Prevalence of Some Food-borne Pathogens in Cooked Liver Sandwich

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Abstract

A total of 30 cooked liver sandwiches were randomly collected from different street vendor shops at Ismailia city for evaluation of some food-borne pathogens. The obtained results revealed that the mean values of total aerobic plate and total Bacillus Cereus counts were $7.8 \times 10^5 \pm 7.8 \times 10^5$ and $1.3 \times 10^3 \pm 7.4 \times 10^2$ cfu/g respectively. The results showed that the incidence of Escherichia Coli serotypes in the same samples were $O_1$ (33%) and $O_{26}$ (67%). Enterobacteriaceae strains as Enterobacter Aerogenes, Proteus Mirabilis, Serratia Odorifera and Yarsinia Intermedia could be identified by 1 (16%), 1 (16%), 2 (34%) and 2 (34%) respectively. It’s cleared that 1(3%) of samples were positive for shigella meanwhile salmonella failed to be detected from all samples under inspection. It could be concluded that the liver sandwiches were exposed to inadequate hygienic measures during preparation represented as inadequate cooking temperature and cross contamination from different sources by food-borne pathogens. In addition, some suggestions and recommendations were given for improving the sanitary condition of cooked liver sandwiches to ensure its safety for human consumption.

Introduction

Food habits of sociality have substantially changed due to rapid urbanization and hurried way of living result to increase demand for fast food meals (Sherihan et al., 2014). Not only the meat and edible offal have long been considered as highly desirable, nutritious and protein-rich food, but they are also highly perishable because they provide the nutrients needed to support the growth of many types of microorganisms, due to their unique biological and chemical nature, their quality attributes deteriorate from the time of slaughter until consumption (Kalalou et al., 2004). Street foods are perceived to be a major public health risk due to lack of basic infrastructure and services, difficulty in controlling the large numbers of street food vending operations because of their diversity, mobility and temporary nature (Ghosh et al., 2007 and de Sousa, 2008). The offal is rich in mineral and vitamin content (Oztan, 2005). Liver consider on of the highly nutritive edible offal which contain vitamin A, vitamin B, vitamin C, vitamin D, Iron, Zinc and copper and mostly eaten as takeaway sandwiches. Fast food become probably the source of most diseases caused by bacteria due to uncovered food, areas infected by flies and others insects also using poor quality water for washing the material and equipment (Barro et al., 2002). True food poisoning or food intoxication caused by eating food that contains a toxin or poison due to bacterial growth in food while food infection is the second type of food-borne illness, It's caused by eating food that contain certain types of live bacteria which are present in the food, once the food is consumed, the bacterial cells is continue to grow and illness can result (Lubna and Ghada, 2012).

Most Pathogenic bacteria such as Bacillus Cereus, Escherichia Coli, salmonella species and shigella have been implicated in a number of food borne illnesses and cause a variety of zoonotic infection in human and animals (Nouichi and Hamdi, 2009). Bacillus Genera are gram-positive rods able to produce endospores resistant to unfavorable external conditions (Logan and DeVos, 2009). Escherichia coli considered as reflection of environmental contamination during slaughter processing and product handling and its count is an index of sanitary quality of examined samples high number can easily give rise to public health hazards (Mercuri & Cox, 1976).
and Kanpelancher, 1981). Salmonella are disseminated by the carrier animals and the infected animals cannot readily be detected at abattoir (McEvoy et al., 2003).

Therefore, the present study was conducted to evaluate the prevalence of Bacillus cereus; Escherichia coli, salmonella and shigella in cooked liver sandwiches traded at Ismailia city.

Material and Methods

1. Samples Collection: A total of 30 cooked liver sandwiches samples were purchased from different street vendor shops in Ismailia City. All collected samples were immediately transferred in icebox container, aseptically handled and moved promptly to microbiology laboratory at Animal Health Institute, Ismailia Lab., to be evaluated bacteriologically.

