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PREVALENCE OF SOME ANTIMICROBIAL RESISTANCE GENES IN MULTIDRUG RESISTANT SALMONELLA ISOLATED FROM BROILER CHICKENS

WALID H. HASSAN[1], AHMED H. ABED[1], ABD EL RADY THABET[2] and EMAN A.M. EL NADY[2].

[1] Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Beni-Suef University.
[2] Department of Bacteriology, Animal Health Research Institute, Assiut Branch.

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ABSTRACT

Salmonellosis is one of the major bacterial problems in the poultry industry in Egypt and worldwide. Resistance to antimicrobial agents within nontyphoidal Salmonellae is a serious problem. The present study aimed to analyze some β-lactamase resistance genes in Salmonella isolates from broiler chicken. Five hundred samples were collected from diseased broiler chickens of different ages (3-6 weeks) from different farms in Assiut Governorate during the period from January 2015 to December 2015. Bacteriological examination showed that 26 salmonella isolates were recovered with a prevalence rate of 5.2% Serotyping of Salmonella isolates showed that *S. Enteritidis S. Typhimurium*, and *S. Kentuky* were identified at rates of 50%, 30.8% and 19.2%, respectively. Results of antibiogram showed that 18 salmonella isolates (92.3%) were multidrug resistant. All isolates were screened for the presence of 2 β-lactamase resistance genes (bla_{CTX} *and* bla_{CMY}) using multiplex PCR. The overall prevalence was 14/26 (53.9%) for bla_{CTX} and 9/26 (34.6%) for bla_{CMY}.

Key words*: Multidrug resistance, Broiler chicken, Salmonellae, β-lactamases.*

INTRODUCTION

Salmonellosis is one of the major bacterial problems in the poultry industry worldwide. salmonella species are responsible for a variety of acute and chronic diseases in both poultry and humans (Okwori *et al.*, 2013).

Although more than 2500 serotypes of salmonella have been identified, in recent years, *S. enterica* serovar Enteritidis (*S.* Enteritidis) and *S. enterica* serovar Typhimurium (*S.* Typhimurium) have been recognized as the two major causative agents of salmonellosis in birds, mammals and humans (Darwin and Miller, 1999).

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 of animal origin 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 on the basis of its sensitivity which could be detected by laboratory examination. The recovery of antimicrobial-resistant salmonella in foods of animal origin has raised concerns that the treatment of human salmonellosis may be compromised because antimicrobial-resistant strains appear to be more often associated with severe disease than are susceptible isolates. Of significant concern is the isolation of salmonella exhibiting decreased susceptibility to fluoroquinolones (e.g., ciprofloxacin) and extended-spectrum cephalosporins (e.g., ceftiofur and ceftriaxone), because these two antimicrobial classes are important in treating salmonella infections in adults and children, respectively (Gupta *et al*., 2003).

Resistance to antimicrobial agents within nontyphoidal salmonella serotypes is considered a serious problem worldwide. Surveillance data demonstrates an obvious increase in overall antimicrobial resistance among salmonellae from 20-30% in the early 1990s to as high as 70% in some countries in the 2000s (Su *et al.*, 2004). More than 340 beta-lactamases have been described in salmonella and the prevalence of genes encoding for them varies region by region (Hasman *et al.*, 2005).

Corresponding author: Dr. EMAN A.M. EL NADY
E-mail address: elnadyeman@yahoo.com
Present address: Department of Bacteriology, Animal Health Research Institute, Assiut Branch.

The purpose of this study was genetic analysis of some β-lactamase resistance genes in salmonellae isolates from broiler chicken.

MATERIALS AND METHODS

1 - Samples

Five hundred samples were collected from diseased broiler chickens (suspected to harbor salmonella) of different ages (3-6 weeks). Sampling were carried out from different farms in Assiut Governorate during the period from January 2015 to December 2015. These chickens were subjected to clinical and postmortem examinations. Samples were collected aseptically from the lesions in the internal organs including liver, heart, lung, air sacs, and kidney.

