Bacteriological and molecular studies on antibiotic resistant *Escherichia coli* isolated from meat and its products in Kaliobia, Egypt

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**ABSTRACT**

The present study was performed on 175 random samples (about 250 g for each) of fresh meat and meat products viz: Sausage, beef burger, luncheon and Kofta (35 for each), were collected from different shops at Kaliobia Governorate, Egypt, for detection of *E. coli* and phenotypic characterization as well as virulence and antibiotic resistant genes in them. Bacteriological examination indicated the isolation of 11 (6.3%) isolates of *E. coli* from 175 samples as kofta, sausage samples (3= 8.6% for each) followed by fresh meat, beef burger samples (2=5.7% for each) and luncheon (1=2.9%). Accordingly, *E. coli* strains were serotyped as one O26:H11 from beef burger samples; four O55:H7 (one from each samples of fresh meat, luncheon, kofta and sausage); two O111:H4 (one from each samples fresh meat and kofta); one O125:H4 from sausage samples and three O25:H48 (one from each samples beef burger, kofta and sausage). The antibiotic sensitivity profile revealed that, the isolated *E. coli* strains were highly resistant for methicillin and oxacillin followed by amoxicillin; ampicillin; oxytetracycline; streptomycin; erythromycin; Nalidixic acid. Meanwhile, they were highly sensitive to enrofloxacin and gentamycin followed by norfl oxacin; cefotaxime and ciprofloxacin. PCR declared that *eaeA*, *bla*TEM; *tetA(A)* and *aadA1* genes were amplified in all four studied *E. coli* strains giving product of 248 bp; 516 bp; 576 bp and 484 bp, respectively. The study concluded that; antibiotic resistances *E. coli* is meat-borne pathogen of public health important.

**Key words:** Meat products, *E. coli*, antibiotic resistant genes


1. **INTRODUCTION**

Meat and meat products serve as important source of proteins for humans. However, recently the emerging antibiotic resistant foodborne pathogens combined with the injudicious use of antibiotics in animals bears considerable public health threats worldwide (Messele et al., 2017). *Escherichia coli* is commonly non-virulent but some strains have adopted pathogenic or toxigenic virulence factors that make them virulent to human and animals. It has become recognized as a serious food borne pathogen and has been associated with numerous out breaks of disease resulting from contaminated meat products (Gi et al., 2009 and Datta et al., 2012). Pathogenic *E. coli* strains have been divided into intestinal pathogenic *E. coli* and extra intestinal pathogenic *E. coli* (ExPEC) depending on the location of the infection they are causing. EPEC strains are responsible for a variety of infections, including bacteremia, urinary tract infections, neonatal meningitis, pneumonia, deep surgical wound infections, endovascular infections, vertebral osteomyelitis, and septicemia (Russo and Johnson, 2000 and Kaper et al., 2004).

The wide spread and imprudent use of antibiotics in food animals is thought to be
accountable for the emergence and wider spreading of antimicrobial resistant (AMR) bacteria in humans (Aslam et al., 2009 and Messele et al., 2017). Antimicrobial resistant may be acquired or intrinsic resistance. Acquired antibiotic resistance, in which a previously sensitive bacterium becomes resistant and the majority of them are propagated through horizontal or lateral gene transfer between bacteria often due to the polymicrobial nature of infections and proximity of pathogens (Rodríguez et al., 2013 and Juhas, 2015). In addition; intrinsic resistance relates to the unique physiological properties of a microorganism, in which their metabolic activity is substantially unaffected by the presence of an antimicrobial compound. Such resistances are generally chromosomally encoded, and are typically responsible for observed differences in resistance observed between genera, species and strains of bacteria. It can be associated with differences in cell wall structures, the ability to pump antimicrobial compounds out of the bacterial cell using efflux pumps, or the production of enzymes capable of inactivating antimicrobial compounds within the bacterial cell (Russell, 2001 and Gilbert and McBain, 2003). Consumption of contaminated and/or uncooked meat poses the risks of acquiring foodborne E. coli strains (Frye and Jackson, 2013) causing a serious public health hazard. Such strains easily harbor antibiotic resistant genes from one another. This is because genes encoding AMR determinants that are carried on mobile genetic elements such as plasmids and transposes of some bacterial strains could be transferred to other bacteria strains during contact causing a threat to cure acute infections in man and animals (Van den Bogaard and Stobberingh, 2000). Beside the antimicrobial resistance among E. coli strains, the pathogenicity of them could be attributed to their virulence factors including those encoding for adhesions (F1, P, and stg fimbriae, curli, and eaeA); anti-host defense factors (ompA, iss, lipopolysaccharide, and K1); iron acquisition systems (aerobactin, Iro proteins, yersiniabactin, and the Sit iron acquisition locus); auto transporters (tsh, vat and aatA); the phosphate transport system, sugar metabolism, and the ibeA protein (Germon et al., 2005; Zhao et al., 2009; Bisi-Johnson et al., 2011; Le Bouguenec and Schouler, 2011 & Van der and Bragg, 2012).

