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DETECTION OF BIOFILM FORMATION AND ANTIBIOTIC RESISTANCE OF SALMONELLA IN BROILER CHICKEN

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ABSTRACT

Two hundred fifty samples from different broiler chicken flocks were subjected for isolation of salmonella species (2017-2018). The percentage for isolated Salmonella was 14.4% (36/250). The obtained Salmonlla strains were obtained (*Salmonella bardo*, *Salmonella norwich*, *Salmonella* brancaster, *Salmonella sekondi II, Salmonella lamberhurst*, *Salmonella belgdam*, *Salmonella kentucky*, *Salmonella enteritidis*, *Salmonella* goetebory, *Salmonella kisii*, *Salmonella* nigeria, *Salmonella* grampian, *Salmonella* newport, *Salmonella noyo*, *Salmonella colindale*, *Salmonella seremban*, *Salmonella remo*, *Salmonella lindenburg, Salmonella* a natum, *Salmonella virchow*, *Salmonella tamiland*, Salmonella york. In vitro antimicrobial sensitivity testing carried out on isolated salmonella strains revealed different antimicrobial resistance variation, high resistance rate were observed with lomefloxacin (77.7%), tetracycline (61%), kanamcin (50%) and trimethoprime and levofloxacin (47.2%). Also strains were subjected for detection of biofilm formation using glass tube test and detection of *fim*A gene was used for biofilm confirmation, 61.11% (22/36) of strains was having ability to produce biofilm, while 38.88 % (14/36) have no ability for biofilm production. Both positive and the negative biofilm formation of salmonella strains revealed the same degree of antibiotic resistance (100%). No great significance between biofilm formation, multidrug resistance and the intensity of clinical signs and postmortem lesions were observed, so no relation between biofilm formation and antimicrobial resistance.

Key words: Biofilm, salmonella, antibiotic resistance, broiler.

INTRODUCTION

Salmonella can cause disease in domestic animals, differ in severity of a signs, diarrhea and enteritis to systemic syndrome, lead to great economic losses in poultry industry. Salmonellosis is of public health concern in both the developed and developing countries, it is one of the most important pathogens transmitted by food, especially poultry, which cause food poisoning, it has the ability to form biofilms on surfaces and It′s adhesion can be influenced by different physicochemical properties of these surfaces, while *Salmonella* uses fimbriae and produces cellulose as the main matrix components of biofilms.

Salmonella infections are a serious medical and veterinary problem worldwide and there is an increasing need for new strategies for prevention and control (Majowicz *et al*., 2010).

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Biofilms are bacterial association that attach to a biological or non-biological surface and are enveloped by a bacterial-initiated matrix. This structure promote bacteria to survive in hostile conditions such as exposure to UV light, metal toxicity, acid exposure, dehydration and salinity, phagocytes, and several antibiotics and antimicrobial agents (Hall-Stoodley *et al*., 2004), as well as they can also form biofilms on chicken intestinal epithelium Ledeboer and Jones, (2005).

Microorganisms may be naturally resistant to antimicrobial agent or a specific category of antimicrobials but resistance may also be acquired. (Kadlec *et al*., 2012).

Many bacteria are able to attach and tocolonize environmental surfaces by producing biofilms (Donlan, and Costerton, 2002). Surface-associated community forming microcolonies surrounded by a matrix of exopolymers that trap other bacteria, nutrients, and debris is known as bacterial biofilm (Chavant *et al*., 2002).

The biofilm formation is required several developmental steps that included several

distinguishable steps: (a) attachment to the carrier surface, reversible, (b) irreversible attachment, binding to the surface with the participation of adhesions or exopolysaccharides, (c) the development of microcolonies, a distinct mushroom shape, (d) the maturation of biofilmarchitecture (Donlan, and Costerton, 2002) and (Barnhart and Chapman, 2006), (e) under favorable conditions, the synthesis of martrix compoundsdecline and biofilm dispersion due to enzymatic cleavage of the matrix Gjermansen *et al*. (2005).

The *fim* A gene encodes the major structural subunit, while the *fim* H gene encodes the adhesin protein that is located at the tip of the assembled fimbrial structure and mediates binding to the receptor. The *fim* H adhesin is involved in biofilm formation on HEp-2 tissue culture cells, murine intestinal epithelium, and chicken intestinal epithelium (Boddicker *et al.*, 2002).

The aim of the work was to detect relation between antibiotic resistance of Salmonella strains and biofilm formation in broiler chicken.

MATERIALS AND METHOD

1- Sampling

Two hundred fifty samples obtained from different broiler flocks at different age and from different sources (farms, back yard, shops) were subjected for isolation of salmonella from (2017 to2018) as shown in table (1).

Table 1: Sampling for salmonella isolation from broiler flocks.

