Occurrence of Enterobacteriaceae in Raw Meat and in Human Samples from Egyptian Retail Sellers

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The present study was performed to assess the presence of Enterobacteriaceae in raw meat and handlers in Egypt using cultivation and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS). A total of 100 raw meat samples (chicken and beef meat, 50 each) were randomly purchased from butchers and local meat retailers located at Mansoura city, Egypt. Fifty human samples were collected from meat handlers (hand swabs and stool specimens, 25 each). 228 bacterial isolates were recovered from these samples. Unidentified isolates were characterized by partial 16S rRNA gene sequencing. Escherichia coli isolates were further typed using a DNA microarray system. Proteus spp. (60.0%) were found to be the most abundant followed by Escherichia coli (38.7%), Klebsiella spp. (17.3%), and Citrobacter spp. (13.3%). The presence of different Enterobacteriaceae in locally produced retail raw meat demonstrates the risk of infection of people through consumption of raw or undercooked meat and the risk for cross-contamination of other food products. Harmonized and concerted actions from veterinary and public health authorities are needed to reduce the risk of infection.

1. Introduction

Most of the pathogens that play a role in foodborne diseases are of animal origin [1]. Foodborne diseases pose a serious threat to the health of people in Africa and cause huge economic losses [2]. Up to one-third of the population in developing countries is affected by foodborne diseases each year. It is assumed that foodborne and waterborne diarrheal diseases kill more than 2.2 million people each year [3]. A major problem in food hygiene is the fecal contamination of beef and chicken meat with Enterobacteriaceae such as Salmonella spp., Escherichia (E.) coli, Proteus (P.), and Klebsiella (K.) species [4, 5]. To minimize the prevalence of foodborne diseases and to reduce the microbial contamination of food, effective monitoring of the occurrence and reliable identification of zoonotic bacterial pathogens in food is essential. Currently, routine detection of foodborne pathogens relies on cultivation and biochemical identification [6]. These methods are laborious and time-consuming, and may lead to false identifications [7]. In recent years, MALDI-TOF MS has been implemented in microbiological routine laboratories for broad-spectrum identification of bacteria [8–11]. The present study was performed to assess the presence of Enterobacteriaceae in raw meat and handlers in Egypt using cultivation of bacteria and MALDI-TOF MS. When MALDI-TOF MS lead to doubtful results, partial sequencing of 16S rRNA genes was used to verify the identification. Furthermore, E. coli isolates were characterized by microarray analysis.

2. Material and Methods

2.1. Sample Collection. The study was conducted between October 2012 and May 2013. A total of 100 fresh raw meat samples (chicken and beef meat, 50 each) were randomly purchased from butchers and local meat retailers located in Mansoura city, Egypt. In addition, 50 human samples (hand
swabs and stool specimens from meat handlers, 25 each) were collected. The samples were placed on ice and transported immediately to the Hygiene and Zoonoses laboratory, Faculty of Veterinary Medicine, Mansoura University, Egypt. An informed consent was obtained from all persons involved in this study.

2.2. Sample Preparation. Twenty-five grams of each raw beef or chicken meat was transferred to a blender bag and homogenized with 225 mL of 0.1% buffered peptone water (BPW; Oxoid, Wesel, Germany). Pre-enrichment was done for 24 h at 37°C. A loop full of the enriched broth was streaked on MacConkey agar plates (Oxoid) and Eosin Methylene Blue Lactose plates (Oxoid) and incubated at 37°C for 24 h. For human samples, hand swabs and stool specimens were directly inserted into sterile tubes containing 10 mL BPW under aseptic conditions and incubated at 37°C for 24 h. Then, the samples were cultivated as described previously.