2. Preparation of samples (ISO, 2003): Twenty five grams (±0.5) of cooked liver were weighed and transferred to into a high sterile stomacher bags containing 225 ml 0.1% (W/V) sterile buffered peptone water whereas homogenized using a lab blender (Seward stomacher lab system 400 R\UK) for 2 minutes to obtain the original homogenate fluid of a dilution rate 10\1. From original homogenate, 1 ml was transferred to a series of sterile test tubes containing 9 ml of 0.1% sterile buffered peptone water and well mixed to prepare decimal serial dilutions of sample homogenate up to 10\7. Experimental trials were repeated twice.

3. Determination of total Bacillus Cereus count: Determination of Bacillus Cereus is carried out according to the method described by BAM (2012).

4. Detection of Escherichia coli: Determination of Escherichia coli is carried out according to the method described by APHA (2002).

5. Serological identification of Escherichia Coli isolates: All E. coli isolates were subjected to serological typing by slide agglutination test according to Lee et al., (2009) using standard polyvalent and monovalent E. coli antiseras (Seiken, Japan). Only fresh bacterial culture from 24 hours age colonies onto nutrient agar media were used.

6. Detection of salmonella and shigella: Detection of salmonella and shigella is carried out according to the method described by USA/FSIS (2004) with a slit modification in the type of the used selective media.

7. Serological identification of salmonella species: All salmonella isolates were subjected to serological typing by slide agglutination test according to Grimont and Weill (2007) using standard polyvalent and monovalent salmonella antiseras (Seiken, Japan). Only fresh bacterial cultures from 24 hours age colonies onto nutrient agar media were used.

Results and Discussion

Poor storage conditions besides the high nutritive value of meat might be favoring growth and rapid multiplication of bacteria hence the high counts (Bogere and Baluka, 2014).

Total aerobic plate count: Aerobic plate count is generally accepted as a criterion for microbial contamination of carcasses and a useful indicator of hygienic conditions of abattoir (Cohen et al., 2007).

It's evident from results recorded in table (1) that total aerobic plate counts of street vended liver sandwich were ranged from 8.1 × 10\2 to 9.7 × 10\6 (cfu/g) with an average 7.8 × 10\5 ± 7.8 × 10\5. The obtained results were nearly similar to those reported by Kirralla (2007), Adam (2009) and Dalia et al., (2013); they lower than Abou (1995), Ghada (2001), Abd El-Raheem (2013) and Abd-El-Malek (2014) and they higher than Mohamed et al., (2004) and El Mossalami (2008).

According to Microbiological Guidelines for Food (2014) criteria of 16 samples considered satisfactory, 10 samples were border line and none of samples were unsatisfactory. The lower result agrees with the fact that heating
of the foods kill most of bacteria leading to decrease total count of bacteria.

**Total Bacillus Cereus count:**

Bacillus Cereus is important for food hygiene because of their hydrolytic activities on food components and the ability of strain to produce toxins or grow at refrigerated temperatures (Saad and Ahmed, 2013).

It's evident from results recorded in table (2) and table (3) that Bacillus Cereus counts of street vended liver sandwiches were detected in 7 (24%) of the total 30 samples and the results ranged from $1 \times 10^3$ to $2 \times 10^4$ (cfu/g) with an average $1.3 \times 10^3 \pm 7.4 \times 10^2$ (cfu/g). According to *Microbiological Guidelines for Food* (2014) criteria the results were categorized as 2 (6%) satisfactory, 4 (13%) border line and 0.00% unsatisfactory.

The obtained results were nearly similar to those reported by Ghada (2001) and El-Mossalami, *et al.*, (2008); while lower than Abd-El-Malek (2014) and higher than Nassar (1999).

The number of Bacillus Cereus cells present in fresh offal reflecting the degree of contamination of the carcass with soil and dirt at abattoir level.

The presence of the Bacillus Cereus in cooked food may due to the heat resistant nature of Bacillus spores allows them to survive in foods, which have undergone moderate heat processing and normal cooking processes (Rasooly *et al.*, 2015) and also the contribution made from additives such as spices and fillers.

