Phenotypic characterization of some food poisoning bacteria isolated from meat and meat products in Kaliobia, Egypt

Ashraf A. Abd El-Tawab1, Ahmed A. A. Maarouf2, Fatma I. El Hofy1, Nesma M. G. Ahmed2

1 Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Benha University
2 Department of Bacteriology, Animal Health Research Institute, Benha Branch, Egypt

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ABSTRACT

The present study was performed on 250 random samples of fresh meat and meat products. Beef burger, kofta, minced meat and sausage (50 each) were collected from different shops (25 gm of each sample) at Kaliobia Governorate, Egypt, to detect the prevalence of some toxigenic food-borne bacteria, besides the phenotypic characterization and detection of some virulence genes. Bacteriological examination of the collected samples resulted in an isolation of Staph. aureus isolates (41/16.4%), E. coli (25/10.0%), B. cereus (21/8.4%) and Salmonella (3/1.2%). The antibiotic sensitivity tests for the isolated strains showed multiple antibiotic resistances (ampicillin, methicillin, oxytetracycline, amoxicillin, streptomycin, erythromycin, doxycycline and cotrimoxazole). Therefore, E. coli, Staph. aureus and B. cereus strains especially antibiotic resistances ones are meat-borne pathogens of public health important.

1. INTRODUCTION

Meat and meat products are important sources of easily digestible proteins and other nutrients for humans and considered an ideal culture medium for many microorganisms, especially toxigenic ones like E. coli, Staph. aureus, Salmonellae and B. cereus and that have been linked to major outbreaks of food poisoning, illness and death all over the world (Hamed et al., 2015; Zafar et al., 2016).

Escherichia coli is one of the most important toxigenic bacteria and associated with numerous disease problems from contaminating meat (Datta et al., 2012). It is commonly non-virulent, but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. Pathogenic E. coli strains have been broadly classified into two major categories; extraintestinal pathogenic and diarrheagenic E. coli which classified into six categories including Enteropathogenic E. coli (EPEC), Entero-toxigenic E. coli (ETEC), Enteroinvasive E. coli (EIEC), Enteroaggregative E. coli (EAEC), diffusively adherent E. coli (DAEC) and Enterohemorrhagic E. coli (EHEC)/Shiga toxin-producing E. coli (STEC) (Monaghan et al., 2011).

Staphylococcus aureus is considered an important foodborne disease worldwide due to its ability to produce wide arrays of toxins (Argudin et al., 2010). Staph. aureus main character is the production of heat-stable enterotoxins cause food intoxications. Currently, 20 Staphylococcal enterotoxins (SEs) are known: 5 classical and 15 newly described (Ono et al., 2008).

The enterotoxigenic B. cereus strains, produce haemolysis, phospholipases c and enterotoxins resulting in food-borne diseases with emetic and diarrheal syndromes (Abostate et al., 2006). Salmonella is a food-borne pathogen contaminating food and water. It causes severe acute gastroenteritis and typhoid fever (Vehlner, 2016).

Antimicrobial resistance (AMR) is a major global issue for human and animals due to improper use of antibiotics in food animals (Saud et al., 2019; Messele et al., 2017). The emergence of antimicrobial resistance among E. coli, Staph. aureus, Salmonella and B. cereus strains of animal origin has important public health implications. Several studies showed that drug-resistant of E. coli, Staph. aureus, Salmonella and B. cereus strains infections in human were caused by strains from animals and that those infectious agents harbored the same mobile resistance genes as were found in diverse bacterial species from a variety of animal sources (Jackson, 2013).

As the level of contamination of meat and its products with different food-borne pathogens cause serious problems for consumers, so, the present study was conducted to throw light over the bacterial status of meat and common meat products (beef burger, kofta, minced meat and sausage) at Kaliobia Governorate, Egypt.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 250 random samples from fresh meat and meat products. Beef burger, kofta, minced meat and sausage (50 for each) were collected from different shops (25 gm of each sample) at Kaliobia Governorate, Egypt.

* Corresponding author: Nesma M. G. Ahmed, Department of Bacteriology, Animal Health Research Institute, Benha Branch, Egypt
2.2. Bacteriological examination:
About 25 grams of each sample under examination were prepared for bacteriological examination following APHA (2001).

2.2.1. Isolation and identification of E. coli following ISO16649-3 (2001):
Typical E. coli colonies on Tryptone Bile Glucouronide (TBX) medium which appeared as blue colonies, were picked up for identification morphologically by Gram stain, biochemical tests and serologically by slide agglutination test using E. coli antiser (Table 1) of DENKA SEIKEN CO., LTD.TOKYO, Japan.

