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# Tracking the Sources of Contamination and Evaluating the Effect of Cooking on Bacterial Load in Meat Meals Prepared and Served in Foodservice Establishments

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## Abstract

In this study, we determined the aerobic plate count, *Staphylococcus* (Staph.) *aureus* count, and most probable numbers (MPN) of coliforms, fecal coliforms, and *Escherichia* (E.) *coli*, and the presence of *Listeria monocytogenes* in 120 samples of beef steak and beef burger at various stages of preparation in a randomly selected food serving establishment in Beni-Suef City, Egypt. Additionally, 60 swab samples from knives, cutting boards, and operator's hands were investigated for the same bacteriological criteria. Subsequently, bacterial isolates were subjected to molecular identification by investigating the presence of virulence and antibiotic resistance genes. The results showed that 100 % of meat product samples at various stages of preparation contained aerobic bacterial load and *Staph. aureus*. Conversely, *E. coli* and *L. monocytogenes* were undetectable in meat product samples. Interestingly, 13, 7, and 1 out of 20 swab samples from hands contained coliforms, fecal coliforms, and *E. coli*, respectively, while knives and cutting boards were free from fecal coliforms and *E. coli*. Furthermore, contamination with *Staph. aureus* was reported in 100 % of knives and hand swab samples and 50 % of cutting board swab samples. Furthermore, the only *E. coli* isolate from a worker's hand contained *eae*A gene, but not *stx*1 gene, which indicates that it is an enteropathogenic *E. coli* (EPEC) strain. Moreover, 6 and 5 *Staph. aureus* isolates from operator hands and beef steak after cooking had *coa* and *mec*A genes, respectively, which are responsible for plasma coagulation and methicillin resistance, respectively. To sum up, this food serving establishment does not apply proper food safety guidelines, practices inadequate cleaning and sanitizing and poor personal hygiene, accordingly, it should be faced with a high level of concern and consideration by the legal authorities.

KEYWORDS
Foodservice, Beef, Hygienic practice, *E. coli*, *Staph. aureus*

## INTRODUCTION

Foods contaminated with pathogenic microorganisms had been considered health risks to consumers. Nowadays, every year around two million people die as a result of foodborne diseases (Winias, 2011). Food poisoning and foodborne diseases affect public health globally as they lead to many health problems and uncounted precocious deaths (Joint FAO/WHO Expert Committee, 2007). The difficulties that face the developing countries in performing optimum hygienic food handling practices make the situation more complicated. The growth in the food industry and the development of many fast foods expose the food production process to several microbial contamination sources especially those associated with improper handling practices. Satisfactory hygienic standards in food production are necessary to protect the public health because many foodborne diseases are responsible for most outbreaks of diarrhea, the enteric infections due to Salmonella and typhoid fever infections in many countries (Schlundt *et al*., 2004; Newell *et al*., 2010; Badi *et al*., 2018).

In addition, there are several types of foodborne pathogenic microorganisms such as *Staphylococcus* (Staph.) *aureus*, *Escherichia* (E.) *coli*, and *Listeria* (L.) *monocytogenes*. *Escherichia* coli is a Gram-negative bacterial pathogen that exists as normal microbiota in animals and is responsible for many infections including septicemia, pyelonephritis, and cystitis (Manges *et al*., 2007; Singh *et al*., 2012; Majowicz *et al*., 2014). *Staphylococcus aureus*, Gram-positive bacteria, is one of the most common pathogens related to food intoxication which is manifested by gastroenteritis and vomiting, as well as causes a wide variety of diseases such as skin and soft tissue infections (Gundogan *et al*., 2005; Bortolaia *et al*., 2016). *Staph. aureus* can grow at 15 °C to 45 °C, and a high concentration of salt (15%) (Behling *et al*., 2010). *Listeria* is a gram-positive, facultative intracellular foodborne pathogen. Its infections are associated with approximately a 12% fatality rate, which is the highest rate through foodborne pathogens (Marriott *et al*., 2018).

Nowadays, many individuals tend to eat out, often at foodservice operations (Nyachuba, 2010). Foodservice or catering in-

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dustry defines those businesses, institutions, or companies that prepare and serve meals outside the home. These industries include restaurants, hotels, school and hospital cafeterias, catering operations, fast-food outlets, and other food service establishments. Given the high volume of meals prepared in catering establishments together with meal exposure to several handling and environmental factors, the exposure risk to foodborne illnesses is expected to be considerably higher than eating at home (Hedberg *et al*., 2006). In foodservice environments, various factors may be related to foodborne diseases. According to the Food and Drug Administration, these factors are the food served coming from unsafe sources, poor personal hygiene, improper cooking, time and temperature abuse, or inadequate cleaning and sanitizing ( Alves, *et al*. 2021).

