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ANTIBACTERIAL EFFECT OF CINNAMON AND OREGANIUM OILS ON MULTIDRUG RESISTANT *ESCHERICHIA COLI* AND *SALMONELLAE* ISOLATED FROM BROILER CHICKENS

By

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ABSTRACT

The current study aimed to investigate the prevalence of *Escherichia coli (E. coli*) and *Salmonellae* infections in broiler chickens detecting the genotypic characters of multidrug resistant (MDR) isolates and determining the cinnamon and oreganium oils activities against these isolates. Two hundred samples were collected from 120 diseased broiler chickens of different ages (1-5weeks) from different farms in Beni-Suef and El-Fayoum Governorates during the period from January 2015 up to September 2015. Bacteriological examination showed that 115 bacterial isolates were recovered with a prevalence rate of 57.5% of which 91 *E. coli* isolates (45.5%) and 24 *Salmonella* isolates (12%). Serogrouping of *E. coli* isolates revealed that serogroup O_{78} was the most prevalent (27.5%) followed by serogroups O_{158} (18.7%) and O_{128} (16.5%). Serotyping of *Salmonella* isolates showed that *S.* Kentuky, *S.* Enteritidis and *S.* Infantis were identified at rates of 37.5%, 33.3% and 29.2%, respectively. Results of antiprogram showed that 73 *E. coli* isolates (80.2%) and 18 *Salmonella* isolates (75%) were MDR. PCR was applied on 8 MDR isolates to determine the 7 genes. *BlaTEM* gene was the most prevalent found in all *E. coli* isolates (100%) followed by *sul1* (92.9%), *iutA* (78.6%) and *i*ss (71.4%) while that *invA* gene was the most prevalent in all *Salmonella* serovars (100%) followed by *sul1* (75%) and *tetA* (62.5%). The antibacterial activities of cinnamon and oreganium oils were tested against 50 MDR; 32 *E. coli* and 18 *Salmonella* isolates. Oreganium completely inhibited the growth of all the tested bacterial isolates at a concentration of 1% and at 0.5%, concentration inhibited also all *E. coli* isolates and 83.3%

Salmonellae isolates. Cinnamon oil completely inhibited the growth of all the tested bacterial isolates at a concentration of 3% while at 2% concentration 59.4% of *E. coli* isolates and 33.3% of *Salmonellae* isolates were inhibited.

INTRODUCTION

Colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic *Escherichia E. coli* (APEC), including coli septicemia, coli granuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), cellulitis, swollen-head syndrome, peritonitis, salpingitis, panophthalmitis, and omphalitis/yolk sac infection **(Saif *et al.*, 2003)**. Coli septicemia is the most common form of colibacillosis and is responsible for significant economic losses in aviculture in many parts of the world **(Ewers *et al.*, 2003)**. A wide variety of pathogenic *E. coli* has been identified by somatic O antigen serogrouping, but the most common are serotypes O_1, O_2 and O_{78} **(Peighambari *et al.*, 1995 and Gomis *et al.*, 2001)**. *Salmonella* infections have become of the most important groups of bacterial diseases affecting poultry. *Salmonellosis* caused by *Salmonella enterica* serovar Enteritidis (*S.* Enteritidis) is a disease affecting birds, mammals and humans. Therefore, it is considered as a zoonosis **(Kid *et al.*, 1996)**. It has been said that, the worldwide increase in *S. Enteritidis* outbreaks is due to increased virulence. Virulence varies among different strains and a specific plasmid has been suggested **(Christensen *et al.*, 1992)**. Although infections due to *E. coli* and *Salmonellae* have been costly to the poultry industry, the exact virulence mechanisms used by these organisms to cause disease in birds remain interesting point of research. The presence of several virulence genes has been positively linked to the pathogenicity of APEC strains **(Stordeur *et al.*, 2002)** and *Salmonellae* **(Malorny *et al.*, 2003)**. The establishment of PCR assays was to facilitate determination of the frequency with which the various virulence-associated genes occur in the resident APEC and *Salmonella* populations. Although antimicrobials are valuable tools to treat clinical disease and to maintain healthy and productive birds, antimicrobial drug use has been implicated as a risk factor in the development and dissemination of drug resistance **(Gosh and LaPara, 2007)**. Food animals and their production environments are reservoirs of both resistant bacteria and resistance genes that could be transferred to humans either by direct contact or indirectly via the food production chain **(WHO, 2011)**. Therefore, the appropriate antibiotic should better be selected based on its sensitivity that could be detected by laboratory examination.

