Extremely drug-resistant *Salmonella* in broiler production chain in Egypt

Walid Elmonir[1], Abdelhalim M. Hegazy[2], Wael F. El-Tras[3] and Asmaa Shohiep[1]

[1]Hygiene and Preventive Medicine (Zoonoses) Department, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafrelsheikh 33516, Egypt
[2]Poultry Diseases Department, Animal Health Research Institute, Kafrelsheikh Provincial lab, Kafrelsheikh 33516, Egypt
[3]Faculty of Aquatic and Fisheries Sciences (Zoonoses), Kafrelsheikh University, Kafrelsheikh 33516, Egypt
ashohiep@gmail.com

Abstract: Background and objectives: Antibiotic resistance of *Salmonella* isolated from poultry is an emerging threat for public consumers. A cross-sectional study was conducted for investigating prevalence and antibiotic resistance of *Salmonella* in broiler production chain in Egypt. **Materials and Methods:** Environmental samples and broiler carcasses in 30 premises were examined for detection of *Salmonella*. Antibiotic resistance of the isolates was conducted phenotypically by disk diffusion and genetically by detection of *tetA*, *aacC* and *qepA* genes. **Results:** *Salmonella* was detected in 30% of the investigated premises; broiler farms (20%), abattoirs (40%) and poulterers' shops (40%). Highest percentages of *Salmonella* detection were recorded in litter and water (13.3% per each) in broiler farm environment and spleen in broiler carcasses (26.7%). *S.* Kentucky was the most common serovar (40%) and *S.* Uganda (13.3%) was reported for the first time in chicken in Egypt. Multidrug resistance (MDR) was reported in 86.7% of *Salmonella* isolates. Highest resistances were for tetracyclines (86.7%), ampicillin (86.7%), ciprofloxacin (60%) and cefotaxime (53.3%). Six *Salmonella* isolates, *S.* Kentucky (1), *S.* Reubeuss (2), *S.* Tamilnada (1) and *S.* Untypable (2), were extremely drug resistant (XDR) isolates that showed concomitant resistance for ciprofloxacin and cefotaxime. The *tetA*, *aacC* and *qepA* genes were detected in 50%, 30% and 30% of *Salmonella* isolates, respectively. However, discrepancies were observed between phenotypic and genetic antibiotic resistance. **Conclusions:** As far as we know, this is the first report of XDR-*Salmonella* in broiler production chain in Egypt, which may pose an immense risk for public health and broiler industry.
[Walid Elmonir, Abdelhalim M. Hegazy, Wael F. El-Tras and Asmaa Shohiep. **Extremely drug-resistant *Salmonella* in broiler production chain in Egypt.** *Life Sci J* 2017;14(9):81-87]. ISSN: 1097-8135 (Print) / ISSN: 2372-613X (Online). http://www.lifesciencesite.com. 7. doi:10.7537/marslsj140917.07.

Keywords: *Salmonella*, Broilers, Antibiotic resistance, XDR-*Salmonella*.

1. Introduction

Salmonella is one of the most important pathogen responsible for human food poisoning in the world. About 30% of all *Salmonella* outbreaks with suspected food vehicle, were associated with poultry[1].

Salmonella can enter the chicken meat production chain at several points. Poultry farm is a critical point for transmission of *Salmonella* in the production chain. In the farms, within flock transmission of *Salmonella* occurs either by direct contact between infected and uninfected birds or through contaminated poultry house environment; feed, litter, water, rodent and insects[2, 3]. On the other hand, *Salmonella* cross-contamination in chicken slaughterhouses can occur at several stages as by feces during evisceration, cross contamination between healthy carcasses and infected carcasses or contaminated utensils[4]. Moreover, *Salmonella* transmission can occur by contact between live poultry or their byproducts at poulterer's or retail shops, respectively[5]. *Salmonella* was isolated at several points in the chicken meat production chain with varied frequencies worldwide, however higher frequencies were usually associated with lower biosecurity measures and unhygienic food handling practices[6, 7].