2 - Isolation and identification of Salmonella species

Samples were cultured into selenite-F broth and incubated at 37ºC for 18-24 hrs. Then, a loopful of this culture was streaked out onto MacConkey's agar then the non-lactose fermenter (pale) colonies were streaked onto xylose lysine deoxycholate (XLD) and Salmonella-Shigella (SS) agar media and incubated at 37ºC for 18-24 hours. All isolates were identified as salmonella species based on their colony morphology and biochemical tests according to schemes described by Collee *et al.* (1996) and Quinn *et al*. (2002). The salmonella isolates were also confirmed biochemically by using the API 20E system (BioMérieux, Marcy-l'Étoile, France).

3 - Serotyping of Salmonella species

Salmonella isolates were serotyped by slide agglutination test carried out according to Kauffman-White scheme (Kauffman, 1974) for the determination of Somatic (O) and flagellar (H) antigens using salmonella antiserum (DENKA SEIKEN Co., Japan).

4 - Antimicrobial susceptibility testing

All Salmonella isolates were tested for their antimicrobial susceptibility to 14 different antimicrobial discs including erythromycin (15µg), nalidixic acid (30µg), penicillin (10 IU), amoxicillin (30µg), oxytetracycline (30µg), sulphamethoxazole-trimethoprime (25µg), ampicillin (10µg), streptomycin (10µg), neomycin (30µg), chloramphenicol (30µg), norfloxacin (10µg), ciprofloxacin (5µg), kanamycin (30µg) and gentamicin (10µg) (Oxoid Limited, Basing Stoke, UK). Antimicrobial susceptibility testing was performed using disc diffusion method on Muller Hinton agar according to CLSI (2015). The antimicrobial susceptibility was based on the induced inhibition zones according to the guidelines of the CLSI (2015). Resistance to two/or more antimicrobials of different classes was taken as multidrug resistant (MDR) (Chandran *et al*., 2008).

5 - Multiplex-PCR for detection of β-lactamases resistance genes

5.1- DNA extraction:

DNA was extracted by using bacterial DNA e xtraction kits (Qiagen, Germany, GmbH) according to the manufacturer instructions.

5.2- Screening of β-lactamases resistance genes by multiplex-PCR:

The multiplex-PCR assay was applied on 26 salmonella isolates for detection of 2 β-lactamase resistance genes bla_{CTX} (responsible for cefotaxime resistance) and bla_{CMY} (responsible for cephalosporin resistance) according to (Ahmed *et al*., 2009). Targeted genes and their primer sequences are listed in (Table 1).

Table 1: Primer sequences and amplified products for the β-lactamase resistance genes *blaCTX and blaCMY*.

Gene		Primer Sequence 5'-3'	Amplified product	Reference
Bla_{CTX}	*F*	*CGCTTTGCGATGTGCAG*	550 bp	**Ahmed *et al.* (2009)**
	R	*ACCGCGATATGCTTGGT*		
Bla_{CMY}	*F*	*GACGCCTCTTTCTCCACA*	1007 bp	
	R	*TGGAACGAAGGCTACGTA*		

RESULTS

1 - Bacteriological isolation of salmonella species.

Out of 500 broiler chicken samples, 26 salmonella isolates were recovered with a prevalence rate of 5.2%.

2 - Serotyping of salmonella isolates.

The 26 salmonella isolates were serotyped as 13 S. *Enteritidis* (50%), 8 *S. Typhimurium* (30.8%) and 5 *S. Kentuky* (19.2%).

3 - Antimicrobial susceptibility testing.

Results of *in-vitro* sensitivity tests showed that salmonella isolates were completely resistant to erythromycin and penicillin while they were highly resistant to amoxicillin and streptomycin (92.3% for both), nalidixic acid (80.8%), sulphamethoxazole-trimethoprime (76.9%), ampicillin (69.2%) and oxytetracycline (65.4%). On contrary, salmonella strains were highly sensitive to kanamycin (96.2%) then gentamycin (73.1%) (Table 2). MDR salmonella isolates were 24 isolates (92.3%).

Table 2: Distribution of Antimicrobial susceptibility for 26 salmonella isolates.

