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

Ashraf A. Abd El-Tawab[1], Ahmed A. A. Maarouf[2], Fatma I. El Hofy[1], Nesma M. G. Ahmed[2]

[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 for each) were collected from different shops (25gm of each sample) at Kaliobia Governorate, Egypt, to detect the prevalence of some toxigenic food-borne bacteria, beside 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 cefotaxime). 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 micro-organisms, 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), Entero-invasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC), diffusively adherent *E. coli* (DAEC) and Entero-haemorrhagic *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 Glucournide (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* antisera (Table 1) of DENKA SEIKEN CO., LTD.TOKYO, Japan.

Table1 Antisera used in serological identification of E. coli

Polyvalent Sera	Monovalent sera						
Polyvalent 1	O1	O26	O86a	O111	O119	O127a	O128
Polyvalent 2	O44	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, H4, 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 (PEMBA)) 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* O_{111}:H_4 (one from each samples of fresh meat, kofta and minced meat samples), five (20.0%) *E. coli* O_{125}:H_{18} (two from 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 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%), 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 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), oxytetracycline (76.2%), amoxicillin (66.7%), 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	
Isolates	No.	%[1]	No.	%[1]	No.	%[1]	No.	%[1]	No.	%[1]	No.	%[2]
B. cereus	1	2.0	3	6.0	7	14.0	4	8.0	6	12.0	21	8.4
E. coli	4	8.0	3	6.0	6	12.0	7	14.0	5	10.0	25	10.0
Salmonella	0	0.0	0	0.0	1	2.0	1	2.0	1	2.0	3	1.2
Staph. aureus	8	16.0	4	8.0	12	24.0	9	18.0	8	16.0	41	16.4
Total	13	26.0	10	20.0	26	52.0	21	42.0	20	40.0	90	36.0

[1] % Percentage in relation to total number of each sample (50). [2] %Percentage in relation to total number of samples (250)

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

Samples	Fresh meat		Beef Burger		Kofta		Minced meat		Sausage		Total	
E.coli serotype	NO.	%[1]	NO.	%[1]	NO.	%[1]	NO.	%[1]	NO.	%[1]	NO.	%[1]
O_{55}:H_7	1	4.0	1	4.0	2	8.0	2	8.0	1	4.0	7	28.0
O_{111}:H_4	1	4.0	0	0.0	1	4.0	1	4.0	0	0.0	3	12.0
O_{125}:H_{18}	1	4.0	0	0.0	1	4.0	2	8.0	1	4.0	5	20.0
O_{126}:H_7	0	0.0	0	0.0	1	4.0	1	4.0	1	4.0	3	12.0
O_{128}:H_{27}	1	4.0	1	4.0	0	0.0	0	0.0	0	0.0	2	8.0
O_{142}:H_2	0	0.0	1	4.0	0	0.0	0	0.0	1	4.0	2	8.0
O_{158}:H_2	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	25µg	3	12.0	5	20.0	17	68.0	R
Ampicillin	20 µg	2	8.0	6	24.0	17	68.0	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
Florphenicol	30 µg	14	56.0	6	24.0	5	20.0	S

No.: Number of isolates. AA: Antibiogram 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	25µg	6	14.6	8	19.5	27	65.9	R
Cefotaxime	30 µg	6	14.6	9	22.0	26	63.4	R
Streptomycin	S/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
Florphenicol	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: Antibiogram activity. %: Percentage in relation to total number of isolates (41)

Table 6 *In vitro* anti-microbial sensitivity test for isolated *B. cereus* strains

Antimicrobial agents	Disk concentrations	Sensitive		Intermediate		Resistant		AA
		No.	%	No.	%	No.	%	
Ampicillin	20 µg	0	0.0	3	14.3	18	85.7	R
Methicillin	5 µg	1	4.8	2	9.5	18	85.7	R
Oxytetracycline	30 µg	1	4.8	4	19.0	16	76.2	R
Amoxicillin	25µg	3	14.3	4	19.0	14	66.7	R
Erythromycin	15 µg	5	23.8	3	14.3	13	61.9	R
Cefotaxime	30 µg	3	14.3	7	33.3	11	52.4	R
Neomycin	30 µg	3	14.3	13	61.9	5	23.8	IS
Doxycycline	30 µg	3	14.3	12	57.1	6	28.6	IS
Streptomycin	S/10	2	9.5	11	52.4	8	38.1	IS
Gentamicin	10 µg	17	80.9	1	4.8	3	14.3	S
Norfloxacin	10 µg	17	80.9	3	14.3	1	4.8	S
Ciprofloxacin	5 µg	15	71.4	0	0.0	6	28.6	S
Meropenem	10 µg	15	71.4	5	23.8	1	4.8	S
Florphenicol	30 µg	13	61.9	2	9.5	6	28.6	S

No.: Number of isolates. AA: Antibiogram activity. %: Percentage in relation to total number of isolates (21)

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; Zafar *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 a&b), 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 (2018), El-Sayed (2019) and El-Shora (2019). Meanwhile, these results disagreed with those of Gwida *et al.* (2014), Abd El-Tawab *et al*. (2015b), Adwan *et al.* (2015) and Abd El Salam (2019), who isolated *E. coli* from raw meat and meat products with high incidence. In addition, the results disagreed with Hamed *et al.* (2015), who failed to isolate *E. coli* from beef burger and sausage samples. The colonial appearance and the biochemical profile of recovered *E. coli* isolates were similar to those previously reported such as the fermentation of certain sugars or enzymatic reaction (Surendraraj *et al.*, 2010; Markey *et al*., 2013; Abd El-Tawab *et al*., 2015b; El-Sayed, 2019). The serological examination of 25 isolated *E. coli* isolates (Table 3) came in harmony with those of Abd El-Tawab *et al*. (2015b), Tarabees *et al.* (2015), El-Rais (2018) and El-Sayed (2019), who detected the same serotypes of *E. coli* from meat and meat product samples. The recovery of *E. coli* from meat and its products samples indicates fecal contamination and implies that other pathogens of fecal 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), Armany *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 Tarabees *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 (Staphyloxathine) 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 Ghanyem (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.

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