The emerging increase in antibiotics resistance is a global public health threat. Reckless use of antibiotic in poultry industry for prevention, growth promotion or therapy have resulted in continuous emerging of multidrug resistant (MDR) poultry isolates. These MDR-*Salmonella* isolates can occasionally transfer to human by food or contact limiting therapeutic options and menace patients' life[8-10]. Alarming emergence of extremely drug resistant (XDR) *Salmonella* isolates that showed concurrent resistance for Fluoroquinolones and extended spectrum cephalosporins were reported in sporadic studies from human patients in China, Taiwan and Zambia[8]. However, first detection of XDR-*Salmonella* in chicken was reported in China[11].

Resistances to antibiotics are genetically encoded and can be attributed to either mutations in antimicrobial target genes, or horizontally transferred resistance genes by genetic vehicles as plasmids. Ciprofloxacin, tetracyclines and gentamicin are very

popular antibiotics in poultry production. Resistance to ciprofloxacin has been associated with either mutation in the *gyrA* and *parC* genes or plasmid mediated quinolone resistance (PMQR) genes and sometimes combination of both[12]. However, recent reports showed that the acquisition of PMQRs more likely has increased the prevalence of ciprofloxacin-resistant *Salmonella* from both human and animal cases[8, 9]. The resistance to tetracycline is associated with *tet* (*A-G*) genes, yet *tetA* is one of the most common *tet* genes detected in animal *Salmonella* isolates[12]. The aminoglycoside acetyl transferase (*aac*) genes which were reported in several *Salmonella* isolates can confer resistance to aminoglycosides as gentamicin[12].

This study was conducted to investigate the prevalence of *Salmonella* in chicken meat production in Egypt and determine their antibiotic resistance phenotypes. The genetic determinants of resistance to ciprofloxacin (*qepA*), tetracycline (*tetA*) and gentamicin (*aacC*) were also studied.

2. Material and Method

Samples collection

The samples were collected during the period between February 2014 and February 2015 from a total of 30 premises in chicken meat production chain in the Nile-Delta of Egypt; 15 broiler farms, 10 poulterers' shops and 5 abattoirs. Poulterers' shops are live bird shops, where birds are slaughtered, defeathered and eviscerated then sold as fresh carcasses with giblets to consumers. Poultry farms were randomly selected with a single flock for each farm. The flocks ranged between 10 to 15 thousand birds and 2 to 6 weeks of age. Inside the farm, samples were collected in pools of five for each of litter, water and feed. In poulterers' shops and abattoirs, ten carcasses were randomly selected and samples from spleen, liver, gall bladder and intestinal content were pooled per each shop or abattoir. Samples were transported on ice to the laboratory and microbiological analysis was immediately conducted.

Isolation and identification of *Salmonella* pathogens

For pre-enrichment, a total of 25 gm or ml of each pooled sample was incubated with 225 ml of buffered peptone water (Oxoid) at 37^{o}C for 18 to 20 hrs. One and 0.1 ml of the pre-enrichment were inoculated in 10 ml of Selenite-F broth (Oxoid) and 10 ml Rappaport–Vassiliadias Soya Peptone (RVS) broth (Oxoid), respectively. The *Salmonella* enrichment tubes were incubated for 18 hrs. at 37^{o}C and 42^{o}C for the Selenite-F and RVS broth, respectively. A loopful from each enrichment broth was streaked on XLD and SS agar plates (Oxoid), which were incubated at 37^{o}C for 24 to 48 hrs. Suspected colonies were picked and furtherly identified by biochemical tests using API 20E kit (BioMérieux, France). Pure suspected *Salmonella* isolates were shipped to central laboratories of Ministry of Public Health, Cairo, Egypt for serological confirmation and serotyping.

Antimicrobial susceptibility testing

Antimicrobial resistance of the *Salmonella* isolates was tested using the disc diffusion method on Mueller Hinton agar according to the guidelines of the Clinical and Laboratory standards Institute[13]. Antibiotic disks (Oxoid) were chosen based on commonly used antibiotics for human and poultry therapy as follow: ciprofloxacin (5µg), cephotaxime (30µg), cefoxitin (30µg), cephalothin (30µg), ampicillin (10µg), amoxicillin/clavulanic acid (20/10µg), gentamicin (10µg), chloramphenicol (30µg), tetracycline (30µg) and sulfamethoxazole/trimethoprim (23.7/1.25µg). The inhibition zones were interpreted using standard break points described by CLSI[13].

