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

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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), Enterotoxigenic E. coli (ETEC), Enteroinvasive E. coli (EIEC), Enteroaggregative E. coli (EAEC), diffusively adherent E. coli (DAEC) and Enterohaemorrhagic 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.

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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 antiserum (Table 1) of DENKA SEIKEN CO., LTD.TOKYO, Japan.

<table>
<thead>
<tr>
<th>Polyvalent Serotypes</th>
<th>Monovalent Sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvalent 1</td>
<td>O1, O2, O6a, O11, O119, O127a, O128</td>
</tr>
<tr>
<td>Polyvalent 2</td>
<td>O4, O5, O123, O126, O146, O166</td>
</tr>
<tr>
<td>Polyvalent 3</td>
<td>O14, O114, O142, O151, O157, O158</td>
</tr>
<tr>
<td>Polyvalent 4</td>
<td>O6, O27, O78, O148, O159, O168</td>
</tr>
<tr>
<td>Polyvalent 5</td>
<td>O20, O25, O63, O153, O167</td>
</tr>
<tr>
<td>Polyvalent 6</td>
<td>O8, O15, O115, O169</td>
</tr>
<tr>
<td>Polyvalent 7</td>
<td>O28ac, O112ac, O124, O136, O144</td>
</tr>
<tr>
<td>Polyvalent 8</td>
<td>O29, O143, O152, O164</td>
</tr>
</tbody>
</table>

H-sera: H2, H4, H6, H17, H11, H18 and H2

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 (PMBBA) 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%) and fresh 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 O125: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%), oxacillin (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 florphenicol (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%), oxacillin (68.3%), amoxicillin (65.9%), cefoxime and streptomycin (63.4% for each), doxycycline (56.1%) and erythromycin (51.2%). They were intermediate sensitive to florphenicol (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), oxacillin (76.2%), amoxicillin (66.7%), erythromycin (61.9%) and cefotaxime (52.4%).
Table 2 Prevalence of foodborne pathogens in examined samples

<table>
<thead>
<tr>
<th>Samples</th>
<th>Fresh meat</th>
<th>Beef Burger</th>
<th>Kofta</th>
<th>Minced meat</th>
<th>Sausage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>55%</td>
<td>42%</td>
<td>55%</td>
<td>55%</td>
<td>55%</td>
<td></td>
</tr>
<tr>
<td>Salmonella</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
</tbody>
</table>

1) % Percentage in relation to total number of examined samples (250)

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

<table>
<thead>
<tr>
<th>E. coli serotype</th>
<th>NO.</th>
<th>%</th>
<th>NO.</th>
<th>%</th>
<th>NO.</th>
<th>%</th>
<th>NO.</th>
<th>%</th>
<th>NO.</th>
<th>%</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>O157:H7</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>2</td>
<td>8.0</td>
<td>2</td>
<td>8.0</td>
<td>1</td>
<td>4.0</td>
<td>7</td>
<td>28.0</td>
</tr>
<tr>
<td>E. coli H11</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>0.0</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>E. coli H11</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>0.0</td>
<td>5</td>
<td>20.0</td>
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<tr>
<td>O26: 11</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>4.0</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>O157:H7</td>
<td>1</td>
<td>4.0</td>
<td>0</td>
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<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
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<td>0.0</td>
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<tr>
<td>O157:H7</td>
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<td>0</td>
<td>0.0</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>1</td>
<td>4.0</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>Total</td>
<td>4</td>
<td>16.0</td>
<td>3</td>
<td>12.0</td>
<td>6</td>
<td>24.0</td>
<td>7</td>
<td>28.0</td>
<td>5</td>
<td>20.0</td>
<td>25</td>
<td>100.0</td>
</tr>
</tbody>
</table>

1) % Percentage in relation to total number of examined samples (250)

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

Antimicrobial agents | Disk concentrations | Sensitive | Intermediate | Resistant | AA |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
</tr>
<tr>
<td>Methicillin</td>
<td>5 µg</td>
<td>4</td>
<td>1</td>
<td>4.0</td>
<td>3</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>25µg</td>
<td>3</td>
<td>12.0</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20 µg</td>
<td>2</td>
<td>8.0</td>
<td>6</td>
<td>24.0</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>4</td>
<td>16.0</td>
<td>8</td>
<td>32.0</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 µg</td>
<td>4</td>
<td>16.0</td>
<td>15</td>
<td>60.0</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 µg</td>
<td>5</td>
<td>20.0</td>
<td>13</td>
<td>52.0</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10 µg</td>
<td>20</td>
<td>80.0</td>
<td>4</td>
<td>16.0</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 µg</td>
<td>18</td>
<td>72.0</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>17</td>
<td>68.0</td>
<td>3</td>
<td>12.0</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>16</td>
<td>64.0</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>Flohexacil</td>
<td>30 µg</td>
<td>14</td>
<td>56.0</td>
<td>6</td>
<td>24.0</td>
</tr>
</tbody>
</table>

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 |
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
<td>%</td>
<td>NO.</td>
</tr>
<tr>
<td>Methicillin</td>
<td>5 µg</td>
<td>2</td>
<td>4.9</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>20µg</td>
<td>4</td>
<td>9.8</td>
<td>6</td>
<td>14.6</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>17</td>
<td>71.1</td>
<td>13</td>
<td>51.7</td>
</tr>
<tr>
<td>Flohexacil</td>
<td>30 µg</td>
<td>17</td>
<td>71.1</td>
<td>24</td>
<td>58.5</td>
</tr>
<tr>
<td>Neomycin</td>
<td>30 µg</td>
<td>7</td>
<td>17.1</td>
<td>23</td>
<td>56.1</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>33</td>
<td>80.5</td>
<td>5</td>
<td>12.2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>28</td>
<td>68.3</td>
<td>8</td>
<td>19.5</td>
</tr>
<tr>
<td>Meropenem</td>
<td>10 µg</td>
<td>26</td>
<td>63.4</td>
<td>13</td>
<td>31.7</td>
</tr>
</tbody>
</table>

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 florphenicol (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. (2015), 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). 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. (2015), 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). 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).
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


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


43. 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.