The hands of foodservice employees can be vectors in the spread of foodborne disease because of poor personal hygiene or cross-contamination. For example, an employee might contaminate his hands when using the toilet, or bacteria might be spread from raw meat to salad greens by the food handler's hands (Boleij and Tjalsma 2012). Due to the high risk of food contamination in a foodservice establishment, many topics should be included in food handler training such as personal hygiene, hand care, and sanitation, body hygiene, work attire, and food hygiene (Lee *et al*., 2017).

Despite there being many studies that surveyed the bacterial status of meat products, to the best of the authors' knowledge, there are no previous research works that tracked the sources of contamination and the effect of different preparation processes on the bacteriological status in meat meals in foodservice establishments in Egypt. Therefore, the current study was conducted to determine the aerobic plate count, *Staph. aureus* count, and most probable numbers (MPN) of coliforms, fecal coliforms, and *E. coli* and detect pathogenic *E. coli*, *Staph. aureus*, and *L. monocytogenes* in beef steak at various stages of preparation (before cutting, after cutting, and after cooking), as well as in beef burger at various stages of preparation (before meat chopping, after chopping, and after cooking) in a randomly selected foodservice establishment in Beni-Suef City, Egypt. Additionally, swab samples from knives, cutting boards, and the operator's hands were investigated for the same bacteriological criteria. Subsequently, *Staph. aureus* and pathogenic *E. coli* isolates were subjected to molecular identification by investigating the presence of virulence genes (*eae*A and *stx*1) in the case of *E. coli*, and coagulation gene (*coa*) and methicillin-resistance gene (*mec*A) in the case of *Staph. aureus*.

## MATERIALS AND METHODS

*Sample collection*

The samples analyzed in this study were collected from a randomly selected food serving establishment in Beni-Suef City. A total of 120 random meat samples were collected at different stages of preparation including beef steak before cutting, after cutting, and after cooking (20 samples each), as well as beef burger before mincing, after mining, and after cooking (20 samples each). In addition, contact surface swab samples from cutting knives, cutting boards, and operator hands (20 samples each) were collected. The collected samples were separately placed in sterile plastic bags, identified, and transferred in an insulated icebox to the laboratory of Food Safety, Faculty of Veterinary Medicine, Beni-Suef University (Egypt) under complete aseptic conditions without delay for further preparation and bacteriological examination. To obtain consent from the operators to participate in this study, first, adequate and clear information was given to them about the hand swabbing procedure, then they were gently asked to participate in the hand swabbing step if they agree. All operators who participated in this study provided informed verbal consent before the collection of hand swabs.

*Preparation of meat samples for bacteriological analysis*

The collected samples were prepared according to the method recommended by the American Public Health Association (APHA, 1992). Twenty-five grams of each meat sample, either raw or cooked, were transferred to a sterile homogenizer flask containing 225 mL of 0.1% sterile peptone water (Oxoid, Hampshire, UK) under complete aseptic conditions. Each sample was homogenized for 2-4 min at 2000 rpm in a homogenizer and then allowed to stand for 5 min at room temperature to make the first dilution ($10^{-1}$). Then tenfold serial dilution was done, as the flask contents were vortexed, and one mL was transferred to a separate sterile test tube containing 9 mL of 0.1% sterile buffered peptone water to make the 2nd serial dilution ($10^{-2}$), and the later step was repeated till obtaining the dilution $10^{-6}$. While in the case of cotton swab samples from contact surfaces, each cotton swab was transferred to a separate test tube containing 10 mL of 0.1% sterile buffered peptone water, and tenfold serial dilution was carried out as above-mentioned in meat samples.