Table 1. Antiseras used in serological identification of E. coli

| Polyvalent | Monovalent |
|------------|------------|
| Sera       |            |
| Polyvalent 1 | O1 O26 O66a O111 O119 O127a O128 |
| Polyvalent 2 | O4 O55 O125 O126 O146 O166 |
| Polyvalent 3 | O18 O114 O142 O151 O157 O158 |
| Polyvalent 4 | O6 O27 O78 O148 O159 O168 |
| Polyvalent 5 | O20 O25 O63 O153 O167 |
| Polyvalent 6 | O8 O15 O115 O169 |
| Polyvalent 7 | O28ac O112ac O124 O136 O144 |
| Polyvalent 8 | O29 O143 O152 O164 |

H-sera: H2, H3, H6, H7, H11, H18 and H21.

2.2.2. Isolation and identification of Staph. aureus strains following FDA (2001):
Suspected Staph. aureus colonies that appeared as circular, smooth, convex, gray to jet-black, frequently with light-colored (off-white) margin, surrounded by opaque zone and frequently with an outer clear zone on Baird-Parker agar were identified morphologically by Gram stain, biochemically, and coagulase activities.

2.2.3. Isolation and identification of B. cereus strains following Rhodehamel and Harmon (2001): Typical B. cereus colonies (blue, turquoise to peacock blue, about 5 mm in diameter and surrounded by a zone of egg yolk precipitation on Polymyxin –pyruvate-Egg yolk-Mannitol- Bromothymol blue agar (PEBMA)) were picked up for identification morphologically by Gram stain and biochemical tests following Paul et al. (2009).

2.2.4. Isolation and identification of Salmonella strains following ISO 6579 (2002): Typical Salmonella colonies grown on XLD agar medium had a pink color with black center. Meanwhile, typical Salmonella colonies onto Salmonella-Shigella agar were pale color colonies indicated non-lactose fermenting with black centers were identified morphologically by Gram stain, biochemically, and coagulase activities.

2.3. In-Vitro anti-microbial sensitivity test:
E. coli, Staph. aureus and B. cereus isolated strains were subjected to the sensitivity test against different antibiotics using the disc and agar diffusion method (Koneman et al., 1997) and interpretation of results were carried out according to CLSI (2018).

3. RESULTS

The results of bacteriological examination of meat and meat product samples and in vitro sensitivity test for E. coli, Staph. aureus and B. cereus isolated strains (Tables 2-6).

The prevalence of E. coli strains isolated from minced meat samples (7/14%) followed by kofta (6/12.0%), sausage (5/10.0%), fresh meat (4/8.0%) and beef burger samples (3/6.0%). The prevalence of Staph. aureus strains isolated from kofta samples (12/24.0%) followed by minced meat (9/18.0%), sausage, fresh meat (8/16.0% for each) and beef burger samples (4/8.0%). The prevalence of B. cereus strains isolated from kofta (7/14.0%) followed by sausage (6/12.0%), minced meat (4/8.0%), beef burger (3/6.0%) and fresh meat samples (1/2.0%). The prevalence of Salmonella strains isolated from kofta (1/2.0%) followed by sausage (1/2.0%), minced meat (1/2.0%).

The results of serological examination Table (3) showed that seven strains (28.0%) were typed as E. coli O55:H7 (two from each samples of kofta and minced meat, and one from each samples fresh meat, beef burger and sausage).

Three (12.0%) E. coli O111:H4 (one from each samples of fresh meat, kofta and minced meat samples), five (20.0%) E. coli O25:H11 (two from each minced meat, and one from each samples of fresh meat, kofta and sausage samples), three (12.0%) E. coli O126:H7 (one from each samples of kofta, minced meat and sausage samples), two (8.0%) E. coli O128:H27 (one from each samples of fresh meat and beef burger), two (8.0%) E. coli O142:H2 (one from each samples of beef burger and sausage) three (12.0%) E. coli O158:H2 (one from each samples of kofta, minced meat and sausage samples).

The in vitro sensitivity tests for the isolated E. coli (Table 4) showed that they were highly resistant for methicillin (84.0%), oxytetracycline (72.0%), amoxicillin and ampicillin (68.0% for each), streptomycin (60.0%) and erythromycin (52.0%). Meanwhile, they were intermediate sensitive to doxycycline (60.0%), cefotaxime (56.0%) and neomycin (52.0%). Moreover, they were highly sensitive to meropenem (80.0%), norfloxacin (72.0%), gentamycin (68.0%), Ciprofloxacin (64.0%) and florfenicol (56.0%).

