Global Veterinaria 18 (5): 322-331, 2017
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2017.322.331

Molecular Characterization of Class 1 Integrons and Antibiotic Resistance Genes in *Salmonella enterica* Isolated from Chicken

[1]*Rania M. Orady,* [2]*Salwa M. Helmy,* [3]*Ahmed M.A. Ammar,*
[1]*Wafaa M. Hassan,* [2]*Etab M.Abo Remela and* [4]*Azza S. EL-Demerdash*

[1]Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQCP).
Animal Health Research institute, Dokki, Giza, Egypt
[2]Department of Microbiology, Faculty of Veterinary Medicine,
Kafr EL Sheikh University, Kafr EL Sheikh, Egypt
[3]Department of Microbiology, Faculty of Veterinary Medicine, Zagazig University, Zagazig, Egypt
[4]Animal Health Research Institute, Zagazig, Egypt

Abstract: The use of antimicrobial agents for therapeutics is strongly associated with a prevalence of antimicrobial resistance through several poultry farms in Egypt. Thus, we aimed to characterize integrons in Salmonella isolates from chicken in correlation with their antimicrobial resistance pattern. A total of 300 clinical tissue specimens collected from different poultry farms in Dakahlia governorate were examined for the presence of Salmonella followed by phenotypic antibiotic susceptibility testing and detection of class I integron and associated gene cassettes. Twenty isolates exhibited features of multidrug resistance (MRD). Class 1 integron was found in19 of 20 MRD Salmonella isolates (95%). Sequencing of variable parts revealed that class 1 integrons harbored genes encoding resistant determinants to trimethoprim (*dfrA1* and *dfrA15*), chloramphenicol (*catB*) and aminoglycoside (*aadA1*). Thus, this study was planned to detect antibiotic resistance genes in Salmonella to select the highly effective antimicrobial agents for treatment and prevention.

Key words: Salmonella · Multi drug resistance · Class 1 integrons

INTRODUCTION

Avian salmonellosis is an important disease causing serious impediment to the development of poultry industry especially in developing countries of Asia and Africa. Since no "effective" immunoprophylactic measures are available for the disease till date, strict biosecurity is the only alternative to preclude the disease [1].

The uncontrolled usage of antimicrobials has resulted in the emergence of multiple drug resistant *Salmonella* species in the food production continuum [2]. Multidrug resistance (MDR), is a serious and growing phenomenon that has emerged as one of the pre-eminent public health concerns of the 21st century [3]. Several of the antibiotic resistance genes observed in Gram-negative microorganisms are part of a gene cassette inserted in an integron [4, 5]. The most common cassettes contain genes that confer resistance to a range of antimicrobial agents, including aminoglycosides, B-lactams, chloramphenicol and trimethoprim, as well as genes that confer resistance to antiseptics and disinfectants [4, 5]. Several classes of integrons related to antibiotic resistance have been identified that can be distinguished by the nucleotide sequence of their respective integrase [5].

Integrons are mobile DNA elements with the ability to capture genes, notably those encoding antibiotic resistance, by site specific recombination and they have an integrase gene (*int*I), a nearby recombination site (*att*I) and a promoter, *Pant* [6].

Integrons are considered to be the most important contributors to multidrug resistance in Gram-negative bacteria especially class 1 integrons which are mostly found on bacterial plasmid [7]. These integrons comprise two conserved segments (5° CS and 3° CS) separated by a variable region that usually contains one or more gene

Corresponding Author: Rania M. Orady, Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQCP). Animal Health Research Institute, Dokki, Giza, Egypt. E-mail: raniaorady84.ro@gmail.com.

cassettes. [8]. The 5° CS contains a promoter (*Pant*), an integration site (*att*I1) and integrase gene (*int*I1). The 3° CS usually consists of *sul*1 that encodes resistance to sulphonamides, *qac*EÄ1 (Attenuated variant of *qac*E gene) and *ORF5* of unknown function. The gene cassettes located in the variable regions are mobile and generally encode for antibiotic resistance [9].

Therefore, the aim of our study was planned to detect antibiotic resistance profile of *Salmonella enterica* isolated from chickens and their relevant resistance genes carried on class 1 integron in Egypt to select the highly effective antimicrobial agents for treatment and prevention.

MATERIALS AND METHODS

Sampling and Isolates Characterization: A total of 300 samples (118 ceca, 85 liver, 55heart and 42 spleen) were aseptically collected from 250 chicken apparently healthy 50, diseased 150 and freshly dead 50, from different localities in Dakahlia Province, Egypt and were submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQCP) Animal Health Research institute, Dokki, Giza, Egypt. All samples were subjected to conventional methods for isolation and identification according to ISO 6579[10].Identified isolates were serotyped by a standard slide and tube agglutination test in serology unit using commercial polyvalent and monovalent O and H antisera (SIFIN. Berlin, Germany. Catalogue number: TR 1141, TR 1145) to identify Salmonella serovars.

Antimicrobial Susceptibility Testing: The antimicrobial susceptibility testing was done by the agar disc diffusion method on Muller Hinton agar as described by Finegold and Martin [11]. The used antimicrobial agents were Nalidixic acid (NA) (30 µg), chloramphenicol(C)(30 µg), Flumequine(UB) (30 µg), Ciprofloxacin (CIP) (5 µg), Enrofloxacin (ENR) (5 µg), Norfloxacin (NOR) (10 µg), Levofloxacin (LEV) (5 µg), Amoxicillin (AML) (10 µg), Ampicillin – sulbactam (SAM) (20 µg), Cefotaxime (CTX)(30 µg), Ceftriaxone (CRO)(30 µg), Ceftazidime (CAZ) (30 µg), Neomycin (N) (30 µg), Streptomycin (S) (10 µg), Amikacin(AK) (30 µg), Gentamycin (CN) (10 µg), Oxytetracycline (OT)(30 µg) and Sulfamethoxazole-Trimethoprim (SXT) (25 µg). The inhibition zones, in millimeters, were measured in duplicate and scored as sensitive, intermediate and resistant categories in accordance with the critical breakpoints recommended by the Clinical and Laboratory Standards Institute [12].