*Bacteriological examination*

Aerobic plate count (APC) at 35°C

To determine the aerobic plate count (APC) in meat and swab samples, the pouring plate technique was applied (APHA, 1992). Briefly, one mL of each previously prepared serial dilution from each ample was inoculated into duplicate sterile Petri dishes. Then, about 12 - 15 mL of previously melted standard plate count agar (Oxoid, Hampshire, UK) at 44°C to 47°C was poured into each Petri dish. The plate content was carefully mixed by rotating the Petri dishes and allowed to solidify by leaving the Petri dishes standing on a cool horizontal surface. The inoculated, as well as the control Petri dishes, were inverted and incubated at 36±1°C for 24±3 h. The plates with 30-300 colonies were selected and counted using the colony counting equipment and APC was calculated according to the following equation:
Aerobic plate count = average number of colonies × dilution factor

Most Probable Number (MPN) of coliforms

The three-tube method (APHA, 1992) was applied for the determination of the MPN of coliforms. From each dilution, one mL was separately transferred to three Lauryl Sulfate Tryptose broth (LST) (Oxoid) tubes containing inverted Durham's tubes. The inoculated LST tubes were incubated at 35°C and then examined after 24 h. Gas negative tubes were re-incubated for another 24 hrs. After that, a loopful from each positive (gas and turbidity) tube was transferred into tubes containing Brilliant Green Bile Lactose Broth (BGBL) (Oxoid) containing inverted Durham's tubes. The tubes were incubated at 35° C for 24 h then examined for gas production. Positive tubes showing gas production and turbidity were recorded. The MPN of coliforms per g or cm2 was estimated and recorded according to the table recommended by (APHA, 1992) using the following equation:
MPN / g or cm2 = No. from the table × middle dilution factor/100

MPN of fecal coliform

For estimation of fecal coliform MPN using the method of APHA (1992), a loopful from each positive BGBL tube was transferred to a sterile test tube containing EC broth (Oxoid). EC broth tubes were incubated at 44.5±0.5°C for 24 h. The negative EC broth tubes were re-incubated and re-examined again after 48 hrs. The fecal coliform MPN was calculated using the same previously mentioned method of coliforms.

MPN of *E. coli*

For estimation of *E. coli* MPN, a loopful from each positive EC broth tube was streaked on Eosin Methylene Blue Agar (EMB, Oxoid) plate (APHA, 1992). The inoculated plates were incubated at 35°C for 24 hrs. The suspected *E. coli* colonies appeared as greenish metallic nucleated with a dark purple center with or without sheen. Two typical colonies from each suspected EMB plate were transferred into nutrient agar slants and incubated at 35°C for 24 h and kept refrigerated for further identification. MPN of *E. coli* was calculated using the same previously mentioned method of coliforms.

Enumeration of *Staph. aureus*

One hundred microliters of each prepared decimal dilution were spread onto the surface of duplicate Baird Parker agar (Oxoid) plates using a sterile bent glass rod until the surface appear dry (APHA, 1992). The plates were allowed to dry in an upright position for about 10 min at laboratory temperature until the inoculum was absorbed by the agar. The inoculated and control plates were inverted and incubated at 35°C for 24-48 h. Suspected colonies showing black shiny with narrow white margin surrounded by outer clear zone were counted. Suspected colonies were picked up and stabbed in semisolid agar tubes for further biochemical and molecular identification. *Staph. aureus* count per g or $cm^2$ was calculated as follows:
*Staph. aureus* /g or $cm^2$ = average number of colonies × dilution factor × 10

Isolation of *Listeria monocytogenes*

Twenty-five grams of each meat sample were separately homogenized with 225 mL of half Fraser broth (Oxoid) with its selective supplement (Oxoid) and then incubated at 30°C for 18 - 24 h (Tiwari and Aldenrath, 1990). One hundred microliters of previously incubated flasks were inoculated in a tube containing 10 mL of the Fraser broth (Oxoid) with its selective supplement (Oxoid). The inoculated Fraser broth was incubated at 37°C for 24-48 h. After that, a loopful from each selective Fraser broth tube was streaked on the surface of Oxford agar medium (Oxoid) with its selective supplement (Oxoid). The inoculated plates were incubated at 30°C for 24 h. The incubated plates were examined for the suspected colonies of *Listeria monocytogenes* (black colonies, approximately one mm in diameter and surrounded by black halos) and positive tubes were recorded.

*Molecular identification of bacterial isolates*

*E. coli* isolates were molecularly identified for the presence of two virulence genes (*stx*1 and *eae*A), as well as *Staph. aureus* isolates were molecularly identified for the presence of two genes (*mec*A and *coa*). Genomic DNA was extracted using the QIAamp DNA Mini Kit. Specific primers obtained from Metabion (Germany) for each target gene, were used for DNA amplification using uniplex PCR, Emerald Amp GT PCR mastermix (2x premix), and PCR grade water (Sambrook *et al*., 1989). The specific sequences and length of amplified segments are shown in Table 1.