As food-borne bacteria specially antimicrobial resistant ones constitutes serious problems for consumers, therefore, the present study was conducted to detect E. coli in meat and meat products (luncheon; kofta; beef burger and sausage) at Kaliobia Governorate, beside the phenotypic characterization of the isolates and determination of virulence and their antibacterial resistant genes.

2. MATERIALS AND METHODS

2.1. Samples

A total of 175 random samples (about 250 g for each) of fresh meat and meat products viz: Sausage, beef burger, luncheon and Kofta (35 for each), were collected from different shops at Kaliobia Governorate.

2.2. Bacteriological examination

Twenty five grams of each sample were prepared for bacteriological examination according to APHA (2001).

2.2. Isolation and identification of E. coli (Quinn et al., 2002)

One ml of prepared sample was inoculated into nutrient broth and incubated aerobically at 37°C for 12 hours. A loopful from incubated nutrient broth was streaked on MacConkey’s agar plates and incubated for 24 hours at 37°C. Suspected lactose fermented colonies were picked up and streaked on the following media: Eosin methylene blue (EMB); Brilliant Green agar (BG) and Xylose Lysine Deoxycholate (XLD) agar and incubated for another 24-48 hours at 37°C. Suspected colonies (colonies with metallic green sheen on EM; yellow colonies on BG and bright yellow colonies and agar on XLD) were picked up and kept in Semi-solid nutrient agar. The purified isolates of E. coli were morphologically identified by Gram stain; biochemical tests and serologically by slide agglutination test using E. coli antisera of DENKA SEIKEN CO., LTD.TOKYO, Japan according to Edward and Ewing (1972) and Quinn et al. (2002).

2.3. In-Vitro anti-microbial sensitivity test

The isolated E. coli 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 NCCLS (2007).

2.4. Detection of Virulence and antibiotic resistant genes of E. coli by PCR

PCR was applied by using four sets of primers for detection of four virulence and antibiotic resistant genes that may play a role in virulence of E. coli. These genes were intimin or E. coli attaching and effacing gene (eaeA); β-lactamase ampicillin resistance gene (blaTEM); tetracycline resistant A gene (tetA) and streptomycin resistant gene (aada1).

It was applied on four random isolated E. coli that showed antibiotic resistant by disk diffusion method to the same studied strains following QIAamp® DNA Mini Kit instructions (Catalogue no. 51304), Emerald Amp GT PCR mastermix (Takara) with Code No. RR310A and 1.5% agarose gel electrophoreses (Sambrook et al., 1989) using the Primers sequences, target genes, amplicons sizes and cycling conditions showed in Table (2).

3. RESULTS

The recorded results in Table (3) revealed a total of 11 (6.3%) isolates of E. coli were recovered from 175 samples and were isolated from, kofta, sausage samples (3= 8.6% for each) followed by fresh meat, beef burger samples (2=5.7% for each) and luncheon (1=2.9%). The recovered isolates are Gram-negative, medium sized rods, arranged singly, pairs and groups and motile. They grow well on different media and showed rounded, non-pigmented colonies on Nutrient agar medium, while on MacConkey’s agar medium showed rounded, non-mucoid bright pink colonies (lactose fermenter) on the surface of the medium. On Eosin methylene blue agar (EMB) showed a distinctive greenish metallic sheen colonies; yellow colonies on Brilliant Green agar (BG) and bright yellow colonies and agar on Xylose Lysine Deoxycholate (XLD) agar.

The results of biochemical identification showed that, all isolates had characteristic biochemical reaction to be E. coli, where, all the 11 isolates were positive for indole test; Methyl red test; catalase test; sugar fermentation test; nitrate reduction test; Eijkman test . Meanwhile, they were negative for oxidase; Voges-Proskauer; Urease; citrate utilization and gelatin hydrolysis tests. The results of serological examination revealed that, E. coli strains were typed as one O26:H11 (9.1%) from beef burger samples; four O55:H7 (36.4%) from fresh meat, luncheon, kofta and sausage samples; two O111:H4 (18.2%) from fresh meat and kofta samples; one O119:H4 (9.1%) from sausage samples and three O125:H18 (27.3%) from beef burger, kofta and sausage samples.