Table 1: oligonucleotide primers

Primer	Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
Hep35	Int	TGCGGGTYAARGA-TBTKGATTT	491	[13]
Hep36		CARCACATGCGTRTARAT		
Hep58	Int1	TCATGGCTTGTTATGACTGT	Variable*	
Hep59		GTAGGGCTTATTATGCACGC		
Int1-F	Int1	CCTCCCGCACGATGATC	280	[14]
Int1-R		TCCACGCATCGTCAGGC		

Variable: Its variable and not detected at seperate region as it specific for integron cassettes as mentioned in perivious research.

PCR Screening of Class 1 Integron: Based on antimicrobial resistance profiles, MDR *Salmonella* species (Resistant to three or more class of antimicrobial agents) were screened for presence of integrase gene, class 1 integron and their associated genes cassettes. Plasmid DNAs of bacterial isolates were extracted usingQIAprep SpinMiniprep Kit, Catalogue no. 27104 and using oligonucleotide primers in Table (1).

Characterization of Inserted Gene Cassettes by Sequencing: A purified PCR product was sequenced in the forward and/ or reverse directions on an Applied Biosystems 3130 automated DNA Sequencer (ABI, 3130, USA). Using a ready reaction Big dye Terminator V3.1 cycle sequencing kit. (Perkin-Elmer/Applied Bios stems, Foster City, CA), with Cat. No. 4336817 and A BLAST® analysis (Basic Local Alignment Search Tool) [15] was initially performed to establish sequence identity to Gene Bank accessions.

RESULTS

Incidence of the Isolated Salmonellae from Different Internal Organs: Examination of 300 chicken samples aseptically collected from 250 chicken apparently healthy (50), diseased (150) and freshly dead (50) chickens revealed that 32 isolates of *Salmonella* species were isolated with an overall percentage of (12.8%).

The highest isolation rate of *Salmonella* species was in the highest isolation rate of *Salmonella* species was in Spleen (14.29%) followed by Cecum (12. 71%), Liver (10. 59%) and heart (3. 64%).

Twenty serovars were identified including *S. typhimurium* and *S. Enteritidis* which predominated and accounted for 15. 6% of total Salmonella isolates for each serovar. Other serotypes isolated were *S.*Newport with incidence (12.5%), *S.* Kentucky and *S.* Tamale with incidence (6.2% for each) and *S.* Apeyemene*, S.* Daula,

Table 2: Results of antibiotic susceptibility testing of Salmonella isolates to different antibiotics

		Salmonella isolates(no = 32)		
Antimicrobial class	Antimicrobials	R	I	S
Penicillins	Amoxicillin (AM)	24 (75.0%)	1 (3.1%)	7 (21.8%)
B-lactam inhibitors	Ampicillin/Sulbactam (SAM)	24 (75%)	4 (12.5%)	4 (12.5%)
Cephalosporins	Cefotaxime (CTX)	6 (18. 7%)	9 (28.1%)	17 (53.1)
	Ceftriaxone (CRO)	5 (15. 6%)	8 (25%)	19 (59.3%)
	Ceftazidime (CAZ)	5 (15. 6%)	13 (40. 6%)	14 (43. 7%)
Aminoglycosides	Gentamicin (CN)	10 (31.25%)	7 (21.8%)	15 (46.8%)
	Neomycin (N)	14 (43. 7%)	12 (37.5%)	6 (18. 7%)
	Streptomycin (S)	18 (56.2%)	5 (15. 6%)	9 (28.1%)
	Amikacin (AK)	0	0	32 (100%)
Quinolones	Nalidixic acid (NAL)	30 (93. 7%)	1(3.1%)	1(3.1%)
Fluoroquinolones	Flumequine (UB)	30 (93)7%)	1 (3.1%)	1(3.1%)
	Ciprofloxacin (CIP)	11 (34.3%)	7 (21.8%)	14 (43. 7%)
	Enrofloxacin (ENR)	17 (53.1%)	8 (25%)	17 (53.1%)
	Norfloxacin (NOR)	5 (15. 6%)	7 (21.8%)	20 (62.5%)
	levofloxacin (LEV)	5 (15. 6%)	8 (25%)	19 (59.3%)
Phenicols	Chloramphenicol (C)	12 (37.5%)	6 (18. 7%)	14 (23.3%)
Tetracyclines	Oxytetracycline (OT)	23 (71. 8%)	1 (3.1%)	8 (25%)
Sulphonamides, inhibitors and combinations	Trimethoprim–Sulfamethoxazole (SXT)	17 (53.1%)	1 (3.1%)	14 (23.3%)

S. Tamale, *S*. Bargny, *S*. Papuana, *S*. Labadi, *S*. Amersfoort, *S*. Hindmarsh, *S*. Rechovot, *S*. Cremieuz, *S*. Heistopdenberg, *S*. Agona, *S*. Molade, *S*. Lexingtonand *S*. Takoradi with incidence (3.1%) for each one.

Drug Resistance Analysis of Bacterial Isolates: Results of antibiotic susceptibility testing of Salmonella isolates to different antibiotics are shown in Table (2).

Regarding antibiogram of Salmonella isolates, the obtained results showed that 100% of the isolates were susceptible to amikacin, while, high resistance rates were observed against Flumequine (93.7%) and Nalidixic acid (93.7%), Ampicillin/Sulbactam (75%), Oxytetracycline (71. 8%), Amoxicillin (75.0%), Trimethoprim–Sulfamethoxazole(53.1%)andStreptomycin (56.2%) followed by Enrofloxacin (53.1%), Neomycin (43. 7%) and Chloramphenicol (37.5%). Such increased resistance to the aforementioned antibiotics could be attributed to the wide and misuse of these drugs in veterinary field.