Nowadays, plenty of spices and herbs are valued for their antimicrobial activities and medicinal effects in addition to their flavor and fragrance qualities **(Shan *et al.*, 2007)**. The essential oils (EOs) from many plants are known to possess antibacterial, antifungal, antiviral and antiparasitic activities **(Pinto *et al.*, 2009)**. EOs are concentrated, hydrophobic liquid containing volatile aromatic compounds extracted from plants **(Isman,2000)**.They have started to be recognized for their potential antimicrobial role only in recent years, but the spectrum of activity and mechanisms of action remain unknown for most of them. The antimicrobial activity of these natural extracts is due to their high hydrophobicity, which enables them to cross the bacterial cell membranes loss of function or damage of proteins, lipids, and organelles within the bacterial cell **(Bakkali *et al.*, 2008)** leading to cell death. The purpose of this study was to investigate the prevalence of *E. coli* and *Salmonella* infections in broiler chickens detecting their genotypic characters and to detect the activity of cinnamon and oreganium oils against these isolates and possibility for their application in the veterinary medicine as alternatives to antibiotics.

MATERIAL AND METHODS

2.1. Chickens Samples.

Two hundred samples were collected from 120 diseased broiler chickens of different ages (1-5 weeks) from different farms in Beni-Suef and El-Fayoum Governorates during the period from January 2015 up to September 2015. These chickens were subjected to clinical and postmortem examinations. Samples were collected from the lesions in the internal organs including liver, heart, lung, air sacs, and yolk sac.

2.2. Bacteriological isolation.

The collected samples were cultivated twice under aseptic condition. For isolation of *Salmonellae*, the specimens were inoculated into buffered peptone water and incubated aerobically at 37ºC for 24 hrs. Transfer 0.1 of buffered peptone water to 10 ml of Rappaport Vassiliadis soy peptone broth (RV) and incubated at 41.5°Cfor 18-24hrs. Then, a loopful of this culture was streaked out onto MacConkey's agar, xylose lysine deoxycholate (XLD) and *Salmonella*-Shigella (SS) agar media and incubated at 37°C for 18-24 hours. Suspected colonies were subjected to biochemical testing according to **Collee *et al.* (1996)**. For isolation of *E. coli*, the specimens were inoculated into MacConkey's broth and incubated aerobically at 37ºC for 24 hrs. Then, a loopful of this culture was streaked out onto MacConkey's agar

and incubated at 37ºC for 18-24 hours. Lactose fermenter (pink) colonies were streaked onto and eosin methylene blue agar and confirmed as *E. coli* using the standard biochemical tests according to **Collee *et al*. (1996)**. All the recovered isolates were identified morphologically and biochemically according to schemes described by **Collee *et al.* (1996) and Quinn *et al.* (2002)**.

2.3. Serological identification:

2.3.1. Serogrouping of *E. coli* isolates. *E. coli* isolates were serogrouped by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to **Quinn *et al.* (2002)**.

2.3.2. Serotyping of *Salmonella*. *Salmonella* isolates were serotyped by slide agglutination test using diagnostic polyvalent and monovalent O and H *Salmonella* antisera according to Kauffman-white scheme **(Kauffmann, 1974)**.

2.4. Antimicrobial susceptibility testing.

All *E. coli* and Salmonella isolates were tested for their antimicrobial susceptibility to 12 different antimicrobial discs. They included amoxicillin (10µg), rifampicin (5µg), enrofloxacin (5µg), colistin sulphate (25µg), gentamicin (10 µg), fosfomycin (300µg), spectinomycin (100µg), lincomycin (10µg), ceftriaxone (30µg), amoxycillin+clavulinic acid (30µg),doxycyclineHCl (30µg),sulphamethoxazole-trimethoprime (25µg) (Oxoid, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to **CLSI (2012)**. The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the **CLSI (2012)**. Resistance to three/or more antimicrobials of different categories was taken as multidrug resistance (MDR) **(Chandran *et al*., 2008)**.

2.5. Polymerase chain reaction.

PCR was applied on 22 MDR isolates (14 *E. coli* and 8 *Salmonella* serovars) for detection of 7 genes; 4 resistance genes (*tetA*, *blaTEM*, *sulI* and *dfrA*) as well as 3 virulence genes; *tsh*, *iss* and *iutA* in *E. coli* and *invA*, *avrA* and *sopB* in *Salmonellae*, (Table 1).