**Escherichia Coli**

The obtained result in table (4) revealed that incidence of *E. coli* were serotyped to $O_1$ 1(33%) and $O_{26}$ 2(67%), in the examined cooked liver samples. These results were nearly similar to those reported by Lubna and Ghada (2012) while lower than Abd El Rahman *et al.* (2011), Ahmadi *et al.* (2012), Pavithra and Ghosh (2013) and Hassanin *et al.* (2014) and higher than Gormely *et al.* (2010). The reheating is often inadequate to destroy bacteria that may be present as this would allow the food-borne pathogens that germinate from spores which survived cooking or that contaminate the food after cooking, to survive and proliferate (Rane, 2011).

Heating food at high temperature 51°C decrease the survival of *E. coli* cells after 10 min. and no colonies were observed after heating for 180 minute, viable *E. coli* were not found after at 53 °C for 60 min (Nakano *et al.*, 2012).

The obtaind of enterobacteriaceae out lined in table (5) revealed that the incidence of Enterobacter Aerogenes, Proteus Mirabilis, Serratia Odorifera and Yarsinia Intermedia were 1 (16%), 1 (16%), 2 (34%) and 2 (34%), respectively in cooked liver sandwiches.

The result were varied from results recorded by Ziino *et al.*, (2009) and nearly similar to Faten *et al.*, (2013) and El-Seady (1997). The high incidence of enterobacteriaceae in edible offal may be attributed to the unsanitary conditions of offal collection after evisceration; putting offal on floor contaminated with fecal matters and delayed transportation of offal to special hygienic place (Hemmat *et al.*, 2013).

**Salmonella:**

Salmonella failed to be isolated from cooked liver sandwiches, these result agreed with Abou (1995), Ghada (2001), Büyükyörük *et al.*, (2014) while disagreed with Abd-El-Malek (2014).

According to *Microbiological Guidelines for Food* (2014) criteria all samples considered satisfactory. Low incidence of salmonella isolation may be attributed to the fact that
most pathogenic bacteria destroyed between 72°C to 83°C so the cooking method should be effectively applied to produce temperature sufficient to kill all these pathogens (Murphy et al., 2001).

Shigella:

The obtained results in table (6) revealed that incidence of shigella were 1(3%) in cooked liver sandwiches. The obtained results were not agree with Abou (1995) who could isolate shigella from 30 samples of roasted liver and Abd-El-Malekf (2014) could detect shigella in (23%) of samples. According to Microbiological Guidelines for Food (2014) all positive samples were not applicable.

Shigella species have the potential to cause large out breaks because of their low infectious dose (10 cells), and cannot isolate it from different meat product (Cetinkaya et al., 2008). The presence of shigella indicate a human fecal contamination shigella transmission by the fecal-oral route, including direct person-to-person contact and may be indirect through ingestion of contaminated food or water (Jomezadeh, 2014), so it is most likely to occur in children and those who neglect to clean hands thoroughly.

From the results it could be concluded that cooked liver sandwich samples that traded in Ismailia city were exposed to fecal contamination and environmental contamination. Also prepared under bad sanitary condition. It is necessary to have a strict validation rules for consumer rights, also rules for street vendor shops as they exposed to exhaust from vehicles, microbial loads, insufficient cooking and absence of health certificate (which is not enough for ensures the product safety). Finally, cooperation should be done between observer veterinarian and Ministry of Interior by doing regularly campaigns on products of animal origins and street vendors.

References
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of shigella species from stool samples among hospitalized children in Abadan, Iran. Gastroenterology and Hepatology from Bed to Bench, 7(4): 218–223.


Microbiological Guidelines for Food (For ready-to-eat food in general and specific food items) (2014): Published by Centre for Food Safety, Food Safety Food and Environmental Hygiene Department, Available online at : http://www.cfs.gov.hk/english/foodleg/files/food_leg_Microbiological_Guidelines_for_Food_e.pdf.