Antimicrobial agent	Sensitive		Intermediate		Resistant	
	NO.	%	NO.	%	NO.	%
Erythromycin (E)	-	-	-	-	26	100
Penicillin(P)	-	-	-	-	26	100
Amoxicillin (AMX)	-	-	2	7.7	24	92.3
Streptomycin (S)	2	7.7	-	-	24	92.3
Nalidixic acid (NA)	2	7.7	3	11.5	21	80.8
Sulphamethoxazol-trimethoprime (SXT)	4	15.4	2	7.7	20	76.9
Ampicillin (AMP)	5	19.2	3	11.5	18	69.2
Oxytetracycline (T)	5	19.2	4	15.4	17	65.4
Chloramphenicol (C)	7	26.9	6	23.1	13	50.0
Norfloxacin (NOR)	11	42.3	5	19.2	10	38.5
Neomycin (N)	13	50.0	7	26.9	6	23.1
Ciprofloxacin (CIP)	14	53.8	8	30.8	4	15.4
Gentamycin (G)	19	73.1	5	19.2	2	7.7
Kanamycin (K)	25	96.2	-	-	1	3.8

4 - Multiplex-PCR for detection of β-lactamases resistance genes (*bla$_{CTX}$ and bla$_{CMY}$*).

Multiplex-PCR assay was done for 26 salmonella strains for detection of 2 β-lactamase resistance genes *bla$_{CTX}$* and *bla$_{CMY}$*. The results revealed that *bla$_{CTX}$* gene was found in 14/26 isolates (53.9%) arranged as follow; 8 *S. Enteritidis* (30.8%), 4 *S. Typhimurium* (15.4%) and 2 *S. Kentucky* (7.7%). Meanwhile, *bla$_{CMY}$* gene was found in 9/26 isolates (34.6%) arranged as follow; 4 *S. Enteritidis* (15.4%), 3 *S. Typhimurium* (11.5%) and 2 *S. Kentucky* (7.7%) (Tables 3& 4 and Fig. 1& 2).

Table 3: Multiplex-PCR results for β-lactamase resistance genes in salmonella isolates.

NO	Serotype	*bla$_{CTX}$*	*bla$_{CMY}$*	NO	Serotype	*bla$_{CTX}$*	*bla$_{CMY}$*
1	*S. Enteritidis*	+	-	14	*S. Typhimurium*	+	-
2	*S. Enteritidis*	+	+	15	*S. Typhimurium*	-	+
3	*S. Enteritidis*	-	-	16	*S. Typhimurium*	-	-
4	*S. Enteritidis*	-	-	17	*S. Typhimurium*	+	-
5	*S. Enteritidis*	+	-	18	*S. Typhimurium*	+	+
6	*S. Enteritidis*	-	-	19	*S. Typhimurium*	-	+
7	*S. Enteritidis*	+	-	20	*S. Typhimurium*	+	-
8	*S. Enteritidis*	+	+	21	*S. Typhimurium*	-	-
9	*S. Enteritidis*	+	+	22	*S. Kentucky*	-	+
10	*S. Enteritidis*	+	-	23	*S. Kentucky*	-	-
11	*S. Enteritidis*	-	-	24	*S. Kentucky*	+	-
12	*S. Enteritidis*	-	+	25	*S. Kentucky*	-	-
13	*S. Enteritidis*	+	-	26	*S. Kentucky*	+	+

Table 4: Resistance phenotype and prevalence of β-lactamase resistance genes in salmonella isolated from diseased broilers.