Molecular detection of antibiotic resistance genes:

Genomic DNA extraction of *Salmonella* isolates was performed using the QIAamp DNA Mini kit (Qiagen) according to manufacturer's recommendations. Primers (Metabion, Germany) used for amplification of *tetA*[14], *aacC*[15] and *qepA*[16] genes that confer resistance for ciprofloxacin, tetracycline and gentamicin, respectively were investigated in this study (Table 1). PCR mixture contained 12.5 µl of Emerald Amp GT PCR Master Mix (Takara, Japan), 1 µl of each primer (20 pmol), 6 µl of DNA template and PCR grade water up to a 25 µl reaction volume. The PCR cycling conditions were illustrated in Table 1. The reaction was performed in an applied biosystem 2720 thermal cycler and the PCR products were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany). Gel pilot 100 bp DNA Ladders (Qiagen, Germany) were used to determine the fragment sizes and the gel was photographed by a gel documentation system (Alpha Innotech, Biometra).

3. Result

Salmonella was detected in 20% of the broiler farms in this study (Table 2). Prevalences of *Salmonella* in broiler farm environment samples were as follow: 13.3%in litter, 6.7% in feed and 13.3%in water samples. The prevalence of *Salmonella* in poultry abattoirs and poulterers' shops was 40% per each (Table 2). Spleen (26.6%) followed by liver (20%) showed the highest percentages of *Salmonella* detection in the examined broiler carcasses' samples (Table 3). A total of 15 *Salmonella* isolates were detected in this study, 12 isolates belonged to 6 serovars and 3 isolates was untypable (Table 4).*S.* Kentucky was the most common serovar among isolated *Salmonellae* (40%).*S.* Uganda was detected for the first time from chicken in Egypt and

represented 13.3% of the isolates. Multidrug resistance to at least two classes of antibiotics was recorded by 86.7% of *Salmonella* isolates. Major resistances were for tetracyclines and ampicillin (86.7% per each) (Table 5). In addition, *Salmonella* isolates showed high resistance rates to ciprofloxacin (60%) and cefotaxime (53.3%). Six of 15 (40%) *Salmonella* isolates were XDR-*Salmonella*, *Salmonella* showed concomitant resistance for ciprofloxacin and cefotaxime, and were distributed as follows: (1) *S.* Kentucky, (2) *S.* Reubeuss, (1) *S.* Tamilnada and (2) *S.* UT (Table 4). Molecular detection of resistance genes revealed that *qepA*, *tetA* and *aacC* genes were detected in 30%, 50% and 30% of *Salmonella* isolates, respectively (Table 5).

Table 1: Targeted genes and PCR cycling conditions used in this study

PCR cycling conditions					Targeted gene		
Final Extension	Amplification (35 cycle)			Primary Denaturation	Amplicon size	Sequence	Primer
	extension	annealing	denaturation				
72˚C (10 min.)	72˚C (45 sec.)	50˚C (45 sec.)	94˚C (30 sec.)	94˚C (5 min.)	576 bp	F: GGTTCACTCGAACGACGTCA	*tetA*
						R: CTGTCCGACAAGTTGCATGA	
72˚C (10 min.)	72˚C (45 sec.)	60˚C (45 sec.)	94˚C (30 sec.)	94˚C (5 min.)	448 bp	F: GGCGCGATCAACGAATTTATCCGA	*Aacc*
						R: CCATTCGATGCCGAAGGAAACGAT	
72˚C (10 min.)	72˚C (45 sec.)	50˚C (45 sec.)	94˚C (30 sec.)	94˚C (5 min.)	403 bp	F: CGTGTTGCTGGAGTTCTTC	*qepA*
						R: CTGCAGGTACTGCGTCATG	

Table 2: Frequency distribution of *Salmonella* in different premises of broiler production chain in Egypt.

Examined premises (%)	+ve	N	Premises
(20)	3	15	**Broiler Farms**
(40)	4	10	**Poulterers' shops**
(40)	2	5	**Abattoirs**
(30)	**9**	**30**	**Total**

N: number of samples; *: pooled samples.

Table 3: Frequency distribution of *Salmonella* in different samples of broiler production chain in Egypt.