The in vitro sensitivity tests for the isolated Staph. aureus (Table 5) revealed that they were highly resistant for methicillin (82.9%), ampicillin (75.6%), oxytetracycline (68.3%), amoxicillin (65.9%), cefotaxime and streptomycin (63.4% for each), doxycycline (56.1%) and erythromycin (51.2%). They were intermediate sensitive to flornfenicol (58.5%) and neomycin (56.1%). Meanwhile, they were highly sensitive to norfloxacin (80.5%), gentamycin (73.2%), ciprofloxacin (68.3%) and meropenem (63.4%).

The in vitro sensitivity tests for the isolated B. cereus (Table 6) revealed that they were highly resistant for ampicillin and methicillin (85.7% for each), oxytetracycline (76.2%), amoxicillin (67.7%), erythromycin (61.9%) and cefotaxime (52.4%).
Table 2 Prevalence of foodborne pathogens in examined samples

| Samples         | Fresh meat | Beef Burger | Kofta | Minced meat | Sausage | Total |
|-----------------|------------|-------------|-------|-------------|---------|-------|
| E. coli         | 4          | 8.0         | 6     | 12.0        | 7       | 14.0  |
| Salmonella      | 0          | 0.0         | 1     | 2.0         | 1       | 2.0   |
| Staph. aureus   | 8          | 16.0        | 12    | 24.0        | 9       | 18.0  |
| Total           | 13         | 26.0        | 20    | 52.0        | 21      | 42.0  |

1: % Percentage in relation to total number of each sample (30). 2: Percentage in relation to total number of samples (250).

Table 3 Serological typing of E. coli strains isolated from different examined samples

| E. coli serotype | NO. | %1 | NO. | %1 | NO. | %1 | NO. | %1 | NO. | %1 | NO. | %1 | Total |
|------------------|-----|----|-----|----|-----|----|-----|----|-----|----|-----|----|-------|
| O11:H2           | 1   | 4.0| 1   | 4.0| 2   | 8.0| 2   | 8.0| 1   | 4.0| 7   | 28.0|
| O12:H11          | 1   | 4.0| 0   | 0.0| 1   | 4.0| 1   | 4.0| 0   | 0.0| 3   | 12.0|
| O15:H12          | 1   | 4.0| 0   | 0.0| 1   | 4.0| 2   | 8.0| 1   | 4.0| 5   | 20.0|
| O16:H12          | 0   | 0.0| 0   | 0.0| 1   | 4.0| 1   | 4.0| 1   | 4.0| 3   | 12.0|
| O18:H12          | 0   | 0.0| 1   | 4.0| 0   | 0.0| 0   | 0.0| 0   | 0.0| 2   | 8.0 |
| O26:H12          | 0   | 0.0| 0   | 0.0| 1   | 4.0| 1   | 4.0| 1   | 4.0| 3   | 12.0|
| Total            | 4   | 16.0| 3   | 12.0| 6   | 24.0| 7   | 28.0| 5   | 20.0| 25  | 100.0|

1: Percentage in relation to total number of examined E. coli (25).

Table 4 In vitro anti-microbial Sensitivity test for E. coli isolates

| Antimicrobial agents | Disk concentrations | Sensitive | Intermediate | Resistant | AA |
|----------------------|---------------------|-----------|--------------|-----------|----|
|                      | No. | %     | No. | %     | No. | %     |
| Methicillin          | 5 μg | 1     | 4.0 | 3     | 12.0 | 21   | 84.0 | R   |
| Amoxicillin          | 25 μg | 3     | 12.0 | 5     | 20.0 | 17   | 68.0 | R   |
| Ampicillin           | 20 μg | 2     | 8.0  | 6     | 24.0 | 18   | 72.0 | R   |
| Oxytetracycline      | 30 μg | 1     | 4.0  | 6     | 24.0 | 18   | 72.0 | R   |
| Streptomycin         | 10 μg | 2     | 8.0  | 8     | 32.0 | 15   | 60.0 | R   |
| Erythromycin         | 15 μg | 4     | 16.0 | 8     | 32.0 | 13   | 52.0 | R   |
| Doxycycline          | 30 μg | 4     | 16.0 | 15    | 60.0 | 6    | 24.0 | IS  |
| Cefotaxime           | 30 μg | 6     | 24.0 | 14    | 56.0 | 5    | 20.0 | IS  |
| Neomycin             | 30 μg | 5     | 20.0 | 13    | 52.0 | 7    | 28.0 | IS  |
| Meropenem            | 10 μg | 20    | 80.0 | 4     | 16.0 | 1    | 4.0  | S   |
| Norfloxacin          | 10 μg | 18    | 72.0 | 5     | 20.0 | 2    | 8.0  | S   |
| Gentamicin           | 10 μg | 17    | 68.0 | 3     | 12.0 | 5    | 20.0 | S   |
| Ciprofloxacin        | 5 μg  | 16    | 64.0 | 5     | 20.0 | 4    | 16.0 | S   |
| Florfenicol          | 30 μg | 14    | 56.0 | 6     | 24.0 | 5    | 20.0 | S   |

No. Number of isolates. AA: Antibiotic activity. %: Percentage in relation to total number of isolated E. coli (25).

