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Predominance and Antimicrobial Resistance Profiles of *Salmonella* and *E. coli* From Meat and Meat products

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Abstract

This study was conducted to investigate the prevalence, serotypes, and antimicrobial resistance of *Salmonella* and *E. coli* isolates recovered from 200 meat and meat products samples (fresh beef, frozen Brazilian meat, frozen beef liver, minced beef, beef burger, Egyptian luncheon, kofta and fresh beef sausage), collected randomly from supermarkets and grocery stores at El Fayoum governorate, between September 2019 to July 2020. *E. coli* was detected in 3% (n= 6/200) and *Salmonella* in 1.5% (n=3/200) of the total samples, respectively. *Salmonella* isolates were serotyped as *S.* Infantis from frozen Brazilian meat, *S.* Typhimurium from fresh beef sausage and *S.* Tsevie from fresh minced beef. Serotyping of *E. coli* showed that *E. coli* O26:H11, *E. coli* O91:H21, *E. coli* O128:H2, and *E. coli* O124 were isolated from fresh minced beef, frozen beef liver, kofta and beef sausage, respectively. Antimicrobial resistance phenotyping revealed that the isolated *Salmonella* and *E. coli* strains were resistant to 60-86.66% and 66.66- 93.33% of the total tested antibiotics, respectively. *E. coli* strains were positive for bla_{CTX-M}, *aadB*, *Sul1*, *dfrA*, *tetA*(A), bla_{SHV}, and *qnrA*, while bla_{TEM}, bla_{CTX-M}, *qnrA* and *aadB* virulence genes were detected in *Salmonella*. The antimicrobial resistance genotypic and phenotypic data evaluated in this study would be helpful to estimate the transmission of multilinked antimicrobial resistance genes to human pathogens and the likelihood of consumer exposure to resistant strains.

KEYWORDS
Meat products, *Salmonella*, *E. coli*, Antimicrobial resistance, Antimicrobial resistance genes

INTRODUCTION

Meat and meat products serve as essential sources of protein, essential amino acids, minerals, vitamins, and other nutrients for most people in different parts of the world. However, they are an ideal medium for growth and multiplication of microorganisms (Al-Mutairi, 2011). However, foodborne diseases, especially those caused by the emerging antibiotic-resistant foodborne pathogens, bear considered public health threats, and their public health impact is widely recognized due to the economic impact of morbidity and mortality (Jansen *et al*., 2019; Messele *et al*., 2017). Most foodborne bacteria often cause self-limiting gastroenteritis, but invasive disease and various complications can also occur. Among them, *Salmonella* causes systemic salmonellosis and *E. coli* causes bloody diarrhea and hemolytic- uremic syndrome (Bantawa *et al*., 2019). *Salmonella* is one of the most common and representative pathogens isolated from confirmed foodborne illness cases worldwide and pose a serious threat to human health (DaCunha-Neto *et al*., 2017). Compared to other foodborne pathogens, *Salmonella* has the highest rate of infection, making it a greater economic burden for many countries (Ramirez-Hernandez *et al*., 2018). The transmission of *Salmonella* to humans is usually induced by the consumption of contaminated foods or water, cross- contamination, and the consumption of raw or uncooked foods that contains *Salmonella* (Yang *et al*., 2014). *E. coli* is a commensal organism found the human gut. It is not usually virulent, but some strains have acquired pathogenic or toxigenic virulence factors that make them virulent in human and animals. *E. coli* has become recognized as a serious food borne pathogen and has been linked to numerous outbreaks caused by contaminated meat products (Datta *et al*., 2012). Consumption of contaminated and/or raw meat carries the risk of infection with foodborne strains of *E. coli* strains (Frye *et al*., 2013). Recently, the widespread and misuse of antibiotics in animal husbandry and human medicine has contributed to an increase in antibiotic resistance, particularly in multi-drug resistant bacteria (MDR) (Arslan and Eyi, 2010). Resistant *Salmonella* and *E. coli* isolates and their antimicrobial resistance can spread rapidly among human throught the consumption of contaminated meat products (Zhu *et al*., 2019). In addition, the growth of global trade and travel contribute to the spread of antimicrobial resistance between countries and continents. Therefore, antimicrobial resistance is recognized as one of the major public health problems of the 21st century worldwide (Doménech *et al*., 2015). As food-borne bacteria especially antimicrobial-resistant ones constitute severe problems for consumers. Furthermore, meat and meat products are the main source of foodborne infections and the most important link between livestock and humans; therefore, this study

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was conducted to investigate the prevalence, phenotyping and genotyping antimicrobial resistance and virulence genes of *Salmonella* and *E. coli* in meat and meat products in El Fayoum Governorate, Egypt from September 2019 to July 2020.

MATERIALS AND METHODS

Samples collection

A total of 200 samples of meat and meat products including fresh beef, frozen Brazilian meat, frozen beef liver, minced beef, beef burger, beef luncheon, kofta and fresh sausage (25 each) were randomly collected from various supermarkets and grocery stores at El Fayoum governorate, Egypt between 2019-2020. The samples were aseptically packed into polyethylene bags and transferred in an ice box to the laboratory of the Department of Botany, Faculty of Science, and El Fayoum University for testing within 3 h.

Bacteriological examination

Isolation and identification of *Salmonella*

The enrichment and isolation of *Salmonella* from meat and meat products samples was done according to ISO 6579 (2002). In brief, 25 g of each sample was homogenized for at least 2 min. at high speed in a sterile stomacher (Stomacher® 400, Seward, UK) with 225 mL of 0.1% sterile buffered peptone water (Himedia, India) and incubated at 37 °C for 24 hours. Then, 0.1 mL of the suspension was added to 10 mL of Rappaport–Vassiliadis (RV) broth (Oxoid, England) and incubated at 42 °C for 24 h. After selective enrichment, the suspensions were streaked onto Xylose Lysine Deoxycholate Agar (XLD, Oxoid, England) and incubated at 37 °C for 18-24 h. Single typical *Salmonella* colonies were selected for purification on nutrient agar plates and secondary purification was performed on XLD plates for biochemical and serological identification.