Examined samples/premises (%)	+ve	N*	Type	Premises
(13.3)	2	15	Litter	**Broiler Farms' Environment**
(13.3)	2	15	Water	
(6.7)	1	15	Feed	
(11.1)	**5**	**45**	**Total**	
(26.7)	4	15	Spleen	**Broiler's carcasses**
(20)	3	15	Liver	
(6.7)	1	15	Gall bladder	
(13.3)	2	15	Intestinal content	
(16.7)	**10**	**60**	**Total**	
(14.3)	**15**	**105**		**Total**

N: number of samples; *: pooled samples.

Table 4: Frequency distribution of *Salmonella* serovars in different samples from broiler production chain in Egypt.

Total	Broiler carcasses				Broiler farm environment			Serovars
N (%)	Gall B.	Intestine	Liver	Spleen	Feed	Water	Litter	
5 (40)	0 (0)	0 (0)	0 (0)	3 (60)	0 (0)	0 (0)	2 (40)	***S.* Kentucky**
2 (13.3)	0 (0)	0 (0)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	***S.* Uganda**
2 (13.3)	0 (0)	1 (50)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	***S.* Reubeuss**
1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	***S.* Emek**
1 (6.7)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	***S.* Tamilnadu**
1 (6.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	***S.* Aesch**
3 (20)	1 (33.3)	1 (33.3)	0 (0)	0 (0)	0 (0)	1 (33.3)	0 (0)	***S.* UT**

Table 5: Phenotypic and genetic antibiotic resistance profiles of *Salmonella* serovars isolated from broiler production chain in Egypt.

Genetic resistance profile			Phenotypic resistance profile										Isolates		
qepA	*aacC*	*tetA*	SXT	TE	C	CN	AMC	AMP	KF	FOX	CTX	CIP	ID	Serovars	No.
		+[a]	+	+[a]	+	+	+	+			+	+	o	*S.* Kentucky	**1***
	+		+	+	+			+	+			+	7		**2**
		+[a]	+	+[a]	+		+	+	+				8		**3**
+[c]		+[a]		+[a]			+	+				+[c]	2		**4**
ND	ND	ND		+			+	+				+			**5**
									+	+			5	*S.* Uganda	**6**
+													10		**7**
		+[a]	+	+[a]	+	+	+	+	+		+	+	6	*S.* Reubeuss	**8***
ND	ND	ND	+	+	+	+	+	+	+		+	+			**9***
+			+	+	+		+	+	+		+		4	*S.* Emek	**10**
	+[b]	+[a]	+	+[a]		+[b]		+	+		+	+	1	*S.* Tamilnada	**11***
	+			+				+	+				3	*S.* Aesck	**12**
ND	ND	ND	+	+	+	+		+	+	+	+	+		*S.* UT	**13***
ND	ND	ND	+	+	+	+	+	+	+		+	+			**14***
ND	ND	ND	+	+	+	+	+	+	+		+				**15**
3 (30)	3 (30)	5 (50)	10 (66.7)	13 (86.f7)	9 (60)	7 (46.7)	9 (60)	13 (86.7)	11 (73.3)	2 (13.3)	8 (53.3)	9 (60)	N (%)	Total	

positive; N: number; CIP: ciprofloxacin; CTX: cephotaxime; FOX: Cefoxitin; KF: Cephalothin; AMP: ampicillin; AMC: amoxicillin-clavulanic acid; CN: gentamicin; C: Chloramphenicol; TE: tetracycline; SXT: sulfamethoxazole/trimethoprim; UT: Untypable; *: XDR-isolates; [a]: matched phenotypic and genetic tetracycline resistance; [b]: matched phenotypic and genetic gentamicin resistance; [c]: matched phenotypic and genetic ciprofloxacin resistance; ID: PCR ID of tested Salmonella isolates; ND: not done.