2.3. Bacterial Identification. Colonies were picked and streaked onto nutrient agar plates, incubated at 37°C for 18–24 h and then stored at −20°C as glycerol cultures until shipping to the Friedrich-Loeffler-Institut, Institute of Bacterial Infections and Zoonoses, Jena, Germany. In Germany, the bacterial isolates were identified using MALDI-TOF MS as described by Karger et al. [12]. Briefly, bacteria were cultivated on Columbia sheep blood agar at 37°C for 24 h. Single colonies were picked, suspended in 300 μL of water, and precipitated by addition of 900 μL of ethanol p.a. (Carl Roth GmbH, Karlsruhe, Germany). Samples were centrifuged at 10,000 g for two minutes. The supernatant was carefully removed and the sediment was resuspended in 50 μL of 70% (v/v) formic acid (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany). After mixing with 50 μL acetonitrile (Carl Roth GmbH), the suspension was centrifuged as described above and the supernatant was transferred into a fresh tube. One μL of the supernatant was spotted two times onto a MALDI target plate (polished steel MTP 384 plate, Bruker Daltonik GmbH, Bremen, Germany) and allowed to dry at room temperature. Finally, the dried spots were overlaid with one μL of matrix, which was a saturated solution of α-cyano-4-hydroxycinnamic acid (Sigma-Aldrich Chemie GmbH) in 50% acetonitrile and 2.5% trifluoroacetic acid (Sigma-Aldrich Chemie GmbH). As soon as the samples were air-dried-measurement was started within 10 min.

Spectra were acquired with 300 laser shots with an Ultraflex I instrument (Bruker Daltonik GmbH) in the linear positive mode in the range of 2,000 to 20,000 Da. Acceleration voltage was 25 kV and the instrument was calibrated in the range between 3,637.8 and 16,952.3 Da using the IVD Bacterial Test Standard Calibrant (BTS; Bruker Daltonik GmbH). For species identification, the BioTyper database 3.0 (Bruker Daltonik GmbH) was used. An identification (ID) score >2.30 is regarded a highly probable species identification; scores 2.0–2.29 indicate secure genus and probable species identification; scores 1.70–1.99 allow probable genus identification and lower scores provide no reliable results (Table 3). Unidentified bacteria and bacteria with a score <2.0 were identified using partial 16S rRNA gene sequencing.

2.4. DNA Extraction and Partial 16S rRNA Gene Sequencing. Isolation of DNA was carried out with a High Pure PCR Template Preparation Kit (Roche Diagnostics, Mannheim, Germany) according to the manufacturer’s instructions. Partial 16S rRNA genes of unidentified bacterial isolates were amplified by PCR with 16SUNI-L (5’−AGA GTT TGA TGG CTC AG-3’) as the forward primer and 16SUNI-R (5’−GTG TTA CCG GCG TTG TGT AC-3’) as the reverse primer (Jena Bioscience GmbH, Jena, Germany) to generate approximately 1,400-bp ampiclon as published by Kuhnert et al. [13]. PCR products were analyzed by agarose gel electrophoresis, ethidium bromide staining, and visualization under UV light. Bands were cut out, and DNA was purified using a Gel Extraction Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations. Cycle sequencing of partial 16S rRNA genes was done in both directions by using forward and reverse amplification primers with a BigDye Terminator Version 1.1 Cycle Sequencing Kit (Applied Biosystems, Darmstadt, Germany) according to the recommendations of the manufacturer. Sequencing products were analyzed with an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Identification of isolates was done by a BLAST search (http://www.ncbi.nlm.nih.gov/blast/) using 16S rRNA gene sequences.

2.5. Genotype Characterization by E. coli Genotyping Microarray. Sixteen E. coli isolates were selected for the genotype characterization using a DNA microarray system. DNA was extracted as described above. DNA concentration was determined spectrophotometrically. Miniaturized E. coli oligonucleotide arrays in the ArrayStrip format (Alere Technologies GmbH, Jena, Germany), E. coli Genotyping Kit (Kit for DNA-based detection of virulence genes in E. coli isolates, Cat. number 205400050) containing gene targets for the identification of virulence genes [14], antimicrobial resistance genes [15], and DNA-based serotyping [16] were used for the genetic characterization of the E. coli isolates.

For labeling and biotinylation of the genomic DNA, a site-specific labeling approach was used as published by Monecke and Ehrlich [17]. Primer elongation, hybridization, washing, and staining of array strips were described previously by Geue et al. [18]. The array strips were photographed using an Array Mate instrument (Alere Technologies GmbH) and automatically analyzed. After automated spot detection, mean signal intensity (mean) and local background (lbg) were measured for each probe position and values were calculated by the formula value = 1−mean/lbg. Resulting values below 0.1 were considered negative and above 0.3 were considered positive. Values between 0.1 and 0.3 were regarded as ambiguous. Validation was performed using a collection of sequenced control strains (GeneBank Accession numbers AE005174 (E. coli EDL933 O157:H7), FM180568 (E. coli E2348/69 O127:H6), U00096 (E. coli K-12 MG1655), AP009048 (E. coli K-12 W3110), CP000247 (E. coli O6:K15:H31), CP001509
Table 1: Identification of Enterobacteriaceae in meat and meat handler samples by MALDI-TOF MS.