Isolation and identification of *E. coli*

It was carried out in accordance with ISO 4833-1 (2013). Briefly, 225 mL of 0.1% sterile peptone water was accurately applied to 25 g of the sample and well blended for 2 mins using a sterile blender. Lauryl Tryptose Selective Broth (LST) and *Escherichia coli* (EC) broth (Himedia, Indian) were used as selective media for *E. coli*. Positive EC tubes were streaked onto Eosin Methylene-Blue (EMB) Himedia, India) agar and incubated at 37 °C. Typical *E. coli* colonies (green with metallic sheen) were picked up and placed to Tryptone Soya Agar (TSA) slants and incubated at 37 °C for 24 h for biochemical and serological identification. *Salmonella* serological identification was performed according to Kauffman – White scheme (Kauffman, 1974) for the determination of somatic (O) and flagellar (H) antigens using *Salmonella* antiserum (Denka Seiken Co., Japan). *E. coli* isolates were serologically tested according to Kok *et al*. (1996) using rapid diagnostic *E. coli* antisera sets (Denka Seiken Co., Japan).

Table 1. Cycling conditions of the different primers during PCR (fast-cycling PCR).

Gene	Primer sequence (5'-3')	Length of amplified product (bp)	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
phoA	CGATTCTGGAAATGGCAAAAG CGTGATCAGCGGTGACTATGAC	720	95˚C 5 min.	94˚C 30 sec.	55˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
InvA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGG*AacC*	284	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
bla_{TEM}	ATCAGCAATA*AacC*AGC CCCCGAAG*Aac*GTTTTC	516	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
bla_{SHV}	AGGATTGACTGCCTTTTTG ATTTGCTGATTTCGCTCG	392	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
bla_{CTX-M}	ATG TGC AGY ACC AGT AAR GTK ATG GC TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
AadB	GAGCGAAATCTGCCGCTCTGG CTGTTAC*Aac*GGACTGGCCGC	319	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.
QnrA	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	94˚C 5 min.	94˚C 30 sec.	53˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
bla_{OXA}	ATATCTCTACTGTTGCATCTCC A*AacC*CTTCA*AacC*ATCC	619	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
aac(6′)-Ib-cr	CCCGCTTTCTCGTAGCA TTAGGCATCACTGCGTCTTC	113	94˚C 5 min.	94˚C 30 sec.	52˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
AacC	GGCGCGATC*Aac*GAATTTATCCGA CCATTCGATGCCGAAGGA*Aac*GAT	448	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
TetA(A)	GGTTCACTCG*Aac*GACGTCA CTGTCCGACAAGTTGCATGA	576	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
Sul1	CGG CGT GGG CTA CCT GAA CG GCC GAT CGC GTG AAG TTC CG	433	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
aac(3)-Ia	TTGATCTTTTCGGTCGTGAGT TAAGCCGCGAGAGCGCC*Aac*A	150	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec	72˚C 30 sec	35	72˚C 7 min.
DfrA	TGGTAGCTATATCGAAGAATGGAGT TATGTTAGAGGCGAAGTCTTGGGTA	425	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
TetB	CCTTATCATGCCAGTCTTGC ACTGCCGTTTTTTCGCC	773	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing for all isolates was performed according to the recommendations of the clinical and laboratory standard institute (CLSI, 2019) guidelines. Briefly, swabs of a standardized colony suspension (equivalent to 0.5 McFarland standard) were streaked onto Mueller Hinton Agar plate (Oxoid, UK) and the following antibiotic discs were evenly distributed and firmly pressed on the agar plates: [imipenem (tienam) (10 µg), tetracycline (30 µg), sulfamethoxazole/trimethoprim (sutrim) (25 µg), ceftriaxone (rocephin) (30 µg), cefotaxime (claforan) (30 µg), amoxicillin /ciavulnic acid (augmentin) (20 + 10 µg), ampicillin / sulbactam (unasyn) (10 µg), ciprofloxacin (ciprinol) (5 µg), gentamicin (10 µg), ceftazidime (30 µg), meropenem (meronem) (10 µg), linezolid (30 µg), erythromycin (erythrin) (15 µg), penicillin (10 µg) and clindamycin (dalacin c) (2 µg) (Oxoid, UK). The plates were then inverted and incubated for 18 h at 37 °C. the diameter of the zone of inhibition was measured to the nearest millimeter with a ruler and interpreted as susceptible, intermediate, and resistant strains according to CLSI guidelines.

Molecular characterization and antimicrobial resistance genes of Salmonella and E. coli

Salmonella and *E. coli* isolates were confirmed by polymerase chain reaction (PCR) for the presence of virulence genes (*inva* and *phoA*). The confirmed isolates were tested for the presence of genes associated with resistance to β-lactams (bla_{TEM}, bla_{OXA}, bla_{SHV}, bla_{CTX-M}), tetracycline (*tetA* (A), tetB), Aminoglycosides (*AacC*, *aadB*, *aac*(6')Ib-cr, *aac*(3)-Ia), Quinolones (*qnrA*, *aac*(6')Ib-cr), Sulfonamides (*Sul1*), and trimethoprim (*dfrA*), by polymerase chain reaction (PCR) according to the instructions of QIAamp® DNA Mini Kit instructions (Catalog no. 51304), Emerald Amp GT PCR master mix (Takara), Code No. RR310A and electrophoreses in 1.5% agarose gel (Sambrook *et al*., 1989). The primers (Metabion, International AG, Germany) used to amplify the resistance and virulence associated genes in this study are listed in Table 1.

