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Integrons and antibiotics resistance genes in *Escherichia coli* and *Salmonella* strains isolated from diarrheic chicks

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Abstract

Many species belonging to family *Enterobacteriaceae* which present in the intestinal tract of human and animals are frequently exposed to different antimicrobials, creating the potential to disseminate genes of resistance to antimicrobials. Twenty–four *Escherichia coli* and13 *Salmonella* isolates were recovered from diarrheic chicks, and the resistance patterns of the isolates were determined using disc diffusion method. The isolates were serologically identified and screened molecularly by PCR for the presence of *bla*TEM and *aada1*resistance genes and presence of *intI1* gene (class 1 integron). The serological examination of *E coli* strains revealed that there were 10 serotypes, the most predominant serotype was O158 (5 isolates), followed by O78, O55:H7 and O91:H21 (3 isolates), O26 : H11, O86, O142 and O128:H2 (2 isolates), O111 : H4 and O2:H6 (1 isolates). Serotyping of *Salmonella* isolates resulted, *S. Enteritidis* (5) isolates, *S. Typhimurium* (3) isolates, *S. Kentukey* (3) isolates *S. Molade* and *S. Larochelle* (1) isolate.

E.coli and *Salmonella* isolates showed marked variations in their antibiotic resistance patterns. *bla*TEM and *aada*1 genes were identified in 100% of examined *E. coli* and *Salmonella* isolates. On the other hand, *intI1* gene (Class 1 integrons) were found in 100% of the *E. coli* isolates whereas it was found in20% of *Salmonella* isolates only.

The higher incidence of multidrug resistant *E.coli* and *Salmonella isolates* harboring resistance genes in this study constituting a devastating problem for poultry industry.

Keywards: *Multidrug resistant (MDR), E.coli, Salmonella, intI1 gene, Diarrhae, blaTEM and aada1 genes.*

Introduction

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, **(Hsu *et al.,* 2006).** Microbial resistance is the loss of sensitivity by a microorganism to an antimicrobial to which it was originally susceptible. This resistance can be acquired by mutations in chromosomal DNA or by the acquisition of extra-chromosomal genetic material by means of plasmids and transposons **(Vazquez *et al.,* 2005).** Many antibiotic resistant Gram-negative bacteria contain integrons, which are genetic elements that mediate drug resistance. The use of a single antibiotic as treatment can also select for resistance to other antibiotics whose genes reside in the same integrons **(Arestrup *et al.,* 2001).** Class 1 integrons are found extensively in clinical isolates, and most of the known antibiotic-resistance gene cassettes belong to this class. To date, and considering only those cassettes that differ in nucleotide sequence by more than 5% over 80 different gene cassettes from class 1 integrons have been described between them, these elements confer resistance to all known β -lactams, all minoglycosides, chloramphenicol, trimethoprim, streptothricin, rifampin, erythromycin, fosfomycin, lincomycin and antiseptics of the quaternary-ammonium-compound fami-

ly, **(Fluit and Schmitz 2004).** Multidrug resistant (MDR) encoded by linked resistance genes occurs on integrons, which are potentially mobile genetic elements considered to be involved in the transfer of MDR, **(Leverstein *et al.,* 2003)**. Antimicrobial resistance is accomplished by Integrons that play important role in the dissemination of antimicrobial resistance among Gram negative bacteria,4 classes of integrons are known (1, 2, 3, and 4), with class 1 being predominant among the members of this family both in the normal and pathogenic microbiota of animals (**Goldstein *et al.,* 2001).**

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High level of resistance to beta-lactam antibiotics among *Enterobacteriaceae* was conferred by Extended Spectrum Beta-Lactamase (ESBL) in poultry farms, thereby classic *blaTEM* genes were present in *E. coli* and *Salmonella spp*. from poultry samples **(Sumalee 2008)**. *aadA1* gene which confer resistance to aminoglycoside adenyltransferase was most frequently associated with MDR *E. coli* harbored class 1 integrons **(Alieda *et al.,* 2007, Hala *et al.,* 2018),** also *aadA1* gene was also reported in MDR *Salmonella spp*. from poultry in Egypt by **Sahar *et al.,* (2018)**

Antibiotic usage selects for resistance not only in pathogenic bacteria but also in the endogenous flora of exposed individuals or populations. Therefore; the antibiotic selection pressure for resistance in bacteria in poultry is high and consequently, their fecal flora contains a relatively high proportion of resistant bacteria **(Piddock 1996)**.