Table 5 In vitro anti-microbial Sensitivity test for Staph. aureus isolated strains

| Antimicrobial agents | Disk concentrations | Sensitive | Intermediate | Resistant | AA |
|----------------------|---------------------|-----------|--------------|-----------|----|
|                      | No. | %     | No. | %     | No. | %     |
| Methicillin          | 5 μg | 2     | 4.9 | 5     | 12.2 | 34   | 82.9 | R   |
| Ampicillin           | 20 μg | 4     | 9.8 | 6     | 14.6 | 31   | 75.6 | R   |
| Oxytetracycline      | 30 μg | 2     | 4.9 | 11    | 26.8 | 28   | 68.3 | R   |
| Amoxicillin          | 25 μg | 6     | 14.6 | 8     | 19.5 | 27   | 65.9 | R   |
| Cefotaxime           | 30 μg | 6     | 14.6 | 9     | 22.0 | 26   | 63.4 | R   |
| Streptomycin         | 5/10 | 3     | 7.3  | 12    | 29.3 | 26   | 63.4 | R   |
| Doxycycline          | 30 μg | 6     | 14.6 | 12    | 29.3 | 23   | 56.1 | R   |
| Erythromycin         | 15 μg | 7     | 17.1 | 13    | 31.7 | 21   | 51.2 | R   |
| Florfenicol          | 30 μg | 7     | 17.1 | 24    | 58.5 | 10   | 24.4 | IS  |
| Neomycin             | 30 μg | 7     | 17.1 | 23    | 56.1 | 11   | 26.8 | IS  |
| Norfloxacin          | 10 μg | 33    | 80.5 | 5     | 12.2 | 3    | 7.3  | S   |
| Gentamicin           | 10 μg | 30    | 73.2 | 6     | 14.6 | 5    | 12.2 | S   |
| Ciprofloxacin        | 5 μg  | 28    | 68.3 | 8     | 19.5 | 5    | 12.2 | S   |
| Meropenem            | 10 μg | 26    | 63.4 | 13    | 31.7 | 2    | 4.9  | S   |

No. Number of isolates. AA: Antibiotic activity. %: Percentage in relation to total number of isolates (41).
B. cereus isolates were intermediate sensitive to neomycin (61.9%), doxycycline (57.1%) and streptomycin (52.4%). Despite that they were highly sensitive to gentamycin and norfloxacin (80.9% for each), ciprofloxacin and meropenem (71.4% for each) and florfenicol (61.9%).

4. DISCUSSION

Pathogenic, mainly toxigenic bacterial species of E. coli, Salmonellae, coagulase positive Staph. aureus and B. cereus have been linked to major outbreaks of food poisoning, illness and death all over the world (Son et al., 2014; Hamed et al., 2015; Zatar et al., 2016).

The results of bacteriological examination of examined samples (Table 2) revealed that, Staph. aureus isolates; E. coli; B. cereus and Salmonella were recovered from 250 examined samples with a total of 90 (36.0%) to all isolated bacteria. Nearly similar results were recorded by Abd El-Tawab et al. (2015a, 2015b), Binsy et al. (2016), El-Rais, Eman (2018) and El-Sayed (2019). These bacterial pathogens in meat and its products are of public health importance for consumers (Bennett et al., 2013; Son et al., 2014; Binsy et al., 2016). Pathogenic strains of E. coli affecting humans are responsible for intestinal diseases (gastroenteritis) and extra intestinal infections, which include urinary tract infections, bacteremia, and neonatal meningitis. E. coli accounts for more than 90% of all uncomplicated UTIs (Binsy et al., 2016). Twenty-five E. coli strains were isolated from minced meat, kofta, sausage, fresh meat and beef burger samples. Nearly similar results were obtained by Tarabees et al. (2015), Armany et al. (2016), El-Rais, Eman (2018) and El-Sayed (2019). These bacterial pathogens in meat and its products are of public health importance for consumers (Bennett et al., 2013; Son et al., 2014; Binsy et al., 2016).

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The results disagreed with those of ectal origin may be present. The increased incidence of E. coli in the examined samples may be due to mishandling during production, processing, and distribution or to the use of contaminated water during evisceration and slaughtering (Gwida et al., 2014).