Additionally, 20 out of 32 isolates (62.5%) showed multidrug resistance phenotypes to at least three classes of antimicrobials.

Class 1 Integrons and Associated Cassette Arrays: In this approach, integrase gene was detected in 19 out of the 20 MDR isolates with percentage (95%) giving characteristic bands at 491 bp (Fig. 1). Also, class 1 integron (Conserved segment) were detected in those isolates with percentage (95%) isolates giving

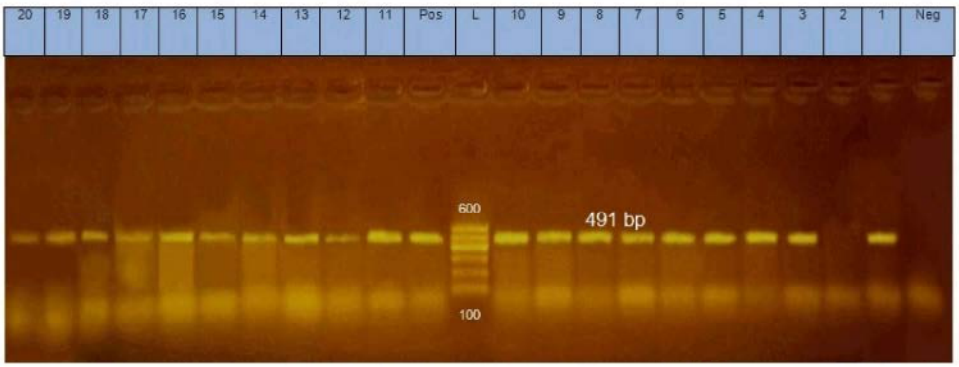

Fig. 1: Agarose gel electrophoresis showing PCR amplification at 491bp fragment for integrase gene among DNA products of 20 multidrug resistant salmonella isolates. L):100 bp DNA ladder, Neg.: Negative Control, Pos.: Positive control, lane 2: negative sample.

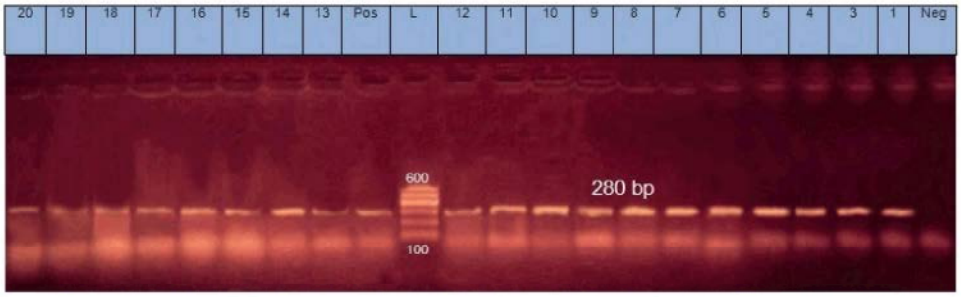

Fig. 2: Agarose gel electrophoresis showing PCR amplification at 280 bp fragment for class 1 integron (Conserved segment) among DNA products of 20 multidrug resistant salmonella isolates. L):100 bp DNA ladder, Neg.: Negative Control, Pos.: Positive control, lane 2: negative sample.

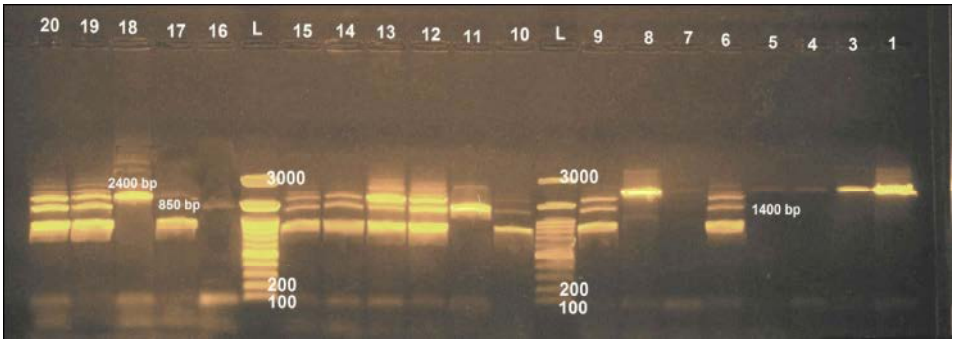

Fig. 3: Agarose gel showing amplified products of class 1 integrons at variable sizes of Salmonella isolates. Lanes (1-20): amplicons of Salmonella isolates, L: 100 bp DNA ladder