Table (1): Primers used in PCR

Gene		Primer Sequence5'-3'	Amplified product	Reference
invA	*F*	*GTGAAATTATCGCCACGTTCGGGCAA*	284 bp	Oliveira *et al.* (2003)
	R	*TCATCGCACCGTCAAAGGAACC*		
tsh	*F*	*GGT GGT GCA CTG GAG TGG*	620 bp	Delicato *et al.* (2003)
	R	*AGT CCA GCG TGA TAG TGG*		
iss	*F*	*ATGTTATTTTCTGCCGCTCTG*	266 bp	Yaguchi *et al.* (2007)
	R	*CTATTGTGAGCAATATACCC*		
iutA	*F*	*GGCTGGACATGGGAACTGG*	300 bp	
	R	*CGTCGGGAACGGGTAGAATCG*		
sopB	*F*	*tcagaagRcgtctaaccactc*	517 bp	Huehn *et al.* (2010)
	R	*taccgtcct cat gcacactc*		
avrA	*F*	*CCT GTA TTG TTG AGC GTC TGG*	422 bp	
	R	*AGA AGA GCT TCG TTG AAT GTC C*		
bla_{TEM}	*F*	*ATCAGCAATAAACCAGC*	516 bp	Colom *et al.* (2003)
	R	*CCCCGAAGAACGTTTTC*		
dfrA	*F*	*AGC ATT ACC CAA CCG AAA GT*	817 bp	Huovinen *et al.* (1995)
	R	*TGT CAG CAA GAT AGC CAG AT*		
sul1	*F*	*CGGCGTGGGCTACCTGAACG*	443 bp	Sabarinath *et al.* (2011)
	R	*GCCGATCGCGTGAAGTTCCG*		
tetA(A)	*F*	*GGTTCACTCGAACGACGTCA*	576 bp	Randall *et al.* (2004)
	R	*CTGTCCGACAAGTTGCATGA*		

2.6. Agar dilution method for detection of antibacterial activity of essential oils.

According to the method of **Jeff-Agboola *et al.* (2012)** the antibacterial activity of cinnamon and oreganium against 50 MDR isolates (32 *E. coli* and 18 *Salmonella* serovars) was tested. Briefly, the tested bacteria were grown on tryptone soya agar at 37˚C for 24 hr, then cells were suspended in physiological saline (0.9% NaCl), and the suspension was adjusted to 1×10^6 CFU. Tryptone soya agar was prepared and autoclaved at 121˚C for 15 minutes and kept at 55˚C and then the tested oils were sterilized by filtration (pore size, 0.45 µm), and were mixed with TSA according to the tested concentrations. Cinnamon oil (Herbal Global co.) was prepared at concentrations of 1, 2, and 3% while, oreganium oil (Herbal Global co) was prepared at concentrations of 0.5 and 1%. The oil-agar medium (10 ml) was then poured into sterile petri dishes and was solidified. Equal amounts of the bacterial suspensions were inoculated and speared onto the agar plates. The plates were then incubated at 37˚C for 24 - 48hr.

RESULTS

3.1. Prevalence of *E. coli* and Salmonella infections in broiler chickens. Out of 200 broiler chicken samples, 115 bacterial isolates were recovered with a prevalence rate of 57.5% of which isolates, 91 isolates (45.5%) were *E. coli* while 24 isolates (12%) were *Salmonella* spp. (Table 2).

Table (2): Prevalence of *E. coli* and Salmonella isolation in broiler chickens

Total No. of samples	Positive isolation						Negative isolation	
	E. coli		*Salmonella*		Total			
	No.	%	No.	%	No.	%	No.	%
200	91	45.5	24	12	115	57.5	85	42.5

% was calculated according to total No. of the examined samples

3.2. Serological identification

3.2.1. Serogrouping of *E. coli* isolates. Out of 91 *E. coli* isolates, 6 O-serogroups were obtained. The serogroup O_{78} was the most prevalent represented 25 isolates (27.5%) followed by serogroups O_{158}; 17 (18.7%) and O_{128}; 15 (16.5%). Then, the serogroups O_{55}, O_1 and O_{168} represented as 12 isolates (13.2%), 9 isolates (9.9%) and 4 isolates (4.4%), respectively. Moreover, there were 9 isolates (9.9%) were untyped with the available antisera.

3.2.2. Serotyping of *Salmonella* isolates. The 24 *Salmonella* isolates were serotyped as 9 *S.* Kentuky (37.5%), 8 *S.* Enteritidis (33.3%) and 7 *S.* Infantis (29.2%).

3.3. Antimicrobial susceptibility testing. Results of *in-vitro* sensitivity tests showed that *E. coli* isolates were highly sensitive to colistin sulphate only (91.2%) while they were moderately sensitive to amoxycillin+clavulinic acid (57.1%). On the other hand, they were highly resistant to lincomycin, rifampicin (97.8% for each), amoxicillin (96.7%) and sulphamethoxazole-trimethoprim (84.6%). MDR *E. coli* isolates were 73 isolates (80.2%). On the other hand, *Salmonella* isolates were completely sensitive to colistin sulphate while they were highly sensitive to enrofloxacin (91.7%). On the other hand, they were completely resistant to leucomycin, rifampicin. MDR *Salmonella* isolates were 18 isolates (75%).