The present study was aimed to detect class 1integron gene (*intI1*), *bla*TEM and *aada1* genes in a selection of MDR *Salmonella* and *E. Coli* serotypes and to investigate the association of reduced susceptibility to antimicrobial agents with the presence of integrons as verified the plate inhibition test (antibiogram).

Materials and Methods

Sample collection:

A total of 100 samples (intestines) were collected from diseased and freshly dead broilers chickens suffering from diarrhea from different poultry farms located in Assiut Governorates in separate zipper lock bag, kept in ice box and immediately transported to the laboratory.

Isolation and Identification of *E. coli* and *Salmonella spp*:

E. coli and *Salmonella* isolation was conducted according to **Quinn *et al.,* (2002**). 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:

Serological identification of *Salmonella spp* (13isolates)was carried out by slide agglutination technique according to **Kauffman (1974)** for the determination of Somatic (O) and Flagellar (H) antigen using Salmonella antiserum (DENKA SEIKEN Co., Japan). *E.c*oli (24 isolates) were serologically identified according to **Kok *et al.,* (1996)** by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for detection *E. coli* serotypes.

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done according to **Finegold and Martin (1982)** using agar disc diffusion method on Mueller Hinton agar. The isolated strains were tested against 13 antibiotic discs of commonly used chemotherapeutic agents, which were Amoxicillin (25µg) Ampicillin (10 µg) Cefoxitin (30 µg) Cefadroxil (30 µg) Ciprofloxacin(5 µg) Enrofloxacin (10 µg)Streptomycin(10 µg) Neomycin (30 µg) Gentamycin (10 µg) Sulfamethoxazol 25 µg) Tetracycline (30µg) Colistin (25 µg) Florphenicol (l5 µg) from Oxoid Hampshire, U K. The interpretation of inhibition zones of tested isolates was carried out according to **CLSI (2015).**

Multidrug resistant Index (MDRI)
Resistance to more than three antibiotics groups was considered as multidrug resistance (MDR). MDR index (MDRI) of individual isolates was calculated by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics to which the isolate was exposed (**Chandran *et al.*, 2008**). Isolates with MDRI values of more than 0.2 or 20% were considered highly resistant.

MDR index = Number of antibiotics resisted x 100
Total number of antibiotics used

PCR Procedures:-
The isolated *E. coli* and *Salmonella spp* strains (5 isolates of each) were sent to the Reference Laboratory for Veterinary Quality Control on Poultry Production in Animal Health Research Institute, Dokki, Giza, Egypt, for detection of *bla*TEM, *aada1*, and *Int1* genes. According **to Colom *et al.*, (2003), Randall *et al.*, (2004) and Kashif *et al.*, (2013)** as follows:

DNA extraction. DNA extraction from pure isolates of both organisms (5 of each) was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56C for10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer. Primers used were supplied from Metabion (Germany) are listed in table (1).PCR amplification. Primers were utilized in a 25- µl reaction containing 12.5µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primerof 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products. The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. A gel pilot 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (AlphaInnotech, Biometra) and the data was analyzed through computer software.

Table (1). Primers sequences, target genes, amplicon sizes and cycling conditions

Target gene	Primers sequences (5′ -- 3′	Amplified segment (bp	Primary denaturation	Amplification (35 cycles)			Final extension	Reference
				Secondary denaturation	Annealing	Extension		
***bla*TEM**	ATCAGCAATAAACCA GC CCCCGAAGAACGTTTTC	516	94˚C 5 min	94˚C 30 sec	54˚C40 sec.	72˚C 40 sec	72˚C 10 min	**Colom *et al.*, (2003)**
aada1	TATCAGAGGTAGTT-GGCGTCAT GTTCCA-TAGCGTTAAGGTTTCATT	484	94˚C 5 min	94˚C 30 sec.	54˚C40 sec.	72˚C40 sec.	72˚C 10 min	**Randall *etal.* (2004)**
Int1	CCTCCCGCACGATGATC TCCACGCATCGTCAGGC	280	94˚C 5 min.	94˚C 30 sec.	54˚C40 sec.	72˚C40 sec.	72˚C 10 min	**Kashif *et al.*, (2013)**

Results

The overall prevalence of *E. coli* and *Salmonella* isolates in the total examined 100 diarrheic chicks samples were 24 isolates (24%), and 13 isolates (13%) respectively.