RESULTS

Prevalence and serotypes of Salmonella and E. coli

Phenotypic characteristics of *Salmonella* and *E. coli* isolates were identified based on their morphology and biochemical characteristics. Microscopically, *Salmonella* appeared as Gram negative, medium size, evenly bacilli in shape. On XLD, the bacteria gave pink colonies with a black precipitation of iron in the middle of the colonies and H_2S production. All isolates were positive for citrate-utilization, Lysine decarboxylase, H2S production, and methyl-red, tests. Simultaneously, they were negative for Urease, Voges-Proskauer, and Indole tests.

E. coli appeared as Gram-negative moderate size, motile, and evenly coco-bacilli. Colonies appear on EMB agar as shiny metallic-green colonies. Biochemically, *E. coli* isolates were positive for catalase, Lysine decarboxylase, lactose fermentation, indole, and methyl-red, tests. Simultaneously, they were negative for Voges-Proskauer, citrate-utilization, H_2S production, and urease tests.

The bacteriological examination of the collected meat and meat products samples revealed that *E. coli* was more predominant than *Salmonella*. Three *Salmonella* isolates with a percentage of 1.5% (n=3/200) of the total samples were isolated from fresh minced beef, fresh beef sausage in addition to frozen Brazilian meat samples (Table 2).

Regarding the serological examination (Table 4), *Salmonella* isolates (n=3) were typed as *S.* Infantis, *S.* Typhimurium, and *S.* Tsevie which isolated from frozen Brazilian meat, fresh beef sausage and fresh minced beef, respectively.

Six *E. coli* isolates were detected in 3% (n = 6/200) of all meat and meat products samples (Table 3). The highest prevalence of *E. coli* isolates was observed in kofta, frozen beef liver, fresh minced beef and fresh beef sausage samples.

Regarding serotyping of *E. coli*, three isolates were serotyped as enterohemorrhagic *E. coli*; where two of them (O26: H11) were isolated from fresh minced beef and the third (O91: H21) was isolated from frozen beef liver. Two enterotoxigenic *E. coli* (O128: H2) were isolated from kofta and finally one enteroinvasive *E. coli* (O124) was isolated from fresh beef sausage (Table 4)

The incidence of *invA* virulence gene among three *Salmonellae* serotypes (numbered as 1, 2 and 3) as detected by multiplex PCR revealed the prevalence of *invA* gene in all *Salmonellae* serotypes with a percentage of 100% (Figure 1A). Similarly, the *phoA*, the *E. coli* virulence-determining gene, was detected in all *E. coli* serotypes (Figure 1B).

Table 2. Prevalence of *Salmonella* in different meat products (n=25).

Type of samples (n.=25 for each)	*No. (%) of Salmonella*		
	S. Infantis	*S.* Typhimurium	*S.* Tsevies
Fresh beef	0	0	0
Frozen Brazilian meat	1 (4)	0	0
Frozen beef liver	0	0	0
Fresh minced beef	0	0	1 (4)
Beef burger	0	0	0
Egyptian luncheon	0	0	0
Fresh beef sausage	0	1 (4)	0
Kofta	0	0	0
Total (n=200)	1(0.5)	1(0.5)	1(0.5)

Table 3. Prevalence of *E. coli* in different meat products (n=25).

Type of samples (n.=25 for each)	No. (%) of *E. coli*			
	O128 H2	O124	O26:H11	O91:H21
Fresh beef	0	0	0	0
Frozen Brazilian meat	0	0	0	0
Frozen beef liver	0	0	0	1 (4)
Fresh minced beef	0	0	2 (8)	0
Beef burger	0	0	0	0
Egyptian luncheon	0	0	0	0
Fresh beef sausage	0	1 (4)	0	0
Kofta	2 (8)	0	0	0
Total (n=200)	2 (1)	1 (0.5)	2 (1)	1 (0.5)

Antimicrobial susceptibility testing

The antimicrobial susceptibility test of *Salmonella* and *E. coli* isolates against 15 antimicrobial agents are presented in Table 5. All *Salmonella* strains were resistant to Ampicillin/Sulbactam, Penicillin, Amoxyciliin/Ciavulnic acid, Ceftriaxone, Ceftazidime, Cefotaxime, Erythromycin, Clindamycin, Linezolid, with a rate of 100% followed by Gentamicin (66.66%), Imipenem (33.3%), Meropenem (33.3%), Ciprofloxacin (33.3%). However, the same strains showed a high sensitivity to Sulfamethizole/ Trimethoprim

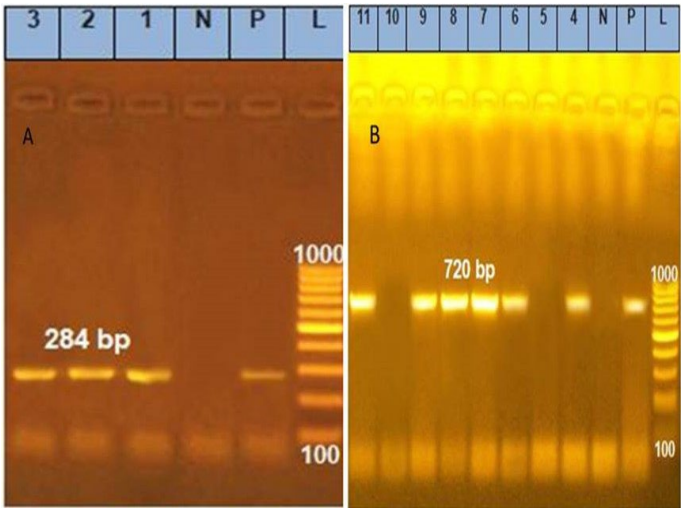

Fig. 1. Agarose gel electrophoresis of multiplex PCR of, (A) *invA* (284 bp) virulence genes for characterization of *Salmonella* species. Lane 1,2 and 3 showed positive amplifications, P and N indicate positive and negative control and ladder=100 bp.(B) *phoA* (720 bp) virulence gene for characterization of *E. coli* species Lane 4,5,6,7,8,9,10 and 11 showed positive and negative amplifications, P and N indicate positive and negative control and ladder=100 bp