2.4. Polymerase chain reaction. The results of PCR of *E. coli* isolates revealed that bla_{TEM} gene was the most prevalent found in all isolates (100%) followed by *sul1*, *iutA* and *i*ss genes which were found in 13 (92.9%), 11 (78.6%) and 10 isolates (71.4%), respectively. Meanwhile *tetA*, *tsh* and *dfrA* genes were found in 5(35.7%), 4 (28.6%) and 3 isolates (21.4%), respectively (Table 3), Fig. (1, 2, 3& 4). On the other hand, the results of PCR of

Salmonella isolates revealed that *invA* gene was the most prevalent found in all isolates (100%) followed by *sul1*and *tetA* genes found in 6 (75%) and 5 isolates (62.5%), respectively. Then, both bla_{TEM} and *avrA* genes were found in 4 isolates (50%) while neither *dfrA* nor *sopB* was found in any isolate (Table 3) , Fig.(5 , 6).

Table (3): Prevalence of resistance/virulence-associated genes among the examined *E. coli* and *Salmonella* serovars.

***E. coli* isolates (n=14)**			***Salmonellae* isolates (n=8)**		
Tested gene	**Positive**		**Tested gene**	**Positive**	
	No.	**%**		**No.**	**%**
tetA	**5**	**35.7**	***tetA***	**5**	**62.5**
sul1	**13**	**92.9**	***sul1***	**6**	**75**
blaTEM	**14**	**100**	***blaTEM***	**4**	**50**
dfrA	**3**	**21.4**	***dfrA***	**0**	**0**
iutA	**11**	**78.6**	***invA***	**8**	**100**
Iss	**10**	**71.4**	***avrA***	**4**	**50**
Tsh	**4**	**28.6**	***sopB***	**0**	**0**

% was calculated according to Number (n.) of examined isolates

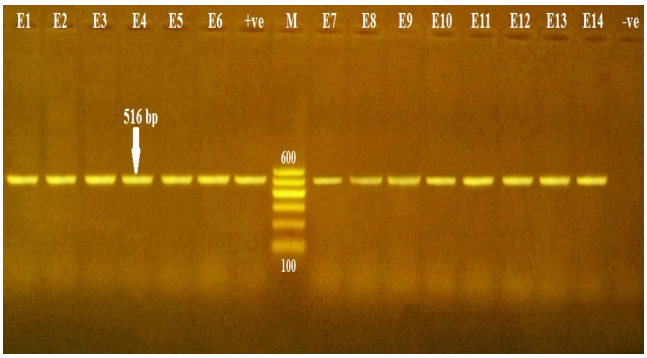

Fig. (1): PCR amplification of the 516bp fragment of *blaTEM* gene from 14 *E. coli* (E1-E14), +ve (control positive), -ve (control negative).

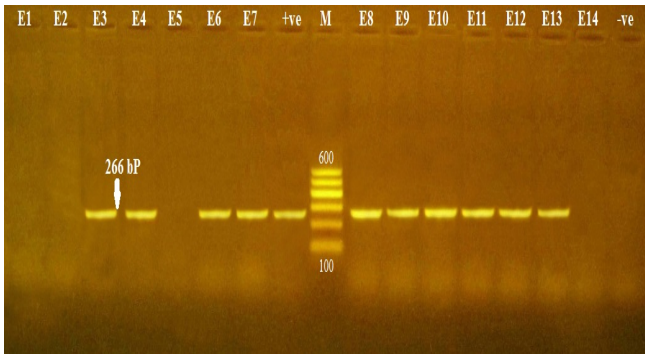

Fig. (2): PCR amplification of the 266bp fragment of *iss* gene from 14 *E. coli* (E1-E14), +ve (control positive), -ve (control negative).

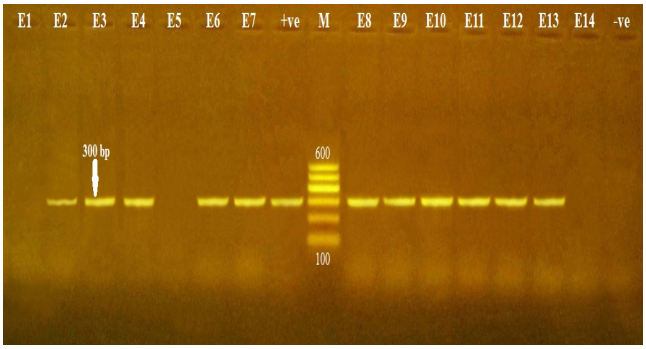

Fig. (3): PCR amplification of the 300bp fragment of *iutA* gene from 14 *E. coli* (E1-E14), +ve (control positive), -ve (control negative).