With regard to serotyping of *E. coli* isolates, the most predominant serotype was O158(5 isolates), followed by O78, O55:H7 and O91:H21 (3 isolates), on the other hand, the most predominant Serotype of *Salmonella* isolates was *S. Enteritidis* (5) isolates, *S. Typhimurium* and *S. Kentukey* (3) isolates as shown in tables (3) & (4)

Table (2). Serotyping of *E. coli* isolates recovered from different examined samples

Number of strains (n=24)	Serodiagnosis	Percentages %
5	O158	20.8%
3	O78	12.5%
3	O55:H7	12.5%
3	O91:H21	12.5%
2	O26 : H11,	8.33%
2	O86	8.33%
2	O142	8.33%
2	O128:H2	8.33%
1	O111 : H4	4.2%
1	O2:H6	4.2%

Table (3). Serotyping of *Salmonella* isolates recovered from different examined samples

Number of strains (n=13)	Serodiagnosis	Percentages %
5	*S. Enteritidis*	38.5%
3	*S. Typhimurium*	23.1%
3	*S. Kentukey*	23.1%
1	*S. Molade*	7.6%
1	*S. Larochelle*	7.6%

Antibiotic resistance patterns *of E. coli* and *Salmonella spp:*

The results showed a higher resistance rate of *E.coli* isolates to ampicillin and Cefoxitin (100%), followed by Amoxicillin, Neomycin and Tetracycline (95.8%), while the isolates were highly sensitive for Colistin, Ciprofloxacin (4.2%). *Salmonella* spp. were resistant to Ampicillin and Neomycin with 100%, followed by Cefadroxil and Amoxicillin (92.3%) , respectively while it was sensitive for Ciprofloxacin and Colistin with 7.6% and 8% respectively as shown in table (4) . The study recorded multidrug resistant index (MDR) among the isolated bacteria at least for 3 chemotherapeutic agents as **0.605** in *E. coli*, **0.573** in *Salmonella spp.*
more than (0.2).

Table (4). In vitro antibiotic resistance pattern

Antibiotic discs	*E. coli* (24)				*Salmonella spp.*(13)			
	Sensitive %n		Resistant %n		Sensitive %n		Resistant %n	
Amoxicillin(25µg)	1	4.2	23	95.8	1	7.6	12	92.4
Ampicillin (10 µg)	0	0	24	100	0	0	13	100
Cefoxitin (30 µg)	0	0	24	100	2	15.4	11	84.6
Cefadroxil (30 µg)	2	8.4	22	91.6	1	7.6	12	92.4
Ciprofloxacin(5 µg)	23	95.9	1	4.1	12	92.4	1	7.6
Enrofloxacin (10 µg)	22	91.7	2	8.3	11	85	2	15
Neomycin (30 µg)	1	4.2	23	95.8	0	0	13	100
Streptomycin(10 µg)	21	87.5	3	12.5	10	77	3	23
Gentamycin (10 µg)	20	83.4	4	16.6	11	85	2	15
Sulfamethoxazol25 µg	5	21	19	79	3	23	10	77
Tetracycline(30µg)	1	4.2	23	95.8	4	30	9	70
Florphenicol (l5 µg)	4	16.7	20	83.3	5	39	8	61
Colistin (25 µg)	23	95.8	1	4.2	12	92.4	1	7.6
MDR Index	0.605				0.573			

PCR results of detecting antimicrobial resistant genes *blaTEM*, *aada1* and class 1 integron *(int1)* gene

In this approach *bla*TEM gene and *aada1* were detected in100% of all representative isolates(5 isolates of each bacterial strain), of *E. Coli* and *Salmonella* isolates giving characteristic bands at 516 bp and 484 bp as shown in fig.(1,2).

The multi-drug resistant *E.coli and Salmonella spp.* isolates were screened for the presence of genes related to class 1 integron ***(int1***). It was found in all tested *E. coli* isolates, resulting in 280 bp amplicons as showed in figure **(3)** on the other hand, in *Salmonella spp*. class 1 integron***(Int1)*** gene was detected in one isolate of the examined 5 isolates.