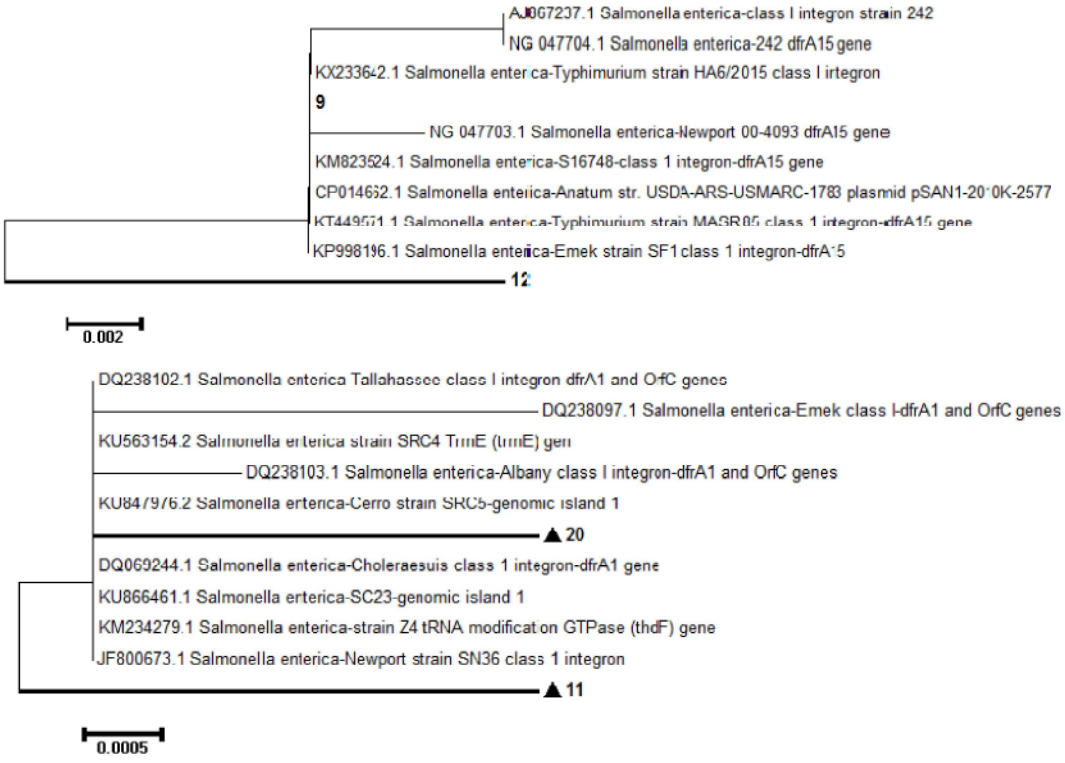

Fig. 4: Phylogenetic tree of sequenced samples

Table 3: Distribution of resistance genes in the examined 20 MDR Salmonella isolates

Code	Serovars	Antimicrobial resistance pattern	Class 1 integron	Integron amplicon size	Gene cassette
1	*S*. Typhimurium	NA-SAM-N-S-OT	+	2400 bp	*dfrA1- catB2- aadA1*
2	*S*. Apeyeme	C-NA-UB-CIP	-	-	-
		ENR-NOR-LEV-AML-SAM-S-OT-SXT			
3	*S*. Kentucky	C-NA-UB-ENR-AML-SAM-SXT	+	2400 bp	*dfrA1- catB2- aadA1*
4	*S*. Daula	UB- ENR-SAM	+	2400 bp	*dfrA1- catB2- aadA1*
5	*S*. Newport	C-NA-UB-AML-SAM-CAZ-N-CN-OT-SXT	+	2400 bp	*dfrA1- catB2- aadA1*
6	*S*. Tamale	C-NA-S-SXT	+	850bp-1400 2400	*dfrA15 dfrA1*
					dfrA1- catB2- aadA1
7	*S*. Bargny	C-NA-UB-CIP-ENR-AML-SAM-OT-SXT	+	2400 bp	*dfrA1- catB2- aadA1*
8	*S*. Enteritidis	C-NA-UB-SAM-S-OT-SXT	+	2400 bp	*dfrA1- catB2- aadA1*
9	*S*. Papuana	NA-UB-CIP-ENR-	-	850bp-1400	*dfrA15 dfrA1*
		NOR-AML-SAM-CTX-CRO-N-CN-S-OT-SXT	+	2400	*dfrA1- catB2- aadA1*
10	*S*. Labadi	C-NA-UB-AML-SAM-N-S-OT-SXT	+	850 bp	*dfrA15*
11	*S*.Amersfoort	NA-UB-ENR-LEV-AML-OT	+	1400 bp -	*dfrA1*
12	*S*. Hindmarsh	NA-UB-CIP-ENR-AML-SAM-		850bp-1400	*dfrA15 dfrA1*
		CTX-CRO-N-CN-S-OT	+	2400	*dfrA1- catB2- aadA1*
13	*S*. Rechovot	C-NA-UB-ENR-LEV-SAM-	+	850bp-1400 2400	*dfrA15 dfrA1*
		CRO-N-CN-S-OT-SXT			*dfrA1- catB2- aadA1*
14	*S*.Cremieuz	C-NA-UB-AML-SAM-S-SXT	+	850bp-1400 2400	*dfrA15 dfrA1*
					dfrA1- catB2- aadA1
15	*S*.Heistopdenberg	C- NA-UB-AML-SAM-OT	+	850bp-1400 2400	*dfrA15 dfrA1*
					dfrA1- catB2- aadA1
16	*S*.Agona	NA-UB-SAM-CN	-	-	-
17	*S*. Santiago	NA-UB-AML-SAM-N-S-OT-SXT	+	850	*dfrA15*
18	*S*. Molade	C-NA-UB-CIP-ENR-AML-SAM-	+	2400bp	*dfrA1- catB2- aadA1*
		CTX-CRO-N-CN-S-OT			
19	*S*. Lexington	NA-UB-ENR-LEV-SAM-CRO-N-CN-S-OT-SXT	+	850bp-1400 2400	*dfrA15 dfrA1*
					dfrA1- catB2- aadA1
20	*S*. Takoradi	C-NA-UB-AML-SAM-S-SXT	+	850bp-1400 2400	*dfrA15 dfrA1*
					dfrA1- catB2- aadA1

(NA): Nalidixic acid,(C): chloramphenicol, (UB): Flumequine, (CIP): Ciprofloxacin, (ENR): Enrofloxacin, (NOR): Norfloxacin, (LEV): Levofloxacin,: (AML): Amoxicillin, (SAM): Ampicillin – sulbactam, (CTX): Cefotaxime, (CRO): Ceftriaxone, (CAZ):Ceftazidime(N): Neomycin(S): Streptomycin, (AK): Amikacin, (CN): Gentamycin, (OT): Oxytetracycline and (SXT): Sulfamethoxazole- Trimethoprim. *dfr*: dihydrofolate reductase gene. *aad:*: aminoglycoside adenyltransferase, *Cat*: chloramphenicol adenyltransferase.

characteristic bands at 280 bp (Fig. 2) and PCR amplification of the variable regions of integron positive isolates revealed three different fragment sizes of approximately 0.85kb, 1.4kb and 2.4kb. The resistance gene cassettes were detected in15 out of the 19class 1 integron positive isolates.DNA sequence analysis revealed that gene cassette fragment gave 100% homology with *(dfrA15, dfrA1 and dfrA1- catB2- aadA),* respectively conferring resistance to trimethoprim, chloramphenicol and aminoglycosides as illustrated in Fig. 4.