A total of 41 Staph. aureus isolates were mostly isolated from kofta, minced meat, sausage, fresh meat and beef burger samples. These results came in accordance with those obtained by Goja et al. (2013), Abd El-Tawab et al. (2015), Armamy et al. (2016), El-Rais (2018) and El-Shora (2019). Meanwhile, these results disagreed with those of Abd El-Hady (2015), Adwan et al. (2015) and Tarabeeb et al. (2015), who isolated Staph. aureus from fresh meat and meat products with high incidence. Also, disagreed with Kalantari et al. (2012), who failed to isolate Staph. aureus from beef burger and beef sausage samples. The colonial appearance and the biochemical profile of isolated Staph. aureus strains were similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction as lipase, extracellular pigmentation production (Staphyloxaehne and Staphylocoagulase (Chandrakanth et al., 2010; Markey et al., 2013; Bahbah, 2018; El-Rais 2018). Moreover, the in vitro sensitivity tests for the isolated Staph. aureus (Table 5) agreed with those reported by Abd El-Tawab et al. (2015), Rahimi and Karimi (2015), Bahbah (2018) and El-Rais (2018). The presence of Staph. aureus in meat and its products indicates poor hygiene of meat handlers as well as lack of sterilization of utensils. They grow without pronounced change in odour or taste in the products and producing heat stable enterotoxins which lead to food poisoning with severe diarrhea and gastroenteritis among consumers (Plaatjies et al., 2004). B. cereus is one of the potential spoilage bacteria associated with meat products and the presence of them with high levels indicates a potential risk of producing toxins. In this study 21 strains of B. cereus were isolated mostly from kofta, sausage, minced meat, beef burger and fresh meat samples. Nearly similar results were obtained by Tewari et al. (2012) and Ibrahim et al. (2014b). But disagreed with those obtained by Samir et al. (2012), Abd El-Tawab et al. (2015a), Mohamed and Ghanem (2015),
Salim, Dalia et al. (2015), Soleimani et al. (2017) and El-Shora (2019), who isolated B. cereus from fresh meat and meat products with high incidence. The colonial appearance and the biochemical profile of recovered B. cereus isolates were similar to those previously reported (Abd El-Tawab et al., 2015a; Savic et al., 2015; Bashir et al., 2017; El-Sayed, 2019; El-Shora 2019). The in vitro sensitivity tests for the isolated B. cereus (Table 6) Nearly similar were recorded by Tahmasebi et al. (2014), Merzougui et al. (2014), Savic et al. (2015) and El-Sayed (2019). The results of Salmonella isolation cleared that, three isolates were recovered from one sample of each minced meat, kofta and sausage samples (1/2.0%). Meanwhile, failed to be isolated from fresh meat and beef burger samples. The colonial appearance and the biochemical profile of isolated Salmonella strains was like those previously reported by Kumar et al. (2010), Ozkalp (2012) and Abd El-Salam (2014). The results of in vitro sensitivity tests for the isolated strains proved that, multiple antibiotic resistances are widely spread among isolated E. coli, Staph. aureus and B. cereus strains. These observations agreed with the reports of Shrestha (2013), Abd El-Tawab et al. (2015 a & b) and El-Rais (2018), and it is of serious concern because these drugs are still considered the most recommended for the treatment of both animal and human.

5. CONCLUSION

Finally, the recorded results showed high rate of pathogens, this may be due to poor hygienic aspects. Moreover, the results proved that multiple antibiotic resistances are widely spread among isolated strains.

6. REFERENCES

1. Abd El-Hady, M. A. (2015): Bacteriological and Molecular characterization of Staphylococcus aureus Isolated from Beef Meat Products in El Gharbia Governorate. M.V.Sc. Thesis (Microbiology) Fac. Vet. Med. Cairo Univ.

2. Abdalslam, S.A., Hassan, M.A., Kaheel, H.A., Abobaker, T.M.; Alnourain, T. H., Hamdan, H. A., Gokul Shankar, S. and Thambirajah, J. (2014): Isolation of Escherichia coli O157 and other food borne pathogens from meat products and their susceptibility to different antimicrobial agents. Curr. Res. Microbiol., Biotechnol.2 (3): 391-397.

3. Abd El Salam, Marwa (2019): Effect of Some natural and chemical preservatives on Shiga toxin producing E. coli in minced meat. Ph. D. V. Sc. Fac Vet. Med. Benha Univ.

4. Abd El-Tawab, A.A., El-Hofy, F.I., Khater, D.F. and Al-Baaly, Y.M. (2015a): Molecular studies on toxicigenic strains of Bacillus cereus isolated from some meat products. Benha Vet. Med. J., 29(1): 129–133.

