INTRODUCTION

Broiler carcasses have relatively high frequency of contamination with pathogenic bacteria of public health significance including *E. coli*, *salmonella*, *campylobacters*, *Staphylococcus aureus* (Abu-Ruwaied *et al.*, 1994 and Althaus *et al.*, 2017). Special attention in poultry meat processing is paid to the fact that live birds enter the processing plant are heavy loaded with large number of different microorganisms residing externally on their skin, feathers or internally in the alimentary tract (Kotula and Pandya, 1995).

Poultry slaughter’s is a multi-stage operation; commercial broiler processing operations include scalding, defeathering, evisceration, washing, chilling and packaging. During these various processing operations; opportunities exist for the contamination of the carcass from different sources such as the environment, contamination via scalding water and tanks, chilling tanks, knives, processing equipment such as defeathering machines, the hands of workers and also by cross-contamination from carcass to another (Afshin *et al.*, 2013 and Nidaullah *et al.*, 2017).

Poultry meat remains an important and probably the major source of human infection with *campylobacters*, *salmonella*, *Escherichia coli* and *Staphylococcus aureus* which are currently recognized as the major bacterial pathogens associated with poultry and implicated in significant number of human foodborne diseases (Antunes *et al.*, 2016). Moreover, *Campylobacter jejuni* and salmonella are important human pathogens and recognized as the leader of bacterial caused gastroenteritis in humans and foodborne illness ranging from self-limiting gastroenteritis to a number of severe sequelae (Berrang *et al.*, 2000 and Foley and Lynne, 2008). Human infections with these pathogens can occur during the improper handling of raw chicken carcasses, by eating insufficiently cooked chicken and via cross-contamination of other foods by contact with knives.
or cutting boards used to prepare contaminated raw chickens (Keener *et al*., 2004).

Fecal indicator bacteria, especially fecal coliforms, are good microbial indicators of the potential presence of disease causing bacteria and also show the general sanitary quality of the food. Food contamination by *Escherichia coli* is closely associated with fecal contamination (Cason *et al*., 2000). Coliforms, *Escherichia coli* and *Staphylococcus aureus* have been used in poultry products to assess microbiological safety and sanitation conditions during processing (Bean and Griffin, 1990).

Although foodborne hazards may be of physical, chemical or microbiological origin, there is currently widespread recognition that microbial foodborne hazards represent the greatest risk to consumers (Schirone *et al*., 2017) therefore the aim of the current study is to investigate the potential microbial hazards contaminating broiler carcasses in semi-automated small scale poultry abattoir in Fayoum Governorate during processing through examination of broiler carcasses at different processing steps for coliforms (MPN), faecal coliforms (MPN), *E. coli* (MPN), *Staphylococcus aureus* count as well as isolation of *Salmonellae* and *Campylobacter jejuni*.

**MATERIALS AND METHODS**

**Sampling procedure:**

On each of 15 replicate survey days, 6 broiler carcasses (35-40 days of age) were collected after 6 processing steps, one carcass was removed from the shackle line after each step namely scalding, defeathering, evisceration, washing, chilling and packaging. A total of 90 carcasses were examined (six on each replicate). The abattoir use non-chlorinated tape water during different processing steps.

Carcasses were removed from the line at random using a clean pair of latex gloves for each carcass and individually placed into a separate sterile plastic bag. Individual carcasses were subjected to whole-carcass rinse technique with 400 ml of 0.1% sterile buffered peptone water which added to each bag and shaken vigorously by hand for 1 min. After shaking, carcasses was removed aseptically and the collected rinsate was poured out of the bags into sterile specimen cups and placed in an ice box and transported to the reference laboratory for veterinary quality control on poultry production- Fayoum branch within 1 h for examination.

**Microbiological analysis:**

For microbiological analyses, serial dilutions of the collected rinsate of each carcass were performed in 0.1% buffered peptone water up to $10^{-6}$. The collected rinsate was tested for:

1- Coliforms (MPN), faecal coliforms (MPN), *E. coli* (MPN) according to the three tube method technique recommended by FDA (2002).