Primers were utilized in a 25 µL reaction tube containing 12.5 µL of Emerald Amp Max PCR Master Mix (Takara, Japan), one µL of each primer of a 20 pmol concentration, 6 µL of DNA template, and 4.5 µL of nuclease-free water. The reactions were performed in an Applied Biosystem 2720 thermal cycler. Briefly, the initial denaturation step was done at 94°C for 5 min, then followed by 35 cycles of 94°C for 45 sec, afterwards, annealing was applied. The time of annealing was 30 sec at 50˚C for *mec*A and *eae*A genes, while for *coa* was 40 sec at 55˚C and for *stx*1 was 40 sec at 58˚C. Subsequently, an extension step at 72°C for 45 sec and a final extension step at 72°C for 10 min was conducted for all genes. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. Twenty µL of the PCR products were loaded in each gel slot. The fragment sizes were determined using Gelpilot 100 bp and 100 bp plus DNA Ladders (Qiagen, Germany, GmbH) and gene ruler 100 bp ladder (Thermo Scientific, Germany). Afterward, the gel was photographed by a gel documentation system (Alpha Innotech, Biometra) (Sambrook *et al*., 1989).

*Statistical analysis*

The statistical analysis was carried out using the Minitab 20 (Minitab statistical software). One-way analysis of variance (one-way ANOVA) had been applied. Tukey's test was used as the post hoc test for the separation of means ($P < 0.05$).

Table 1. Primer sequences of target genes, length of the amplified product, and annealing temperatures.

| Targeted pathogen | Targeted genes | Gene role (virulence/resistance) | Primer sequences (5'-3') | Length of amplified segments | References |
|---|---|---|---|---|---|
| *Staph. aureus* | *mec*A | Resistance (methicilin-resistance) | GTA GAA ATG ACT GAA CGT CCG ATA A<br>CCA ATT CCA CAT TGT TTC GGT CTA A | 310 bp | McClure *et al*. (2006) |
| | *coa* | Virulence (coagulation) | ATA GAG ATG CTG GTA CAG G<br>GCT TCC GAT TGT TCG ATG C | 570 bp | Iyer and Kumosani (2011) |
| *E. coli* | *stx*1 | Virulence (Shiga-toxin production) | ACACTGGATGATCTCAGTGG<br>CTGAATCCCCCTCCATTATG | 614 bp | Dipineto *et al*. (2006) |
| | *eaeA* | Virulence (Attaching and effacing intimin) | ATGCTTAGTGCTGGTTTAGG<br>GCCTTCATCATTTCGCTTTC | 248 bp | Bisi-Johnson *et al*. (2011) |

## RESULTS AND DISCUSSION

When eating outside, consumers expect to obtain safe food with an adequate food hygiene level, which reduces the incidence of food-borne diseases. As well, the consumers always rely on legal authorities to regulate and inspect restaurants in order to assure that hygiene requirements are applied. In this study, we aimed to investigate the food hygiene level in a randomly selected food-service establishment, as well as track the main sources of bacterial contamination and assess the efficiency of cooking steps in reducing the bacterial load in the ready-to-eat meat meals.

For these objectives, we collected beef steak samples at different stages of preparation (before meat cutting, after meat cutting, and after cooking) and examined them for the following bacteriological criteria; APC, Coliforms MPN, Fecal coliforms MPN, *E. coli* MPN, *Staph. aureus*, and *Listeria monocytogenes* as shown in Table 2. The APC values were about 3.55, 4.23, and 3.55 $log_{10}$ at the sampling points, respectively. This indicates that handling of meat during cutting significantly added ($p<0.05$) about 0.68 log CFU of bacteria per g from operator handlers, cutting boards, and knives. (Ehuwa *et al*., 2021).

Despite the cooking step significantly reduced ($p<0.05$) the aerobic bacterial load by the same amount, this reduction rate might be not enough as about 3.55 $log_{10}$ CFU/g of bacteria were remaining in the ready-to-eat beef steak. A similar scenario was noticeable in the case of coliforms MPN and fecal coliforms MPN, additionally, the cooking step eliminate any coliforms including fecal ones from cooked beef steak. Concerning *Staph. aureus*, 100 % of examined beef steak samples at each sampling point (before cutting, after cutting, and after cooking) were contaminated with *Staph. aureus* (Table 2). As well, there was not a significant difference between the levels of *Staph. aureus* in the three stages of beef steak sampling, which confirms the improper cooking procedure or the post-cooking contamination by *Staph. aureus* from food handlers since human is the main source of this bacteria (Bencardino *et al*., 2021). On the contrary, we could not isolate either *E. coli* or *L. monocytogenes* from the examined beef steak samples at any stage of preparation (Table 2).