In-vitro sensitivity test (Table, 4) revealed that the E. coli isolates were highly resistant for methicillin and oxacillin (90.9%) followed by amoxicillin; ampicillin and oxytetracycline (81.8% for each); streptomycin (72.7%) and erythromycin, Nalidixic acid (63.6% for each). Meanwhile, they were highly sensitive to enrofloxacin and gentamycin (90.9%) followed by norfloxacin (81.8%) and cefotaxime, ciprofloxacin (72.7% for each). Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol (63.6%).

PCR results showed that, eaeA; blaTEM; tetA (A) and aada1 genes were amplified in all four studied E. coli strains giving product of 248 bp.; 516 bp.; 576 bp. and 484 bp., respectively as shown in Fig. (1-4).

Table (1): Antisera used in serological identification of E. coli

<table>
<thead>
<tr>
<th>Polyvalent Sera</th>
<th>Monovalent sera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyvalent 1</td>
<td>O1</td>
</tr>
<tr>
<td>Polyvalent 2</td>
<td>O44</td>
</tr>
<tr>
<td>Polyvalent 3</td>
<td>O18</td>
</tr>
<tr>
<td>Polyvalent 4</td>
<td>O6</td>
</tr>
<tr>
<td>Polyvalent 5</td>
<td>O20</td>
</tr>
<tr>
<td>Polyvalent 6</td>
<td>O8</td>
</tr>
<tr>
<td>Polyvalent 7</td>
<td>O28ac</td>
</tr>
<tr>
<td>Polyvalent 8</td>
<td>O29</td>
</tr>
</tbody>
</table>
Bacteriological and molecular studies on antibiotic resistant *Escherichia coli* isolated from

Table (2): Oligonucleotide primers sequences source Metabion (Germany)

<table>
<thead>
<tr>
<th>Target M.O.</th>
<th>Gene</th>
<th>Primer sequence (5’-3’)</th>
<th>Length of amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>TetA(A)</td>
<td>GGTTCACCTCGAACGACGTCA</td>
<td>576 bp.</td>
<td><em>Randall et al.</em> ,2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CTGTCGACAAGTTGACATGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>AadaI</td>
<td>TATCAGAGGTAGTGGCGCTCAT</td>
<td>484 bp.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>GTTCCATTAGCTTAAAGTTTTCATT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>blaTEM</td>
<td>ATCAGCAATAAACCAGC</td>
<td>516 bp.</td>
<td><em>Colom et al.</em>, 2003</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCCGAAGAAGTTTTC</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>eaeA</td>
<td>ATG CTT AGT GCT GGT TTA GG</td>
<td>248 bp.</td>
<td><em>Bisi-Johnson et al.</em>, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>GCC TTC ATC ATT TCG CTT TC</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table (3): Prevalence of *E. coli* strains in the examined samples (n=35 for each sample)

<table>
<thead>
<tr>
<th>Samples</th>
<th><em>E. coli</em> strains NO.</th>
<th>%*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fresh meat</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>luncheon</td>
<td>1</td>
<td>2.9</td>
</tr>
<tr>
<td>Kofta</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Beef Burger</td>
<td>2</td>
<td>5.7</td>
</tr>
<tr>
<td>Sausage</td>
<td>3</td>
<td>8.6</td>
</tr>
<tr>
<td>Total (175)</td>
<td>11</td>
<td>6.3</td>
</tr>
</tbody>
</table>

* Percentage in relation to total No. of each examined samples (35 for each).