4. Discussion

The prevalence of *Salmonella* in broiler farms was 20%. Varied higher (90.9%) and lower (4.3%) prevalences were reported in USA by Berghaus et al.[6] and Alali et al.[17], respectively. *Salmonella* transmission within the broiler farms can be mediated by contaminated feeds, water or litter[2]. The prevalence of *Salmonella* in litter was 13.3%, which was higher than another report (5%) in Brazil[18]. *Salmonella* was recorded in 6.7% of feed samples, which was higher than that (5%) reported in USA[17]. Prevalence of *Salmonella* in water samples was 13.3% but lower rate (0.2%) was reported in Kuwait[19]. The recorded high rates of *Salmonella* in broiler farm environment in this study, highlights unhygienic production especially in small-scale farms in Egypt and it poses a critical point for *Salmonella* transmission in the broiler production chain.

Salmonella was detected in 40% of the examined abattoirs. This finding was comparable with a report (45.2%) in China[11] and lower than another report (63.8%) in Korea[20]. Prevalence of *Salmonella* in broiler poulterers' shops was 40% and this was almost similar to a report (38%) from retail shops in Pakistan[21]. Liver and spleen provide niches where *Salmonella* multiplication can occur without interruption by host defense mechanisms[2]. Spleen followed by liver showed the highest percentages of *Salmonella* detection in 26.6% and 20% of examined broiler carcasses, respectively. Lower rates in spleen and liver samples (6.25% per each) were reported in India[22]. Intestinal contents are usually incriminated in carcasses contamination during evisceration of broiler carcasses. Prevalence rates of *Salmonella* in intestinal content was 13.3% which was comparable with those reported in Kuwait[19]. Diversity in the prevalence rates between different studies are attributed to differences in sampling methods, seasons, geographical distributions and isolation techniques.

A total of 15 *Salmonella* isolates were detected in this study. Among the isolated serovars, *S.* Kentucky, *S.* Uganda, and *S.* Emek are zoonotic serovars that were associated with several cases of human food poisoning worldwide[23-25]. *S.* Kentucky was the most common serovar among isolated *Salmonellae* (40%). This finding agreed with Foley et al.[26] who reported an emerging increase of *S.* Kentucky serovar in commercial broilers since 1990s. Recently, there was an emerging increase of human cases caused by *S.* Kentucky. During the period between 2007 and 2012, 12 European countries reported 1301 cases of *S.* Kentucky infection and most of these cases were related to traveling to northern Africa (including Egypt) and southern Asia[25]. *S.* Uganda represented 13.3% of the isolates. This may be the first report of *S.* Uganda isolation from chicken in Egypt. This serovar was predominantly isolated from pigs and was responsible of 4 pork-associated outbreaks in humans between 1998 and 2008 in USA[24]. A single isolate of *S.* Emek (6.7%) was recorded in this study, yet this serovar was very common in samples collected from poultry environment and human cases during 1993–2002 in Thailand[23].

In our result, 86.7% of *Salmonella* isolates were multidrug resistant to at least two classes of antibiotics. Lower rates of multidrug resistance in *Salmonella* isolates from broilers were recorded in other countries including USA (39.7%) and Korea (21.6%) by Alali et al.[17] and Kidie et al.[27], respectively. The most common resistance was for tetracyclines and ampicillin (86.7% per each), which was in line with a report in South Africa[10]. However high sensitivity for ampicillin were reported in Korea[27] and in Iran[7].

Fluoroquinolones, and extended spectrum cephalosporins are drugs of choice in human and animal salmonellosis. Despite of retaining sensitivity for cefoxitin by most of *Salmonella* isolates in this study, they showed very high resistance rates to ciprofloxacin (60%) and cefotaxime (53.3%). Much lower resistance rates (8.6 - 17%) in chicken isolates from China[9, 11], respectively. Also, Chinese *Salmonella* isolates showed lower cefotaxime resistance (5.5%) than reported in this study[9, 11]. Emerging increase in ciprofloxacin resistance in *Salmonella* was recorded during the last decade[9, 28]. In addition, Le Hello et al.[28] suggested that the ciprofloxacin-resistant *S.* Kentucky in France originated in Egypt and spread throughout Africa, the Middle East and eventually reached Europe by travelers during 2002 to 2008.