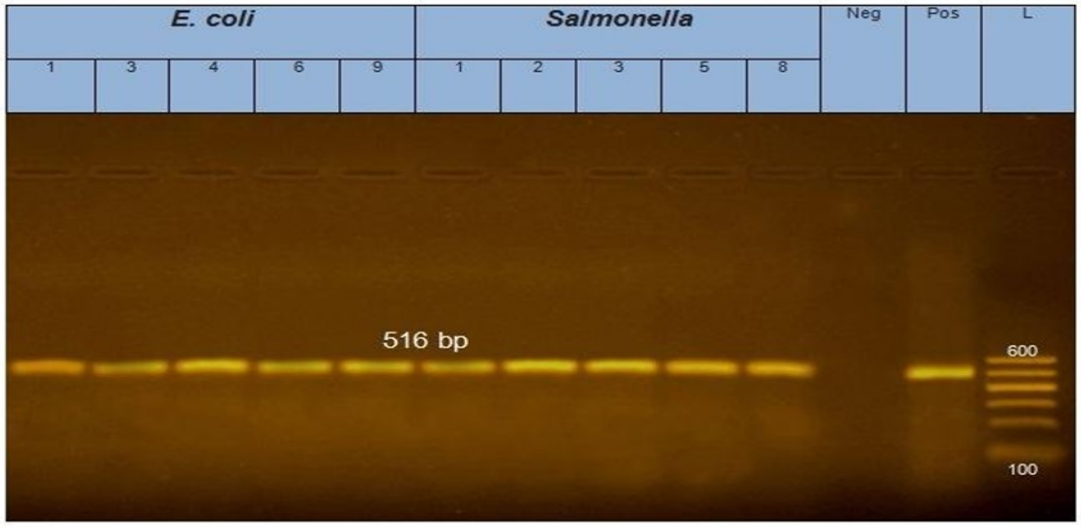

Figure (1). Agarose gel electrophoresis of products obtained by PCR for *E. coli* strains to detect gene *bla*TEM): lane no 1, 3, 4, 6, 9 in *E. coli* strains and lane no 1, 2, 3, 5, 8 in *Salmonella* were positive at 516 bp. Fragment Pos.: positive control Neg: negative control. (L):100bp DNA ladder

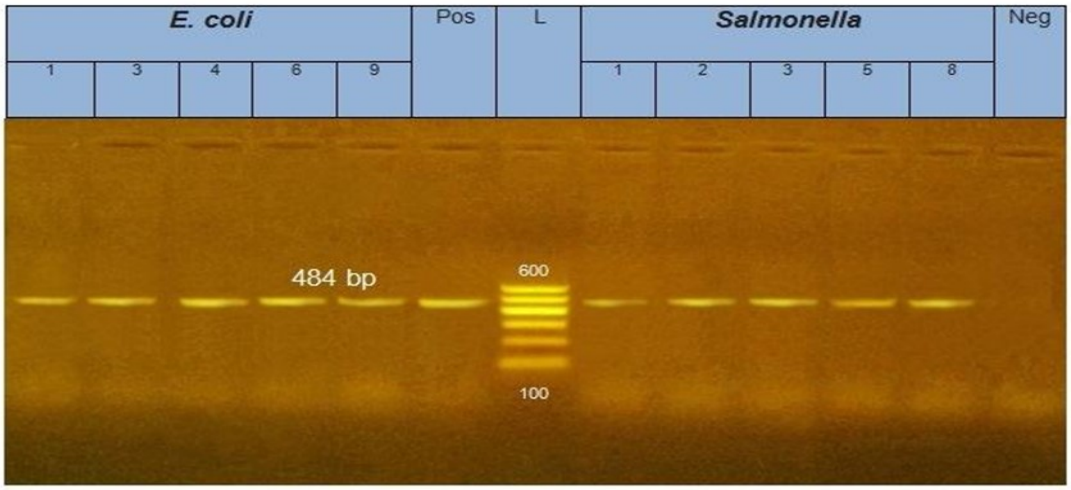

Figure (2). Agarose gel electrophoresis of products obtained by PCR for *E. coli* and *Salmonella* strains to detect gene (*aada1)*, lane 1, 3, 4, 6, 9 in *E. coli* strains and lane 1, 2, 3, 5, 8 in *Salmonella* were positive at 484 bp. fragment. Pos.: positive control Neg.: negative control. (L):100bp DNA ladder