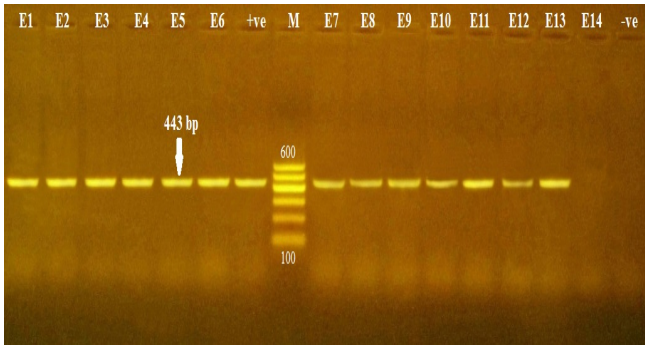

Fig. (4): PCR amplification of the 443bp fragment of *sul1* gene from 14 *E. coli* (E1-E14), +ve (control positive), -ve (control negative).

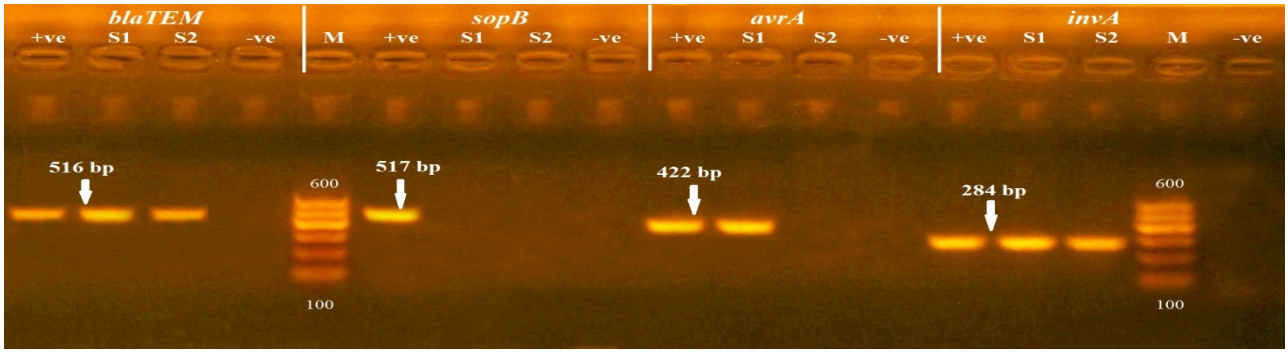

Fig. (5): PCR amplification of the 516bp, 217bp, 422bp and 284bp fragments of *blaTEM*, *sopB, avrA* and *invA* genes, respectively, from two *Salmonella* isolates (S1-S2), +ve (control positive), -ve (control negative).

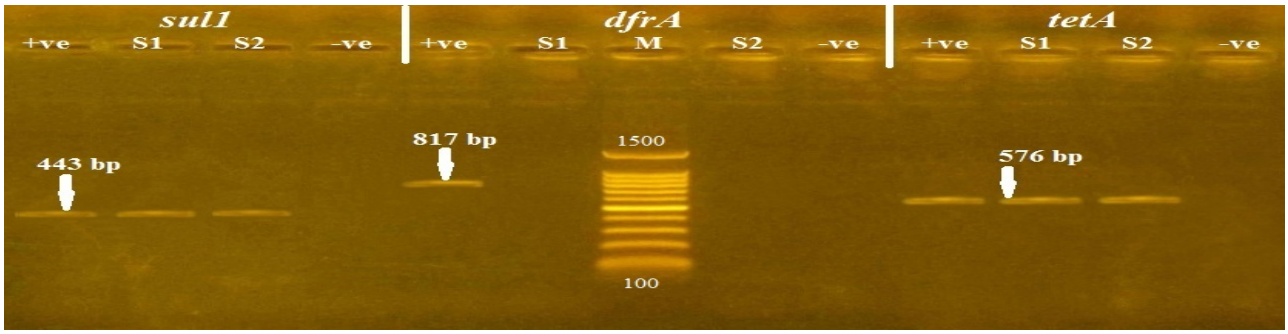

Fig. (6): PCR amplification of the 4243bp, 817bp and 576bp fragments of *sul1*, *sopB, dfrA* and *tetA* genes, respectively, from two *Salmonella* isolates (S1-S2), +ve (control positive), -ve (control negative).