3- *Salmonella* isolation was performed according to the technique recommended by ISO 6579 (ISO, 2002).

4- *Campylobacter* isolation was done according to the technique recommended by ISO 10272-1 (ISO, 2006).

All the identified *E. coli* and salmonella isolates were serologically identified by the slide agglutination test using polyvalent antisera for O and H antigens for salmonellae and O antigen for *E. coli*.

**Statistical analysis:**

The data were statistically analyzed by one way analysis of variance (ANOVA) using SPSS program (SPSS version 20, IBM Inc. Chicago, IL and USA). Group means of data were compared to determine significant differences in the number of bacteria recovered. All significant differences were determined at $P \leq 0.05$.

**RESULTS**

Data presented in table (1) revealed that contamination level for coliforms was 5.17, 5.47, 5.77, 5.54, 5.47 and 5.3 log $10$ cfu/ml of carcass rinse in scalding, defeathering, evisceration, washing, chilling and packaging, respectively. Moreover, the count of faecal coliforms was 5, 5.3, 5.54, 5.47, 5.3 and 5 log $10$ cfu/ml of carcass rinse. In addition, the *E. coli* (MPN) was 3.6, 4.6, 4.54, 4.3, 4, and 4.3 log $10$ cfu/ml of carcass rinse. The rate of *Staphylococcus aureus* contamination in the examined stages was 4.39, 4.9, 4.54, 4.17, 3.9 and 4.17 log $10$ cfu/ml of carcass rinse in scalding, defeathering, evisceration, washing, chilling and packaging, respectively.
Table 1: Bacterial counts of broiler carcasses at different processing steps (n= 15 examined carcasses in each step)

<table>
<thead>
<tr>
<th>Processing Step</th>
<th>Coliforms (MPN)</th>
<th>Faecal coliforms (MPN)</th>
<th>E. coli count (MPN)</th>
<th>Staphylococcus aureus count (MPN)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalding</td>
<td>5.17 ± 3.8</td>
<td>5 ± 4.9</td>
<td>3.6 ± 4.6</td>
<td>4.39 ± 3.3</td>
</tr>
<tr>
<td>Defeathering</td>
<td>5.47 ± 4.9</td>
<td>5 ± 4.5</td>
<td>4.6 ± 4.3</td>
<td>4.9 ± 3.9</td>
</tr>
<tr>
<td>Evisceration</td>
<td>5.77 ± 5.54</td>
<td>5.54 ± 5.47</td>
<td>4.54 ± 4.57</td>
<td>4.54 ± 4.57</td>
</tr>
<tr>
<td>Washing</td>
<td>5.54 ± 5.3</td>
<td>5 ± 4.9</td>
<td>4.3 ± 4.3</td>
<td>3 ± 3.3</td>
</tr>
<tr>
<td>Chilling</td>
<td>5.47 ± 5.3</td>
<td>5 ± 4.9</td>
<td>3.9 ± 4.3</td>
<td>3.3 ± 3.3</td>
</tr>
<tr>
<td>Packaging</td>
<td>5.3 ± 3.95</td>
<td>5 ± 4.9</td>
<td>3.77 ± 3.9</td>
<td>3.4 ± 3.4</td>
</tr>
</tbody>
</table>

Counts expressed as mean ± S.E/ml of carcass rinse

Means within the same row with no common superscript are significantly different at p ≤ 0.05

Based on the results from table (2) there was evidence for multiple contamination of the examined broiler carcasses in all the sampling points with E. coli, Salmonella spp, Staphylococcus aureus and Campylobacter jejuni with different percentages.