Another meat product always served in foodservice establishments in Egypt is the grilled beef burger was also investigated at different stages of preparation (Table 3). Similar to what was observed in beef steak, the step of meat mixing/chopping with ingredients significantly ($p<0.05$) increased APC, coliforms MPN, fecal coliforms MPN, and *Staph. aureus* counts in beef burger mix after mixing/chopping than in beef samples before chopping. This could be attributed to the contamination coming from unclean operator hands, mincing machines, and other contact surfaces, this is due to the food handlers do not always take accurate hygiene measures to prevent cross-contamination. It was expected that food-handling practices contribute to 40–60% of the cases of food-borne illness (De Jong *et al*., 2008; Ebert, 2018; Ulusoy *et al*., 2021).

In contrast, the grilling process significantly ($p<0.05$) eliminated the coliforms and fecal coliforms to zeros. While this cooking step reduced the APC and *Staph. aureus* by 1.37 and 2.56 $log_{10}$ CFU/g, respectively (Table 3). Inadequate cooking is one of the main factors causing foodborne illness (de Jong *et al*., 2012), and a big proportion, 40–60%, of foodborne illness outbreaks are originating from private households and catering (Cogan *et al*., 2002). This is partially caused by the consumption of improperly cooked meat. Most consumers and restaurants do not use a food thermometer but determine the doneness of meat most often by cutting the meat to evaluate changes in color and texture, or by other subjective techniques (Fischer *et al*., 2006). Similar to beef steak samples, *E. coli* and *L. monocytogenes* were absent from beef burger samples at all stages of sample collection. Similar

Table 2. Bacterial counts in beef steak samples collected from a food service establishment at different stages of preparation ($log_{10}$ CFU/g).

| Beef steak samples | APC | Coliforms MPN | Fecal coliforms MPN | *E. coli* MPN | *Staph. aureus* |
|---|---|---|---|---|---|
| Raw beef steak before cutting (n=20) | $3.55\pm2.10^{b}$ | $1.48\pm0.50^{b}$ | $0.46\pm0^{b}$ | 0±0 | $4.05\pm3.64^{a}$ |
| Raw beef steak after cutting (n=20) | $4.23\pm3.67^{a}$ | $2.59\pm1.97^{a}$ | $0.62\pm 0^{a}$ | 0±0 | $4.11\pm2.38^{a}$ |
| Beef steak after cooking (n=20) | $3.55\pm2.10^{b}$ | $0.06\pm0^{c}$ | $0\pm0^{c}$ | 0±0 | $4.08\pm2.19^{a}$ |

Data are represented by Mean ± standard errors.
Where, APC = Aerobic plate counts, MPN= most probable number
Different small letters superscripts (a, b, c) in the same column indicate significant differences between means at $P < 0.05$.

Table 3. Bacterial counts in beef burger samples collected from a food service establishment at different stages of preparation ($log_{10}$ CFU/g

| Beef burger samples | APC | Coliforms MPN | Fecal coliforms MPN | *E. coli* MPN | *Staph. aureus* |
|---|---|---|---|---|---|
| Raw beef before chopping (n=20) | $3.58\pm2.23^{b}$ | $1.35\pm0^{b}$ | $0.24\pm0.24^{b}$ | 0±0 | $3.80\pm2.72^{b}$ |
| Raw beef after mixing/chopping (n=20) | $3.91\pm2.76^{a}$ | $2.04\pm1.36^{a}$ | $1.53\pm0.83^{a}$ | 0±0 | $5.20\pm3.50^{a}$ |
| Beef burger after cooking (n=20) | $2.54\pm1.16^{c}$ | $0\pm0^{c}$ | $0\pm0^{c}$ | 0±0 | $2.64\pm1.68^{b}$ |

Data are represented by Mean ± standard errors.
Where, APC = Aerobic plate counts, MPN= most probable number
Different small letters superscripts (a, b, c) in the same column indicate significant differences between means at $P < 0.05$.

