Japanese Journal of Veterinary Research 64(Supplement 2): S173-180, 2016

Occurrence, phenotypic and genotypic characterization of multidrug resistant zoonotic bacteria isolated from poultry slaughterhouses

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Abstract

A total of 125 swab samples were collected from tables, knives, rinsing water, carcasses' surfaces and workers' hands (25, each) in five poultry slaughter houses at Sharkia Province, Egypt. These samples were examined for the presence of *E. coli* and *Salmonella* spp. and the resistance patterns of the isolates were determined using disc diffusion method. The isolates were serologically, molecularly identified and screened for the presence of antibiotic resistance genes using PCR. The overall prevalence of E.coli was 58.4% compared to 4.8% for *Salmonella* spp. *E. coli* isolates were serologically identified into 10 different serotypes with the predominance of serotype O125:K70 (7 isolates). Moreover, *Salmonella* isolates were serotyped into S. Enteritidis (3 isolates), S. Typhimurium, S. Emek and S. Agona (one isolate, each). *E.coli* and *Salmonella* isolates showed marked variations in their antibiotic resistance patterns. QRDRs of the *gyr*A, *sul*1 and *tet*A genes were identified in 60, 62 and 68 % of E. coli isolates, respectively. On the other hand, the respective prevalence of the former genes in *Salmonella* isolates was 50, 50 and 66.7%. The higher incidence of multidrug resistant *E.coli* and *Salmonella* harboring resistance genes in this study constituting a devastating problem for poultry industry and poultry consumers.

Keywords: *E. coli, Salmonella* spp., drug resistance, resistance genes

Introduction

Poultry meat has become the main source of animal protein in urban and rural populations. Poultry slaughterhouses are one of the major critical points that have potential effect on the hygiene of poultry meat consumed by these populations. During slaughter operations especially in small scale poultry slaughterhouses in particular during skinning, scalding, evisceration, dressing, transport and meat cutting a smaller or high number of the bacteria can be found on carcasses as a contaminant from alimentary tracts of birds, water, working tables, draining boards, utensils and poultry handlers[10)]. Incidences of zoonoses originating from poultry products and processing environments have been reported[11)]. Of importance, *Escherichia coli* and *Salmonella* are the most common pathogenic microorganisms present in poultry meat and have been

incriminated as the leading causes of food-borne illnesses worldwide[22]. Human infection can occur either through handling of raw poultry carcasses and products or the consumption of undercooked poultry meat. Recently, there is a dramatic increase in the antimicrobial resistance in different species of bacteria, particularly multidrug resistance in *Salmonella* and *Escherichia coli* which continue to emerge throughout the world because antimicrobials are extensively used for therapeutic and prophylactic purposes in animals and humans[17]. The emergence of resistance among these pathogens in food animals such as poultry is of increasing concern due to the potential for transfer to the human population[19]. Assessing the distribution of resistance genes responsible for acquisition and spread of the antibiotic resistance in the bacterial population represents a potential useful tool in understanding antimicrobial resistance epidemiology and could facilitate development of effective prevention and control strategies[12]. The present study was conducted to determine the occurrence and the antibiotic resistance patterns of *E. coli* and *Salmonella* spp. isolated from poultry slaughter houses in Sharkia Province, Egypt. Also, molecular identification of the isolates and the detection of drug resistance genes were performed using PCR assay.

Materials and methods

Sample collection: A total of 125 swab samples were collected from tables, knives, rinsing water, carcasses' surfaces and workers' hands (25, each) in five poultry slaughter houses at Sharkia Province, Egypt during the period from May to December, 2014. The swab samples were obtained using sterile cotton swabs which were immersed in sterile test tubes containing buffered peptone water (BPW). All samples were transported as soon as possible in an insulated ice box to the Laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig, Egypt for bacteriological analysis.