Table (4): In-Vitro anti-microbial Sensitivity test for isolated *E. coli*

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>Disk concentrations</th>
<th>Sensitive No.</th>
<th>Intermediate No.</th>
<th>Resistant No.</th>
<th>%</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxicillin</td>
<td>25 µg</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>9.1</td>
<td>81.8</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>20 µg</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>9.1</td>
<td>81.8</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>30 µg</td>
<td>8</td>
<td>72.7</td>
<td>2</td>
<td>18.2</td>
<td>9.1</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>5 µg</td>
<td>8</td>
<td>72.7</td>
<td>3</td>
<td>27.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Enrofloxacin</td>
<td>5 µg</td>
<td>10</td>
<td>90.9</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>15 µg</td>
<td>2</td>
<td>18.2</td>
<td>2</td>
<td>18.2</td>
<td>7</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10 µg</td>
<td>10</td>
<td>90.9</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
</tr>
<tr>
<td>Methicillin</td>
<td>5 µg</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>9.1</td>
<td>10</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>30 µg</td>
<td>3</td>
<td>27.3</td>
<td>1</td>
<td>9.1</td>
<td>7</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>10 µg</td>
<td>9</td>
<td>81.8</td>
<td>2</td>
<td>18.2</td>
<td>0</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1 µg</td>
<td>0</td>
<td>0.0</td>
<td>1</td>
<td>9.1</td>
<td>10</td>
</tr>
<tr>
<td>Oxytetracycline</td>
<td>30 µg</td>
<td>1</td>
<td>9.1</td>
<td>1</td>
<td>9.1</td>
<td>9</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>S/10</td>
<td>1</td>
<td>9.1</td>
<td>2</td>
<td>18.2</td>
<td>8</td>
</tr>
<tr>
<td>Trimethoprim/</td>
<td>(1.25/)</td>
<td>2</td>
<td>18.2</td>
<td>7</td>
<td>63.6</td>
<td>2</td>
</tr>
<tr>
<td>Sulphamethoxazol</td>
<td>23.75 mcg</td>
<td>7</td>
<td>63.6</td>
<td>2</td>
<td>18.2</td>
<td>8</td>
</tr>
</tbody>
</table>

No.: Number of isolates
% Percentage in relation to total number of isolated E. coli (11)

AA: Antibiogram activity

Fig. (1): Agarose Gel electrophoresis of Intiman or E. coli attaching and effacing (eaeA) gene.
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 248 bp).
Lane 1 - 4: E. coli (Positive).

Fig. (2): Agarose Gel electrophoresis of β-lactamase ampicillin resistance gene (blaTEM).
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 516 bp).
Lane 1 - 4: E. coli (Positive).

Fig. (3): Agarose Gel electrophoresis of tetracycline resistant (tetA(A)) gene.
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 576 bp).
Lane 1 - 4: E. coli (Positive).

Fig. (4): Agarose Gel electrophoresis of streptomycin resistant (aadA1) gene.
Lane L: 100-600 bp DNA Ladder.
Neg.: Negative control.
Pos.: Positive control (at 484 bp).
Lane 1 - 4: E. coli (Positive).

4. DISCUSSION

Escherichia coli is one of the most important bacterial pathogens in meat and its products that are responsible for food-borne infections, illness and death all over the world especially antimicrobial resistant ones (Binsy et al., 2016). The results of bacteriological examination of examined samples (Table, 3) revealed that E. coli were isolated from, kofta, sausage samples (8.6% for each), fresh meat, beef burger samples (5.7% for each) and luncheon (2.9%).

Nearly similar results were obtained by Abd El-Salam-Azza (2014); Armany et al. (2016); Tarabees et al. (2015) and El-Rais, Eman(2018). Meanwhile, these results were disagreed with those of Phillips et al. (2006); Nychas et al. (2008); Abdaslam et al. (2014); Gwida et al. (2014); Abd El-Tawab et al. (2015) ; Adwan et al. (2015) and Ramadan (2015) who isolated E. coli from raw meat and meat products with high incidence. In addition, the results were disagreed with Wehab and Hegazy (2007) who could not isolate E. coli from beef burger samples with Siriken et al. (2006) who failed to isolate E. coli from beef sausage samples.

The colonial appearance and the biochemical profile of E. coli isolated was similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction (Burbutashvili et al., 2007; Raji et al., 2007 and Surendraraj et al., 2010). In addition, the results of serological examination revealed that, E. coli strains were typed as one O26:H11 (9.1%) from beef burger samples; four O55:H7 (36.4%) from fresh meat, luncheon, kofta and sausage samples; two O111:H4 (18.2%) from fresh meat and kofta samples; one O119:H4 (9.1%) from sausage samples and three O125:H18 (27.3%) from beef burger, kofta and sausage samples. These results came in harmony with
those of Kalchayanand et al. (2012); Mansour (2013); Mohammed et al. (2014); Abd El-Tawab et al. (2015) and Tarabees et al. (2015) who detected the same serotypes of *E. coli* from meat and meat product samples. In-vitro sensitivity tests for the isolated *E. coli* (Table, 4) showed that, they were highly resistant for methicillin and oxacillin followed by amoxicillin; ampicillin; oxytetracycline; streptomycin; erythromycin and Nalidixic acid. Meanwhile, they were highly sensitive to norfloxacin and gentamycin followed by cefotaxime and ciprofloxacin. Moreover, they were intermediate sensitive to trimethoprim/ sulphamethoxazol. Nearly similar were obtained by Althalhi et al. (2010); Li et al. (2011); Amosun et al. (2012); Zhao et al. (2012) and Abd El-Tawab et al. (2015).