NO	Serotype	Resistance phenotypes	Resistance gene
1	*S. Enteritidis*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP, G	bla_{CTX}
2	*S. Enteritidis*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR, N	bla_{CTX}, bla_{CMY}
5	*S. Enteritidis*	E, P, AMX, S, NA, SXT, AMP, T, C	bla_{CTX}
7	*S. Enteritidis*	E, P,AMX, S, NA, SXT, AMP, T	bla_{CTX}
8	*S. Enteritidis*	E, P,AMX, S, NA, SXT, AMP, T	bla_{CTX}, bla_{CMY}
9	*S. Enteritidis*	E, P,AMX, S, NA, SXT	bla_{CTX}, bla_{CMY}
10	*S. Enteritidis*	E, P,AMX, S, NA, SXT	bla_{CTX}
12	*S. Enteritidis*	E, P,AMX, S	bla_{CMY}
13	*S. Enteritidis*	E, P	bla_{CTX}
14	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP, G, K	bla_{CTX}
15	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP	bla_{CMY}
17	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR	bla_{CTX}
18	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR	bla_{CTX}, bla_{CMY}
19	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C	bla_{CMY}
20	*S. Typhimurium*	E, P,AMX, S, NA, SXT, AMP, T, C	bla_{CTX}
22	*S. Kentucky*	E, P,AMX, S, NA, SXT, AMP, T, C, NOR, N, CIP	bla_{CMY}
24	*S. Kentucky*	E, P, AMX, S, NA, SXT, AMP	bla_{CTX}
26	*S. Kentucky*	E, P	bla_{CTX}, bla_{CMY}

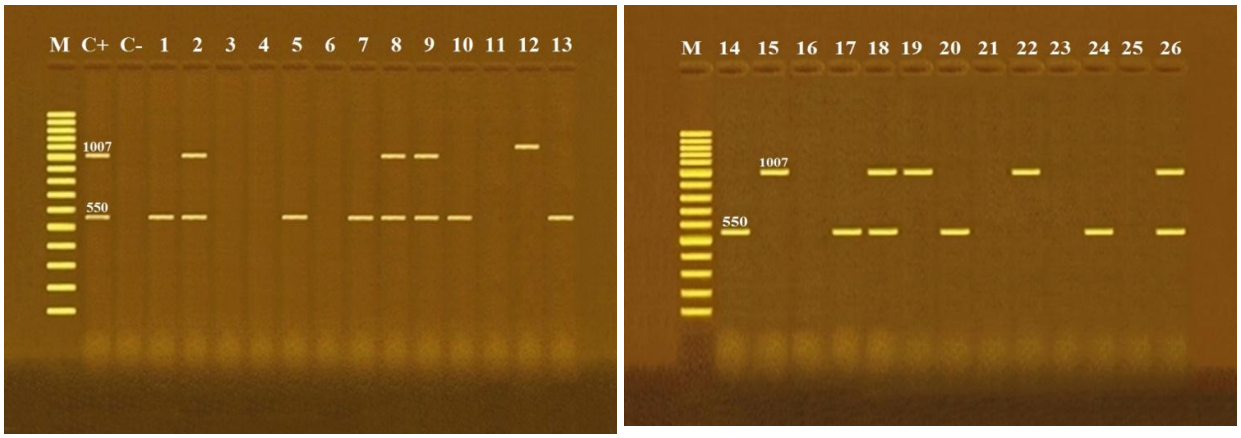

Fig. (1& 2): PCR amplification of the 550bp and 1007bp fragments of bla_{CTX} and bla_{CMY} genes, respectively, from 26 *Salmonella* isolates (1-26) showing positive amplicons migrates with the molecular 100 bp ladder marker (M)., C+ (control positive), C- (control negative). *S.Enteritidis* (1-13), *S.Typhimurium* (14-21), *S.Kentucky* (22-26)

DISCUSSION

Salmonellosis causes great mortalities and various morbidity changes as well as economic losses in poultry industry and a serious public health problem throughout the world (Pedersen *et al*., 2002). Identification and genotyping of salmonella isolates are essential for epidemiological surveillance and investigations of outbreak.

In the current work, 26 salmonella species were recovered from the 500 samples of internal organs (liver, heart, lung, airsacs, and kidney) of broiler chickens with prevalence rate of 5.2%. This result was similar to that obtained by reported by Abd El-Galil *et al.* (1995); 6%, and nearly similar to those of Abd El Fattah (2014); 8.72%, and Radwan *et al.* (2016); 12%, while it was much lower than that reported by Sharada *et al*. (1999); 30.5%.