(100%) and Tetracycline (100%). All strains of *Salmonella* tested were resistant to one or more antibiotics. *S.* Tsevie showed resistance to 66.66% of the total tested antibiotics (Table 6) while it was sensitive only for sulfamethoxazole/ trimethoprim and tetracycline and intermediate for ciprofloxacin, meropenem and imipenem. *Salmonella* Infantis was a resistant isolate for most antibiotics with a percentage of 86.66% except sulfamethoxazole/ trimethoprim and Tetracycline. *S.* Typhimurium showed resistance to the tested antibiotic in a percentage of 60% but it was sensitivity to meropenem, imipenem, ciprofloxacin, tetracycline, gentamycin, and sulfamethoxazole/ trimethoprim.

The antibiotic resistance patterns of *E. coli* are presented in Table 5. All strains of *E. coli* recoverd from meat and meat product samples developed resistance against ampicillin/sulbactam, penicillin, amoxyciliin/ciavulnic acid, ceftriaxone, ceftazidime, cefotaxime, erythromycin, clindamycin with a percentage of 100%, followed by gentamicin (87.5%), linezolid (87.5%), sulfamethizole/ trimethoprim (62.5 %), imipenem (62.5 %), meropenem (62.5 %), ciprofloxacin (62.5%). However, 87.5% of isolated strains were sensitive to tetracycline.

E. coli O91: H21 showed resistance for many antibiotics (11/15) with a percentage of 73.33%. However, *E. coli* O26: H11

Table 4. Serotyping of *Salmonella*e and *E. coli* in different meat products (n=25).

Sample type	*Salmonella*				
	Identified strains	No (%)	Group	Antigenic structure	
				O	H
Frozen Brazilian meat	*S.* Infantis	1 (4)	C1	6, 7, 14	r:1, 5
Fresh beef sausage	*S.* Typhimurium	1 (4)	B	1, 4, 5, 12	i:1, 2
Fresh minced beef	*S.* Tsevie	1 (4)	B	4, 5	i: e, n, z15
Sample Type	*E. coli*				
	No (%)	Serovars		Strain characterization	
Kofta	2 (8%)	O128:H2		ETEC	
Fresh beef sausage	1 (4%)	O124		EIEC	
Fresh minced beef	2 (8%)	O26:H11		EHEC	
Frozen beef liver	1 (4%)	O91:H21		EHEC	

Table 5. Breakpoint values of antimicrobial agents according to CLSI (2019) and phenotypic antimicrobial susceptibility profiles of *Salmonella* and *E. coli* isolates used in this study.

Antibiotic class	Type of antimicrobial	*Conc. (µg)*	*Salmonella*			*E. coli*		
			R	*I*	*S*	*R*	*I*	*S*
Penicillins	Ampicillin /Sulbactam	10	100	0	0	100	0	0
	Penicillin	10	100	0	0	100	0	0
	Amoxicillin/Clavulanic acid	20+10	100	0	0	100	0	0
Cephalosporins	Ceftriaxone	30	100	0	0	100	0	0
	Ceftazidime	30	100	0	0	100	0	0
	Cefotaxime	30	100	0	0	100	0	0
Macrolides and Triamilides	Erythromycin	15	100	0	0	100	0	0
Aminoglycosides	Gentamicin	10	66.7	0	33.33	80	0	20
Tetracyclines	Tetracycline	30	0	0	100	20	0	80
Lincomycins	Clindamycin	2	100	0	0	100	0	0
Quinolones	Ciprofloxacin	5	33.3	33.3	33.3	60	0	40
Oxazolidinones	Linezolid	30	100	0	0	80	0	20
Sulfonamides	Sulfamethizole/ Trimethoprim	25	0	0	100	60	0	40
Carbapenems	Imipenem	10	33.3	33.3	33.3	60	0	40
	Meropenem	10	33.3	33.3	33.3	60	0	40

S, Susceptible; I, Intermediate resistance; R, Resistant.