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**Table 1. Statistical analytical results of total aerobic plate count (cfu/g) of the cooked liver sandwich from street vendors (n=30):**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cooked Liver samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>8.1×10²</td>
</tr>
<tr>
<td>Maximum</td>
<td>9.7×10⁶</td>
</tr>
<tr>
<td>Mean</td>
<td>7.8×10⁵ ± S.E</td>
</tr>
<tr>
<td>± S.E</td>
<td>±7.8×10³</td>
</tr>
</tbody>
</table>

**Table 2. Incidence of Bacillus Cereus in cooked liver sandwich (n=30):**

<table>
<thead>
<tr>
<th>Samples</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Negative (ND)*</td>
<td>23</td>
<td>76</td>
</tr>
</tbody>
</table>

*ND: Not detected<10⁻²

**Table 3. Statistical analytical results of total Bacillus Cereus count (cfu/g) of the cooked liver sandwich from street vendors (n=30):**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Cooked Liver samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>1×10</td>
</tr>
<tr>
<td>Maximum</td>
<td>2×10⁴</td>
</tr>
<tr>
<td>Mean</td>
<td>1.3×10³ ± S.E</td>
</tr>
<tr>
<td>± S.E</td>
<td>±7.4×10²</td>
</tr>
</tbody>
</table>

**Table 4. Incidence of E. coli Serotypes isolated from cooked liver sandwiches (n=30):**

<table>
<thead>
<tr>
<th>E. coli Serotypes</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>O₁</td>
<td>1 33</td>
</tr>
<tr>
<td>O₂₆</td>
<td>2 67</td>
</tr>
</tbody>
</table>
### Table 5. Frequency distribution of enterobacteriacea isolated from the cooked liver sandwiches:

<table>
<thead>
<tr>
<th>Cooked liver sandwich</th>
<th>Strains</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enterobacter aerogenes</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Proteus mirabilis</td>
<td>1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>Serratia odorifera</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Yersinia intermedia</td>
<td>2</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>6</td>
<td>100</td>
</tr>
</tbody>
</table>

### Table 6. Incidence of shigella species isolated from cooked liver samples (n=30)

<table>
<thead>
<tr>
<th>Samples</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Negative</td>
<td>29</td>
<td>97</td>
</tr>
</tbody>
</table>

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دراسات عن انتشار بعض مسببات الأمراض المنقولة عن طريق ساندويتشات الكبد المطهي

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المخصّص العربي

تم في هذه الدراسة فحص بكتيرولوجيًا عدد ثلاثون عينة من عينات ساندويتشات الكبد المطهي المجمعة عشوائيًا من محلات بيع الساندويتشات الجاهزة بمدينة الإسماعيلية لاسيما في انتشار بعض مسببات الأمراض البكتيرية بها؛ وأوضحت النتائج المحققة عليها أن كثافة البكتيريا الهولاندية والبكتيريا الشاذة لميكروبات SS سيريس هي

\[
7.8 \times 10^5 \pm 7.8 \times 10^3 \text{ خلية} / جرام على التوالي. 
\]

وتبين أن نسبة وجود ميكروب الإشريشنا كولي هو (10%) وقد تم تصنيف بعض العينات المعزولة من عينات الكبد المطهي وهي O_1 وO_26 وO_10 و10^5 -10^7 خلية / جرام على التوالي. وتم توصيف عينات انتروبايكرتار أرجنوسا وبرتيس ميريلس وسيرياتا لومينيا وبرسينيا إنترمينيا من نفس العينات. وكانت نسبة وجود ميكروب الشيجيليا هي 3% من إجمالي عدد العينات بينما لم يتمكن من عزل جرثومة السالمونيلا بالطرق المستخدمة. واستنادًا إلى النتائج فقد توافر الاشتباطات الصحية في محلات تجهيز الساندويشات مختلفة في عدد الطهي الحدي لعجالة عند تجهيزها للاستهلاك الأممي. وأيضاً حتمية التثوّث النعتي بالجراثيم المسببة للتمتّم الغذائي أثناء التجهيز وبعد عملية الطهي هذا وقد تذكر بعض التوصيات التي من شأنها تحسين الحالة الصحية لسандويتشات الكبد المطهي لضمان سلامتها للاستهلاك الأممي.

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