| Identified microorganism          | Raw beef meat $(n = 50)$ | % | Raw chicken meat $(n = 50)$ | % | Total | % | Stool specimens $(n = 25)$ | % | Hand swabs $(n = 25)$ | % | Total | % |
|----------------------------------|--------------------------|---|----------------------------|---|-------|---|--------------------------|---|--------------------------|---|-------|---|
| Citrobacter spp.                 | 12                       | 24.0 | 1                         | 2.0 | 13    | 13.0 | 4                         | 16.0 | 3                        | 12.0 | 7     | 14.0 |
| C. amalonaticus                  | 0                        | 0    |                           | 0   | 0     | 0   | 1                         | 4.0  | 1                        | 4.0  | 2     | 8.0 |
| C. freundii                      | 5                        | 10.0 | 1                         | 2.0 | 6     | 6.0  | 3                         | 12.0 | 1                        | 4.0  | 4     | 8.0 |
| C. braakii                       | 3                        | 6.0  | 0                         | 0   | 3     | 3.0  | 1                         | 4.0  | 1                        | 4.0  | 2     | 8.0 |
| C. koseri                        | 3                        | 6.0  | 0                         | 0   | 3     | 3.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| C. youngae                       | 1                        | 2.0  | 0                         | 0   | 1     | 1.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| Enterobacter cloacae             | 0                        | 0    |                           | 0   | 0     | 0   | 2                         | 8.0  | 0                        | 0    | 2     | 4.0 |
| Escherichia coli                 | 27                       | 54.0 | 8                         | 16.0 | 35    | 35.0 | 17                        | 68.0 | 6                        | 24.0 | 23    | 46.0 |
| Klebsiella spp.                  | 3                        | 6.0  | 13                        | 26.0 | 16    | 16.0 | 1                         | 4.0  | 9                        | 36.0 | 10    | 20.0 |
| K. pneumoniae                    | 3                        | 6.0  | 11                        | 22.0 | 14    | 14.0 | 0                         | 0    | 6                        | 24.0 | 6     | 12   |
| K. oxytoca                       | 0                        | 0    | 2                         | 4.0  | 2     | 2.0  | 1                         | 4.0  | 3                        | 12.0 | 4     | 8.0 |
| Morganella morganii              | 16                       | 32.0 | 2                         | 4.0  | 18    | 18.0 | 0                         | 0    | 2                        | 8.0  | 2     | 4.0 |
| Proteus spp.                     | 29                       | 58.0 | 39                        | 78.0 | 68    | 68.0 | 15                        | 60.0 | 7                        | 28.0 | 22    | 44.0 |
| P. vulgaris                      | 22                       | 44.0 | 0                         | 0    | 22    | 22.0 | 4                         | 16.0 | 0                        | 0    | 4     | 8.0 |
| P. mirabilis                     | 6                        | 12.0 | 39                        | 78.0 | 45    | 45.0 | 11                        | 44.0 | 7                        | 28.0 | 18    | 36.0 |
| P. penneri                       | 1                        | 2.0  | 0                         | 0    | 1     | 1.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| Providencia spp.                 | 1                        | 2.0  | 1                         | 2.0  | 2     | 2.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| Providencia stuartii             | 0                        | 0    | 1                         | 2.0  | 1     | 1.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| Raoultella spp.                  | 5                        | 10.0 | 2                         | 4.0  | 7     | 7.0  | 0                         | 0    | 3                        | 12.0 | 3     | 6.0 |
| R. planticola                    | 5                        | 10.0 | 0                         | 0    | 5     | 5.0  | 0                         | 0    | 1                        | 4.0  | 1     | 2.0 |
| R. ornithinolytica               | 0                        | 0    | 2                         | 4.0  | 2     | 2.0  | 0                         | 0    | 2                        | 8.0  | 2     | 4.0 |
| Serratia liquefaciens            | 1                        | 2.0  | 0                         | 0    | 1     | 1.0  | 0                         | 0    | 0                        | 0    | 0     | 0    |
| Total number of isolates         | 94                       | 66   | 160                       | 39   | 29    | 29   | 68                        | 39   |

(E. coli BL21), AE014075 (E. coli CFT073), and CP000946 (E. coli ATCC 8739)).