Interestingly, 6 of 15 *Salmonella* isolates (1 *S.* Kentucky, 2 *S.* Reubeuss, 1 *S.* Tamilnada and 2 *S.* UT) showed concomitant resistance for ciprofloxacin and cefotaxime, which can be described as XDR-*Salmonella*[8]. XDR-*Salmonellae* were recorded in sporadic studies from human patients in China, Taiwan and Zambia[8] and a single study from chicken in China[11]. As far as we know, this the first report of XDR-*Salmonella* isolates from chicken in Egypt. All XDR-*Salmonella* in this study were also resistant to ampicillin, gentamicin, tetracycline and sulfamethoxazole/trimethoprim, which agreed with findings of Bai et al.[11] who reported resistance to all these drugs in *Salmonella* isolates with co-resistance to ciprofloxacin and cefotaxime in China. Around 75% of broiler farms in Egypt are small-scale type that share lack of adequate biosecurity measures and unsupervised use of antibiotics[29]. Both managemental risks may enhance emerging of XDR-*Salmonella* by increasing chances of gene-transfer in concurrent multi-serovars infection and selective pressure caused by excess of antibiotics administered. XDR-*Salmonella* will be difficult to treat and therefore could potentially raise morbidity and mortality among animal and human population.

Resistances to antibiotics are genetically encoded. Acquisition of PMQRs facilitated development of fluoroquinolone resistance in *Salmonella*[9]. The *qepA* gene, one of the PMQRs, was detected in 30% of *Salmonella* isolates. This was much higher than the previous records in human (2.4%) in Spain[30] and animal products (1.15%) in Tunisia[31], while another study reported the absence of this gene in *Salmonella* isolates in Korea[32]. The *qepA* gene was detected in three different serotypes (1 *S.* Kentucky, 1 *S.* Uganda and 1 *S.* Emek) in this study, however it was only reported in *S.* Typhimurium in Tunisia and Spain[30, 31]. The *qepA* was associated with phenotypic resistance to ciprofloxacin in *S.* Kentucky isolate, which may imply its role in the emerging quinolone resistance of *Salmonella* spp. It is not clear whether this increase in detection rate of *qepA* in *Salmonella* recently is due to higher rate of plasmid transfer of this gene, probably from other Enterobacteriaceae spp. as *E. coli* or simply due to few studies of *qepA* prevalence have been performed in *Salmonella*[30].

The *tetA* gene, one of the *tet* genes responsible for tetracycline resistant, was recorded in 50% of *Salmonella* isolates in this study, which agreed with Zishiri et al.[10] who reported *tetA* in 44% of *Salmonella* isolates from chicken in South Africa. All *Salmonella* isolates with *tetA* gene showed phenotypic resistance for tetracycline, however tetracycline resistance was also noted in 3 isolates without *tetA* gene. This could be attributed to possible involvement of another non-tested genes of the *tet* complex (i.e. *tetB-G*). The *aacC* gene that confer resistance to aminoglycosides, was reported in 30% of *Salmonella* isolates in this study. The *aacC* gene was shown to play an important role in gentamicin resistance in USA *Salmonella* isolates[12], yet our result showed that only one isolate with this gene was associated with phenotypic resistance to gentamicin.

Discrepancies in phenotypic-genetic antibiotic resistance observed in *Salmonella* isolates of this study was also reported in other studies[10, 30] and was usually attributed to either existence of alternative mechanisms of resistance or defect in expression of resistant genes.

In conclusion, this study showed high prevalence of *Salmonella* in investigated broiler production chain. Majority of detected serovars were zoonotic and showed high percentages of multidrug resistance. Additionally, XDR-*Salmonella* with concomitant resistance for ciprofloxacin and cefotaxime emerged for the first time in broiler production chain in Egypt as well as Africa. These findings imply unhygienic production and misuse of antibiotics in broiler production in Egypt, which warrant continuous surveillance for the emerging antibiotic resistance and improve of biosecurity measures especially for small-scale production sector.

Acknowledgements

The authors are grateful to the veterinarians, owners and workers of broiler farms, poulterers and abattoirs who helped in the samples collection.