2.5. Antibacterial activity of essential oils. The antibacterial activities of cinnamon and oreganium were tested against 50 MDR isolates including 32 *E. coli* serogroups and 18 *Salmonella* serovars. Cinnamon oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 3% while at 2% concentration 19 *E. coli* isolates (59.4%) and 6 *Salmonella* isolates (33.3%) were inhibited. On contrary, 1% concentration had no effect on all the tested isolates. On the other hand, oreganium oil completely inhibited the growth of all the tested bacterial isolates (100%) at a concentration of 1%. All *E. coli* isolates (100%) and 15 *Salmonella* isolates (83.3%) were inhibited at 0.5% concentration **(**Table 4).

Table (4): Antibacterial activity of cinnamon and oreganium essential oils against *E. coli* and *Salmonella* isolates.

Tested Bacteria	No.	Cinnamon activity						Oreganium activity			
		1%		2%		3%		0.5%		1%	
		No.	%	No.	%	No.	%	No.	%	No.	%
E. coli	**32**	**0**	**0**	**19**	**59.4**	**32**	**100**	**32**	**100**	**32**	**100**
Salmonella	**18**	**0**	**0**	**6**	**33.3**	**18**	**100**	**15**	**83.3**	**18**	**100**

DISCUSSION

One of the major problems in poultry farms is the control of bacteria that causing diarrhea, respiratory manifestation and omphalitis **(McKissick, 2006)**. Accordingly, great attention and efforts should be paid to eliminate these microorganisms causing different affections in poultry. Avian colibacillosis is one of the most important diseases of chickens, resulting in significant losses among baby chicks, broilers and laying hens **(Ibrahim *et al.*, 1998)**. This syndrome characterized by multiple organ lesions with airsacculitis and associated pericarditis, perihepatitis and peritonitis being most typical **(Ewers *et al.*, 2003)**. In addition, *Salmonella* infection causes great mortalities and various morbidity changes as well as economic losses in poultry industry **(Pedersen *et al.*, 2002)**. In the current study, the prevalences of *E. coli* and *Salmonella* isolation from the internal organs (liver, heart, lung, air sacs, and yolk sac) of broiler chickens were detected in 200 broiler chickens (Table 2). The prevalence of *E. coli* isolation was 45.5% while the prevalence of *Salmonella* isolation was 12% with a total prevalence of 57.5%. Concerning *E. coli* this results agreed with those obtained by **Radwan *et al.* (2014)** who reported nearly the same prevalence. **El-Sukhon *et al.* (2002)** obtained higher prevalences while **Sripoernomo *et al.*, (1992),** reported a lower prevalence. The variations in the prevalence rates of *E. coli* in cases of colisepticaemia may be due to the difference in the pathogenicity, virulence of the strains, the severity of the cases and the immunological status of the host **(Heba *et al.*, 2012)**. Stress may cause invasion of pathogenic *E. coli* from intestine into blood stream, spreads into various internal organs, and typically causes pericarditis, perihepatitis, peritonitis, salpingitis and other extra intestinal diseases **(Leitner and Heller 1992)**. Regarding the prevalence of the isolated Salmonellae in the current study, it was similar to that obtained by **Abd El- Fattah (2014)**; 8.72%, and nearly similar to those reported by **Abd El-Galil *et al.* (1995)**; 6%, while it was much lower than that reported by **Sharada *et al.* (1999)**; 30.5%. Serogrouping of 91 *E. coli* isolates revealed that6 O-serogroups were obtained. The serogroup O_{78} was the most prevalent with a rate of 27.5% followed by serogroups O_{158} (18.7%) and O_{128} (16.5%). In addition, the serogroups O_{55}, O_1 and O_{168}were identified at rates of 13.2%, 9.9% and 4.4%, respectively, while 9.9% of *E. coli* isolates were untyped with the available antisera. These results were the same to those obtained by many authors **(Gomis *et al.*, 2001; Abed, 2007 and Abd El-Fattah, 2014)** who achieved that *E. coli* serogroups O_{78} was the most