Table 2: Prevalence of isolated microorganisms in broiler carcass rinses collected at different steps of broiler processing (n = 15 examined carcasses in each step)

<table>
<thead>
<tr>
<th>Processing Step</th>
<th>E. coli</th>
<th>Salmonella spp</th>
<th>Staphylococcus aureus</th>
<th>Campylobacter jejuni</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalding</td>
<td>13 86.7 %</td>
<td>5 33.3 %</td>
<td>14 93.3 %</td>
<td>9 60 %</td>
</tr>
<tr>
<td>Defeathering</td>
<td>13 86.7 %</td>
<td>7 46.7 %</td>
<td>15 100 %</td>
<td>11 73.3 %</td>
</tr>
<tr>
<td>Evisceration</td>
<td>13 80 %</td>
<td>8 53.3 %</td>
<td>11 73.3 %</td>
<td>10 66.7 %</td>
</tr>
<tr>
<td>Washing</td>
<td>13 86.7 %</td>
<td>7 46.7 %</td>
<td>11 73.3 %</td>
<td>10 66.7 %</td>
</tr>
<tr>
<td>Chilling</td>
<td>13 86.7 %</td>
<td>5 33.3 %</td>
<td>13 86.7 %</td>
<td>9 60 %</td>
</tr>
<tr>
<td>Packaging</td>
<td>13 86.7 %</td>
<td>5 33.3 %</td>
<td>13 86.7 %</td>
<td>9 60 %</td>
</tr>
</tbody>
</table>

The data reported here (table 3) showed the serotypes of isolated Salmonellae and E. coli. During scalding stage the isolated serotypes of salmonella were S.Virchow, S. Aba, S. Kentucky while the isolated serotypes of E. coli were O1, O127, O26. Furthermore, these serotypes were S.Virchow and O119 in defeathering. Moreover, in evisceration stage; S. Aba and O44 were the isolated serotypes. Similarly, in washing stage the isolated serotypes were S. Aba while O44 and O26 were the isolated E. coli serotypes. Meanwhile, chilling stage yield S. Aba, S.Virchow, S. Kentucky for salmonella and O26, O78 for E. coli. In addition, in packaging; S. Infantis and O78 were the isolated serotypes during this stage.

Table 3: Isolated serotypes of salmonella and E. coli during different processing steps

<table>
<thead>
<tr>
<th>Processing Step</th>
<th>Salmonella</th>
<th>E. coli</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scalding</td>
<td>S.Virchow, S. Aba, S. Kentucky</td>
<td>O1, O127, O26</td>
</tr>
<tr>
<td>Defeathering</td>
<td>S.Virchow</td>
<td>O119</td>
</tr>
<tr>
<td>Evisceration</td>
<td>S. Aba</td>
<td>O44</td>
</tr>
<tr>
<td>Washing</td>
<td>S. Aba</td>
<td>O44, O26</td>
</tr>
<tr>
<td>Chilling</td>
<td>S. Aba, S.Virchow, S. Kentucky</td>
<td>O26, O78</td>
</tr>
<tr>
<td>Packaging</td>
<td>S. Infantis</td>
<td>O78</td>
</tr>
</tbody>
</table>
DISCUSSION

Contamination of broiler carcasses during processing can occur at numerous points such as scalding, plucking, defeathering, evisceration or chilling operations. These stages are more important with respect to cross-contamination during processing and have been linked to increase of prevalence or numbers of pathogens on carcasses.

Coliforms, Faecal coliforms and E. coli counts:

It was noted from table (1) that coliforms (MPN), faecal coliforms (MPN) and E. coli (MPN) levels increased after scalding (during defeathering and evisceration) stages then decreased during washing stage as the washing after evisceration subsequently removed some contaminant from the carcasses. The contamination was further decreased after chilling of the carcasses; this finding is agreed with that reported by Bashor et al. (2004) and Althaus et al. (2017).

There was a significant differences between the processing steps at which the evisceration process has the highest coliform and faecal coliform counts (5.77 log_{10} cfu/ml and 5.54 log_{10} cfu/ml), respectively while, there was no significant difference in relation to E. coli (MPN) between the different processing steps.