Serotyping of 26 salmonella isolates showed that *S.Enteritidis*, *S.Typhimurium* and *S.Kentuky* were identified at rates of 50%, 30.8% and 19.2%, respectively. The obtained results run parallel to that obtained by Hegazy (2002) who 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 diseases therefore reducing their enormous losses in the poultry industry (Radwan *et al.*, 2016). Increasing antimicrobial resistance is an important public health concern, and the emergence and spread of antimicrobial resistance is a complex problem driven by numerous interconnected factors. *In-vitro* antimicrobial susceptibility testing of veterinary pathogens can provide valuable guidance to the veterinarian in the choice of appropriate chemotherapy (Radwan *et al.*, 2016). Moreover, it is very useful to detect the multidrug resistant isolates.

In the present study, the recovered salmonella isolates (*n=26*) were subjected to *in-vitro* antimicrobial susceptibility tests against 14 different antimicrobial drugs to detect MDR isolates for further analyses of the isolates. Results illustrated in table (2) revealed that *Salmonella* isolates showed complete resistance against erythromycin and penicillin while they were highly resistant to amoxicillin and streptomycin (92.3% for both), nalidixic acid (80.8%), sulphamethoxazole-trimethoprime (76.9%), ampicillin (69.2%) and oxytetracycline (65.4%). On the other hand, they were highly sensitive to kanamycin (96.2%) then gentamycin (73.1%). MDR salmonella isolates were 24 isolates with prevalence rate of 92.3%. Comparable results have been reported worldwide; in Egypt (Ahmed *et al.*, 2009), in USA (Frye and Fedorka-Cray, 2007), and in Italy (Mammina *et al*., 2002), and in Portugal (Antunes *et al.*, 2006).

Beta-lactams belong to a family of antibiotics, the members of which have a β-lactam ring. Penicillins, cephalosporins, clavams (or oxapenams), cephamycins and carbapenems are members of this family. In Gram-negative bacteria, resistance to β-lactam antibiotics is primarily mediated by β-lactamases, which hydrolyze the β-lactam ring and thus inactivate the antibiotic. Many different β-lactamases have been described, but *TEM*-, *SHV*-, *OXA*-, *CMY*- and *CTX-M*-β-lactamases are the most predominant in Gram-negative bacteria. bla_{CTX-M}arise resistance to penicillins, extended-spectrum cephalosporins, and monobactams, and the enzymes are inhibited by clavulanate, sulbactam, and tazobactam. Typically, the CTX-M-ases hydrolyze cefotaxime more efficiently than ceftazidime, which is reflected in substantially higher MICs to cefotaxime than to ceftazidime (Livermore and Woodford, 2006).

In the current study 2 β-lactamase resistance genes (bla_{CTX} and bla_{CMY}) were evaluated in all salmonella isolates (*n=26*) using multiplex-PCR assay. The results illustrated in tables (3& 4) and Fig. (1& 2) revealed that bla_{CTX} gene was found in 14/26 isolates (53.9%) arranged as follow; 8 *S. Enteritidis* (30.8%), 4 *S. Typhimurium* (15.4%) and 2 *S. Kentucky* (7.7%). The resistance gene bla_{CTX} has previously been identified and reported increasingly in Gram-negative rods (Bradford, 2001; Bonnet, 2004; Eckert *et al*., 2004; Lartigue *et al*., 2005; Naas *et al*., 2005 and Pitout *et al*., 2005) and was characterized and isolated in Germany and Italy (Barthelemy *et al*., 1992 and Bauernfeind *et al.*, 1996). On the other hand, bla_{CMY} gene was found in 9/26 isolates (34.6%) arranged as follow; 4 *S. Enteritidis* (15.4%), 3 *S. Typhimurium* (11.5%) and 2 *S. Kentucky* (7.7%). The gene bla_{CMY} has previously been identified in *S*. Typhimurium and other serovars isolated from animals in Canada (Allen and Poppe, 2002), Egypt (Ahmed *et al*., 2009) and USA (Doublet *et al.*, 2004; Frye and Fedorka-Cray, 2007 and Zhao *et al*., 2007).