DISCUSSION

Salmonella cause diseases in animals and people. It is one of the most significant causes of food poisoning in humans. Salmonellosis is an important zoonosis associated with food consumption of animal origin. Poultry eggs, meat and their products are the commonest vehicles for the transmission of human salmonellosis constituting an important threat to public health [16].

The aim of this work was to throw light on molecular detection of antimicrobial resistant genes carried on class 1 integron among MDR *Salmonella* species which may transfer through chicken to human.

The obtained results in the current study showed that on examination of 300 chicken samples aseptically collected from 250 apparently healthy 50, diseased 150and freshly dead 50chickens. 32 *Salmonella* isolates were obtained with an overall percentage of (12.8%) with highest isolation rate in spleen.

Nearly similar results were obtained in Egypt by Draz*et al.* [17] who reported 11.4% of *Salmonella* species were isolated from living layer flocks in poultry farms at Alexandria, Egypt. Moreover, in Sharkia Governorate, El-Azzouny *et al.* [18] recorded Salmonella with

percentage of 10% in broiler internal organs (Liver, spleen and heart) with a previous history of diarrhea. Meanwhile, Al-Zenki *et al.* [19] isolated *Salmonella* species with the percentage of 12.6% in ceca collected from poultry farms.

Different percentages were reported by Sadoma [20] and Mohammed *et al.* [21] that isolated salmonellae from chicken farms in Gharbia and Kafr-Elsheikh with an overall prevalence of 2% and 2.5%, respectively. Meanwhile, in Sharkia, a percentage of 1.7% of Salmonella isolation rate was reported by Ahmed *et al.* [22] from chicken reared in rural houses.

Many studies showed different prevalence rates of Salmonella isolates in broilers worldwide as in North Vietnam [23] where *Salmonella* spp. were found in 3.1% of organ samples of chickens. However, higher percentage was recorded by Molla *et al.* [24] who isolated salmonella from chicken liver with the percentage of 34.5% in Ethiopia. These differences in prevalence rates may reflect considerable disparity in the sampling scheme, sample types, Salmonella detection protocol and geographic location.

Poultry are commonly infected by a wide variety of Salmonella serovars; one serovar may be a predominant isolate in a country for several years before it is replaced by another serovar. Serovars vary geographically, but clinically significant S. Typhimurium and S. Enteritidis were identified as the most common serovars reported globally and accounted for 15. 6% of total Salmonella isolates for each serovar. Other serotypes isolated were *S.* Newport with percentage (12.5%), *S.* Kentucky and *S.* Tamale with percentage (6.2%) and *S.*Apeyeme*, S.* Daula*S. S.* Tamale, *S.* Bargny, *S.* Papuana, *S.* Labadi, *S.*Amersfoort, *S.Hindmarsh, S.* Rechovot, *S.*Cremieuz, *S.*Heistopdenberg, *S.*Agona, *S.* Molade, *S.* Lexington and *S.*Takoradi with percentage (3.1%).

These results agreed with Verma *et al.* [25] who isolated *S.* Typhimurium with a percentage of (18.10%) While these results differ from Edel *et al.* [26] who recorded *S.* Enteritidis with an incidence of 1.4% for laying flocks and 1.1% for broiler breeder flocks in Netherland. While, lower percentages of *S.* Typhimurium isolation were reported in Nigeria (6.7%) and Taiwan (7.7%) by Orji *et al.* [27] and Tsai *et al* [28], respectively.

Another study in Egypt reported a predominance of S. Enteritidis and S. Typhimurium from chicken (58.33% and 41.66%, respectively [29]. Also, in Saudi Arabia, S. Enteritidis and S. Typhimurium dominated among the recovered Salmonella serovars from chicken (55.56% and 22.22%, respectively) [30].

Recently, incidence of multiple antimicrobial resistances in Salmonella spp. isolated from poultry has increased as a result of extensive use of antibiotics in human and veterinary medicine [31].

In this study, all strains were sensitive to amikacin which was the most effective chemotherapeutic agent against Salmonella infection which is in parallel with that recorded by snow *et al* [32]. Also Higher rates of sensitivity were observed to Neomycin and chloramphenicol with percentages comparable to those found in many developing countries, especially Bangladesh, Nigeria and Pakistan [33] and [34].

While high levels of resistance were observed against Flumequine and Nalidixic acid, Ampicillin/ Sulbctam, Amoxicillin, Trimethoprim–Sulfamethoxazole, Enrofloxacin and Oxytetracycline. These results are nearly similar to the results obtained by Chuanchuen *et al* [35] reported that *Salmonella enterica* isolates from poultry were resistant to the antibiotics: Ampicillin (53.3%); Chloramphenicol, (36.1%); Gentamicin (17.2%); Tetracycline (59%); Trimethoprim (39.3%); Streptomycin (55.7%) and Sulfamethoxazole (68.9%). Such increased resistance to the aforementioned antibiotics could be attributed to the wide and miss use of these drugs in veterinary field.

Furthermore, 20 isolates (62.5%) showed multidrug resistance phenotypes to at least three classes of antimicrobials. The percentage of multidrug-resistant Salmonella strains is differ from that reported in Italy (2.3%) [36] Iran (23.5%) [37] and that found (100%) by in Turkey [38], Spain (100%) [39], Brazil (100%) [40], Nepal (100%) [41], the United States (92%) [42].but nearly agree with[43] in Morocco (75.43%) There is good evidence for an association between presence of class1 integron and emerging MDR.

