sample were 7.4×10^2 cfu/ml and in internal sample were 5.3×10^2 cfu/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 Campylobacter 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.

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