Isolation of E. coli and Salmonella spp:

E. coli isolation was conducted according to Quinn *et al.*[23]. On the other hand, *Salmonella* spp. was isolated following the standard methods described in the ISO 6579 [18].

Identification of E. coli and Salmonella spp:

Biochemical identification: The isolated pure cultures of *E. coli and Salmonella* spp. were biochemically identified using the following tests; oxidase, indole, methyl red, voges proskauer, citrate utilization, urea hydrolysis, triple sugar iron agar and lysine decarboxylase.

Serological identification: Serotyping of *E.coli* and *Salmonella* spp. was performed by slide agglutination test using polyvalent and monovalent antisera according to Kauffmann-White scheme.

Molecular identification using polymerase chain reaction (PCR): Biochemically confirmed *E. coli* and *Salmonella* isolates were molecularly identified by conventional PCR assay[25] using primers specific for *E. coli uid*A gene (UAL-F:5'- TGG TAA TTA CCG ACG AAA ACG GC-3', UAR-R: 5'-ACG CGT GGT TAC AGT CTT GCG-3')[28] and invasion gene (invA) of Salmonella (invA-F: 5'-ACA GTG CTC GTT TAC GAC CTG AAT-3', invA-R: 5'-AGA CGA CTG GTA CTG ATC GAT AAT-3') [8].

Antibiotic susceptibility test:

All *E.coli* and *Salmonella* isolates were tested for their susceptibility toward 10 antibiotics provided by BioMerieux, F6980 Marcy Etoite, France using disk diffusion method according to NCCLS[21]. The antibiotics used were as follows: chloramphenicol (30 μg), ciprofloxacin (5 μg), enrofloxacin (10 μg), tetracycline (30 μg), erythromycin (15 μg), trimethoprim- sulfamethoxazole (25 μg), amoxicillin (25 μg), ampicillin (10 μg), gentamycin (10 μg) and neomycin (30 μg). The sensitivity of the microorganism to different antibiotic discs was measured by the diameter of inhibitory zone and compared with antibiotic susceptibility testing sheet.

Molecular detection of antimicrobial resistance

genes in multidrug resistant strains: Phenotypically resistant E. coli and Salmonella isolates were screened for the presence of the genes coding for drug resistance using PCR[25]. The primers synthesized by NWG- Biotech AC targeting *tet*A gene (*tet*A-F: 5'-GCT GTC GGA TCG TTT CGG-3', *tet*A-R: 5'-CAT TCC GAG CAT GAG TGCC-3')[14], *sul*1 (*sul*1-F: 5'-CGG ACG CGA GGC CTG TATC-3', *sul*1-R: 5'-GGG TGC GGA CGT AGT CAGC-3')[15] and QRDRs of the *gyr*A gene (*gyr*A-F: 5'-ATG AGC GAC CTT GCG AGA GAA ATT ACA CCG-3', *gyr*A-R:5'- TTCCATCAGCCCTT CAATGCTGATGTCTTC-3')[2] were used.

Results and discussion

Occurrence of E.coli and Salmonella spp. in the examined samples:

The overall prevalence of *E. coli* in the total examined samples was 58.4% with prevalence of 68, 40, 76, 56 and 52% in tables, knives, rinsing water, carcasses' surfaces and workers' hands, respectively (Table 1). These results are nearly similar to Bonyadian *et al.*[9] who isolated *E. coli* from poultry carcasses with a percentage of 57.3%. However, Tuhin-Al-Ferdous *et al.*[29] identified *E. coli* in 73.3% of rinsing water samples. Concerning *Salmonella* spp. it was clear from Table 1 that 4.8% of the total samples were positive. The prevalence in tables and workers' hands was (4%, each). Moreover, *Salmonella* spp. was isolated from rinsing water and carcasses' surfaces with a percentage of 8% for each but wasn't identified in knives. Nearly similar results were recorded by Chotinum *et al.*[13] in Thailand who isolated *Salmonella* spp. from 7.3% of the carcass-rinse samples. In contrast, our findings are lower than Álvarez-Fernández and García-Fernández[3]

Table 1. Occurrence of *E. coli* and *Salmonella* spp. in the examined samples.

