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Original Research Article

PREVALENCE OF SHIGA TOXIGENIC AND MULTI DRUG RESISTANT *Escherichia coli* IN READY TO EAT CHICKEN PRODUCTS' SANDWICHES

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Abstract: Ready to eat (RTE) chicken products are known for their popularity among people from different age groups in Egypt. Two hundred and fifty samples of RTE chicken sandwiches represented by chicken fajitas, shawarma, burger, pane and luncheon (50 for each) were collected and examined for prevalence, virulence and resistance of *Escherichia coli* being one of the most important enteropathogens worldwide. The obtained results declared the presence of *E. coli* in 42, 34, 30, 26 and 14% of the examined samples, respectively. The isolates were found to belong to different *E. coli* pathotypes such as enteropathogenic, enterohemorrhagic, enterotoxigenic and enteroinasive and were positive for serious virulence genes (*Stx*1, *Stx*2 and *eae*A). Moreover, the isolates were tested for their resistance against fourteen commonly used antimicrobials in order to determine their resistance patterns which consequently would reflect their public health significance as well as the degree of drug misuse within the food production chain.

Key words: *E. coli*; antimicrobial resistance; STEC; chicken products; diarrheal diseases

Introduction

Chicken meat is considered as a good source of high quality animal protein, especially in Egypt, where shortages in red meat production are common. Chicken meat is introduced to processing sector and sold as chicken products; further heat treatments are applied in restaurants or at street vending sites, then sold as ready to eat (RTE) chicken meat sandwiches. Ready to eat chicken sandwiches are appreciated for their unique flavors and accessibility. They also provide food security for low income population and livelihood for a significant percentage of the populace in many developing countries (1). The importance of RTE food as a carrier of several microbes has been established, especially street vended food where hygienic standards of preparation are not strictly followed or enforced (2). The fact that very few illnesses can be linked to RTE food with certainty makes it not easy to estimate the problem of foodborne diseases, and these links are usually made only during outbreak situations (3). *Escherichia coli* is one of the most predominant facultative anaerobes of both human and other warm-blooded mammals and often remains harmlessly in the intestinal lumen. Usually, it is considered as an indicator of fecal contamination in food and water;

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however, several *E. coli* clones have the ability to cause diseases within the intestinal tract and in other organs of the host. Pathogenic *E. coli* could be classified into six main pathotypes, including shiga-toxin producing *E. coli*, enteropathogenic *E. coli,* enterotoxigenic *E. coli,* enteroaggregative *E. coli*, enteroinvasive *E. coli,* and diffusively adherent *E. coli* (4). *E. coli* has the ability to transmit, acquire and conserve resistance genes from other bacteria in the environment (5). The rate of antimicrobial resistance in *E. coli* acts as an indicator of resistance transmission in bacterial populations and also as an indicator for the antimicrobials used in the treatment of slaughter animals and humans (6). Therefore, this study was conducted to detect the prevalence of shiga toxin-producing and multi drug resistant *E. coli* in RTE chicken sandwiches collected from different restaurants and street vendors in Zagazig, Sharkia, Egypt.

Material and methods

Samples collection and preparation

Two hundred and fifty RTE chicken sandwiches were collected randomly from different restaurants at Zagazig city, Sharkia Governorate, Egypt, at different sanitation levels during the period from September 2017 till June 2018. The collected samples represented by 50 chicken sandwiches each of fajitas, shawarma, burger, pane and luncheon. Twenty five grams of sandwich core plus 225 ml of sterile peptone water (0.1%) were added and thoroughly homogenized in a sterile blender at 200 rpm for 1-2 min. to provide a homogenate of 1\10 initial dilution (7).