Table 4. Bacterial counts in swab samples from meat cutting tools and operator hands collected from a food service establishment before use/work ($log_{10}$ CFU/$cm^2$).

| Swab samples | APC | Coliforms MPN | Fecal coliforms MPN | *E. coli* MPN | *Staph. aureus* |
|---|---|---|---|---|---|
| Knives (n=20) | $1.89\pm2.16^{b}$ | $0\pm0^{c}$ | $0\pm0^{b}$ | $0\pm0^{b}$ | $2.16\pm1.57^{b}$ |
| Meat cutting boards (n=20) | $3.94\pm3.49^{a}$ | $1.52\pm0.76^{a}$ | $0\pm0^{b}$ | $0\pm0^{b}$ | $2.83\pm2.38^{b}$ |
| Operator hands (n=20) | $3.62\pm2.17^{a}$ | $0.91\pm0.36^{b}$ | $0.15\pm0^{a}$ | $0.57\pm0.27^{a}$ | $4.05\pm3.17^{a}$ |

Data are represented by Mean ± standard errors.
Where, APC = Aerobic plate counts, MPN= most probable number
Different small letters superscripts (a, b, c) in the same column indicate significant differences between means at $P < 0.05$.

results were previously reported by (Wehab and Hegazy, 2007) who did not isolate *E. coli* from raw beef steak samples. As well, a study by (Cagney *et al*., 2004) reported that unpackaged beef samples were free of *E. coli*.

In order to evaluate the steps of meat cutting in beef steak preparation and meat mincing in beef burger preparation as possible sources of meat contamination, cotton swab samples were collected from knives, cutting boards, and operator hands before their use or work. The swab samples were examined for the same bacteriological criteria as meat samples (Table 4). Cutting boards and operator's hands had significantly ($p<0.05$) higher levels of APC than knife surfaces. Additionally, cutting boards contained the highest values of coliforms MPN, followed by operator hands ($p<0.05$), whereas knives were free from coliforms.

Surprisingly, 13, 7, and 1 out of 20 swab samples from hands contained coliforms, fecal coliforms, and *E. coli*, respectively, while knives and cutting boards were free from fecal coliforms and *E. coli* (Table 4 and 5). This confirms the role of the operator's hands in adding extra contamination to meat during preparation. The presence of *E. coli* in hands of workers was similar to Ramadan (2015) who found *E. coli* in workers' hands and with high rates in frozen beef burger samples. The appearance of *E. coli* in hand swab samples may be due to contamination by water during hand washing or improper handwashing after using the toilet (Duffy *et al*., 2003).

Furthermore, contamination with *Staph. aureus* was reported in 100 % of knives and hand swab samples (20 out of 20 samples, each) and 50 % (10 out of 20) of cutting board swab samples (Tables 4 and 5). Similar contamination levels in beef burgers, beef meat, and minced meat with *Staph. aureus* was detected by Shaltout *et al*. (2017) and Abd El Tawab *et al*. (2015). Since human is the main reservoir of *Staph. aureus* (Seo and Bohach, 2012), the contamination of these cutting tools with *Staph. aureus* indicates the poor personal hygiene in this establishment. Conversely, we failed to isolate *L. monocytogenes* from all swab samples of knives, cutting boards, and operator hands (data are not shown).

In this regard, it was reported that carcass transportation to butchers' shops or food service establishments and further mincing/cutting steps under unsanitary conditions including poor cleaning and sanitizing and poor personal hygiene could increase the contamination level of beef steak and beef burger (Mrema *et al*., 2006).

In the current study, we isolated only one *E. coli* isolate, which was from a hand swab sample. To investigate the virulence of this isolate, it was subjected to molecular identification for the presence of *eae*A (Table 6, Fig. 1), which is a gene necessary for the characteristic intimate attachment of enteropathogenic *E. coli* to epithelial cells of the host (Donnenberg *et al*., 1993). The obtained results showed that the isolated *E. coli* strain from the operator's hands contained *eae*A, which is able to induce diarrhea, fever, and systemic and gastrointestinal disturbances in humans (Donnenberg *et al*., 1993). These findings indicate the poor personal hygiene of some food handlers in this food establishment, and their hands before work may be contaminated with enteropathogenic *E. coli* (EPEC) which can contaminate the food during preparation. Consequently, consumers eating in that food establishment may be at risk of exposure to foodborne illness.