Source of samples	No.	*E. coli*	*Salmonella* spp.
Tables (T)	25	17(68%)	1(4%)
Knives (K)	25	10(40%)	0(0%)
Rinsing water(RW)	25	19(76%)	2(8%)
Carcasses' surfaces(CS)	25	14(56%)	2(8%)
Workers' hands(WH)	25	13(52%)	1(4%)
Total	125	73(58.4%)	6 (4.8%)

With regard to serotyping, Table 2 showed the predominance of *E. coli* serotype O125:K70 (7 isolates), followed by O142:K86, O124:K72, untypable (3 isolates, each), O126:K71, O78:K80 (2 isolates, each), O25:K11, O127:K63, O91: K-, O86:K71, O119:K69 (one isolate, each). O125:K70, O86:K71 and O119:K69 were previously isolated from chicken meat in Egypt by Saad *et al.*[24]. On the other hand, serotyping of *Salmonella* isolates (Table 3) revealed that S. Enteritidis was the most common serovar (3 isolates), *S*. Typhimurium, *S*. Emek and *S*. Agona (one isolate, each). Similarily, Chotinum *et al.*[13] identified S. Enteritidis, S. Typhimurium and S. Agona in samples collected from different sources in small scale poultry slaughter houses in Thailand.

Antibiotic resistance patterns of E. coli and Salmonella spp: Table 4 showed a higher resistance rate of *E.coli* isolates to ampicillin (87.7%), followed by tetracycline, gentamycin (64.4%, each), trimethoprim-sulfamethoxazole (54.8%), amoxicillin (53.4%), erythromycin (52%), neomycin (42.5%), enrofloxacin (38.4%), ciprofloxacin (32.9%) and chloramphenicol (31.5%). These patterns of antibiotic resistance for *E.coli* from poultry slaughter houses substantiate the findings of Álvarez-Fernández *et al.*[4]. Concerning the resistance pattern of *Salmonella* spp., Table 4 verified that 33.3% of the isolates were resistant to each of amoxicillin, ampicillin and

neomycin, 16.7% for enrofloxacin, ciprofloxacin and erythromycin. In contrast none of the isolates exhibited resistance to each of trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline and gentamycin. The resistance patterns of *Salmonella* in this study contrast the findings of Sodagari *et al*.[27] in Iran.

Table 2. *E.coli* serotypes recovered from poultry slaughter houses

Serovars \ Source	T	K	R W	CS	WH	Total
O125:K70	3	0	2	1	1	7
O142:K86	1	2	0	0	0	3
O126:K71	1	0	0	0	1	2
O124:K72	0	3	0	0	0	3
O25:K11	0	0	1	0	0	1
O127:K63	0	0	0	1	0	1
O91:K-	0	0	0	1	0	1
O86:K71	0	0	0	1	0	1
O119:K69	0	0	0	1	0	1
O78:K80	0	0	0	0	2	2
Untypable	0	0	2	0	1	3
Total	5	5	5	5	5	25

Table 3. *Salmonella* serotypes recovered from poultry slaughter houses

Serovars \ Source	T	K	RW	CS	WH	Total
S. Enteritidis	1	0	1	0	1	3
S.Typhimurium	0	0	1	0	0	1
S. Emek	0	0	0	1	0	1
S. Agona	0	0	0	1	0	1
Total	1	0	2	2	1	6

The resistance to more than three drugs was exhibited by a higher percentage of E.coli (64.4%) and *Salmonella* isolates (50%) (Table 5). Moreover, none of the isolates were resistant to all drugs. Multidrug resistance in *E. coli* and *Salmonella* was previously reported by Álvarez-Fernández *et al.*[5] and Chotinum e*t al.*[13], respectively.