Isolation and identification of E. coli

One ml of the homogenate was added to 9 ml of MacConkey`s broth containing durham's tube then incubated at 44±0.5 °C for 48 hs. A loopfull from each positive tube (acid and gas) of MacConkey`s broth was streaked onto eosin methylene blue (EMB) agar. The inoculated plates were incubated at 37°C for 24 hs. Typical colonies of *E. coli* appear greenish, metallic with dark purple center. Suspected colonies were purified and subcultured onto nutrient agar slopes and incubated for further investigations (8). Morphological and biochemical identification of *E. coli* were then performed as described previously (9-11). The isolates were serologically identified (12) using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the enteropathogenic types.

Multiplex PCR for detection of toxin producing genes of E. coli

DNA was extracted from *E. coli* isolates using QIAamp DNA Mini kit (Qiagen, GmbH, Germany) according to the manufacturer's instructions. Oligonucleotide primers (Pharmacia Biotech, Sweden) used for amplification of *E. coli* virulence genes (*stx*1, *stx*2 and *eae*A) are shown in Table (1). PCR assay was carried out in 1 µl of nucleic acid template (approximately 30 ng of DNA), 10 mM Tris-HCl (pH 8.4), 10 mM KCl, 3 mM MgCl2; 2 mM concentrations of each primer, 0.2 mM concentrations of each 29-deoxynucleoside 59-triphosphate, and 4 U of Ampli*Taq* DNA polymerase (Perkin-Elmer). PCR cycling protocol was performed in a programmable Thermal Cycler (Master cycler, Eppendorf, Hamburg, Germany) as following: an initial denaturation at 95°C step for 3 min. followed by 35 cycles of 95°C for 20 sec, 58°C for 40 s, and 72°C for 90 sec then final extension at 72 °C for 5 min (13). *E. coli* O157:H7 Sakai (positive for *stx*1, *stx*2 and *eae*A) and *E. coli* K12DH5α (a nonpathogenic negative control strain) were used as reference strains. Amplified DNA fragments were analyzed by 2% of agarose gel electrophoresis (Applichem, Germany, GmbH) in 1x TBE buffer stained with Ethedium bromide and captured as well as visualized on UV transilluminator. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes.

Table 1: PCR primers used for multiplex PCR for detection of shiga toxin genes in isolated *E.Coli* from ready to eat chicken sandwitches

Oligonucleotides sequence (5′–′3)	Specificity	Amplicon Size (bp)	Reference
F-**ACACTGGATGATCTCAGTGG** **R-CTGAATCCCCCTCCATTATG**	Shiga toxin type 1	614	(14)
F- **CCATGACAACGGACAGCAGTT** **R-** **CCTGTCAACTGAGCAGCACTTTG**	Shiga toxin Type 2	779	
F- **GTGGCGAATACTGGCGAGACT** **R- CCCCATTCTTTTTCACCGTCG**	Intimin	890	(15)

Antimicrobial susceptibility testing of E. coli isolates

Antimicrobial susceptibility of *E. coli* isolates was performed according to Kirby-Bauer disc-diffusion procedure as described elsewhere (16). The following antimicrobial discs were tested: Penicillin (P), Erythromycin (E), Oxytetracycline (OT), Nalidixic acid (NA), Ampicillin (AM), Sulphamethoxazol (SXT), Cephalothin (KF), Enrofloxacin (ENR), Oxacillin (OX), Neomycin (N), Chloramphenicol (C), Kanamycin (K), Ciprofloxacin (CIP) and Gentamicin (CN) (Oxoid Limited, Basingstoke, Hampshire, UK). The interpretation of the results was done according to Clinical and Laboratory Standards Institute (CLSI) (17). Multiple antibiotic resistance (MAR) index for each isolate was determined according to the following formula (18):

MAR index= No. of antimicrobials to which the isolate is resistant (isolates classified as intermediate were considered sensitive for MAR index) / Total No. of tested antimicrobials.

Results and discussion

Diarrheal diseases are imposing a great hurdle to public health universally, being included in the list of the top ten global causes of death worldwide (19). That fact has attracted our attention to perform a study in order to assess the prevalence, resistance and virulence of *E. coli* in some RTE foods, considering the fact that this microorganism is one of the most important causes of gastrointestinal tract (GIT) disturbance and diarrhea throughout the world (20).