3. Results and Discussion

Rapid, accurate, and reliable detection and identification of bacterial foodborne pathogens are critical for food safety. The gold standard is bacterial isolation followed by microscopic and biochemical identifications, which is time-consuming and laborious [6].

In recent years, MALDI-TOF MS has been introduced in microbiological routine laboratories, because it provides results within only a few hours. The instruments are still expensive, but reagent costs are low, and identification of bacteria can be largely automated. The bacteria are identified by comparing the obtained mass spectra to those from a reference library [8]. Limitations have been observed for bacteria that require special sample preparation and some very closely related species. The following bacteria were identified only by partial 16S rRNA gene sequencing: Staphylococcus sciuri, Lysinibacillus spp., and Macrococcus caseolyticus.

The highest number of strains was isolated from raw beef, followed by raw chicken meat, seller stool specimens, and hand swabs from sellers. The high number of Proteus isolates from chicken meat (78.0%) and beef meat (58.0%) was remarkable. The presence of Proteus spp. in the meat samples can obviously be attributed to unhygienic food processing. Proteus spp. were isolated and identified by researchers from raw meat and its products in other studies in Egypt [19–23]. Twenty-seven bacterial isolates out of 50 (54.0%) and 8 out of 50 (16.0%) samples of raw beef and chicken meat, respectively, were identified as E. coli. In several studies, E. coli was isolated in a high percentage from raw meat and unprocessed ready-to-eat products [24–30]. Contamination may occur due to bowel rupture or use of contaminated water during evisceration and slaughtering [31, 32]. However, neither MALDI-TOF MS analyses nor 16S rRNA gene sequencing allows differentiation between Shigella spp. and E. coli. A total of 16 presumptively identified E. coli isolates were characterized for the genoserotypes, E. coli virulence associated genes, and antibiotic resistance genes using a DNA microarray. A complete DNA-based assigned serotype was determined only in five E. coli isolates. Only serotype O103:H7 could be characterized completely, because the number of O antigens detectable by this method is currently limited. In 10 other isolates, fliC genes for H2 (5 isolates), H49 (found twice), and H19, H31, or H38 were detected, respectively. In one isolate, neither the O-antigen nor the fliC gene could be found (Table 2). From 68.0% of the stool samples and 24.0% of the hand swabs of meat sellers, E. coli was isolated (Table 1). This high prevalence could
be attributed to inadequate sanitary conditions and poor general hygiene. Stephan et al. [33] detected *E. coli* verotoxin encoding genes in 3.5% of healthy employees in the meat industry. Numerous *E. coli* virulence associated markers were tested using the oligonucleotide microarrays. The *stx2* gene was found in one of the 16 isolates only. The isolates were characterized as *stx2a* or *stx2c* or *stx2d* subtypes in accordance with the nomenclature published by Lewis [34]. The microarray does not allow more differentiated subtyping. The *ehxA* gene encoding the EHEC hemolysin was detected in the same isolate. The plasmid encoded virulence genes *esp P* (encoding for a serine protease), *saa* (encoding for the STEC autoagglutinating adhesin), *subA* (encoding for a subtilase cytotoxin), and *iss* (increased serum resistance) were also demonstrated in the same isolate. However, intimin genes were not found. A non-LEE-encoded effector protein gene (*espI*) and the gene for enterobactin siderophore receptor/adhesin (*iha*) were also obtained (Table 2). In the other *E. coli* isolates, the genes for fimbria adhesion (*lpfA*), 14 isolates and *iss* (increased serum resistance, 7 isolates) were demonstrated frequently. The gene for a glutamate-1-semialdehyde aminotransferase (*hemL*, 5 isolates), the *tsh* gene (encoding for a hemoglobin binding protein, 5 isolates), and the gene for an outer membrane siderophore receptor (*iroN*, 4 isolates) were found rarely. The *cba* gene (encoding for a bacteriocin, 2 isolates), the *cdtB* gene (also encoding for a colicin, 2 isolates), the *cdtB* gene (encoding for a cytotoxicid distending toxin, 3 isolates), and the *cnf1* gene (encoding for a cytotoxic necrotizing factor type 1, 3 isolates) were amplified in some isolates. Additional genes for major fimbrial subunit proteins (*flj7-A* and *flj7-G*) were found in 3 isolates. It is noteworthy that isolates belonging to the same serotype were nearly identical regarding the virulence markers (Table 2).