Integron, a novel DNA element has great impact on human health because of its responsibility about bacterial multidrug resistance [44].

In the current study, class 1 integron was screened among the obtained multidrug resistant Salmonella isolates. Class 1 integrons were detected in almost isolates (95 %). Thus, class 1 integrons contribute significantly to antibiotic resistance in Salmonella isolates [45] and [46].

The results of the current study are nearly agree with data of several countries as in Portugal [47] 99% and in India [48] 69.9% of and in Japan. Class 1 integrons were detected in 94.8% of isolates. On the contrary, low percentage obtained by Francis *et al* [49] in China detected class 1 integron in 24.5% of salmonella species isolated from poultry.

The amplification of the variable region of class 1 integrons revealed that 15 isolates (78.9%) only harboured gene cassettes of fragment size 850 bp, 1400bp and 2400 bp which was probably related to the absence of the 3`-conserved region in a large number of integrase positive isolates and/or attributable to attenuation of the promoters [50, 51].

The sequence analysis of gene cassette arrays revealed a predominance of cassettes that confer trimethoprim (*dfr*A genes) that have been reported worldwide in isolates of different origin [52-54].

Furthermore, a class 1 integron carrying *dfrA1- catB2- aadA1* cassette array was exclusively found in almost isolates and conferring resistance to trimethoprim, chloramphenicol and aminoglycoside. The persistence of these genes, which have been reported worldwide in isolates from different origins, might be associated with the extensive use of streptomycin/spectinomycin, trimethoprims, sulfonamides and chloramphenicol in poultry farms. No significant association between quinolone compounds resistance and integron present was not surprising because resistance to quinolone compounds is derived through chromosomal point mutations rather than being carried on any mobile genetic elements [50].

CONCLUSIONS

Integrons carrying gene cassettes encoding for antibiotic resistance are significantly present among *Salmonella* spp., especially isolated from poultry medicated by antibiotics. Uncontrolled use of antibiotics would increase the numbers of MDR isolates and integrons prevalence, which after a while, it could be a significant public health concern. Hence, functional surveillance of antimicrobial resistance and appropriate& effective measures geared towards curbing indiscriminate and unregulated use of antibiotics are urgently need to prevent outbreaks of MDR bacteria in Egypt.

ACKNOWLEDGMENTS

A lot of thanks are to Department of Microbiology, Faculty of Veterinary Medicine, Kafr EL Sheikh University, Kafr EL Sheikh, Egypt. Bacteriology unit, serology unit and biotechnology unit, Reference Laboratory for Veterinary Quality Control on Poultry Production (RLQCP).Animal Health Research institute, Dokki, Giza, Egypt, supported this study and necessary facilities to carry out this work.

REFERENCES

1. Rajagopal, R., M. Mini and R. Ramanathan, 2013. Outbreaks of salmonellosis in three different poultry farms of Kerala, India. Asian Pac. J. Trop. Biomed., 3(6): 496-500.
2. Chuanchuen, R., K. Ajariyakhajorn, C. Koowatananukul, W. Wannaprasat, S. Khemtong and S. Samngamnim, 2010. Antimicrobial Resistance and Virulence Genes in Salmonellaenterica Isolates from Dairy Cows. Foodborne Pathogens AND Disease., 7(1): 63-69.
3. Food and Drug Administration. NARMS retail meat annual report,2007. Availableat: http:// www.fda.gov/ Animal Veterinary/ Safety Health/Antimicrobial Resistance/National Antimicrobial Resistance Monitoring System/ucm164662.htm. Accessed 29 November 2010.
4. Hall, R.M. and C.M. Collis, 1998. Antibiotic resistance in gram- negative bacteria:The role of gene cassettes and integrons, Drug Resist update, 1: 109-119.
5. Row-Magnus, D.A. and D. Mazel, 2002. The role of integrons in antibiotic resistance gene capture, Int. J. Med. Microbial., 292: 115-25.
6. Glenn, L.M., M.D. Englen, R.L. Lindsey, J.F. Frank, J.E. Turpin and M.E. Berrang, 2012. Analysis of antimicrobial resistance genes detected in multiple-drug resistant Escherichia coli isolates from broiler chicken carcasses. Microb. Drug Resist, 18: 453-463.
7. Chuanchuen, R., S. Khemtong and P. Padungtod, 2007. Occurrence of qacE/qacEÄ1genes and their correlation with class 1 integrons in Salmonellaenterica isolates from poultry and swine. The Southeast Asian Journal of Tropical Medicine and Public Health., 38(5): 855-62.
8. Carattoli, A., 2001. Importance of integrons in the diffusion of resistance. Vet. Res., 32(3-4): 243-259.
9. Daly, M. and S. Fanning, 2000. Molecular characterization of Irish Salmonella enterica serotype Typhimurium: detection of class I integrons and assessment of genetic relationships by DNA amplification fingerprinting. Appl Environ. Microbiol., 66: 614-
10. ISO 6579, 2002. 4thed.: Microbiology -General guidance on methods for the detection of Salmonella, International organize for standardization, Geneva, Switzerland.
11. Finegold, S.M. and W.J. Martin, 1982. Diagnostic microbiology. 6th Ed., The C.V. Mosby Company, St. Louis, Toronto, London.