The results presented in Table (2) showed that 42% of chicken fajitas samples harboured *E. coli*. These results were much higher than those obtained previously (21). On the other hand, about 34% of chicken shawarma samples were *E. coli* positive and these results were lower than those recorded by several authors (22,23). Regarding chicken burger samples, the presented study showed that *E. coli* was detected in 30% of the examined samples and this result was higher than that obtained latter (24). Furthermore, about 26% and 14% of chicken pane and chicken luncheon samples, respectively were positive for *E. coli*. These results were higher than those obtained by Samaha et al. (25), while higher results than those of the current study for chicken pane and chicken luncheon samples, respectively were previously detected (23,26).

Although, *E. coli* is readily inactivated above 55°C, the post cooking cross contamination due to contact of raw ingredient with cooked meat may account for prevalence of *E. coli* in the tested RTE chicken sandwiches (27). Also the obtained results showed that chicken fajita samples had the highest prevalence of *E. coli* among the tested samples, and this may be due to inadequate cooking and post processing contamination.

Table 2: Prevalence and serotypes of *E.coli* in ready to eat chicken sandwiches (N=250)

Chicken products		Fajitas	Shawarma	Burger	Pane	Luncheon
	Prevalence	21 (42)	17 (34)	15 (30)	13 (26)	7 (14)
Serotypes of *E. coli*	**O_{111}:H_2**	2 (4)	3 (6)	1 (2)	3 (6)	2 (4)
	O_{91}:H_{21}	1 (2)	ND	2 (4)	1 (2)	ND
	O_{127}:H_6	3 (6)	3 (6)	ND	1 (2)	1 (2)
	O_{119}:H_6	5 (10)	4 (8)	1 (2)	2 (4)	ND
	O_{113}:H_4	ND	2 (4)	3 (6)	1 (2)	1 (2)
	O_{26}:H_{11}	8 (16)	3 (6)	2 (4)	3 (6)	1 (2)
	O_{55}:H7	2 (4)	ND	3 (6)	ND	1(2)
	O_{103}	ND	ND	1 (2)	1(2)	ND
	O_{124}	ND	2 (4)	2 (4)	1(2)	1(2)

Data were represented by No (%); ND, not detected. The percentages in the table were calculated according to number of examined samples per each product type.

Serological identification of *E. coli* is of a great public health importance in order to shed light on the pathotypes included as well as their potential health hazards. In the current study, different serotypes of *E. coli* were identified (Tables 2 and 3). Twenty nine enteropathogenic isolates were obtained from the examined samples represented by four O91:H21 from fajitas, burger and pane samples, twelve O119:H6 isolates from fajitas, burger, shawarma and pane, seven O113:H4 isolates from shawarma, burger, pane and luncheon and six O55:H7 isolates from fajitas, burger and luncheon. Besides, thirty *E. coli* isolates that belonged to enterohemorrhagic pathotype were isolated from the examined samples and represented by five O111:H2 isolates from fajitas, shawarma, burger, pane and luncheon samples, also five O26:H11 isolates from the same samples and only two O103 isolates were obtained from burger and pane. Another pathotype was also identified among the obtained isolates in this study. Eight O127:H6 isolates of the enterotoxigenic pathotype were identified in fajitas, shawarma, pane and luncheon samples. In addition, the study also revealed the isolation of enteroinvasive pathotype from the examined samples. Six enteroinvasive isolates belonging to serotype O124 were isolated from shawarma, burger, pane and luncheon samples. Similar serotypes were also isolated in previous studies performed on RTE meat and meat products. For example, serotypes like O26, O127:H6, O119:H6 and O111 were previously detected in chicken pane and shawerma samples (26). Also, O111, O55, O26 and O91 *E. coli* serotypes were previously documented in RTE chicken meat products (21,28,29). These different pathotypes of *E. coli* had developed the ability to induce diseases in human's GIT, urinary tract and meninges (30). They were reported to cause about 30 to 40 % of all the diarrheal cases in developing countries (31). Furthermore, the current study also referred to the existence of some essential virulence genes in the isolated strains which play a significant role in their pathogenesis. The results presented in Table (2) showed that *Stx*1 was detected in five *E. coli* isolates including O26:H11, O119:H6, O127:H6, O91:H21 and O103, while only two isolates were positive for *Stx*2 (O119:H6 and O55:H7). In addition, six isolates including O26:H11, O55:H7, O127:H6, O113:H4, O103 and O111:H2 were all positive for *eae*A gene. Previous studies had also declared the detection of such virulence genes (*stx*1, *stx*2 and *eae*A) in *E. coli* strains isolated from RTE chicken meats and their products (28,29).