References

1. Crim, S.M., M. Iwamoto, J.Y. Huang, P.M. Griffin and D. Gilliss *et al.*, 2013. Incidence and trends of infection with pathogens transmitted commonly through food - Foodborne Diseases Active Surveillance Network, 10 U.S. Sites, 1996–2012. Morb. Mortal. Wkly. Rep. (MMWR), 62: 283–287.
2. Gast, R.K., 2003. Paratyphoid infections. Diseases of poultry, 11th ed. (Eds Saif, Y.). Iowa State University Press, USA.
3. Liljebjelke, K.A., C.L. Hofacre, T. Liu, D.G. White and S. Ayers *et al.*, 2005. Vertical and Horizontal Transmission of Salmonella within Integrated Broiler Production System. Foodborne Pathog. Dis., 2: 90-102.
4. Northcutt, J.K., M.E. Berrang, J.A. Dickens, D.L. Fletcher and N.A. Cox, 2003. Effect of broiler age, feed withdrawal, and transportation on levels of coliforms, Campylobacter, Escherichia coli and Salmonella on carcasses before and after immersion chilling. Poult. Sci., 82: 169-173.
5. Alvarez-Astorga, M., R. Capita, C. Alonso-Calleja, B. Moreno, and M. Del *et al.*, 2002. Microbiological quality of retail chicken by-products in Spain. Meat Sci., 62: 45-50.
6. Berghaus, R.D., S.G. Thayer, B.F. Law, R.M. Mild and C.L. Hofacre *et al.*, 2013. Enumeration of salmonella and campylobacter spp.in environmental farm samples and processing plant carcass rinses from commercial broiler chicken flocks. Appl. Environ. Microbiol., 79: 4106–4114.
7. Sodagari, H.R., Z. Mashak and A. Ghadimianazar, 2015. Prevalence and antimicrobial resistance of Salmonella serotypes isolated from retail chicken meat and giblets in Iran. J. Infect. Dev. Ctries, 9: 463-469.
8. Wong, M.H., M. Yan, E.W. Chan, K. Biao and S. Chen, 2014. Emergence of clinical Salmonella enterica serovar Typhimurium isolates with concurrent resistance to ciprofloxacin, ceftriaxone, and azithromycin. Antimicrob. Agents Chemother., **7**: 3752–3756.
9. Lin, D., K. Chen, E. Wai-Chi Chan and S. Chen, 2015. Increasing prevalence of ciprofloxacin-resistant food-borne Salmonella strains harboring multiple PMQR elements but not target gene mutations. Sci. Rep., 5: 14754.
10. Zishiri, O.T., N. Mkhize and S. Mukaratirwa, 2015. Prevalence of virulence and antimicrobial resistance genes in Salmonella spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort J. Vet. Res., 83: 1-11.
11. Bai, L., R. Lan, X. Zhang, S. Cui and J. Xu *et al.*, 2015. Prevalence of Salmonella Isolates from Chicken and Pig Slaughterhouses and Emergence of Ciprofloxacin and Cefotaxime Co-Resistant S. enterica Serovar Indiana in Henan, China. PLoS One, 10: e0144532.
12. Frye, J.G. and C.R. Jackson, 2013. Genetic mechanisms of antimicrobial resistance identified in Salmonella enterica, Escherichia coli, and Enteroccocus spp. isolated from U.S. food animals. Front. Microbiol., 4: 135.
13. Clinical and Laboratory Standards Institute (CLSI), 2014. Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement (M100-S24), Vol. 34 No. 1, PA, USA.
14. Randall, L.P., S.W. Cooles, M.K. Osborn, L.J. Piddock and M.J. Woodward, 2004. Antibiotic resistance genes, integrons and multiple antibiotic resistance in thirty-five serotypes of Salmonella enterica isolated from humans and animals in the UK. J. Antimicrob. Chemoth., 53: 208–216.
15. Lynne, A.M., B.S. Rhodes-Clark, K. Bliven, S. Zhao and S.L. Foley, 2008. Antimicrobial Resistance Genes Associated with Salmonella enteric Serovar Newport Isolates from Food Animals. Antimicrob. Agents Chemoth., 52: 353–356.
16. Cattoir, V., L. Poirel and P. Nordmann, 2008. Plasmid-mediated quinolone resistance pump QepA2 in an Escherichia coli isolate from France. Antimicrob. Agents Chemoth., 52, 3801-3804.
17. Alali, W.Q., S. Thakur, R.D. Berghaus, M.P. Martin and W.A. Gebreyes, 2010. Prevalence and distribution of Salmonella in organic and conventional broiler poultry farms. Foodborne Pathog. Dis., **7**: 1363-1371.
18. Giombelli, A. and M.B. Gloria, 2014. Prevalence of Salmonella and Campylobacter on broiler chickens from farm to slaughter and efficiency of methods to remove visible fecal contamination. J. Food Protect., 77: 1851-1859.
19. Al-Zenki, S., A. Al-Nasser, A. Al-Safar, H. Alomirah and A. Al-Haddad *et al.*, 2007. Prevalence and Antibiotic Resistance of Salmonella Isolated from a Poultry Farm and Processing Plant Environment in the State of Kuwait. Foodborne Pathog. Dis., 4: 367-373.
20. Choi, S.W., J.S. Ha, B.Y. Kim, D.H. Lee and J.K. Park *et al.*, 2014. Prevalence and