Table 4. Antibiotic resistance patterns of *E. coli* and *Salmonella* spp.

Antibiotic	*E. coli*		*Salmonella* spp.	
	No.	%	No.	%
EX	28	38.4	1	16.7
CIP	24	32.9	1	16.7
SXT	40	54.8	0	0
AMX	39	53.4	2	33.3
C	23	31.5	0	0
AMP	64	87.7	2	33.3
T	47	64.4	0	0
G	47	64.4	0	0
N	31	42.5	2	33.3
E	38	52	1	16.7

Enrofloxacin (EX), Ciprofloxacin (CIP), Trimethoprim-Sulfamethoxazole (SXT), Amoxicillin (AMX), Chloramphenicol (C), Ampicillin (A), Tetracycline (T), Gentamycin (G), Neomycin (N) and Erythromycin (E).

Table 5. Distribution of multidrug resistant E.coli and *Salmonella* spp. in this study

Resistance patterns	*E. coli*		*Salmonella* spp.	
	No.	%	No.	%
To one drug	4	5.5	0	0
To only two drugs	10	13.7	1	16.7
To only three drugs	12	16.4	2	33.3
To more than three drugs	47	64.4	3	50
To all drugs	0	0	0	0

Molecular characterization of E. coli and Salmonella isolates:

Figure 1 A& B showed an amplification of *E. coli uid*A gene (147 bp) and *inv*A gene (244 bp) of *Salmonella* spp. Regarding the distribution of the resistance genes in phenotypic resistant *E. coli* and *Salmonella* spp., Tables 6 and 7 showed the predominance of *tet*A gene among *E.coli* (68%) and *Salmonella* spp. (66.7%) (Figure 1 C1&C2). Moreover, 62% of *E. coli* and 50% of *Salmonella* spp. were positive for *sul*1 gene (Figure 1 D1&D2). On the other hand, QRDR of *gyr*A gene was identified in 60 and 50% of *E. coli* and *Salmonella* spp., respectively (Figure1 E& F). Nearly similar

percentages for *tet*A (66%) and *sul*1 (59%) in *E. coli* isolates were recorded by Beatriz *et al.*[7] and Guerra *et al.*[16], respectively. However, a lower percentage for *sul*1 (42%) and mutated *gyr*A (39%) genes were previously recorded[7]. On the other hand, a higher frequency for mutated *gyr*A gene (100%) in Ghana was cited[20]. Consistent with our findings, Ahmed and Shimamoto[1] reported similar percentage (66.7%) for *tet*A gene among *Salmonella* isolates from broilers in Egypt. Conversely, higher percentages for *tet*A and *sul*1 genes (100%, each) in *Salmonella* spp. recovered from poultry in Southern Japan were reported[26].

Table 6. Occurrence of antibiotic resistance genes in phenotypic resistant *E.coli*

Source	No. of isolates	Antibiotic resistance genes					
		*gyr*A		*Tet*A		*sul*1	
		No.	%	No.	%	No.	%
T	10	6	60	7	70	6	60
K	10	6	60	8	80	7	70
RW	10	7	70	6	60	6	60
CS	10	5	50	6	60	5	50
WH	10	6	60	7	70	7	70
Total	50	30	60	34	68	31	62

Table 7. Occurrence of antibiotic resistance genes in phenotypic resistant *Salmonella* spp.

Source	No. of isolates	Antibiotic resistance genes					
		*gyr*A		*Tet*A		*sul*1	
		No.	%	No.	%	No.	%
T	1	1	100	1	100	0	0
K	0	0	0	0	0	0	0
R	2	1	50	1	50	1	50
CS	2	0	0	1	50	1	50
WH	1	1	100	1	100	1	100
Total	6	3	50	4	66.7	3	50

The recorded multidrug resistance in both *E. coli* and *Salmonella* spp. recovered from poultry slaughter houses and the higher frequencies for *tet*A, *sul*1 and QRDR of *gyr*A genes in the isolates may be explained by the indiscriminate use of antibiotics in poultry industry as therapeutic agent or feed additives which contribute to the emergence of multidrug resistant bacteria that pose a significant risk factor for human infection with food-borne bacteria.