Table 3: Occurrence of virulence genes in enteropathogenic *E. coli* isolated from ready to eat chicken sandwiches

E. coli isolates	Type	*Stx*1	*Stx*2	*eae*A
O26:H11	EHEC	+	_	+
O119:H6	EPEC	+	+	_
O55:H7	EPEC	_	+	+
O127:H6	ETEC	+	_	+
O113:H4	EPEC	_	-	+
O91:H21	EPEC	+	_	_
O103	EHEC	+	_	+
O124	EIEC	_	_	_
O111:H2	EHEC	-	-	+

EHEC, Enterohemorrhagic *E. coli*; EPEC, Enteropathogenic *E. coli*;ETEC, Enterotoxigenic *E. coli*, EIEC, Enteroinvasive *E. coli;* +, Positive; -, negative

The presence of such genes in the *E. coli* isolates contributes greatly to their pathogenicity and virulence (32). Intimin gene (*eae*A) is an essential factor for the microbe to attach strongly to intestinal mucosal cells and initiate disease (4). On the other hand, *stx*1 and *stx*2 have the ability to inhibit protein synthesis in the host cells and subsequently cause them to die (33). That's why the presence of such virulent strains in RTE foods actually sets a great concern in the face of public health. It also worth mentioning that the presence of STEC in RTE chicken meat may be due to cross contamination from the hands of food handlers (28) or also due to the possibility of contaminating RTE chicken by mixing it with contaminated leafy vegetables and salads post-processing (34).

The current study tested the antimicrobial resistance of seventy three *E. coli* isolates against fourteen commonly used antimicrobials in order to detect the patterns of resistance for them which consequently would reflect their public health significance. As shown in Table (4), 100% of the tested isolates were resistant to Penicillin, followed by Erythromycin (94.5%), Oxytetracycline (83.56%), Nalidixic acid (79.45%), Ampicillin (76.7%), Sulphamethoxazol (74%), Cephalotin (72.6%), Enrofloxacin (53.42%), Oxacillin (52.02%), Neomycin (42.5%), Chloramphenicol (38.35%) and Kanamycin (35.6%). The lowest resistance was found against Gentamycin and Ciprofloxacin with percentages of 5.5% and 16.44%, respectively. The obtained results were in same line with that obtained in a previous study (35), they found that tetracycline and Nalidixic acid resistance were 84.4% and 74.1% in retailed chicken from china. Our results disagree with a previously published work (36) in which the highest resistance was against Oxytetracycline (92%) followed by Sulphonamide-trimethoprim (84%), Amoxycillin (76%) and Erythromycin (60%) for *E. coli* isolated from chicken meat. On the other hand, the isolates obtained by several authors (37,38) from ready to eat meat were found to be slightly similar to our study in that 100% of isolates were Penicillin resistant while the lowest resistance percentage (6.7%) was for Gentamycin.

The multi antimicrobial resistance (MAR) index was ranged from 0.071 to 1 with an average of 0.533. Moreover, 4/73 (5.74%) of examined *E. coli* resist all tested antimicrobials, 53/73 (72.6%) resist seven antimicrobials or more as shown in Table (5). Multiple antimicrobial resistance patterns showed that 83.65% of the isolates were resistant to the three or more antimicrobials. Such prevalence of multidrug resistance in *E. coli* have been reported in Bangladesh (39), China (40), Korea (41) and Nepal (42). The proportion of the isolates with MAR index higher than 0.2 was 83.65%, and lower than or equal to 0.2 was 16.35%. MAR index value higher than 0.2 indicated high-risk sources of contamination, where several antimicrobials may often use for the control of diseases (37). The higher resistance of *E. coli* isolates, could be attributed to the misuse of antibiotics for therapeutic or wide use as growth promotors in chicken industry.