CONCLUSION

The presence of multidrug resistance pathogens occurred due to the misuse of the antibiotics and it is considered a great problem. In this study, multidrug-resistant strains of *S. Enteritidis*, *S. Typhimurium* and *S. Kentucky* from diseased broilers were recovered and identified. Furthermore, 2 important antimicrobial resistance genes (bla_{CTX} and bla_{CMY}) were characterized using multiplex-PCR. These resistance genes confer resistance to many antimicrobial agents regularly used in poultry farming and hospitals.

REFERENCES

Abd El Fattah, A.A. (2014): Bacteriological studies on the bacteria associated with dead -in shell - and early mortalities in chicks. M. V. Sc. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Abd El-Galil, Y.; El-Kenawy, A.I.; El-Gmiey, S.R. and Abd El-Latif, M.M. (1995): Bacterial causes of lowering hatchabilityand early embryonic chicken deaths in balady hatcheries in Dakahlia Governorate. Assiut Vet. Med. J., 33 (6).

Ahmed, A.M.; Younis, E.E.A.; Ishida, Y. and Shimamoto T. (2009): Genetic basis of multidrug resistance in *Salmonellaenterica* serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. Acta. Trop., 111: 144-149.

Allen, K.J. and Poppe, C. (2002): Occurrence and characterization of resistance to extended-spectrum cephalosporins mediated by β-lactamase *CMY-2* in Salmonella isolated from food-producing animals in Canada. Can. J. Vet. Res., 66: 137-144.

Antunes, P.; Machado, J. and Peixe, L. (2006): Characterization of antimicrobial resistance and class 1 and 2 integrons in *Salmonellaenterica* isolates from different

sources in Portugal. J. Antimicrob. Chemother., 58: 297-304.

Barthelemy, M.; Peduzzi, J.; Bernard, H.; Tancrede, C. and Labia, R. (1992): Close amino acid sequence relationship between the new plasmid-mediated extended spectrum beta-lactamase MEN-1 and chromosomally encoded enzymes of *Klebsiellaoxytoca*. Biochim. Biophys. Acta., 1122:15-22.

Bauernfeind, A.; Stemplinger, I.; Jungwirth, R.; Ernst, S. and Casellas, J.M. (1996): Sequences of beta-lactamase genes encoding *CTX-M-1* (*MEN-1*) and *CTXM-2* and relationship of their amino acid sequences with those of other beta-lactamases Antimicrob. Agents Chemother., 40:509-513.

Bonnet, R. (2004): Growing group of extended spectrum β-lactamases: the CTX-M enzymes. Antimicrob. Agents Chemother., 48:1-14.

Bradford, P.A. (2001): Extended-spectrum β-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin.Microbiol. Rev., 14: 933-951.

Chandran, A.; Hatha, A.; Varghese, S. and Mony Sheeja, K. (2008): Prevalence of multiple drug resistant *E. coli* serotypes in a tropical estuary, India. Microbes Environ. 23 (2):153-158.

CLSI, Clinical and Laboratory Standards Institute (2015): Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement (M100-S25). Vol 35 (3) P. 64, Wayne, PA, USA.

Collee, J.G.; Fraser, A.G.; Marmion, B.P. and Simmons, A. (1996): Practical Medical Microbiology. 14th Ed.

Darwin, K.H. and Miller, V.L. (1999): Molecular basis of the interaction of Salmonella with the intestinal mucous. Clin.Microbiol. Rev., 12: 405-428.

Doublet, B.; Caratolli, A.; Whichard, J.M.; White, D.G.; Baucheron, S.; Chaslus-Dancla, E. and Cloeckaert, A. (2004): Plasmid-mediated florfenicol and ceftriaxone resistance encoded by the *floR* and bla_{CMY-2} genes in *Salmonellaenterica* serovars Typhimurium and Newport isolated in the United States. FEMS Microbiol. Lett., 15: 301-305.