5. Abd El-Tawab, A.A., Maarouf, A. A., El-Hofy, Fatma, I. and El-Said, Aya, A. (2015b): Bacteriological studies on some food borne bacteria isolated from chicken meat and meat products in Kaolobia Governorate. Benha Vet. Med. J., 29(2): 47-59.

6. Abostate, M. A. M., Zahran, D. A. and El-Hifnawi, H. N. (2006): Incidence of Bacillus cereus in some meat products and the effect of gamma radiation on its toxin(s). Int. J. Agric. and Biology, 8(1): 35.

7. Adwan, G. M., Alqarem, B. R. and Adwan, K. M. (2015): Prevalence of foodborne pathogens in meat samples in Palestine. Int. food res. J., 22(5): 1806-1812.

8. Al-Mariri, A. and Safi, M. (2014): In vitro antibacterial activity of several plant extract and oils against some Gram – negative bacteria Iran J. Med. Sci., 39(1): 36-43.

9. Amosun, E. A., Ojo, O. E., Alao, I. K. and Ajuyape, A. T. P. (2012): Antimicrobial resistance among commensal Escherichia coli from cattle faeces and beef in Ibadan, Nigeria. African J. Biotechnol., 11(58): 12240-12245.

10. APHA “American Public Health Association” (2001): Compendium of Methods for the Microbiological examination of Foods. 4th Ed. F.P. Downes and K. Ito (editors), APHA. Washington D.C., USA.

11. Argudín, M.A., Mendoza, M.C. and Rodicio, M.R. (2010): Food Poisoning and S. aureus enterotoxins. J. Toxins (Basel), 2(7):1751-1773.

12. Armany, A. G., Ibrahim-Hemmar, M., Amin- Reham, A. and Ahmed-Hanaa, A. (2016): Detection of some foodborne pathogens in meat products by Polymerase Chain Reaction. Benha Vet. Med. J., 30 (1): 323-330.

13. Bahbah, E.A.I. (2019): prevalence of Staphylococci in meat products with special reference Methicillin-resistant Staphylococcus aureus (MRSA) in Kaolobia Governorate M. V. Sc. Thesis (Meat Hygiene), Fac. Vet. Med., Benha Univ.}

14. Bashir, M., Malik, M.A., Momen Javid, M., Badroo, G.A., Bhat, Altaf, M. and Singh, M. (2017): Prevalence and Characterization of Bacillus cereus in Meat and Meat Products in and around Jammu Region of Jammu and Kashmir, India. Int. J. Curr. Microbiol. App. Sci., 6(12): 1094-1106.

15. Bennett, S. D., Walsh, K. A. and Gould, L. H. (2013): Foodborne disease outbreaks caused by Bacillus cereus, Clostridium perfringens and Staphylococcus aureus in United States, 1998–2008, Clin. Infect. Dis., 5: 425-433.

16. Binsy, M., Nanu, E. and Sunil, B. (2016): Isolation of bacteria of public health significance from market beef. Int. J. Adv. Res. Biol. Sci., 3(4): 160-164.

17. Chandrakanth, K., Virupakshiah, D. B. M., Gavimath, C. M., Udaykumar, M. and Kangalkar, V. A. (2010): Comparative genomics of Staphylococcus aureus coagulase gene. J. Advanced Bioinformatics Applications and Research, 1(1): 31-36.

18. CLSI (2018): Performance Standards for Antimicrobial Disk Susceptibility Tests. 13 th ed. CLSI standard M07-A2. Wayne, PA: Clinical and Laboratory Standards Institute.

19. Datta, S. A., Akter, A., Shah, I. G., Fatema, K., Islam, T. H., Bandypadhyay, A., Khan, Z. U. M. and Biswas, D. (2012): Microbiological quality assessment of raw meat and meat products and antibiotic susceptibility of isolated Staphylococcus aureus. J. Agric. Food Anal. Bacteriol., 2: 187-195.

20. El sayed, A. M. A. (2019): Bacteriological and molecular studies on antimicrobial resistant bacteria isolated from meat and meat products M. V. Sc. Thesis (Bacteriology, Immunology and Mycology), Fac. Vet. Med., Benha Univ.

21. El-Rais, Eman, M.A. (2018): Bacteriological and Molecular Studies on Antibiotic Resistant Bacteria in Some Meat Products. M.V. Sc. Thesis (Bacteriology, Immunology and Mycology), Fac. Vet. Med., Benha Univ.