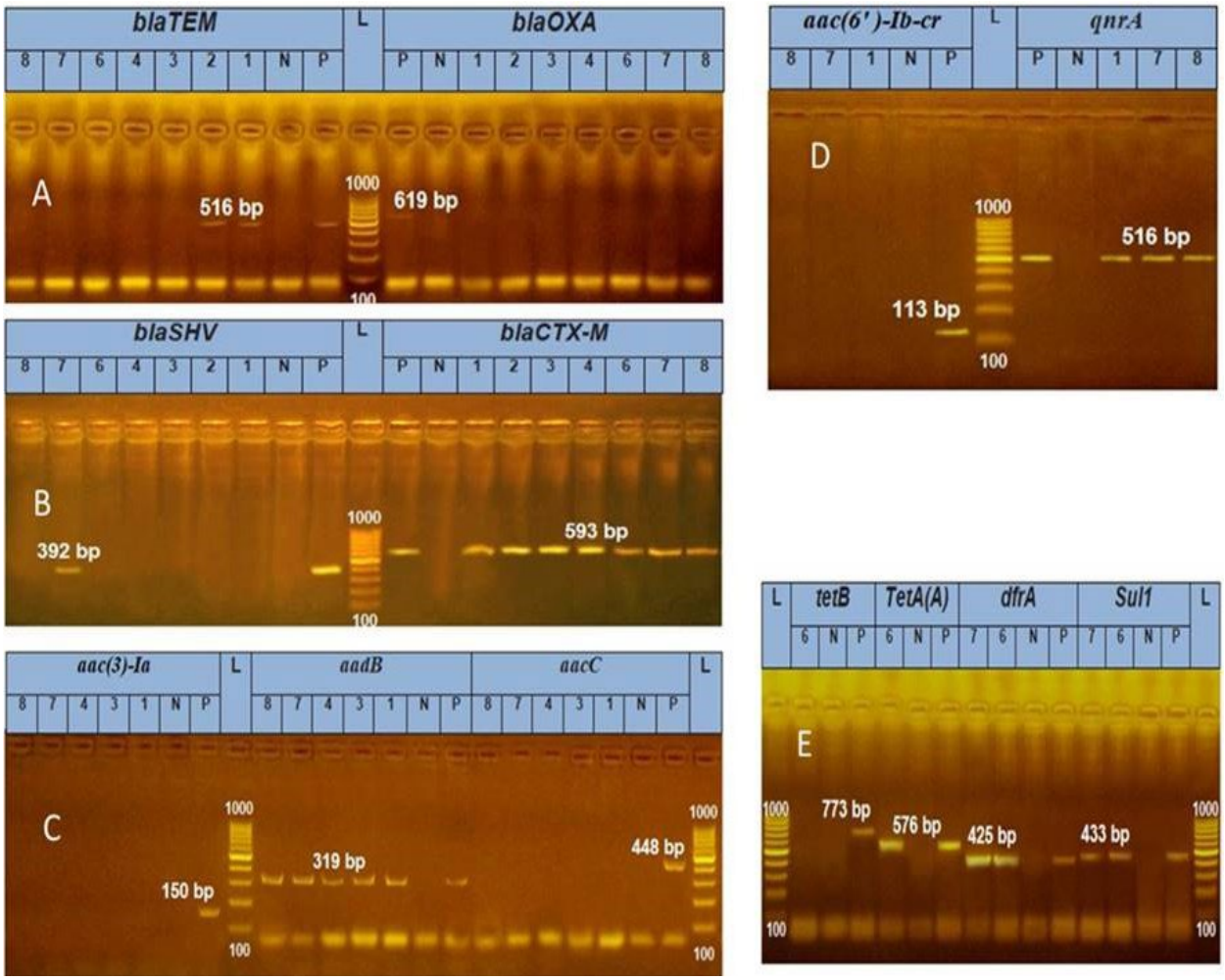

Fig. 2. PCR amplicates of A) bla_{TEM} gene at 516 bp and bla_{OXA} gene at 619 bp. Lane 1,2,3,4,6,7 and 8 showed positive and negative amplifications, P and N indicate positive and negative control and ladder=100 bp. B) bla_{SHV} gene at 392 bp and bla_{CTX-M} gene at 593 bp. Lane 1,2,3,4,6,7 and 8 showed positive and negative amplifications, P and N indicate positive and negative control and ladder=100 bp. C) *aac(3)-Ia* gene at 150 bp, *aadB* gene at 319 bp and *aacC* gene at 448 bp. Lane 1,3,4,7 and 8 showed positive and negative amplifications, P and N indicate positive and negative control and ladder=100 bp. D) *aac(6)-Ib-cr* at 113 bp and *qnrA* at 516 bp. Lane 1,7 and 8 showed positive and negative amplifications, P and N indicate positive and negative control and ladder=100 bp. E) *Sul1* gene at 433 bp, *dfrA* gene at 425 bp, *TetA*(A) gene at 576 bp and *tetB* gene at 773 bp. Lane 6 and 7 showed positive and negative amplications, P = positive control, N = negative control and Ladder = 100 bp.

was resistant to 93.3% of the tested antibiotic (14/15). Meanwhile, *E. coli* O124 showed resistance for 80% (12 /15) of antibiotics tested in this study. *E. coli* O128:H2 showed resistance for many antibiotics (10/15) with a percentage of 66.66% (Table 6).

Antimicrobial resistance genes

The antimicrobial resistance genes (bla_{TEM}, bla_{OXA} ,bla_{SHV}, $bla_{C\text{-}TX\text{-}M}$, *qnrA*, *aac*(6')Ib-cr. *AacC*. *aadB*, *Sul1*, *dfrA*, *tetA*(A), tetB, and *aac*(3)-Ia) conferring resistance to different antimicrobial classes are shown in Table 7. All *Salmonella* strains were positive for $bla_{C\text{-}TX\text{-}M}$, while none carried bla_{OXA} and bla_{SHV} genes. Bla_{TEM} gene was detected only in *S.* Infantis and *S.* Typhimurium. *QnrA* gene was detected in *S.* Infantis only. However, *aac(6)-Ib-cr* gene was detected only in *S.* Infantis but not found. *AacC* and *aac*(3)-Ia genes were detected in *S.* Infantis and *S.* Tsevie only and not complete. Finally, *aadB* gene was detected in *S.* Infantis and *S.* Tsevie. *Sul1*, *dfrA*, *tetA*(A), tetB were absent in all isolates. It was revealed that the superior resistance genes were bla_{CTX-M} (100%), bla_{TEM} (66.6%), *aadB* (66.6%), and *qnrA* (33.3%).

Data in Table 7 indicated that among the thirteen antimicrobial resistance genes detected by PCR, *E. coli* strains were positive for bla_{CTX-M}, *aadB*, *Sul1*, *dfrA*, *tetA*(A), bla_{SHV}, and *qnrA*,. None of *E. coli* isolates carried bla_{TEM} and bla_{OXA} genes, while bla_{CTX-M} gene was detected in all serotypes. Bla_{SHV} gene was detected only in *E. coli* O26: H11. Moreover *qnrA* and *aac*(6)- Ib-cr genes were only detected in *E. coli* O26 and *E. coli* O91 as *qnrA* was found while *aac*(6)- Ib-cr was absent. *AacC*, *aadB* and *aac*(3)-Ia genes were detected in all serotypes except in *E. coli* O124 and the detection revealed absence of *AacC* and *aac(3)-Ia* genes but presence of *aadB* gene. The *Sul1* and *dfrA* genes were detected only in *E. coli* O124 and *E. coli* O26 while *tetA*(A) and tetB were detected only in *E. coli* O124. It was cleared that bla_{CTX-M} was the superior resistant gene among all detected genes.