characterization of Salmonella species in entire steps of a single integrated broiler supply chain in Korea. Poult. Sci., 93: 1251–1257.
21. Soomro, A.H., M. Khaskheli and M.B. Bhutto, 2010. Prevalence and antimicrobial resistance of Salmonella serovars isolated from poultry meat in Hyderabad, Pakistan. Turk. J. Vet. Anim. Sci., 34: 455-460.
22. Selvaraj, R., R. Das, S. Ganguly, M. Ganguli and S. Dhanalakshmi *et al.*, 2010. Characterization and antibiogram of Salmonella spp. from poultry specimens. J. Microbiol. Antimicrob., 2: 123-126.
23. Bangtrakulnonth, A., S. Pornreongwong, C. Pulsrikarn, P. Sawanpanyalert and R.S. Hendriksen *et al.*, 2004. Salmonella serovars from humans and other sources in Thailand, 1993-2002. Emerg. Infect. Dis., 10: 131-136.
24. Jackson, B.R., P.M. Griffin, D. Cole, K.A. Walsh and S.J. Chai, 2013. Outbreak-associated Salmonella enterica serotypes and food Commodities, United States, 1998-2008. Emerg. Infect. Dis., 19: 1239-1244.
25. Westrell, T., D.L. Monnet, C. Gossner, O. Heuer and J. Takkinen, 2014. Drug-resistant Salmonella enterica serotype Kentucky in Europe. Lancet Infect. Dis., 14: 270-271.
26. Foley, S.L., R. Nayak, I.B. Hanning, T.J. Johnson and J. Han *et al.*, 2011. Population dynamics of Salmonella enterica serotypes in commercial egg and poultry production. Appl. Environ. Microbiol., 77: 4273-4279.
27. Kidie, D.H., D.H. Bae and Y.J. Lee, 2013. Prevalence and antimicrobial resistance of Salmonella isolated from poultry slaughterhouses in Korea. Jpn J. Vet. Res., 61: 129-136.
28. Le Hello, S., R.S. Hendriksen, B. Doublet, I. Fisher and E.M. Nielsen *et al.*, 2011. – International Spread of an Epidemic Population of Salmonella enterica Serotype Kentucky ST198 Resistant to Ciprofloxacin. J. Infect. Dis., 204, 675-684.
29. Food and Agriculture Organization (FAO), 2007. Interventions for improving biosecurity of small-scale poultry producers in Egypt' FAO report available at: http://www.fao.org/docs/eims/upload/228408/biosecurity_egy_en.pdf.
30. Lunn, A.D., A. Fàbrega, J. Sánchez-Céspedes and J. Vila, 2010. Prevalence of mechanisms decreasing quinolone-susceptibility among Salmonella spp. clinical isolates. Int. Microbiol., 13: 15-20.
31. Al-Gallas, N., M.S. Abbassi, B. Gharbi, M. Manai and M.N. Ben Fayala *et al.*, 2013. Occurrence of plasmid-mediated quinolone resistance determinants and rmtB gene in Salmonella enterica serovar enteritidis and Typhimurium isolated from food-animal products in Tunisia. Foodborne Pathog. Dis., 10: 813-819.
32. Kim, J.H., J.K. Cho and K.S. Kim, 2013. Prevalence and characterization of plasmid mediated quinolone resistance genes in Salmonella isolated from poultry in Korea. Avian Pathol., 42: 221-229.

9/24/2017