Moreover, the results proved that multiple antibiotic resistances are widely spread among isolated strains of *E. coli*. These observations agreed with the reports of Raji et al. (2007). It is of serious concern because these drugs are still considered the most recommended for the treatment of colibacillosis in both animal and human. In addition, antibiotic resistance in *E. coli* is of particular concern because it is the most common Gram-negative pathogen in humans, the most common cause of urinary tract infections, a common cause of both community and hospital-acquired bacteremia, as well as a cause of diarrhea (Kaper et al., 2004).

In addition, resistant *E. coli* strains have the ability to transfer antibiotic resistance determinants not only to other strains of *E. coli*, but also to other bacteria within the gastrointestinal tract and to acquire resistance from other organisms (Österblad et al., 2000). The PCR technique is capable of identifying the pathogenic *E. coli* isolates based on the fact that virulence and antibiotic resistant genes vary not only among different species but also among strains of the same species. Thus, numerous studies have been conducted to identify virulence factors and antibiotic resistant genes of isolated *E. coli* strains (Kaper et al., 2004; Ryu et al., 2012 and Ahmed and Shimamoto, 2015). These genes were intimin or *E. coli* attaching and effacing gene (*eaeA*); β-lactamase ampicillin resistance gene (*blaTEM*); tetracycline resistant A gene (*tetA*(A)) and streptomycin resistant gene (*aadA1*). The results of PCR cleared that, *eaeA; blaTEM; tetA*(A) and *aadA1* virulence genes were detected in all 4 studied strains.

Regarding to the *E. coli* attaching and effacing *eaeA* gene, the results of PCR amplification of *eaeA* gene in *E. coli* isolates (Fig., 1) showed that, the *eaeA* gene was amplified in all four studied *E. coli* strains giving product of 248 bp. Similar findings were recorded by Kaper et al. (2004); Ayse et al. (2007); Ojo et al. (2010) and Bisi-Johnson et al. (2011). Meanwhile, for β-lactamase ampicillin resistance (*blaTEM*) gene, the results of PCR amplification of *blaTEM* gene in *E. coli* isolates (Fig., 2) showed that, the *blaTEM* gene was amplified in all of the four studied *E. coli* strains giving product of 516 bp. Similar detection of *blaTEM* gene in *E. coli* strains were recorded by Colom et al. (2003); Sunde and Norstrom (2006); Van et al. (2008); Aslam et al. (2009); Ryu et al. (2012); Hemati et al. (2014) and Ahmed and Shimamoto (2015). For tetracycline resistant tetA (A)gene, the results of PCR amplification of tetA(A) gene in *E. coli* isolates (Fig., 3) showed that, the tetA(A)gene was amplified in all four studied *E. coli* strains giving product of 576 bp. Similar detection of tetA(A) gene in *E. coli* strains were recorded by Randall et al. (2004); Sunde and Norstrom (2006); Van et al. (2008); Aslam et al. (2009); Gao et al. (2012); Momtaz et al. (2012) and Ryu et al. (2012). Moreover, for streptomycin resistant aada1 gene, the results of PCR amplification of aada1 gene in *E. coli* isolates (Fig., 4) showed that, the aada1 gene was amplified in all four studied *E. coli* strains giving product of 484 bp. Similar detection of aada1 gene in *E. coli* strains were recorded by Randall et al. (2004); Sunde and Norstrom (2006); Van et al. (2008) ;Aslam et al. (2009) and Ryu et al. (2012).

Finally, the results proved that multiple antibiotic resistances are widely spread among isolated strains of *E. coli* and decided the fact of Shalini and Rameshwar (2005) that the food chain can be considered as the main route of transmission of antibiotic resistant bacteria between the animal and human populations. Therefore, it was concluded that: *E. coli* especially antibiotic resistances ones are meat-borne pathogens of public health important.

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