Birds delivered to slaughter are generally highly contaminated with bacteria such as Coliforms and Salmonella, E. coli, Staphylococcus aureus and Campylobacter which may present on their intestine especially the ceca and the colon or in the surface of the skin and feather (Berrang et al., 2000).

At the scalding stage in the traditional poultry abattoirs a one single tank is used, many bacteria are washed from the carcasses results in the release of a large load of organic matter, microbes, and fecal material to the scalding water, these contaminants become suspended in the scalding water (Kotula and Pandya, 1995 and Cason et al., 2000). In this way the scald tank water becomes rapidly contaminated with organisms of faecal origin increasing the risk of cross-contamination from one carcass to another (Baily et al., 1987 and Reiter et al., 2007).

The increase in bacterial count during defeathering is may be attributable to the escape of highly contaminated gut contents from the vent while carcasses are passing through automated feather picking machines. The external pressure exerted on the lower abdomen by the picker’s rubber fingers causes release of gut contents which still present in the lower bowel and highly contaminated with enteric pathogens such as Coliforms, E. coli, Salmonella, and Campylobacter leading to an increase in the carcasses contamination (Berrang and Dickens, 2000 and Berrang et al., 2001). In this context, Buhr et al. (2003) found that plugging the cloaca prior to scalding and picking decreased coliforms, E. coli in rinse samples. Moreover, Abu-Ruwaïda et al. (1994) reported an increase in E. coli counts in broiler carcasses following evisceration.

Previous research have reported that, in general washing after evisceration step can be effective means to lower the bacterial counts and lessen the bacterial contamination on eviscerated broiler carcasses and be useful for pathogen control (Stopforth et al., 2007 and Berrang and Bailey, 2009).

The non-significant reduction in bacterial count and incidence of isolated microorganisms during washing and chilling steps could be contributed to lack of application antimicrobial during these steps, this agreed with that reported by Northcutt et al. (2003 a) who found that the use of water in the washing stage during broiler carcasses processing without antimicrobial agent may not significantly reduce carcass coliform or E. coli counts. Moreover, Smith et al. (2005) who suggested that chlorine prevents cross contamination in immersion chilled broiler carcasses.

Mead et al. (2000) suggested that microbial cross-contamination can occur during chilling of poultry; one is via the handling of carcasses by operatives during loading of the chiller and another is by physical contact between adjacent carcasses, which is unavoidable in chiller tank. On the other hand, previously published reports suggested that the chilling stage significantly lower the bacterial count (Berrang and Dickens 2000). In this context, Jiménez et al. (2003) found that chilling step significantly lower the E. coli count from 3.44 log_{10} cfu/ml to 2.28 log_{10} cfu/ml and coliforms from 3.91 log_{10} cfu/ml to 2.68 log_{10} cfu/ml in chicken carcasses during processing.

Comparable level of coliforms in defeathering (5.4 log_{10}) and packaging (5 log_{10}) were detected by Abu-Ruwaïda et al. (1994) while lower coliforms were reported by Northcutt et al. (2003 b) after washing (3.9 log_{10}) and after chilling (2.6 log_{10}) also Berrang and Dickens (2000) detected lower figure for coliforms at different broiler carcasses processing steps. Furthermore, lower figure for E. coli was reported by Berrang and Dickens (2000) at different steps. On the other hand, Cason et al. (2004) reported higher E. coli (6.3 log_{10} & 5.4 log_{10}) and coliform counts (6.5 log_{10} & 5.7 log_{10}) after washing and after chilling, respectively.

The numbers of E. coli are given in table (2) were 86.7%, 86.7%, 86.7%, 93.3%, 86.7% and 86.7% in scalding, defeathering, evisceration, washing, chilling and packaging, respectively. The isolated
serotypes were E. coli O1, O127, O44, O78, O119 and O26 (table 3).

Higher E. coli percentage was reported by Althaus et al. (2017) while, lower E. coli percentage was reported by Gabeer et al. (2012) in different processing steps.