Simultaneously, we examined the same isolate for the presence of *stx*1 gene (Table 6, Fig. 1), which is responsible for the production of Shiga toxins by Enterohemorrhagic *E. coli* (EHEC) (Tahamtan *et al*., 2010). *stx*1 gene was not found in this *E. coli* isolate, and this indicates that the isolate might not belong to the group of Shiga toxin-producing *E. coli* (STEC), since we did not investigate the presence of *stx*2 gene, this was not confirmed. EHEC is the most significant recently emerged group of foodborne pathogens and is more frequently detected worldwide. It

Table 5. Percentage of positive samples (%) based on bacteriological examination.

| Bacteriological criteria | Beef steak samples | | | Beef burger samples | | | Swab samples | | |
|---|---|---|---|---|---|---|---|---|---|
| | Before cutting (n=20) | After cutting (n=20) | After cooking (n=20) | Before chopping (n=20) | After chopping (n=20) | After cooking (n=20) | Knives (n=20) | Cutting boards (n=20) | Hands (n=20) |
| APC | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| Coliforms | 100 | 100 | 25 | 100 | 100 | 0 | 0 | 100 | 65 |
| Fecal coliforms | 35 | 80 | 10 | 35 | 100 | 0 | 0 | 0 | 35 |
| *E. coli* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5 |
| *Staph. aureus* | 100 | 100 | 100 | 100 | 100 | 100 | 100 | 50 | 100 |
| *L. monocytogenes* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Table 6. Incidence of virulence and antibiotic resistance-associated genes in isolated pathogens [*E. coli* (n=1) and *Staph. aureus* (n=6)].

| Isolated pathogen | Isolate number | Source | Targeted genes | |
|---|---|---|---|---|
| | | | *eae*A | *stx*1 |
| *E. coli* | 1 | Hand swabs | + | - |
| | Isolate number | Source | Targeted genes | |
| | | | *mec*A | *coa* |
| *Staph. aureus* | 1 | Hand swabs | + | + |
| | 2 | Hand swabs | + | + |
| | 3 | Hand swabs | + | + |
| | 4 | Beef steak after cooking | + | + |
| | 5 | Beef steak after cooking | - | + |
| | 6 | Beef steak after cooking | + | + |

can cause severe gastrointestinal disease, hemorrhagic colitis, and hemolytic uremic syndrome (HUS) including fatal infections (Fagan *et al*., 1999).

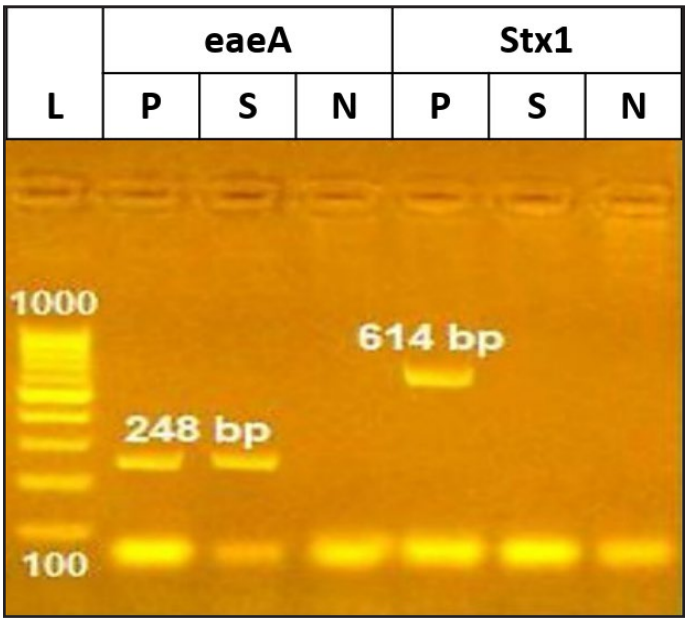


Fig. 1. Electrophoretic profile, in 1.5% agarose gel of polymerase chain reaction (PCR) products, of *stx*1 and *eae*A genes of an *E. coli* isolate from a hand swab sample. *stx*1 and *eae*A genes were detectable at 614 at 248 bp, respectively. L= ladder, P= positive control, S= Sample, N= negative control.