| Number | Serotype* | Virulence marker | Resistance marker |
|--------|-----------|------------------|-------------------|
| 1      | O103:H7   | *hemL*, *lpfA*, *tsh*, *iroN*, *iss* |                    |
| 2      | On.d.:H19 | *blaTEM*         |                    |
| 3      | O103:H7   | *hemL*, *lpfA*, *tsh*, *iroN*, *iss* |                    |
| 4      | O103:H7   | *lpfA*, *tsh*, *iroN*, *iss* |                    |
| 5      | On.d.:H38 | *stx2* (subtype 2a or 2c or 2d), *ehxA*, *espI*, *espP*, *saa*, *iha*, *subA*, *iss* |                    |
| 6      | On.d.:H2  | *cba*, *cdtB*, *cma*, *cnf1*, *flj7-G*, *lpfA*, *iroN*, *iss* |                    |
| 7      | On.d.:H31 | *lpfA*, *cdtB*, *cnf1*, *flj7-A*, *flj7-G* |                    |
| 8      | O103:H7   | *hemL*, *lpfA*, *tsh*, *iroN*, *iss* | *tetA*             |
| 9      | On.d.:H49 | *hemL*, *lpfA* |                    |
| 10     | On.d.:H2  | *lpfA*, *cba*, *cdtB*, *cma*, *cnf1*, *flj7-G*, *iro*, *iss* |                    |
| 11     | O103:H7   | *hemL*, *lpfA*, *tsh*, *iroN*, *iss* | *tetA*             |
| 12     | On.d.:Hn.d.| *lpfA* | *blaTEM* |
| 13     | On.d.:H49 | *lpfA* |                    |
| 14     | On.d.:H2  | *lpfA* |                    |
| 15     | On.d.:H2  | *lpfA* |                    |
| 16     | On.d.:H2  | *hemL*, *lpfA* | *blaMOX-CMY9*, *blaOXA*, *blaVIM* |

* n.d.: not detectable by microarray.

In conclusion, our findings clearly demonstrate that different *Enterobacteriaceae* species are common in retail meat. Insufficient awareness about foodborne zoonoses could...
Table 3: Identification scores for bacteria isolates obtained by Biotyper software (Bruker Daltonik GmbH) for MALDI-TOF MS results. An identification (ID) score >2.30 is regarded as a highly probable species identification; scores 2.0–2.29 indicate secure genus and probable species identification; scores 1.70–1.99 allow probable genus identification and lower scores provide no reliable results.

| Bacterial species             | Number of strains | ID score 1.70–1.99 | ID score 2.0–2.29 | ID score >2.30 |
|-------------------------------|-------------------|--------------------|-------------------|----------------|
| Citrobacter amalonaticus      | 1                 | 1                  | 1                 | 1              |
| Citrobacter braakii           | 7                 |                    | 7                 |                |
| Citrobacter freundii          | 8                 | 1                  | 1                 | 7              |
| Citrobacter koseri            | 3                 |                    | 3                 |                |
| Citrobacter youngae           | 3                 | 1                  | 1                 | 2              |
| Comamonas kerstersii          | 1                 |                    | 1                 |                |
| Enterobacter asburiae         | 2                 | 1                  | 1                 |                |
| Enterobacter cloacae          | 5                 | 2                  | 3                 |                |
| Enterococcus faecalis         | 11                | 1                  | 10                |                |
| Enterococcus faecium          | 3                 |                    | 2                 |                |
| Enterococcus raffinosus       | 1                 |                    | 1                 |                |
| Escherichia coli              | 38                | 11                 | 27                |                |
| Hafnia alvei                  | 2                 |                    | 2                 |                |
| Klebsiella oxytoca            | 2                 | 1                  | 1                 |                |
| Klebsiella pneumoniae         | 17                | 10                 | 7                 |                |
| Klebsiella variicola          | 2                 |                    | 2                 |                |
| Macrococcus caseolyticus      | 4                 | 1                  | 3                 |                |
| Morganella morganii           | 17                |                    | 17                |                |
| Proteus mirabilis             | 40                | 4                  | 36                |                |
| Proteus penneri               | 6                 | 2                  | 4                 |                |
| Proteus vulgaris              | 45                |                    | 9                 | 36             |
| Providencia rettgeri          | 1                 |                    | 1                 |                |
| Providencia stuartii          | 1                 |                    | 1                 |                |
| Pseudomonas putida            | 1                 |                    | 1                 |                |
| Pseudomonas aeruginosa        | 5                 |                    | 5                 |                |
| Raoultella ornithinolytica    | 6                 | 1                  | 5                 |                |
| Serratia liquefaciens         | 1                 |                    | 1                 |                |
| Staphylococcus aureus         | 4                 | 2                  | 1                 | 1              |
| Viridibacillus                | 1                 |                    | 1                 |                |
| Unidentified bacteria         | 4                 |                    | 4                 |                |
| **Total**                     | **242**           | **6**              | **57**            | **175**        |