prevalent serogroup recovered from broilers with enteric and respiratory affections. Serotyping of 24 *Salmonella* isolates showed that *S.* Kentuky, *S.* Enteritidis and *S.* Infantis were identified at rates of 37.5%, 33.3% and 29.2%, respectively. The obtained results run parallel to that obtained by **Abouzeed *et al.* (2000)** who isolated S. Infantis with an incidence 25.6%. **Hegazy (2002)** detected *S.* Enteritidis and *S.* Kentucky at rates of 62.16%, and 5.41% respectively. Antimicrobial therapy is one of the primary control for reducing both the incidence and mortality associated with avian colibacillosis and Salmonellosis therefore reducing their enormous losses in the poultry industry **(Blanco *et al.*, 1997)**. However, resistance to existing antimicrobials is widespread and of concern to poultry veterinarians **(Peighambari *et al.*, 1995)**. *In-vitro* antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy **(Blanco *et al.*, 1997)**. Moreover, it is very useful to detect the multidrug resistant isolates. Moreover, it is very useful to detect the multidrug resistant isolates. In the present work, the recovered *E. coli* (n=91) and *Salmonella* (n=24) isolates were subjected to *in-vitro* antimicrobial sensitivity tests against 12 different antimicrobial drugs to detect the drug of choice for treatment as well as to detect MDR isolates for further analyses of the isolates. The results of antibiogram of *E. coli* isolates showed that a highly sensitivity was observed against colistin sulphate only (91.2%) while moderate sensitivity was detected against amoxicillin +clavulinic acid (57.1%). On the other hand, high resistances were observed against lincomycin, rifampicin (97.8% for each), amoxicillin (96.7%) and sulphamethoxazole-trimethoprime (84.6%). Multidrug resistance was detected in 73 *E. coli* isolates (80.2%). These results agreed with several previous reports **(Peighambari *et al.*, 1995; Gomis *et al.*, 2001 and Radwan *et al.*, 2014)** which have indicated increasing incidences of antibiotic-resistant *E. coli* strains isolated from chickens to several of the antibiotics frequently used in the poultry industry. In addition, **Sharada *et al.* (2001)** found that no single antimicrobial drug was effective by 100% against *E. coli* isolates, which might be due to development of resistance due to indiscriminate use of antibiotics. Concerning MDR ,*E. coli*, the current results were supported those obtained by **Radwan *et al.* (2014)** who reported that MDR was detected in 90.4% of *E. coli* isolates. Moreover, **Blanco *et al.* (1997)** found high levels of resistance to antibacterial drugs in pathogenic strains of *E. coli* isolated from chickens ensuring that multiple drug resistance was common. On the other hand, the results of antibiogram of *Salmonella* isolates showed complete sensitivity to colistin sulphate

while they were highly sensitive to enrofloxacin (91.7%). On the other hand, complete resistances were observed against lincomycin and rifampicin. Multidrug resistance was detected in 18 *Salmonella* isolates (75%). These results coincided with those reported by **Fallah *et al.* (2013)** who reported the high resistance of *Salmonella* isolates chicken against most of these antimicrobials. Belonging MDR *Salmonella* isolates, **Fallah *et al.* (2013)** reported lower values of MDR *Salmonella* recovered from chickens (34.1%). Antimicrobial-resistant *Salmonella* is a public health concern since resistance in *Salmonella* limits the therapeutic options available to veterinarians and physicians in the treatment of human salmonellosis **(Witte, 1998)**. The virulence mechanisms of APEC were summarized in three steps; adhesion, followed by a multiplication in the host's tissues and finally the evasion of its defense systems **(Dho-Moulin, 1993)**. APEC strains may produce temperature sensitive hemagglutinin (*tsh*) which is considered one of the adhesion factors encoded by a *tsh* gene. This gene is located in ColV plasmids that are frequently found in highly pathogenic avian *E. coli* and rarely detected in commensal *E. coli* **(Delicato *et al.*, 2003)**. Because the association of the *tsh* gene with APEC pathogenicity, **Ewers *et al.* (2004)** proposed its utilization as a molecular marker to detect APEC strains. Moreover, APEC possesses a number of other virulence factors that enable them to survive in the extra-intestinal tissues of the host. Serum resistance was found to be an important virulence determinant for *E. coli* in chickens and turkeys **(Delicato *et al.*, 2003)**. The *iss* (increased serum survival) gene is associated with serum resistance and it is significantly more often present in APEC than in commensal *E. coli* **(Vandekerchove, 2004)**. The *iss* gene has been identified as a distinguishing trait of avian but not of human **(Johnson *et al.*, 2008)** and its occurrence in conjugative Col V plasmid can suggest the relationship of *iss* factor to the APEC pathogenicity. APEC strains survive and growth in environments with low iron availability, mainly inside the host, because the expression of iron acquisition systems; like aerobactin, which are associated with virulence in chickens **(Vandekerchove, 2004)**. Most APEC produce aerobactin, while this siderophore is absent in most commensal *E. coli* **(Delicato *et al.*, 2003)**. The gene encoding aerobactin receptor is called *iutA* (iron uptake system). On the other hand, the spread of MDR bacteria has been recognized as an increasing problem in the veterinary and medical fields, and mobile DNA elements, including plasmids, transposons, and integrons, facilitate the proliferation of resistance genes in bacteria **(Liebert *et al.*, 1999)**. Plasmids play an important role in virulence of *E. coli* **(Kovudzhiiski**