12. CLSI, 2011. Clinical and Laboratory Standards Institute performance standard for antimicrobial disc susceptibility tests, approved standards, 11th ed. Wayne: CLSIPublication M2-A11.
13. White, P.A., C.J. McIver, Y. Deng and W.D. Rawlinson, 2000. 19, No. 24 6823 -6831. Characterisation of two new gene cassettes, aadA5 and dfrA17. FEMS Microbiology Letters, 182: 265-269.
14. Kashif, J., R. Buriro, J. Memon, M. Yaqoob, J. Soomro, D. Dongxue, H. Jinhu and W. Liping, 2013. Detection of Class 1 and 2 Integrons, B-Lactamase Genes and Molecular Characterization of Sulfonamide Resistance in Escherichia coli Isolates Recovered from Poultry in China. Pak Vet J., 2013, 33(3): 321-324.
15. Altschul, S.F., W. Gish, W. Miller, E.W. Myersand D.J. Lipmanl, 1990. Basic Local Alignment Search Tool. J. Mol. Biol., 215: 403-410.
16. Zdragas, A., K. Mazaraki, G. Vafeas, V. Giantzi, T. Papadopoulos and L. Ekateriniadou, 2012. Prevalence, seasonal occurrence and antimicrobial resistance of Salmonella in poultry retail products in Greece. Lett. Applied Microbiol., 55: 308-313. CrossRef | Direct Link |
17. Draz, A.A., A.H. El-goharyand H.A. Samahy, 1996. Environmental pollution with certain bacterial pathogens of zoonotic importance in some poultry farms. In The Proceedings of the 7th Sci. Cong., Fac. Vet.Med. Assiut, Egypt. 1997.
18. El-Azzouny, M.M.E., 2014. Occurrence of virulence genes among multidrug resistant Salmonellae isolated from broilers. Ph. D. Thesis, Fac. Vet. Med. Bacteriology, Mycology and Immunology Department. Zagazig University.
19. Al-Zenki, S., A. Al-Nasser, A. Al-Safar, H. Alomirah, A. Al-Haddad, R.S. Hendriksen and F.M. Aarestrup, 2007. Prevalence and antibiotic resistance of Salmonella isolated from a poultry farm and processing plant environment in the state of Kuwait. Foodborne Pathogens and Disease., 4(3): 367-373.
20. Sadoma, A.M., 1997. Salmonella in chicken in connection with human infection. M. V. Sc. Thesis, Fac. Vet. Med. Tanta University.
21. Mohammed, L.N., H.A. Samaha, A.A. Draz and Y.N. Haggag, 1999. Salmonellaeamong birds and human beings. Alex. J. Vet. Sc., 15(1): 147-154.
22. Ahmed, A.M., H. Shimabukuro and T. Shimamoto, 2009. Isolation and molecular characterization of multidrug-resistant strains of Escherichia coli and Salmonella from retail chicken meat in Japan. J. Food Sci., 74(7): M 405-410.
23. Hanh, T.T., N.T. Thanh, H.Q. Thoa, L.T. Thi, L.M. Thuan and N.T.H. Ly, 2006. Prevalence of Salmonella spp. in poultry in Vietnam. Annals of the New York Academy of Sciences., 1081: 266-268.
24. Molla, B., A. Mesfin and D. Alemayehu, 2003. Multiple antimicrobial-resistant Salmonella serotypes isolated from chicken carcass and giblets in DebreZeit and Addis Ababa, Ethiopia. Ethiop. J. Health Dev, 17(2): 131-149.
25. Verma, J.C. and B.R. Gupta, 1995. Occurrence of Salmonella serotypes in animals in India. Ind. J. Comp. Microbiol. Immmnol. Infec. Dis., 16(3-4): 104-108.
26. Edel, W., A. Mijs, K.G. Robinjs, T.S. Vries and J. Smak, 1991. The report of the working group on *Salmonella* Entertidis in poultry in the Netherlands. Surveillance and control in 1990/1991. Tijdschrift voor Diergeneeskunde, 116(15/16): 806-811.
27. Orji, M.U., H.C. Onuigbo and T.I. Mbata, 2005. Isolation of Salmonella from poultry droppings and other environmental sources in Awka, Nigeria. Int. J. Inf. Dis., 9: 86-89.
28. Tsai, H. and P. Hsiang, 2005. The prevalence and antimicrobial susceptibilities of Salmonella and *Campylobacter* in ducks in Taiwan. J. Vet. Med. Sci., 67(1): 7-12.
29. Nagwa S. Rabie, Nashwa O. Khalifa, Mervat E.I. Radwan and Jehan S.A. Afify, 2012. Epidemiological and molecular studies of Salmonella isolates from chicken, chicken meat and human in Toukh, Egypt. Global Veterinaria, 8: 128-132.
30. Moussa, I.M., M.A. Gassem, A.A. Al-Doss, Mahmoud and A.L. Abdel Mawgood, 2010. Using molecular techniques for rapid detection of Salmonella serovars in frozen chicken and chicken products collected from Riyadh, Saudi Arabia. Afr J. Biotechnol., 9: 612-619.
31. Wieliczko, A. and M. Mazurkiewicz, 1999. The incidence of Salmonella infection in poultry in lower Silesia between 1996-1998. Medycyna Weterynaryjna., 55(7): 442-450.