22. El-Shora, Haba, E. (2019): Application of recent techniques for detection of some food borne pathogens isolated from different sources. Ph. D. V. Sc. Thesis (Bacteriology, Immunology and Mycology) Fac. Vet. Med., Benha Univ.

23. Food and Drug Administration “FDA” (2001): Foodborne illness, what consumer need to know. USDA Food Safety and Inspection Service, 1-16.

24. Goja, A.M., Ahmed, T.A.A., Saeed, S.A.M and Dirar, H.A. (2013): Isolation and Identification of Staphylococcus spp. in Fresh Beef. Pakistan J. Nutrition 12, 114-120.

25. Gwida, M., Hetzel, H., Gme, I., and Tomaso, H. (2014): Occurrence of Enterobacteriaceae in Raw Meat and in Human Samples from Egyptian Retail Sellers Hindawi Publishing Corporation International Scholarly Research Notices, Article ID 565671, 6.

26. Hamed, E. A. Ahmed, A.S. and Abd El-Aaty, M. F. (2015): Bacteriological hazard associated with meat and meat products. Egypt J. Agric. Res., 93, 4 (B): 385-393.
27. Ibrahim–Hemmat, M., Amani, M.S., Dalia, A.S. and Ghada, A.A. (2014): Demonstration of aerobic spore formers in some meat products. Benha Vet. Med. J., 26(2):219-226.

28. ISO 16649-2 (2001): Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of _glucuronidase-positive Escherichia coli_ - Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl β-D-glucuronic acid Part 2.

29. ISO 6579 (2002): Microbiology of food and animal feeding stuffs- horizontal method for the detection of Salmonella spp.

30. Jackson, C.R., Davis, J.A. and Barrett, J.B. (2013): prevalence and characterization of Methicillin-resistant Staphylococcus aureus isolates from retail meat and humans in Georgia. J. Clin. Microbiol., 51(4):1199-1207.

31. Kalantari, S., Sepehri, G., Bahrampour, A. and Sepehri, E. (2012): Determination of bacterial contamination isolated from sandwiches in Kerman City and their resistance to commonly used antimicrobials. Arch. Appl. Sci. Res., 4(2):1100-1105.

32. Konemann, E., Allen, S., Janda, W., Schreckenberger, C. and Winiw, W. (1997): Color Atlas and Textbook of Diagnostic Microbiology, Fifth Edition. Lippincott, Philadelphia, New York.

33. Kumar, R., Surendran, P. K. and Thampuran, N. (2010): “Evaluation of culture media for selective enrichment and isolation of Salmonella in seafood”. J. AOAC. Int., 93(5):1468-1471.

34. Li, M. C., Wang, F. and Li, F. (2011): Identification and molecular characterization of antimicrobial-resistant shiga toxin-producing Escherichia coli isolated from retail meat products. Food borne Pathogens and disease, 8(4):489–493.

35. Markey, B.K., Leonard, F.C., Archambault, M., Cullinane, A. and Maguire, D. (2013): Clinical Veterinary Microbiology. Second edition. MOSBY. Elsevier Ltd. Edinburgh London New York Oxford Philadelphia St Louis Sydney Toronto.

36. Merzougui, S., Lkhider, M., Grosset, N., Gaultier, M. and Cohen, N. (2014): prevalence, PFGE typing and antibiotic resistance of _Bacillus cereus_ group isolated from food in Morocco. Food borne pathogen and disease 11(2):145-9.

37. Messele, Y.E., Abdi, R.D., Yalaw, S.T., Tegegne, D.T., Emeru, B.A. and Werid, G.M. (2017): Molecular determination of antimicrobial resistance in Escherichia coli isolated from raw meat in Addis Ababa and Bishofuto, Ethiopia. Ann. Clin. Microbiol. Antimicrob. 16-55.

38. Mohamed, W.S. and Ghanyem, H.R. (2015): Effect of some meat products on _Bacillus cereus_ isolated from different retail meats in the United States, J. Food Sci. L 86:43.

39. Moolenare, A. and Maguire, D. (2013): Clinical Veterinary Microbiology. 2nd Edition. Springer Dordrecht Heidelberg London New York.

40. Oralkal, B. (2012): “Isolation and identification of Salmonella from different samples, Salmonella - A dangerous food borne pathogen

41. Paul, D., George, M.G., Dorothy, J., Krieg, N.R., Wolfgang, Fred A. Ranny, Karl–Henz.S. and Whitman, W.B. (2009): Bergey's manual of systematic bacteriology 2nd Edition springer Dordrecht Heidelberg London New York.

42. Plaatjes, Z., Lues, J. and Buys, E. (2004): Staphylococcal growth in fresh vacuum-packed red meat at various storage conditions. 8th World Congress on Environmental Health. Durban, South Africa.