DISCUSSION

Salmonella and *E. coli* are among the most common foodborne pathogens associated with severe infections that constitute great public health problems worldwide. They are considered a major cause of illness and even death in developing countries, costing billions of dollars in the health and social sectors (Fratamico *et al*., 2005). Therefore, monitoring the presence of foodborne pathogens is an important requirement for detecting the potential problems during food production, processing, preparation, or marketing (Zhang *et al*., 2018).

In our study, the prevalence of *Salmonella* is relatively low compared to several reports which showed high contamination rate of various meat products (Al-Mutairi, 2011; Arslan and Eyi, 2010; Kudaka *et al*., 2006; Saad *et al*., 2018; Schlosser *et al*., 2000). However, *Salmonella* failed to be detected in beef luncheon and

Table 6. Resistance to antimicrobial agents by *Salmonella* and *E. coli* serovars.

Serovars	Antibiotics resistance	No (%)
S. Infantis	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, GEN, CM, CIP, LZD, IMI, MEM	13/15 = 86.66%
S. Typhimurium	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, CM, LZD	9/15 = 60%
S. Tsevie	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, GEN, CM, LZD,	10/15 = 66.66%
E. coli O128: H2	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, GEN, CM, LZD	10/15 = 66.66%
E. coli O124	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, CM, TET, IMI, MEM, TMP/SMX	12/15 = 80%
E. coli O26:H11	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, GEN, CM, CIP, LZD, IMI, MEM, TMP/SMX	14/15 = 93.33%
E. coli O91:H21	AMS, PEN, AMC, CRO, CAZ, CTX, ERY, GEN, CM, CIP, LZD	11/15 = 73.33%

IMI (Imipenem, 10 µg), TET (tetracycline, 30 µg), TMP/SMX (sulfamethoxazole/trimethoprim (sutrim), 25 µg), CRO (ceftriaxone, 30 µg), CTX (cefotaxime ,30 µg), AMC (amoxicillin / ciavulnic acid, 20 + 10 µg), AMS (ampicillin / sulbactam, 10 µg), CIP (ciprofloxacin, 5 µg), GEN (gentamicin, 10 µg), CAZ (ceftazidime, 30 µg), MEM (meropenem,10 µg), LZD (linezolid, 30 µg), ERY (erythromycin, 15 µg), PEN (penicillin, 10µg) and CM (clindamycin, 2 µg).

Table 7. Incidence of virulence and antimicrobial resistance genes of different *Salmonella* and *E. coli* strains isolated from meat and meat products.

Serovars	*invA*	*phoA*	bla_{TEM}	bla_{OXA}	bla_{SHV}	bla_{CTX-M}	*qnrA*	*aac(6')Ib-cr*	*AacC*	*aadB*	*Sul1*	*dfrA*	*tetA(A)*	*tetB*	*aac(3)-Ia*
S. Infantis	+	-	+	-	-	+	+	-	-	+	*ND*	*ND*	*ND*	*ND*	-
S. Typhimurium	+	-	+	-	-	+	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
S. Tsevie	+	-	-	-	-	+	*ND*	*ND*	-	+	*ND*	*ND*	*ND*	*ND*	-
O128	-	+	-	-	-	+	*ND*	*ND*	-	+	*ND*	*ND*	*ND*	*ND*	-
O124	-	+	-	-	-	+	*ND*	*ND*	*ND*	*ND*	+	+	+	-	*ND*
O26	-	+	-	-	+	+	+	-	-	+	+	+	*ND*	*ND*	-
O91	-	+	-	-	-	+	+	-	-	+	*ND*	*ND*	*ND*	*ND*	-

kofta similarly to our findings (Al-Mutairi, 2011; Saad *et al*., 2018). An almost similar result was found in minced meat (6=2.2%) from cattle at butcher houses in Hawassa city, Sidama Regional State, Ethiopia (Worku *et al*., 2022). Furthermore, Doménech *et al*. (2015) obtained a lower percentage of *Salmonella* (0.5%, 7 out of 1264 samples) from dried pork sausages. On the other hand, no *Salmonella* were isolated from beef and minced meat (beef and pork) (Mayrhofer *et al*., 2004). The prevalence of *Salmonella* may be ascribed to the initial level of *Salmonella*, slaughterhouse sanitation, level of food processing, cross-contamination during processing, potential contamination at the retail level, and differences in sampling and culture methods (Uyttendaele *et al*., 1998). In addition, the reasons for differences in the distribution of *Salmonella* serotypes are complex, as they may be due to seasonal or demographic factors, or to the prevalence of a particular species in different products (Ziprin, 1994).

Salmonella isolates in this study were typed as *S.* Infantis, *S.* Typhimurium, and S.Tsevie (n=1 for each). Similarly, one (1.13 %) *S.* Typhimurium was isolated from 75 examined ground beef samples (Arslan and Eyi, 2010). Moawad *et al*. (2017) reported that *S.* Typhimurium was the most common serotype isolated from fresh beef, frozen beef and fresh beef organs.

Foods of animal origin are an important cause of *Salmonella* infections in humans, and *Salmonella*e found in animals are often isolated from humans. *S.* Typhimurium has a higher prevalence than other serotypes worldwide. Food animals harbor serotype Typhimurium in a carrier state (Ellermeier and Slauch., 2006; Ray, 2004).