32. Snow, L.C., R.H. Davies, K.H. Christiansen, J.J. Carrique-Mas, A.D. Wales, J.L. O'Connor, A.J.C. Cook and S.J. Evans, 2007. Survey of the prevalence of Salmonella species on commercial laying farms in the United Kingdom. Veterinary Record, 161: 471-476.
33. Fashae, K., F. Ogunsola, F.M. Aarestrup and R.S. Hendriksen RS 2010. Antimicrobial susceptibility and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. J. Infect. Dev. Ctries, 4: 484-494.
34. Shah, A.H. and N.A. Korejo, 2012. Antimicrobial resistance profile of Salmonella serovars isolated from chicken meat. J. Vet. Anim. Sci., 2: 40-46.
35. ChSuanchuen, R., S. Khemtong and P. Padungtod, 2008. occurrence of *qac*E/*qac*EÄ1genes and their correlation with class 1 integrons in *Salmonellaenterica* isolates from poultry and swine. The Southeast Asian Journal of Tropical Medicine and Public Health., 38(5): 855-62.
36. Nastasi, A., C. Mammina and L. Cannova, 2000. Antimicrobial resistance in *Salmonella* Enteritidis, southern Italy, 1990 -1998. Emerg. Infect. Dis., 6: 401-403.
37. Soltan Dallal, M.M., M. Taremi, L. Gachkar, M.K. Modarressi, M. Sanaei, R. Bakhtiari, M.K. Sharifi Yazdi and M.R. Zali, 2009. Characterization of antibiotic resistant patterns of Salmonella serotypes isolated from beef and chicken samples in Tehran. Jundishapur J. Microbiol., 2: 124-131.
38. Yildirim, Y., Z. Gonulalan, S. Pamuk and N. Ertas, 2011. Incidence and antibiotic resistance of *Salmonella* spp. on raw chicken carcasses. Food Res. Int., 44: 725-728.
39. Carraminana, J.J., C. Rota, I. Agustin and A. Herrera, 2004. High prevalence of multiple resistance to antibiotics in Salmonellae serovars isolated from a poultry slaughterhouse in Spain. Vet. Microbiol., 104: 133-139.
40. Dias de Olivieira, S., C.R. Rodenbusch, G.B. Michael, I.R. Marisa, C.C.W. Canal and A. Brandelli, 2003. Detection of virulence genes in *Salmonella* Enteritidis isolated from different sources. Braz J. Microbiol., 34: 123-124.
41. Shrestha, A., P. Regmi, R.K. Dutta, D.R. Khanal, S.R. Aryal, R.P. Thakur, D. Karki and U.M. Singh, 2010. First report on antimicrobial resistance of Salmonella isolated from poultry in Nepal. Vet. Microbiol., 144: 522-524.
42. Zhao, Y., R. Jansen, W. Gaastra, G. Arkesteijn, B. Van der Zeijst and J. Van Putten, 2002. Identification of genes affecting *Salmonella enterica* serovar Enteritidis infection of chicken macrophages. Infection and Immunity, pp: 5319-5321.
43. Abdellah, C., R.F. Fouzia, C. Abdelkader, S.B. Rachida and Z. Mouloud, 2009. Prevalence and antimicrobial susceptibility of Salmonella isolates from chicken carcasses and giblets in Meknès, Morocco. African J. Microbiol. Res., 3: 215-219.
44. Ren, C., Y. Zhao and Y. Shen, 2013. Analysis of the effect of integrons on drug-resistant *Staphylococcus aureus* by multiplex PCR detection. Molecular Med. Reports, 7(3): 719-724.
45. Ammar, A.M., A.M. Attia, N.K. Abd El-Aziz, M.I. Abd El Hamid and A.S. El-Demerdash, 2016. Class 1 integron and associated gene cassettes mediating multiple-drug resistance in some food borne pathogens. International Food Research Journal, 23(1): 332-339
46. Toleman, M.A., P.M. Bennett and T.R. Walsh, 2006. Common regions e.g. orf513 and antibiotic resistance:IS91-like elements evolving complex class 1 integrons.Journal of Antimicrobial Chemotherapy, 58(1): 1-6.
47. Antunes, P., J. Machado, J.C. Sousa and L. Piexe, 2004. Dissemination amongst humans and food products of animal origin of a *Salmonella* Typhimurium clone expressing an integron-borne OXA-30 ß-lactamase. J. Antimicrob. Chemother, 54: 429-434
48. Raj Kamal Gautam, Aarti S. Kakatkar, Manisha N. Karani, R. Shashidhar and Jayant R. Bandekar, 2017. Salmonella in Indian ready-to-cook poultry: antibiotic resistance and molecular characterization. Microbiology Research, 8(1).
49. Francis Shahada, Takehisa Chuma, Takayuki Tobata and Karoku Okamoto, 2006. Molecular epidemiology of antimicrobial resistance among Salmonella enterica serovar Infantis from poultry in Kagoshima, Japan. International Journal of Antimicrobial Agents, 28(4): 302-307.
50. Martinez-Freijo, P., A.C. Fluit, F.J. Schmitz, J. Verhoef and M.E. Jones, 1999. Many Class I Integrons comprise distinct stable structures occurring in different species of Enterobacteriaceae isolated from widespread geographic regions in Europe. Antimicrobial Agents and Chemotherapy, 43(3): 686-689.

51. Singh, R., C.M. Schroeder, J. Meng, D.G. White, P.F. McDermott, D.D. Wagner, H. Yang, S. Simjee, C. Debroy, R.D. Walker and M. Sunde, 2005. Prevalence and characterization of class 1 and class 2 integrons in Escherichia coli isolated from meat and meat products of Norwegian origin. Journal of Antimicrobial Chemotherapy, 56(6): 1019-1024.
52. Guerra, B., S. Soto, S. Cal and M.C. Mendoza, 2000. Antimicrobial resistance and spread of class 1integrons among Salmonella serotypes. Antimicrobial agents and Chemotherapy, 44(8): 2166-2169.
53. Krauland, M.G., J.W. Marsh and D.L. Paterson, 2009. Integron-mediated multidrug resistance in a global collection of nontyphoidal *Salmonella enterica* isolates. Emerg Infect Dis., 15: 388-396.
54. Vo, A.T., E. Van, Duijkeren and W. Gaastra, 2010. Antimicrobial resistance, class 1 integrons and genomic island 1 in Salmonella isolates from Vietnam. PLoS one., 5: 9440.
55. Martinez Freijo, P., A.C. Fluit, F.J. Schmitz, J. Verhoef and M.E. Jones, 1999. Many class Iintegrons comprise distinctstable structures occurring in different species of Enterobacteriaceae isolated from wide spread geographic regions in Europe. Antimicrob. Agents. Chemother., 43(3): 686-689.