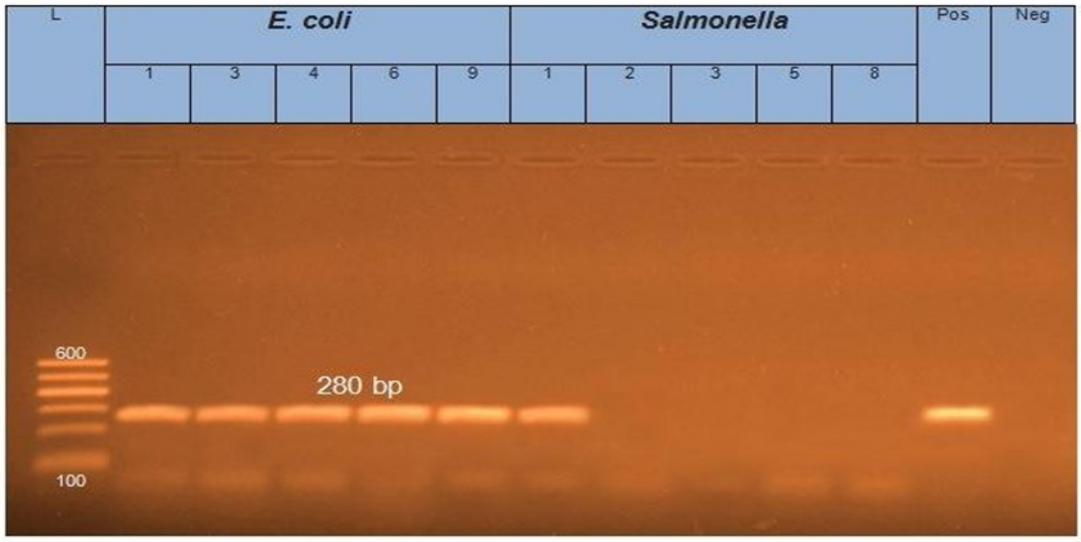

Figure (3). Agarose gel electrophoresis of products obtained by PCR for *E. coli* and *Salmonella* strains to detect integron gene (*Int1)* lane no. 1, 3, 4, 6, 9 in *E. Coli* strains and lane no. 1 in *Salmonella* were positive at 280 bp fragment and lane no. 2, 3, 6, 8 in *Salmonella* were negative**.**

Discussions

The results of *E. coli* isolation and identification from diseased field samples revealed that 24 samples (24%) were positive. Our results agree with **Hussain *et al.*, (2013)** found that the prevalence of *E.coli* was 30.4 % in broilers. Nearly Similar isolation rate was detected by **El Gaber and El-Gohary (1995**) who recovered *E. coli* from 59% of septicemic broilers chickens.

With regard to serotyping, it showed the predominance of *E. coli* serotypes were O158 (5isolates) , followed by and O78 ,O55:H7 and O91:H21 (3 isolates), O26 : H11, O86, O142 and O128:H2 (2 isolates) , O111 : H4 and O2:H6 (1 isolate) .*E. coli* serotypes isolated from broiler farm in Egypt were also reported by **Mohamed *et al.*, (2018) and Yousef *et al.*, (2013)**.

Regarding to the sensitivity pattern of each isolate, all *E. coli* isolates were resistant to Ampicillin and Cefoxitin 100%, Amoxicillin**,** tetracycline and Neomycin 95%**,** sulfamethoxazole (79%), while The effective drugs were ciprofloxacin and colistin where 4.2% of the isolates were resistant. On other hand, **Xia *et al.*, (2009)** observed that avian *E.coli* isolates were resistant to enrofloxacin 99%, ciprofloxacin 100%,norfloxacin 100%, amoxicillin/ clavulanic acid 87.4%, ampicillin 99.5%, gentamicin 97%**.** Likewise, **Suthathip *et al.*, (2016**) reported that antibiotics resistant *E.coli* isolates were resistant to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulfamethoxazole, tetracycline, trimethoprim, cefotaxime, cefpodoxime and ceftazidime were 91%, 14.8%, 5%, 58.4%, 40.9%, 63.4%, 45.7%, 76.1%, 11%, 10% and 9%, respectively. Also, **Alieda *et al.*, (2007)** recorded that Most of the *E. coli* isolates selected were MDR and resistant to sulfamethoxazole (94%), trimethoprim (81%), tetracycline (78%) and amoxicillin (77%). 30% to chloramphenicol, 15% to neomycin, 12% to cefotaxime and 5% to gentamicin.

The development of antibiotic resistance in *E. coli* isolates from poultry is a well-known phenomenon (**Zhao *et al.*, 2005**).

Only one *E.coli* isolate was resistant to all tested antibiotics with a MDR index value 1.Also, one isolate was resistant to 2 antibiotics with MDR index value <0.2 so, out of 24 *E.coli* isolates 23 (95%) were resistant to three or more antibiotics. The MDR index analysis showed 95% of *E. coli* isolates had MDR index value of **0.605**> 0.2 and 5% had MDR index value of

0.153 which less than ≤0.2. The results indicate that all isolates harbor one or more of antibiotic resistance genes. These results agree with **Suthathip *et al.*, (2016**) reported that 98%of the *E.coli* isolates were resistant to at least one antimicrobial agent and 77.4% were MDR. Yet likewise, **Radu *et al.* (2001)** who illustrated that all the *E.coli* isolated from broilers chickens were found to be resistant to three or more of the antimicrobial agents**.** Nearly similar to **Momtaz *et al.*, (2012)** mentioned that Multi-resistance which was defined as resistance to three or more tested agents was found in 64.91% of *E. coli* strains**.** Thereby**,** most *E.coli* isolates recovered from healthy as well as from sick chicken were resistant to multiple classes of antimicrobials, **(Dakic *et al.*, 2011)**.