The high percentage of E. coli isolated in the current investigation could be attributed to the high prevalence of faecal contamination which occur as a result of rupturing of viscera during the evisceration process resulted in faecal contamination of carcasses and further contamination of processing water and that reported by (Mead, 1989).

**Staphylococcus aureus count:**

Data from table (1) showed that Staphylococcus aureus count significantly increased after the carcasses exit the picker (4.9 log \(_{10}\)) then significantly decreased during the chilling stage (3.9 log \(_{10}\)).

It was clearly shown from data in table (2) that contamination level of Staphylococcus aureus in broiler carcasses was 93.3%, 100%, 93.3%, 73.3%, 73.3% and 86.7% in scalding, defeathering, evisceration, washing, chilling and packaging, respectively.

Defeathering is generally considered to be one of the major sites of cross-contamination during broiler processing. The plucking process help in removal of the epidermal layer exposing the skin surface for colonization of bacteria implicating the rubber fingers of the defeathering machine as contamination source (Thomas and McMeekin, 1980 and Geornaras et al., 1997). Direct contact between contaminated and uncontaminated carcasses and the action of the fingers of the picker machines are the possible mechanisms of bacterial cross-contamination during defeathering (Allen et al., 2003). In this context, Allen et al. (2003) found that a marker organism inoculated onto post-scalding carcasses dispersed for ≤ 200 carcasses via feather removal. In this respect, Whittemore and Lyon, (1994) recovered 5.46 to 5.73 log\(_{10}\) Staphylococcus spp. from the rubber picking fingers.

The reason for the high prevalence of Staphylococcus aureus in this study may be attributed to the poor personal hygiene of the workers and non-hygienic practice adopted by workers as handling of carcasses by persons who are harboring staphylococci in their nose, skin, or in an infected lesion. High contamination rate as well as may be due to contamination from skin surface and through contaminated work surfaces and knives (Notermans et al., 1982 and Lambrechts et al., 2014).

Nearly similar results of Staphylococcus aureus count was reported by Abu-Ruwaida et al. (1994) while, Göksoy et al. (2004) detected higher Staphylococcus aureus count ranged from 6.9 log\(_{10}\) to 4.11 log\(_{10}\) in different steps moreover, Whyte et al. (2004) found lower Staphylococcus aureus count after defeathering (3 log\(_{10}\)), after washing (2.48) and after chilling (2.3 log\(_{10}\)).

**Salmonella spp:**

Contamination rate of broiler carcasses with Salmonella spp. observed in this study was 33.3 % in scalding, 46.7 % in defeathering, 40 % in evisceration, 53.3 % in washing, 46.7 % in chilling and 33 % in packaging. The isolated serotypes were S. Virchow, S. Aba, S. Infantis and S. Kentucky (table 3).

Salmonella prevalence has been shown to increase during defeathering (table 2); this is thought to be due to carcass-to-carcass contamination in feather picking machines. In this context, Berrang et al. (2011) observed a significant increase in the prevalence of Salmonella-positive carcasses after defeathering. Moreover, (Smith et al., 2007) declared that evisceration can lead to carcass contamination with Salmonella via crop leakage and intestinal rupture, which are considered major sources of carcass contamination with enteric pathogens.

In this study it was noticed that Salmonella numbers decreased from 46.7% in defeathering to 40 % after evisceration (table 2). In contrast, Lillard et al. (1984) reported a significantly higher Salmonella incidence from fully eviscerated carcasses than from non-eviscerated carcasses.

Nearly similar results for Salmonella were reported by Northcott et al. (2003 b) who recorded that 55 % of broiler carcasses were positive after washing. Also, Cason and Hinton, (2006) detected 50% of carcasses were salmonella-positive after defeathering. Lower incidence of Salmonella (36% after washing) was reported by Cox et al. (2010) also, Rivera-pérez et al. (2014) reported lower figures of salmonella in different steps. On the other side, Abu-Ruwaida et al. (1994) and Carraminana et al. (1997) found a much higher Salmonella incidence ranged from 55% to 100% at different processing steps.