43. Rahimi, F. and Karimi, S. (2015): Characteristics of methicillin resistant _Staphylococcus aureus_ strains isolated from poultry in Iran. Arch. Clin. Infect. Dis., 10(4):1-9.

44. Rohdehamed, E.J. and Harmon, S.M. (2001): _Bacillus cereus_. In Bacteriological Analytical Manual (Jackson, G.J., Merker, R.I. and Bandler, R. eds.) Centre for food safety and Applied Nutrition, U.S; Food and Drug Administration, College Park. M. D. http://www.cfsan.fda.gov/~ebam-14.html.

45. Salim, Dalia A., Amany N. Dapgh, E.I. EL-Toukhy- Dalia M. Mohsen and Ali, G.N. (2015): Detection of _Bacillus cereus_ in some meat products using PCR to differentiate between enterotoxigenic and non- enterotoxigenic isolates. Egypt. J. Agric. Res., 93-4 (B): 393–403.

46. Sambrook, J., Fritsch, E.F. and Montias, T. (1989): Molecular Biology. In: Molecular cloning, Laboratory manual, Second Edition Cold Spring Harbor Laboratory press, USA.

47. Samur, Hanan M. M., M. T. E. and Wafa, F. A. (2012): “Incidence of _Bacillus cereus_ in some raw and cooked meat products and its control by heat treatment”. Proceedings of the 5th Scientific conference of animal Wealth Research in the Middle East and North Africa, Fac. Agric. Cairo univ.: 182-190.

48. Saud, B., Paudel, G., Khichaju, S., Bajracharya, D.andDhungana, G. (2019): Multidrug resistant bacteria from raw meat of Buffalo and chicken, Nepal. Int.Vet.Med.1-8.

49. Savić, D., Josic, D., Stanjkovic-Sebic, A. and Lepasanovic, Z. (2015): Detection of toxin genes andRAPD analysis of _Bacillus cereus_ isolates from different soil types. Genetika, 47(2):627-638.

50. Shrestha, S. (2013): Antibiotic susceptibility pattern of resistant _Escherichia coli_ from poultry waste. BIBECHANA, 9:136-140.

51. Soleimani, M., Hosseini, H., Neyestani, Z., Siadati, S., Pilevar, Z. (2017): Occurrence Of _Bacillus Cereus_ In beef burger marketed in Tehran, Capital Of Iran”, J. Food Quality and Hazards Control, 4(3):70-73.

52. Son, I., Binet, R., Maounounen-Laasri, A., Lin, A., Hammack, T.S.and Kase, J.A. (2014): Detection of five Shiga toxin-producing _Escherichia coli_ genes with multiplex PCR: Food Microbiol.

53. Surendraraj, A., Thampuran, N. and Joseph, T. C. (2010): “Molecular screening, isolation, and characterization of Enterohaemorrhagic _Escherichia coli_ O157:H7 from retail shrimp”. J. Food Prot, 73 (1): 97-103.

54. Tahmasebi, H., Talebi, R. and Zarif, R.B. (2014): isolation of _Bacillus cereus_ in chicken meat and investigation B-Lactamase antibiotic resistant in _Bacillus cereus_ from chicken meat. Advances in life Sciences 4(4):200-206.

55. Tarabees, R. Z., Hassainn, Z. H. and ElBagoury, A.M. (2015): Polymerase Chain Reaction (PCR): An Alternative Rapid Method for Detection of Some Microbial Contamination of Meat Products. Alex. J. Vet. Sciences, 45: 91-98.

56. Tewari, A., Singh, S.P. and Rashmi Singh, R. (2015): Incidence and enterotoxigenic profile of _Bacillus cereus_ in meat and meat products of Uttarakhand, India. J. Food Sci. Technol., 52(3):1796–1801.

57. Vehliner, B. M. (2016): “Mechanisms of resistance to quinolones and epidemiological significance of Salmonella spp. Acta Veterinaria-Beograd 66 (2): 147-159.

58. Zafar, A., Ahmed, E., Wajja, H. and Khan, A. (2016): Microbiological Evaluation of Raw Meat Products Available in Local Markets of Karachi, Pakistan. Pakistan Academy of Sciences B. J. Life. Env. Sci: 53 (2): 103–109.

59. Zhao, S., Bickenstaff, K., Bodeis-Jones, S., Gaines, S. A., Tong, E. and McDermott, P. F. (2012): “Comparison of the prevalence and antimicrobial resistances of _Escherichia coli_ isolates from different retail meats in the United States. J. Appl. Env. Microbiol.: 1701–1707.”