Virulence genes are a set of factors that work synergistically to continue the growth of the microorganism within the host and help it to express virulence leading to the expression of the pathogenic process and the severity of the disease (Murugkar *et al*., 2003). In the present study, the *invA* virulence determinant gene was detected in all *Salmonella* serotypes. The *invA* gene was related to intestinal invasion and widely used for recognizing *Salmonella* spp. in different samples (McWhorter *et al*., 2019).

The highest prevalence of *E. coli* isolates was observed in kofta, frozen beef liver, fresh minced beef and fresh beef sausage samples. In this respect, Abd El-Tawab *et al*. (2019) isolated 11 (6.3%) isolates of *E. coli* from 175 samples of meat and its products; including kofta, sausage (3= 8.6% each) followed by fresh meat, beef burger (2=5.7% each) and luncheon (1=2.9%). Lower incidence of *E. coli* in minced meat (7=2.5%) was recovered by (Worku *et al*., 2022). In several studies, *E. coli* has been isolated from a large proportion of meat and meat products (Al-Mutairi, 2011; Elnawawi *et al*., 2012; Gibbons *et al*., 2006; Hussein, 2007; Messele *et al*., 2017; Saad *et al*., 2018; Shaltout *et al*., 2017; Tavakoli and Riazipour, 2008). Zhao *et al*. (2012) found that 68.9% of ground beef samples were positive for *E. coli*. In addition, twenty-seven bacterial isolates out of 50 (54.0%) samples of raw beef were identified as *E. coli* (Gwida *et al*., 2014). Recently, Adzitey *et al*., (2021) concluded that raw beef (80%) and ready to eat beef (50%) were contaminated by *E. coli*. On the contrary, Siriken *et al*. (2006) failed to isolate *E. coli* from beef sausage. The variation in the prevalence may be due to difference in manufacturing practices, handling, and difference in time of exposure.

In terms of *E. coli* serotyping, the results are consistent with Abd El-Tawab *et al*. (2019); Saad *et al*. (2018) and Tarabees *et al*. (2015) who detected the same *E. coli* serotypes of in samples of meat and meat product. In this respect, Mayrhofer *et al*. (2004) isolated 10 potential pathogenic *E. coli* from 134 beef samples (prevalence 7.5%); of these, seven were categorized as Shiga toxin producing strains (5.2%).

The use of antimicrobials in livestock for treatment, prophylaxis, and growth promotion has raised questions about the development of resistant microbes in food animals. The presence of antimicrobial-resistant bacteria in food animals is a major human health concern due to their potential entry into the meat during animal slaughter and meat processing (Zhu *et al*., 2019). Cross-contamination and consumption of undercooked meat can lead to introduction of resistant bacteria into human body and opportunities may exist in the human gastrointestinal tract for these bacteria to transfer resistance genes to other pathogens.

All *Salmonella* strains were resistant to all tested antimicrobial except Sulfamethizole/ Trimethoprim and Tetracycline. In this respect, Arslan and Eyi (2010) found that all 16 *Salmonella* strains isolated from ground beef showed the highest resistance to cephazolin (100%), ampicillin (93.8%), and amoxicillin–clavulanic acid (68.8%). However, Doménech *et al*. (2015) reported that *Salmonella* isolated from dried pork sausages were sensitive to amikacin, ceftriaxone, ciprofloxacin, gentamicin, and kanamycin, but they were resistant to Tetracycline 85.7%, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole 71.4%, cephalothin (57.1%), and amoxicillin-clavulanate (28.6%). Little *et al*. (2008)

found that the most frequent resistance in *Salmonella* isolates was to ampicillin. Most of the S. enterica isolates demonstrated resistance to ampicillin (87.0%) and cefotaxime (80.0%) and all were susceptible to chloramphenicol, colistin and ciprofloxacin (Moawad *et al*., 2017). Wang *et al*. (2022) found that tetracycline resistance was the most common resistant phenotype of *Salmonella* isolated from retail meat products in Hebei Province, China.

All of the *Salmonella* strains tested were resistant to one or more antibiotics. The existence of *Salmonella* strains resistant to antimicrobial agents can have a potentially negative impact on human health. Arslan and Eyi (2010) found multi resistant to three or more antimicrobial agents in 62% of *Salmonella* strains while about 32% exhibited multiple resistance to four or more antimicrobial drugs. Little *et al*. (2008) showed that 48.1% of the *Salmonella* strains isolated from red meats were multi resistant to at least four antibiotics in the United Kingdom. In a survey conducted in Austria on beef, pork, and poultry meats, a total of 57.7% of *Salmonella* isolates exhibited a resistant phenotype, and most of these strains showed resistance to more than one antimicrobial tested (Mayrhofer *et al*., 2004). Stevens *et al*. (2006) showed that about 16% of the *Salmonella* strains isolated from beef samples in the slaughterhouse and at retailers were multi-resistant to two or more antibacterial gents.

All *E. coli* isolates retrieved from meat and meat products samples developed resistance against all tested antimicrobials. In this context, Messele *et al*. (2017) reported that 50% of the *E. coli* isolates identified from beef meat were resistance to ampicillin. Furthermore, Moawad *et al*. (2017) found that *E. coli* isolates possess resistance to ampicillin, trimethoprim/sulphamethoxazole and amoxicillin–clavulanic acid with 71.4, 61.9 and 61.9%, respectively. However, 10 (47.6%), and 9 (42.8%) isolates were susceptible to ciprofloxacin and ceftriaxone, respectively. Abd El-Tawab *et al*. (2019) fond that *E. coli* were highly resistant for amoxicillin; ampicillin (81.8%); streptomycin (72.7%) and erythromycin (63.6%). On contrary to our findings, Zhao *et al*. (2012) reported tetracycline resistance (50.3%) in *E. coli* isolated from various retail meats in the United States.