Table 4: Antimicrobial susceptibility of *E. coli* isolated from ready to eat chicken sandwiches (N= 73)

Antimicrobial agent	Sensitive	Intermediate	Resistant
Penicillin (P)	0 (0)	0 (0)	73 (100)
Erythromycin (E)	0 (0)	4 (5.5)	69 (94.5)
Oxytetracycline (OT)	9 (12.32)	3 (4.10)	61 (83.56)
Nalidixic acid (NA)	6 (8.21)	9 (12.32)	58 (79.45)
Ampicillin (AM)	0 (0)	17 (23.3)	56 (76.7)
Sulphamethoxazol (SXT)	7 (9.6)	12 (16.44)	54 (74)
Cephalotin (KF)	8 (10.96)	12 (16.44)	53 (72.6)
Enrofloxacin (ENR)	18 (24.65)	16 (21.92)	39 (53.42)
Oxacillin (OX)	26 (35.61)	9 (12.32)	38 (52.05)
Neomycin (N)	32 (43.8)	10 (13.7)	31 (42.5)
Chloramphenicol (C)	38 (52.1)	7 (9.55)	28 (38.35)
Kanamycin (K)	41(56.2)	6 (8.2)	26 (35.6)
Ciprofloxacin (CIP)	55 (75.35)	6 (8.21)	12 (16.44)
Gentamicin (CN)	66 (90.4)	3 (4.1)	4 (5.5)

Data were represented by No (%).

Table 5: Antibiotic resistance pattern and MAR index of *E.coli* isolated from RTE chicken sandwiches (N= 73)

Resistance pattern	Resistance profile	Number of isolates	Number of antibiotics	MAR
I.	P, E, OT, NA, AM, SXT, KF, ENR, OX, N, C, K, CIP, CN	4	14	1
II.	P, E, OT, NA, AM, SXT, KF, ENR, OX, N, C, K, CIP	8	13	0.92
III.	P, E, OT, NA, AM, SXT, KF, ENR, OX, N, C, K	14	12	0.85
IV.	P, E, OT, NA, AM, SXT, KF, ENR, OX, N, C	2	11	0.78
V.	P, E, OT, NA, AM, SXT, KF, ENR, OX, N	3	10	0.714
VI.	P, E, OT, NA, AM, SXT, KF, ENR, OX	7	9	0.642
VII.	P, E, OT, NA, AM, SXT, KF, ENR	1	8	0.571
VIII.	P, E, OT, NA, AM, SXT, KF	14	7	0.5
IX.	P, E,OT, NA, AM, SXT	1	6	0.428
X.	P, E, OT, NA, AM	2	5	0.357
XI.	P, E, OT, NA	2	4	0.285
XII.	P, E, OT	3	3	0.21
XIII.	P, E	8	2	0.142
XIV.	P	4	1	0.071
Average MAR			0.533	

P, Penicillin; E, Erythromycin; OT, Oxytetracycline; NA, Nalidixic acid; AM, Ampicillin; SXT, Sulphamethoxazol; KF, Cephalotin; EN, Enrofloxacin; OX, Oxacillin; N, Neomycin; C, Chloramphenicol; K, Kanamycin; CIP, Ciprofloxacin; CN, Gentamicin; MAR, multiple antibiotic resistance.

Conclusion

In conclusion, RTE chicken sandwiches contaminated with various serotypes of *E. coli* that carry shiga toxin like genes and intimin. The most examined isolates were multiple antimicrobial resistant.

Conflict of interest

None of the authors have any conflict of interest to declare.

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