On the other hand, **Mohamed *et al.*, (2018)** found that *E. coli* isolated from broilers chicken were pathogenic and MDR responsible gene was detected for 6 antibiotics in most of the isolates, but some do not show gene expression, this may be due to few numbers of resistance genes tested or other resistance factors not included in this study.
MDR index value greater than 0.2 indicates high-risk sources of contamination, where several antibiotics may often use for the control of diseases, extensive use of antibiotics in chicken production systems for non-therapeutic purposes such as growth promotion resulted in the resistance of bacteria to these antimicrobial agents. **(Cogliani *et al.*, 2011).**

β-Lactams are among the most clinically important antibiotics in both human and veterinary medicine, and yet resistance to this class of antibiotics is increasing at an alarming rate **Li *et al.*, (2007**).The mechanism of action of β-lactams, e.g (Ampicillin and Cefoxitin) is to disrupt bacterial cell wall synthesis by linking covalently to enzymes, i.e penicillin-binding proteins which conferring resistance to ß-lactamase. In our study the *bla*TEM gene was detected in all multi-drug resistant *E.coli* isolates (100%). The same rate of *blaTEM* gene (100%) was detected by **Hala *et al.*, (2018**) and similarly agree with **Jiang *et al* ., (2011)** detected *blaTEM* gene among 88.9% of avian *E.coli* strains. Also by disc diffusion resistance to β -Lactam group was 100% in Ampicillin and cefadroxil, 95.8% in Amoxicillin and 91.6% in Cefadroxil. likewise, **Zhao *et al.*, (2005)** who recorded that the most common resistance gene detected was *blaTEM* present in 98.2% of *E.coli* isolates. Also, **Colom *et al.*, (2003)** who detected *blaTEM* gene in 45 out of 51 Amoxicillin-clavulanate were resistant *E. coli* isolates with 88.2%. As well as, the high *blaTEM* gene prevalence was recorded by **Brinas *et al.*, (2002)** who detected *blaTEM* gene in 83% of 124 Ampicillin resistant *E. coli* isolates**.** Moreover, *blaTEM* gene was detected in 73% Ampicillin resistance avian *E.coli* isolates, (**Asheraf *et al*., 2015).** Where also wer rate of *blaTEM* gene in amoxicillin resistant *E,coli*(43%) was recorded by **Moussa *et al.*, (2007)** Thereby, **Momtaz *et al.*, (2012)** who recorded that all *E. coli* isolates were susceptible to cephalothin, and ampicillin and No *bla* genes known to be associated with resistance tocephalothin and ampicillin were detected (*bla*SHV & *bla*CMY)**.** In addition**, Ying *et al*., (2008)** who found that 97% of the AMP-*E.coli* resistance mechanism could be explained by the resistance gene *blaTEM*;

Aminoglycoside antibiotics (gentamycin) target the ribosome to inhibit protein translation**.** In the present study the gene conferring resistance to aminoglycoside adenyltransferase *(aadA1)* was detected in 100%of the *E.coli* (5isolates). Similar result recorded by **Hala *et al.*, (2018)** found that (93%) of *E.coli* isolated from broilers chicken were positive for *aadA1 gene*. But lower rate of detection of *aadA1 gene* (17%) was recorded by **Yith *et al.*, (2017**). Similarly, **Alieda *et al.*, (2007)** recorded that *aadA1 gene* was most frequently associated with 76% of MDR *E.coli* harbored class 1integrons. likewise**, Asheraf *et al*., (2015)** mentioned that *aad* (gentamicin resistance gene) was present in 26% in Gentamycin resistant *E.coli*. On the other hand, By disc diffusion (23%*)* of *E.coli* isolates were resistance to Streptomycin whereas 100% of the isolates carried *aadA1* gene, nearly similar to **Moussa *et al.*, (2007)** recorded that *aadA1* gene was detected in (58.9%) streptomycin. –resistant *E.Coli* isolates. Moreover **Mohamed *et al.*, (2018)** detected *aadA1*gene in45% of Streptomycin resistance *E. coli* isolates. Also, they

added that only 15% of tested MDR *E.coli* isolates showed a relationship between phenotype and genotype, whereas17 showed irregular relation including aminoglycoside gene (*aadA*) gene. Thereby, **Hala *et al.*, (2018**) recorded that phenotype is not compatible with genotype in some isolates. Also Presence of gene is not necessary for showing resistance but resistance can occur where no gene for resistance can be detected. Moreover the gene may not be activated. As well, *(aadA1) genes* that encode resistance the remaining amino glycoside-resistant isolates, in which none of the other six genes was detected, suggests the presence of different aminoglycoside resistance determinants, **Dakic *et al.*, (2011).**