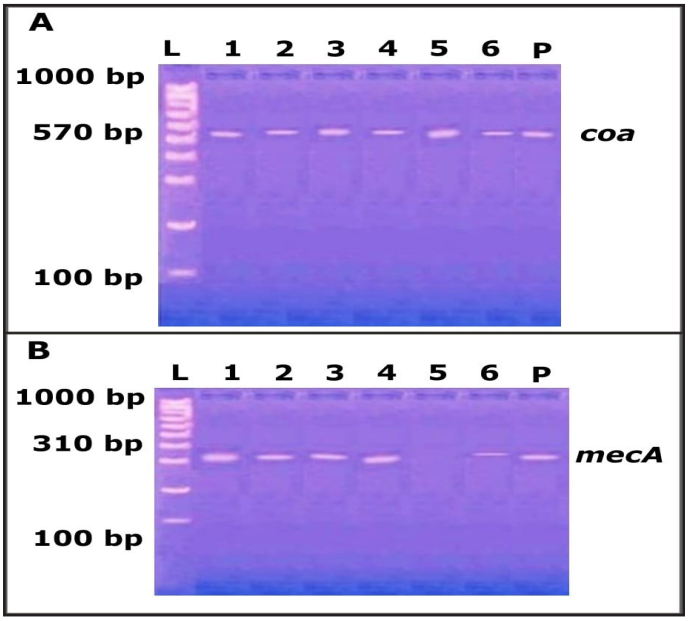


Fig. 2. Electrophoretic profile, in 1.5% agarose gel of polymerase chain reaction (PCR) products of (A) *coa* and (B) *mec*A genes of *Staph. aureus* isolates from hand swab samples and beef steak after cooking. *coa* and *mec*A genes were detectable at 310 and 570 bp, respectively. L= Ladder, 1 – 6 = samples, P= positive control.

Regarding *Staph. aureus* isolates, we selected 6 isolates for molecular identification, which were isolated from operator's hands (isolates 1 - 3) and from ready-to-eat beef steak (isolates 4 - 6), because of their high risk either due to their presence in food workers' hands or ready-to-eat products (Table 6, Fig. 2). The 6 *Staph. aureus* isolates were examined for the existence of *coa* gene, which is one of the main virulence factors and enables the bacteria to coagulate the mammalian plasma (Aarestrup *et al*., 1995). The obtained results presented that the 6 isolates contained *coa* gene (Fig. 1A). It was found that this gene enables *Staph. aureus* to generate abscess, persist in host tissue and resist opsonophagocytic clearance by the host (Chandrakanth *et al*., 2010; McAdow *et al*., 2012). Therefore, it indicates the high pathogenicity of these isolates to consumers.

Another important virulence factor in any pathogen is its antibiotic resistance abilities, therefore, we also identified *Staph. aureus* isolates for the presence of *mec*A gene, which is a characteristic of methicillin resistance in *Staph. aureus* (MRSA) (Wielders *et al*., 2002). The results displayed that 5 out of 6 *Staph. aureus* isolates had *mec*A gene that enables these *Staph. aureus* strains to resist the effect of various antibiotics, especially methicillin (Table 5, Fig. 2B). *Staph. aureus* strains resistant to methicillin and many other antibiotics are major causes of nosocomial infections worldwide. Methicillin resistance is determined by the *mec*A gene, which encodes the low-affinity penicillin-binding protein PBP 2A (Beck *et al*., 1986). The spreading of antimicrobial resistance among foodborne pathogens leads to an increase in the clinical cases of resistant infection (Abdaslam *et al*., 2014).

## CONCLUSION

The handling of beef steak and beef burgers during meat cutting and mincing in a food serving establishment could be an important source of contamination of the end ready-to-eat product. Additionally, the cooking step might not be applied thoroughly, which can leave a high level of bacterial load in the finished product. This risk is maximized if there is contamination with foodborne pathogens such as *E. coli* and *Staph. aureus*. Another important risk that was noticeable, is the improper cleaning and sanitizing and poor personal hygiene, as we found that swab samples from knives, cutting boards, and operator's hands were contaminated with bacteria including coliforms, fecal coliforms, *E. coli*, and *Staph. aureus*. Furthermore, the only *E. coli* isolate from a worker's hand contained *eae*A gene, but not *stx*1 gene, which indicates that it is an EPEC strain. Moreover, 6 and 5 *Staph. aureus* isolates from operator hands and beef steak after cooking had *coa* and *mec*A genes, respectively, which are responsible for mammalian plasma coagulation and methicillin resistance, respectively. In conclusion, this foodservice establishment applies poor food safety measures, practices inadequate cleaning and sanitizing, and food handlers and workers behave poor personal hygiene, accordingly, it should be faced with a high level of concern and consideration by the legal authorities.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest in this study.

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