**Campylobacter jejuni:**

Results in table (2) revealed that Campylobacter jejuni contamination in broiler carcasses was 60%, 73.3%, 80%, 66.6%, 66.7% and 60% in scalding, defeathering, evisceration, washing, chilling and packaging, respectively.
Previous studies stated wide variations of the slaughterhouse prevalence of Campylobacter spp. contamination of broiler carcasses at different processing stages. Several investigators have reported a higher prevalence of Campylobacter spp. through broiler chickens processing than our study (Cason et al., 1997 (94%); Bashor et al., 2004 (80%-93%) and 71% by Franchin et al. (2007) while, others reported lower prevalence (Klein et al., 2007 (51%) and Rahimi et al., 2010 (56%)) at different processing steps. In addition, a comparable level was reported by Berrang et al. (2001) and Figueroa et al. (2009). Broiler carcasses become contaminated with campylobacters because the live bird is frequently a symptomatic intestinal carrier of the organisms and dissemination readily occurs during processing (Baker et al., 1987). In this context, Newel et al. (2001) demonstrated a link between Campylobacter-positive poultry at live receiving and campylobacter-positive carcasses following, scalding, feather removal, evisceration, and chilling. Furthermore, the feathers, skin, crop, and cloaca of birds brought to slaughter are often highly contaminated with campylobacter (Stern et al., 1995 and Berrang et al., 2000).

The high incidence of campylobacter obtained in our study may be attributed to the viscera rupture which is not an uncommon occurrence during manual evisceration technique; this is consistent with Izat et al. (1988) and Stern and Robach (2003) who reported an increase in the Campylobacter concentration on carcasses during the evisceration operation, the increase is a result of viscera rupture, leading to an increased faecal contamination of the broiler carcasses.

Based on our findings, it was noticed that the final packaged carcasses were contaminated by different percentage of pathogenic bacteria; this finding support the earlier reports that demonstrate that the finished product was heavily contaminated (Abu-Ruwaida et al., 1994). Similarly, Izat et al. (1988) and Berrang and Dickens (2000) concluded that final product or fully processed broiler carcasses can be found contaminated with campylobacter after they exit the chill tank or as ready-to-cook carcasses. Moreover, Harrison et al. (2001) demonstrated that chicken package may be a potential source for cross-contamination with Campylobacter and Salmonella which found to be contaminated with 34% and 11% Campylobacter and Salmonella, respectively.

**CONCLUSION**

The present study demonstrates high levels of microbial contamination during processing of broiler carcasses which indicate insufficient sanitation and poor hygienic practices in the abattoir. The high percentage of E. coli, Salmonella, campylobacter, and Staphylococcus aureus obtained in our investigation render the poultry meat as a potential vehicle for transmitting food-borne diseases. Therefore, the application of hygienic measures is very important to reduce the bacterial contamination of broiler carcasses during processing in the abattoir. Furthermore, there is a need for implementation of hazard analysis and critical control point (HACCP) in poultry industry to provide safety food for consumer as it represents a systematic approach for controlling all the potential hazards which may be associated with poultry processing.

**ACKNOWLEDGMENT**

We are extremely grateful to the members of Reference lab for veterinary control on poultry production Fayoum branch for their help in the accomplishment of this work.

**REFERENCES**


The microorganisms investigated in this study were: E. coli O1, O127, O44, O78, O119, W. coli O1, S. Kentucky and S. Infantis, S. Aba, and S. Virchow. The salmonella isolates were E. coli O1, O127, O44, O78, O119, W. coli O1, S. Kentucky and S. Infantis, S. Aba, and S. Virchow.


The microorganisms investigated in this study were: E. coli O1, O127, O44, O78, O119, W. coli O1, S. Kentucky and S. Infantis, S. Aba, and S. Virchow. The salmonella isolates were E. coli O1, O127, O44, O78, O119, W. coli O1, S. Kentucky and S. Infantis, S. Aba, and S. Virchow.