The presence of the *class 1 integron (int1*) *gene* in *E. coli* indicates a potential for lateral antibiotic resistance gene transfer between this bacterium and other chicken gut bacteria. These bacteria have the potential to spread in the environment through the litter (**Nandi *et al.*, 2004)**,

Multi drug -resistant *E.coli* isolates were screened for the presence of genes related to the *class 1 integron (int1*) *gene* in order to investigate the distribution of this resistance disseminating element. *class 1 integron (int1*) *gene* was found in (100%) of the MDR *E. coli* (5 isolates) in our study. The presence of integrons in enteric bacteria from poultry has been previously recorded by **Alieda *et al.*, (2007)** as *class 1 integron (int1*) *gene* were found in 76% of the MDR *E. coli*. Also **Zhao *et al.*, (2005)** detected *class 1 integrons* in (30.9%) of *E.coli* strains. In addition **Sumalee (2008)**recorded that *class 1integrons* was detected in 70.3% and 44.6% of *E.coli* isolated from broilers chicken in Thailand and the US, respectively. As well as**, Moussa *et al.*, (2007**) who found *class 1integron* in 39.4% of the MDR *E. coli* isolates**.** However, all integron-bearing isolates were multi resistant to antibiotics, (*aadA1*) resistance genes were found in100% of the integron-positive isolates whereas The β -lactamase gene resistance was found in 78% of the isolates.
The presence of *class 1 integron (int1)gene* was shown in 42.5% of the *E.coli* isolates . Also association between multidrug resistance and the presence of integrons was significant, (**Dakic *et al.*, 2011)**. Whereas**, Suthathip *et al.*, (2016)** recorded that *class 1 integron (int1*)gene in *E.coli* isolated from broiler were limited and non-*class 1 integron* borne resistance determinants are responsible for resistance phenotype in the majority of MDR *E. coli*.

On the contrary with **Okamoto *et al., (*2009)** recorded that *E.coli* isolates were shown to be sensitive to all the tested antimicrobials and negative for the presence of the antimicrobial resistance gene and *class 1 integron (int1*) gene. Wherefore, among 15 integron-negative isolates, 9 isolates were multidrug resistant while 30 out of 32 integron-positive strains (93.7%) displayed multidrug resistance, (**Dakic *et al.*, 2011)**. These results lead to **Suthathip *et al.*, (2016)** demonstrated the high contamination rate of MDR *E. coli* in broiler carcasses confirmed the role of commensal *E. coli* as carriers of *class 1 integron (int1*)gene and genes encoding broad-spectrum *B*-lactamase *(ESBL*) that have the potential for horizontal transfer.

In our study, concerning salmonella isolates the samples of examined diarrheic cases of broiler chicks revealed 13 % isolation rate of *Salmonella spp*, nearly the same rate of isolation from broilers chicken recorded by **Orji *et al.*, (2005)**. But higher rate of *Salmonella* isolation (62.0%) was detected by **Mohamed and Suelam (2010)** from chicken fecal samples in Egypt. Also, **El-Sharkawy *et al.*, (2017)** isolated *Salmonella spp* at the rate (41.0%) from chicken flock. Variation in the result may be due to samples, volume, hygienic measures in the farms or general health flocks.

Antibiotic disc diffusion revealed that 12\13 of isolated *Salmonella* exhibit extremely high rate of multiple drug resistance (MDR) indexed to all antibiotics used (0.573>0.2) .

Regarding to the sensitivity pattern of each isolate 12 *Salmonella* serovar were multidrug resistant exhibiting resistance to three or more agents of different antibiotic classes.one isolate was resisted to all 13 antimicrobial with MDR value 1>0.2. These result came in ac-

cordance with **Alieda *et al.,* (2007)** found *Salmonella* isolates were resistant to sulfamethoxazole (99%), tetracycline (72%), amoxicillin (61%), or trimethoprim (52%). In contrast 20% were resistant to chloramphenicol, 7% to neomycin and 5% to gentamicin**.**