Eckert, C.; Gautier, V.; Saladin-Allard, M.; Hidri, N.; Verdet, C.; Ould-Hocine, Z.; Barnaud, G.; Delisle, F.; Rossier, A.; Lambert, T.; Philippon, A. and Arlet, G. (2004): Dissemination of *CTX-M*-type beta-lactamases among clinical isolates of Enterobacteriaceae in Paris, France. Antimicrob. Agents Chemother., 48:1249-1255.

Frye, J.G. and Fedorka-Cray, P.J. (2007): Prevalence, distribution and characterization of ceftiofur resistance in *Salmonellaenterica* isolated from animals in the USA from 1999 to 2003. Int. J. Antimicrob Agents, 30: 134-142.

Gosh, S. and LaPara, T.M. (2007): The effects of sub-therapeutic antibiotic use in farm animals on the proliferation and persistence of antibiotic resistance among soil bacteria. ISME J, 1: 191-203.

Gupta, A.; Fontana, J.; Crowe, C.; Bolstorff, B.; Stout, A.; Van Duyne, S.; Hoekstra, M.P.; Whichard, J.M.; Barrett, T.J. and Angulo, F.J. (2003): Emergence of multidrug-resistant *Salmonella enterica* serotype Newport infections resistant to expanded-spectrum cephalosporinsin the United States. J. Infect. Dis. 188:1707-1716.

Hasman, H.; Mevius, D.; Veldman, K.; Olesen, I. and Aarestrup, F.M. (2005): Beta-Lactamases among extended-spectrum beta-lactamase (ESBL)-resistant Salmonella from poultry, poultry products and human patients in The Netherlands. J. Antimicrob. Chemother., 56: 115-121.

Hegazy, A.E. (2002): Epidemiological studies on Salmonellosis in chickens with special reference to Salmonella Enteritidis. Ph. D. Thesis., Fac. Vet. Med., Alex. Univ., Egypt.

Kauffman, G. (1974): Kauffmann white scheme. J. Acta. Path. Microbiol. Sci., 61:385.

Lartigue, M.F.; Poirel, L.; Decousser, J.W. and Nordmann, P. (2005): Multidrug resistant *Shigellasonnei* and *Salmonella enterica* serotype Typhimurium isolates producing *CTX-M* beta-lactamases as causes of community-acquired infection in France. Clin. Infect. Dis., 40:1069-1070.

Livermore, D.M. and Woodford, N. (2006): The β-lactamase threat in Enterobacteriaceae, Pseudomonas and Acinetobacter. Trends Microbiol., 14: 413-420.

Mammina, C.; Cannova, L.; Massa, S.; Goffredo, E. and Nastasi, A. (2002): Drug resistances in Salmonella isolates from animal foods, Italy1998–2000. Epidemiol. Infect., 129: 155-161.

Naas, T.; Lezzar, A.; Bentchouala, C.; Smati, F.; Scheftel, J.M.; Monteil, H. and Nordmann, P. (2005): Multidrug-resistant *Salmonella enterica* serotype Senftenberg isolates producing *CTX-M* beta-lactamases from Constantine, Algeria. J. Antimicrob. Chemother. 56:439-440.

Okwori, A.E.J.; Ogbe, R.J.; Chollom, S.C.; Agada, G.O.A.; Ujah, A.; Okwori, E.; Adeyanju, O.N. and Echeonwu, G.O.N. (2013): Isolation of *Salmonella* Gallinarum from poultry droppings in Jos metropolis, Plateau State, Nigeria.

Pedersen, K.; Hansen, H.C.; Jorgensen, J.C. and Borck, B. (2002): Serovars of Salmonella isolated from Danish turkeys between 1995

and 2000 and their antimicrobial resistance. Vet. Rec., 150(15): 471-474.

Pitout, J.D.; Nordmann, P.; Laupland, K.B. and Poirel, L. (2005): Emergence of Enterobacteriaceae producing extended-spectrum betalactamases (ESBLs) in the community. J. Antimicrob. Chemother., 56:52-59.

Quinn, P.J.; Markey, B.K.; Carter, M.E.; Donnelly, W.J.C.; Leonard, F.C. and Maguire, D. (2002): Veterinary Microbiology and Microbial Disease. Published by Blackwell. PP. 113-116.