***et al.,* 1982)**. The R-plasmids have been extensively studied in view of the prevalence of MDR **(O'Brien *et al.*, 1982)**. Several virulence-associated genes were reported on plasmids of *E. coli* isolated from diseased poultry **(Kelly *et al.*, 2009)** such as genes encoded the resistance to β-lactamase (e.g. *blaTEM*), resistance to sulfonamides (e.g. *sul1*), resistance to tetracycline (e.g. *tetA*) and resistance to trimethoprim (e.g. *dfrA*). In the present work, PCR was applied on14 MDR *E. coli* isolates to determine 7 genes; of which 3 genes responsible for virulence included *tsh*, *iss* and *iutA* as well as 4 genes responsible for antibiotic resistance included bla_{TEM}, *dfrA*, *sul1* and *tetA*. The results illustrated in (Table 3) revealed that bla_{TEM} gene was the most prevalent found in all isolates (100%) followed by *sul1*(92.9%), *iutA* (78.6%) and *i*ss (71.4%) while *tetA*, *tsh* and *dfrA* genes were represented as 35.7%, 28.6% and 21.4%, respectively. *Salmonell*a pathogenicity islands (SPIs) encode a number of virulence factors and play an important role in the pathogenicity of *Salmonella*. Invasion-related gene *invA* and virulence-associated gene; *avrA* and *sopB*, are located within SPI-1 **(Amavisit *et al.*, 2003)**. *Salmonella invA* gene has become one of the most popular PCR target sequences **(Rahn *et al.*, 1992)** and its amplification now has been recognized as an international standard for detection of *Salmonella* and is important in its pathogenesis **(Malorny *et al.*, 2003)**. The *inv*A gene encodes a protein in the inner membrane of bacteria that is responsible for invasion to the epithelial cells of the host **(Darwin and Miller, 1999)**. In addition, *avrA SopB* genes can also be regarded as virulence-associated genes **(Ben-Barak *et al.*, 2006)**. They facilitate induction of apoptosis in macrophages, interleukin production, membrane ruffling and invasion into non-phagocytic host cells **(Mirold *et al.*, 2001)**. In the present work, PCR was applied on 8 MDR *Salmonella* isolates to determine the 7 genes; of which 3 genes responsible for virulence included *invA*, *sopB* and *avrA* as well as 4 genes responsible for antibiotic resistance included bla_{TEM}, *dfrA*, *sul1* and *tetA*.Results revealed that *invA* gene was the most prevalent found in all isolates (100%) followed by *sul1* (75%) and *tetA* (62.5%). while, both bla_{TEM} and *avrA* genes were found in 50% of the isolates. The *dfrA* and *sopB* genes were not found. The uses of plant-derived products as disease control agents have been studied, since they tend to have low mammalian toxicity, less environmental effects and wide public acceptance **(Li *et al.*, 2006)**. Therefore, there has been increasing interest to replace synthetic preservatives with natural, effective and nontoxic compounds. Those are; in the first place, extracts and EOs of spices and herbs **(Smid and Gorris, 1999)**. In the present study, the antibacterial activities of cinnamon and oreganium were tested against 50 MDR *E. coli*

(n=32) and *Salmonella* (n=18) isolates (Table 4). Oreganium showed higher activity than cinnamon. Oreganium oil completely inhibited the growth of all the tested bacterial isolates at a concentration of 1%. In addition, at 0.5% concentration, all *E. coli* isolates while 83.3% of *Salmonellae* isolates were inhibited. On the other hand, cinnamon oil showed complete inhibition at a concentration of 3%. The lower concentrations showed lesser activities where at 2% concentration 59.4% of *E. coli* isolates and 33.3% of *Salmonellae* isolates were inhibited. On contrary, 1% concentration had no effect on all isolates. EOs is more efficient than various artificial antimicrobial agents are. That is due to its low toxicity level and high volatility specific property that is not found in other antimicrobial agents **(Inouye *et al.*, 2003)**. Many authors studied the *in-vitro* antibacterial properties of several EOs **(Burt, 2004 and Bakkali *et al.*, 2008)**. The antibacterial activity of oreganium EO is primarily due to carvacrol and then thymol which are phenols representing the major components comprising 86.5% **(Bharti *et al.*, 2013)**. Carvacrol and thymol are able to disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP **(Burt, 2004)**. On the other hand, the antibacterial activity of cinnamon EO is due cinnamaldehyde; an aromatic aldehyde, which is the major component of cinnamon EO; comprising 65-85%, that exhibit antibacterial properties **(Li *et al.*, 2006)**. It had been suggested that, the carbonyl group of aldehydes can bind to metal ions, sulfhydryl groups, amino groups and proteinspreventing the action of amino acid decarboxylases **(Bowles and Miller, 1993)**. It was concluded that, the presence of multidrug resistance pathogens took place due to the misuse of the antibiotics and it is considered a great problem. EOs exhibit activity against food borne pathogens and it is possible for their application in the veterinary medicine as alternatives toantibiotics.

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