In our study (*blaTEM*) gene was present in 100% of MDR *Salmonella*(5 isolates), the same result was detected by **Chen *et al.,* (2009).** Nearly similar rate (83.3%) was detected by **Sahar *et al., (*2018)**, while **Dilruba *et al.* (2014**) recorded a lower rate (80%) of ß-lactamase genes. Likewise, 94.6% of beta-lactam-resistant *Salmonella* isolates harbored at least one resistance gene of bla_{TEM} or bla_{CTX-M}. (**Zhu *et al.,*2017)**. Also, **Hanem *et al.,* (2017)** demonstrated 65.5% of *Salmonella isolates* were harbored Ampicillin (*bla*TEM) gene whereas class 1 integron gene were detected in 3 *Salmonella* isolates only. Moreover, most serotypes of the genus *Salmonella* isolates obtained from clinical cases were resistant to various antimicrobials and carry class 1 integron, involved in antimicrobial multi resistance **(Vazquez *et al.,* 2005).**

In our study, PCR screening of MDR *Salmonella* isolates detected *class 1 integron* in 20% of the isolates (one isolate). The same result detected by **Dilruba *et al.* (2014**). Nearly similar to **Belgin *et al.*, (2015)** detected *class 1 integron* in 15.47% of *Salmonella* strains isolated from broilers .Also, **Yujuan and Ling (2009)** in China who detected integron class 1 in 13.0% of antibiotic resistant *Salmonella spp***.** But lower rate of Class-I integrons (6.1%) in Salmonella isolates was detected by **Tagelsir *et al.,* (2014**). Whereas **Vinicius *et al.,* (2011)** detected higher rate (45%) of *class 1 integrin* in *Salmonella* strains isolated from broilers. As well **Sumalee (2008)** mentioned that *class 1integrons* were detected in 48.1 and 0.5% of *Salmonella* spp. isolates from Thailand and the US, respectively. In PCR screening of MDR *Salmonella* isolates **Sahar *et al., (*2018)** detected *class 1integron* in (83.3%) of MDR *Salmonella* isolates. In addition **Alieda *et al.,* (2007)** demonstrated the importance of integrons for the occurrence and transmission of multidrug resistance.

On the contrary **Okamoto *et al.,* (2009)** recorded that the gene for resistance to antimicrobials class 1integron (*int1) gene* was not observed in any of the 100 MDR *Salmonella spp*. thereby, presence of gene is not necessary for showing resistance but, the gene may not be activated as well depending on another pathway for resistance**.** Moreover, **(Mathai *et al.,* 2004, Abdallah *et al.,* 2014)** showed that a large number of *Salmonella* isolates did not produce the *int1* gene as it was not detected by PCR. Thereby, presence of other sequence of the enzyme that can be detected by other primer sequence. In our study, 12\13 (92.3%) of *Salmonella* isolates were multi drug resistant whereas 20% of these isolates harbored *class 1 integron*, there fore, no correlation with the presence of class 1 integron and antimicrobial resistance.

The existence of *aadA* gene which confers aminoglycosides resistance, was confirmed in 100% of MDR *Salmonella* isolates (5 isolates), whereas class 1 integron(*int1) gene* was detected in 20% of the isolates. The lower rate (41.7%) of *aadA* gene in MDR *Salmonella* isolates also reported in Egypt by **Sahar *et al.,* (2018)**. The relatively low prevalence of aminoglycoside resistance genes was found in 66.2% of antibiotic-resistant *Salmonella* isolates whereas Class 1 integron was detected in 37.4% of the MDR *Salmonella* isolates **(Zhu *et al.,* 2017), in** addition **Karen *et al., (*2018)** detected only 17.72% of streptomycin-resistant Salmonella had *aadA1*, indicating that another antimicrobial resistance gene (s) were responsible for streptomycin resistance. Moreover, **Hanem *et al.,* (2017)** mentioned that all of the *Salmonella* isolates were sensitive to the streptomycin (100%) despite the presence of streptomycin modifying gene (aadA1) in 50% of the isolates. **Ma *et al.,* (2007)** suggested that some of the antimicrobial resistance genes are silent in bacteria in-vitro however, these silent genes can spread to other bacteria or turn on in -vivo, especially under antimicrobial pressure**.** In addition, **Tagelsir *et al.,* (2014**) revealed that the class-I integrons were identical to each other and contained aminoglycoside adenyltransferase (*aadA1*) genes that encode resistance to trimethoprim, streptomycin.

Conclusion

The higher incidence of multidrug resistant *E.coli* and *Salmonella were* harboring resistance genes in this study constituting a devastating problem for poultry industry and poultry consumers**.** This resistance maybe occur due to use of nontherapeutic antimicrobial growth promotions as feed additives for poultry , unregulated use of antibiotics, usage incomplete therapy. so we in critical need for the regulation of antimicrobial drug usage in poultry production and continuous monitoring of antibiotic resistance for poultry industry and poultry consumers safety.

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