Radwan, I.A.; Abed, A.H.; Abd Al-Wanis, S.A.; Abd El-Aziz, G.G. and El-Shemy. A. (2016): Antibacterial effect of cinnamon and oreganium oils on multidrug resistant *Escherichiacoli* and Salmonellae isolated from broiler chickens. J. Egy. Vet. Med, Ass., 76(2): 169-186.

Sharada, R.; Krishneppa, G.; Raghavan, R.; Gowda, R.N.S. and Upendra, H.A. (1999): Isolation and serotyping of *Escherichia coli* from different pathological conditions inpoultry. Ind. J. Poult. Sci., 34 (3): 336-369.

Su, L.H.; Chiu, C.H.; Chu, C. and Ou, J.T. (2004): Antimicrobial resistance in nontyphoid *Salmonella* serotypes: a global challenge. Clin. Infect. Dis., 39: 546-551.

WHO, World Health Organization (2011): Tackling antibiotic resistance from a food safety perspective in Europe. Available: http://www.euro.who.int/data/assets/pdf_file/0005/136454/ e94889.pdf.

Zhao, S.; McDermott, P.F.; White, D.G.; Qaiyumi, S.; Friedman, S.L.; Abbott, J.W.; Glenn, A.; Ayers, S.L.; Post, K.W.; Fales, W.H.; Wilson, R.B.; Reggiardo, C. and Walker, R.D. (2007): Characterization of multidrug resistant Salmonella recovered from diseased animals. Vet. Microbiol., 123: 122–132.

دراسة عن انتشار بعض الجينات المسببة للمقاومة ضد مضادات الميكروبات فى عترات السالمونيلا متعددة المقاومة للادوية المعزولة من الدجاج اللاحم (بدارى التسمين)

وليد حسن ، أحمد حسين ، عبد الراضى ثابت ، إيمان أبوبكر محمد النادى

Email: elnadyeman@yahoo.com Assiut University web-site: www.aun.edu.eg

السالمونيلا تعتبر واحدة من كبرى المشاكل البكتيرية فى صناعة الدواجن فى مصر وجميع انحاء العالم. وتعتبر المقاومة ضد مضادات الميكروبات فى ميكروب السالمونيلا مشكلة خطيرة. وقد هدفت الدراسة الحالية إلى تحليل بعض جينات البيتا-لاكتاماز المسببة للمقاومة ضد مضادات الميكروبات فى عترات السالمونيلا المعزولة من الدجاج اللاحم (بدارى التسمين). وقد تم جمع خمسمائة عينة من الدجاج اللاحم (بدارى التسمين) المريضة من مختلف الأعمار (٣-٦ أسابيع) من مختلف المزارع فى محافظة أسيوط خلال الفترة من يناير ٢٠١٥ إلى ديسمبر ٢٠١٥. أظهرت نتائج الفحص الشكلى أنه تم عزل ٢٦ عينة من ميكروب السالمونيلا بمعدل انتشار 5.2 %. وأظهرت نتائج التصنيف المصلى للسالمونيلا المعزولة انه تم تحديد النمط المصلى: سيروفار انتيريتيتز ، سيروفار تيفيميوريم ، سيروفار كنتاكى (*S. Enteritidis, S. Typhimurium, S. Kentuky*) بمعدلات 50 % ، 30.8 %، 19.2 % على التوالى. وأظهرت نتائج تحليل المقاومة للمضادات الحيوية أن ١٨ من السالمونيلا المعزولة (92.3%) كانت متعددة المقاومة للأدوية. وأظهرت نتائج الفحص الجينى بواسطة اختبار البلمرة المتسلسلة أن جميع العزلات تحمل ٢ من جينات المقاومة البيتا-لاكتاماز (blaCMY, blaCTX) بمعدل انتشار 26/14 (53.9%) blaCTX (الذى يشفر المقاومة ضد السيفوتاكسيم)، 26/9 (34.6%) blaCMY (الذى يشفر المقاومة ضد الكيفالوسبورن).