endanger both retail sellers and consumers. Education of the traditional meat retailer’s community in Egypt in terms of the importance of hygienic and sanitary precautions would be an important step towards safer food.

**Conflict of Interests**

The authors declare that they have no conflict of interests.

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**References**

[1] L. Busani, G. Scavia, I. Luzzi, and A. Caprioli, “Laboratory surveillance for prevention and control of foodborne zoonoses,” *Annali dell’Istituto Superiore di Sanità*, vol. 42, pp. 401–404, 2006.

[2] A. A. Gajadhar and R. J. Allen, “Factors contributing to the public health and economic importance of waterborne zoonotic parasites,” *Veterinary Parasitology*, vol. 126, pp. 3–14, 2004.

[3] FAO/WHO, “Food safety risk analysis. A guide for national food safety authorities,” Food and Nutrition Paper 87, FAO, Rome, Italy, WHO, Geneva, Switzerland, 2006.

[4] C. Zhao, B. Ge, J. de Villena et al., “Prevalence of *Campylobacter* spp., *Escherichia coli*, and *Salmonella* serovars in retail chicken, turkey, pork, and beef from the Greater Washington, D.C., area,” *Applied and Environmental Microbiology*, vol. 67, no. 12, pp. 5431–5436, 2001.

[5] D. L. Paterson, “Resistance in Gram-negative bacteria: *Enterobacteriaceae*,” *The American Journal of Medicine*, vol. 119, no. 6, supplement 1, pp. S20–S28, 2006.

[6] K. C. Carroll and M. P. Weinstein, “Manual and automated systems for detection and identification of microorganisms,” in *Manual of Clinical Microbiology*, P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfäffler, Eds., pp. 192–217, ASM, Washington, DC, USA, 9th edition, 2007.

[7] I. Abubakar, L. Irvine, C. F. Aldus et al., “A systematic review of the clinical, public health and cost-effectiveness of rapid diagnostic tests for the detection and identification of bacterial intestinal pathogens in faeces and food,” *Health Technology Assessment*, vol. 11, no. 36, pp. 1–216, 2007.

[8] A. Bizzini and G. Greub, “Matrix-assisted laser desorption ionization time-of-flight mass spectrometry, a revolution in clinical microbiological identification,” *Clinical Microbiology and Infection*, vol. 16, no. 11, pp. 1614–1619, 2010.

[9] K. Sogawa, M. Watanabe, K. Sato et al., “Use of the MALDI Biotyper system with MALDI–TOF mass spectrometry for rapid identification of microorganisms,” *Analytical and Bioanalytical Chemistry*, vol. 400, no. 7, pp. 1905–1911, 2011.

[10] A. Croxatto, G. Prod’Hom, and G. Greub, “Applications of MALDI-TOF mass spectrometry in clinical diagnostic microbiology,” *FEMS Microbiology Reviews*, vol. 36, pp. 380–407, 2012.

[11] APHA, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, DC, USA, 3rd edition, 1992.

[12] A. Karger, M. Ziller, B. Bettin, B. Mintel, S. Schares, and L. Geue, “Determination of serotypes of Shiga toxin-producing *Escherichia coli* isolates by intact cell matrix-assisted laser desorption ionization-time of flight mass spectrometry,” *Applied and Environmental Microbiology*, vol. 77, no. 3, pp. 896–905, 2011.

[13] P. Kuhnert, S. E. Capaul, J. Nicote, and J. Frey, “Phylogenetic positions of *Clostridium chauvoei* and *Clostridium septicum* based on 16S rRNA gene sequences,” *International Journal of Systematic Bacteriology*, vol. 46, no. 4, pp. 1174–1176, 1996.