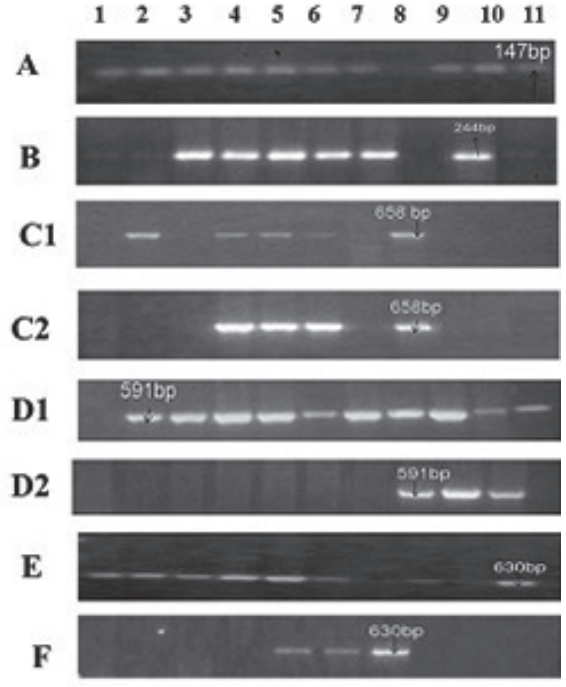

Figure1 A: Representative gel showing an amplification of 147 bp of uidA gene in *E. coli* isolates. Lanes1-11 (*uid*A$^+$), lane 8 (*uid*A$^-$).

Figure1 B: Representative gel showing an amplification of 244 bp of *inv*A gene in *Salmonella* spp. isolates. Lanes 1, 2, 8, 10, 11 (*inv*A$^-$), lanes 3, 4, 5, 6, 7, 9 (*inv*A$^+$).

Figure1 C: Representative gel showing an amplification of 658 bp of *tet*A gene.

C1: *E.coli* isolates (lanes 1, 3, 7, 9, 10, 11: *tet*A-, lanes 2, 4,5, 6,8: *tet*A$^+$).

C2: *Salmonella* isolates (Lanes 4, 5, 6, 8: *tet*A+, lanes 3, 7: *tet*A$^-$).

Figure1 D: Representative gel showing an amplification of 591 bp of *sul*1 gene.

D1: *E.coli* isolates (lane 1: *sul*1-, lanes 2-11: *sul*1$^+$).

D2: *Salmonella* isolates (lane 5, 6, 7: *sul*1-, lanes 8, 9, 10: *sul*1$^+$).

Figure1 E: Representative gel showing an amplification of 630 bp of *gyr*A gene in *E. coli* isolates. Lanes 1-6, 8, 10: gyrA+, lanes 7, 9, 11: gyrA-.

Figure 1 F: Representative gel 630 bp of *gyr*A gene in *Salmonella* spp. isolates. lanes 2, 3, 4: *Salmonella* (*gyr*A$^-$), lanes 5, 6, 7: *Salmonella* (*gyr*A$^+$).

Conclusions

The results from this study emphasize the importance of poultry slaughter houses as a potential source of multidrug resistant *E. coli* and *Salmonella*. In addition, controlling *E. coli* and *Salmonella* in poultry flocks, good hygiene practice, consumer education on topics such as storage temperature and cooking at the right temperature are other appropriate measures that can be practiced and implemented to reduce the risk of antibiotic resistance associated with the consumption of poultry. Thus, much use of antibiotics in poultry industry should be prohibited and completely done under veterinary supervision to minimize the emergence of such resistant strains of *E. coli* & *Salmonella*.

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