All *E. coli* strains from this study showed a multi-drug resistant profile. Moawad *et al*. (2017) reported that 13 *E. coli* isolates (61.9%) were described as multidrug resistant bacteria. Moreover, the results demonstrated that multiple antibiotic resistances are widely spread among isolated *E. coli* in the study conducted by Abd El-Tawab *et al*., 2019. Antibiotic resistance in *E. coli* is of particular concern as it is the most common Gram-negative pathogen in humans; a common cause of both community-acquired and nosocomial-acquired bacteremia, and the main cause of diarrhea as well (Kaper *et al*., 2004). In addition, resistant *E. coli* strains can transfer antibiotic resistance determinants not only to other *E. coli* strains, but also to other bacteria in the gastrointestinal tract and acquire resistance from other organisms.

It was cleared from the previous data that antimicrobial resistance in both *Salmonella* and *E. coli* was extremely high and resistance rates are almost similar in the two pathogens. We theorize that acclimatization to antimicrobials agents used in animal husbandry is an important survival strategy for enteric microbes in the host dominant environment. More striking was the high rate of multidrug resistance in the isolated pathogens. The high level of antimicrobial resistance was directly related to the improper use of broad-spectrum antimicrobials in the livestock and poultry production. The spread of MDR bacteria threatens human health worldwide and could lead to some incurable diseases. Thus, it is necessary to strengthen the monitoring of the antimicrobial resistance of pathogenic bacteria.

The emergence of antimicrobial resistance in meat-borne pathogens and commensals has become a meat safety issue that poses a serious public health risk. Antimicrobial resistance surveillance programs in various nations have been largely based on phenotypic analysis, although genotyping reveals more diversity than phenotypes, allowing for more accurate comparisons between populations of resistant bacteria (Gow *et al*., 2008; Rosengren *et al*., 2009). Regarding, the antimicrobial resistance genes in *Salmonella* isolates, it was revealed that the superior resistance genes were bla_{CTX-M} followed by, bla_{TEM}, *aadB*, and *qnrA*. Wang *et al*. (2021) reported that bla_{TEM} was among the superior resistance genes (62.5%) detected in *Salmonella* isolated from retail meats.

For *E. coli*, it was cleared that bla_{CTX-M} was the superior resistant gene among all detected genes. In addition, data on antimicrobial resistance genes and phenotypes underscore that *E. coli* isolates exhibit high correlation between the phenotypes and genotypes of tetracyclines, β- lactams, aminoglycosides and quinolone. Zhao *et al*. (2012) found that 20% of ceftriaxone and ceftiofur resistant *E. coli* isolates contained bla_{TEM} while no isolates were found to carry bla_{SHV}, bla_{OXA},or bla_{CTX-M}. Abd El-Tawab *et al*. (2019) showed that, the bla_{TEM} gene was amplified in all of the four studied *E. coli* strains. β-Lactam antibiotics are among the most important antibiotic classes including penicillin, cephalosporins, carbapenems and monobactams. β-lactams resistance in Gram-negative bacteria is induced by β-lactamases production, efflux pumps, and alteration of penicillin-binding proteins (Ma *et al*., 2017). Bla_{CTX-M} was distinguished by their utmost activity against cefotaxime than ceftazidime, ceftriaxone, or cefepime. They are mainly found in strains of *Salmonella enterica* serovar Typhimurium and *E. coli* but have also been described in other *Enterobacteriaceae*. In Gram negative bacteria, the most abundant beta-lactamase is bla_{TEM}. Up to 90% of *E. coli* resistance to ampicillin is due to the production of TEM-1, which is also responsible for resistance to ampicillin and penicillin (Cooksey *et al*., 1990). The *aadB* gene confers resistance to kanamycin, gentamicin and tobramycin by a denylating the 2''-hydroxyl group of these antibiotics. The Qnr genes (*qnrA*, qnrB, and qnrS) represent one of the major chromosomal and plasmid-mediated mechanisms of quinolone resistance. These genes encode pentapeptide repeat proteins that block the action of ciprofloxacin (CIP) on bacterial DNA gyrase and topoisomerase IV (Tran and Jacoby, 2002). The resistance to sulfonamide in gram-negative bacilli generally results from the acquisition of the two genes *Sul1* and sul2, which encode forms of dihydropteroate synthase that are not inhibited by the drug (Enne *et al*., 2001). Resistance to these antimicrobials raises concerns about their effectiveness and ultimately therapeutic failure in patients who exposed to these bacteria through the food chain. Antimicrobial resistant *E. coli* can transfer genetic elements of resistance to pathogens in the human gut or while sharing a same niche. Therefore, meat and meat products contaminated with antimicrobial resistant *Salmonella* and *E. coli* can pose a risk to human health if improperly cooked meats is eaten or through poor food handling in the kitchen (i.e., cross-contamination). Of particular concern is the transmission of strains carrying b-lactamase genes.

CONCLUSION

The results of current study showed a low prevalence of *Salmonella* and *E. coli* as foodborne pathogens isolated from meat and meat products in Egypt. Despite the low prevalence, significant multi drug resistant was detected among *Salmonella* and *E. coli* isolates. All *Salmonella* strains were resistant to all tested antibiotic except sulfamethizole/ trimethoprim and Tetracycline. *E. coli* isolates developed a resistance in different patterns against all tested antibiotic, however they were sensitive to Tetracycline. Furthermore, bla_{CTX-M} was the superior resistance gene in both *Salmonella* and *E. coli* isolates. The results of this study also underscore the importance of the impact of antimicrobial resistant pathogens on meat safety. The genotypic and phenotypic antibiotic resistance data assessed in this study would help to assess the transmission of multiple linked antimicrobial resistance genes to human pathogens and possible exposure of consumer to resistant strains.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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