[14] M. E. Anjum, M. Mafoud, P. Slickers et al., “Pathotyping *Escherichia coli* by using miniaturised DNA microarrays,” *Applied and Environmental Microbiology*, vol. 73, pp. 5692–5697, 2007.
[15] M. Batchelor, K. L. Hopkins, E. Liebana et al., “Development of a miniaturized microarray-based assay for the rapid identification of antimicrobial resistance genes in Gram-negative bacteria,” International Journal of Antimicrobial Agents, vol. 31, pp. 440–451, 2008.

[16] K. Ballmer, B. M. Korczak, P. Kuhnert, P. Slickers, R. Ehricht, and H. Hächler, “Fast DNA serotyping of Escherichia coli by use of an oligonucleotide microarray,” Journal of Clinical Microbiology, vol. 45, no. 2, pp. 370–379, 2007.

[17] S. Monecke and R. Ehricht, “Rapid genotyping of methicillin-resistant Staphylococcus aureus (MRSA) isolates using miniaturised oligonucleotide arrays,” Clinical Microbiology and Infection, vol. 11, no. 10, pp. 825–833, 2005.

[18] L. Geue, S. Scharer, B. Mintel, F. J. Conraths, E. Müller, and R. Ehricht, “Rapid microarray-based genotyping of enterohemorrhagic Escherichia coli serotype O156:H25/H- Hnt isolates from cattle and clonal relationship analysis,” Applied and Environmental Microbiology, vol. 76, pp. 5510–5519, 2010.

[19] M. A. M. Ammar, “Spoilage and pathogenic microorganisms in traditional meat products in Assuit [Ph.D. thesis], Assiut University, Assiut, Egypt, 2005.

[20] M. F. Al-Mutairi, “The incidence of Enterobacteriaceae causing food poisoning in some meat products,” Advanced Journal of Food Science and Technology, vol. 3, pp. 116–121, 2011.

[21] A. Von Holy, W. H. Holzapfel, and G. A. Dykes, “Bacterial populations associated with Vienna sausage packaging,” Food Microbiology, vol. 9, no. 1, pp. 45–53, 1992.

[22] C. O. Gill, “Sources of bacterial contamination at slaughtering plants,” in Improving the Safety of Fresh Meat, J. N. Sofos, Ed., pp. 231–243, CRC/Woodhead Publishing, Cambridge, UK, 2005.

[23] I. S. Gibbons, A. Adesiyun, N. Seepersadsingh, and S. Rahaman, “Investigation for possible source(s) of contamination of ready-to-eat meat products with Listeria spp. and other pathogens in a meat processing plant in Trinidad,” Food Microbiology, vol. 23, no. 4, pp. 359–366, 2006.

[24] H. S. Hussein, “Prevalence and pathogenicity of Shiga toxin-producing Escherichia coli in beef cattle and their products,” Journal of Animal Science, vol. 85, pp. E63–E72, 2007.

[25] G. J. E. Nychas, P. N. Skandamis, C. C. Tassou, and K. P. Koutsoumanis, “Meat spoilage during distribution,” Meat Science, vol. 78, pp. 77–89, 2008.

[26] H. R. Tavakol and M. Riazipour, “Microbial quality of cooked meat foods in Tehran University’s restaurants,” Pakistan Journal of Medical Sciences, vol. 24, no. 4, pp. 595–599, 2008.

[27] F. A. Elnawawi, O. A. Attala, and S. Saleh, “Enteropathogens of public health importance in imported frozen meat and chicken,” International Journal of Microbiology Research, vol. 3, pp. 59–63, 2012.

[28] P. S. Mead, L. Slutsker, V. Dietz et al., “Food-related illness and death in the United States,” Emerging Infectious Diseases, vol. 5, pp. 607–625, 1999.

[29] C. M. Schroeder, D. G. White, and J. Meng, “Retail meat and poultry as a reservoir of antimicrobial-resistant Escherichia coli,” Food Microbiology, vol. 21, pp. 249–255, 2004.

[30] T. S. El-Khateib, Sanitary condition of sausage in Assiut [M.S. thesis], Assiut University, Assiut, Egypt, 1982.

[31] F. Scheutz, L. D. Teel, L. Beutin et al., “Multicenter evaluation of a sequence-based protocol for subtyping Shiga toxins and standardizing Stx nomenclature,” Journal of Clinical Microbiology, vol. 50, pp. 2951–2963, 2012.