Age of chicken sample	Number of samples	organ	Clinical signs	Postmortem examination
1 day old	50	Yolk⁄Liver, heart	Apparently healthy	Omphilites /perhepatitis percarditis
One week	25	Internal organs	Diarrhea	Greenish / Percarditis
Two weeks	25	Ceacum	Diarrhea	Typhilitis
Three weeks	25	Ceacum	Diarrhea	Typhilitis
Four weeks	25	Internal organs	Diarrhea	Pale liver / Percarditis
	50	Cloacal swabs	Diarrhea	Diarrhea
	50	Brain	Nervous Signs	Diarrhea / inflammation of brain
Total	250			

2-Isolation

Salmonella isolation and identification was done according to standard methods (ISO 6579:2002) and salmonella serotyping was done according to (Popoff, 2001).

3-Antimicrobial sensitivity test was carried out according to the Clinical and Laboratory Standards Institute (CLSI/NCCLS, 2009). Using disk diffusion method, Table (2).

Table2: Inhibition Zone Diameter Interpretive Standards Breakpoint for Enterobacteriaceae.

Test group	Antimicrobial agent	Disk content	Zone diameter nearest whole mm		
			Resistant(R)	Intermediate(I)	Sensitive(S)
CEPHEMS	**Ceftriaxone (CRO30)**	**30µg**	**≤13**	**14-20**	**≥21**
Aminoglycosides	**Gentamycin (CN 10)**	**10 µg**	**≤12**	**13-14**	**≥15**
	Amikacin (AK30)	**30µg**	**≤14**	**15-16**	**≥17**
	Kanamycin (K30)	**30µg**	**≤13**	**13-14**	**≥15**
	Tobramycin (TOB10)	**10 µg**	**≤12**	**12-14**	**≥15**
Tetracyclines	**Tetracycline (TE30)**	**30µg**	**≤11**	**12-14**	**≥15**
	Doxycycline (DO30)	**30µg**	**≤10**	**11-13**	**≥14**
Fluoroquinoiones	**Ciprofloxacin (CIP5)**	**5µg**	**≤15**	**16-20**	**≥21**
	Levofloxacin (LEV 5)	**5µg**	**≤ 13**	**14-16**	**≥ 17**
	Lomefloxacin (LOM10)	**10 µg**	**≤ 18**	**19-21**	**≥ 22**
	Ofloxacin \ OFX5	**5µg**	**≤ 12**	**13-15**	**≥ 16**
	Norfloxacin \ NOR10	**5µg**	**≤ 12**	**13-16**	**≥ 17**
FOLATE Pathway inhibitors	**Trimethoprime \ TR5**	**5µg**	**≤10**	**11-15**	**≥16**

4- Detection of salmonella biofilm formation

A- Phenotypic test (glass test tube) according to (Daxin Peng, 2016).

The overnight cultures of each bacterium were diluted 1:100 in the diluted TSB. Two milliliters of each bacterial suspension were added into borosilicate glass test tubes and incubated at 28°C for 48 h. Then the liquid was decanted and the tubes were washed gently three times with distilled water. Two ml of 0.4% crystal violet (v/v) were added into each tube and stained at room temperature for 20 min.

B- Conventional PCR technique.
Extraction:
DNA was extracted using commercially available kit, QIAamp® DNA Mini Kit, Catalogue no.51304

PCR Reaction:
The different primers used in this study are described in Table (3).

PCR amplification.
It was done in a 25 µl reaction containing 12.5 µl of Emerald Amp GT PCR master mix (2x premix), 1 µl of each primer (20 pmol conc.), 4.5 µl of PCR grade water, and 6 µl of template. The cPCR reactions were performed in a Biometra T3 thermal cycler. The thermal profiles for *fim* H gene was applied according to (Hojati *et al.,* 2015).

The PCR products were separated by electrophoresis on 1.5% agarose gel stained with ethidium bromide and photographed by a gel documentation system (Alpha Innotech, Biometra) ®.

Table 3: Oligonucleotide primers and sequences encoding for detection of biofilm formation using *Fim H* gene.

Target gene	Primers sequences 5`- 3`	Amplified segment (bp)	Reference
Fim H	*GTGCCAATTCCTCTTACCGTT* *TGGAATAATCGTACCGTTGCG*	**164**	**Hojati *et al., 2015***

RESULTS

Salmonella was detected in apparently healthy one day old broiler chicks that showed (Omphilites, perhepatitis, percarditis), also in diseased broilers that showed diarrhea, nervous signs, unable to walk showed greenish and paleness liver, percarditis, perhepatitis, typhilitis, enlarged cecum, inflammation of brain and oophritis in postmortem examination. Salmoella was representing 14.4 (36/250) in different broiler flocks at different age from different localities (2017-2018).

Different salmonella strains was isolated, (*Salmonella bardo*, *Salmonella norwich*, *Salmonella brancaster*, *Salmonella sekondiП*, *Salmonella lamberhurst*, *Salmonella belgdam*) were demonstrated in one day old, while (*Salmonella kentucky*, *Salmonella enteritidis*, *Salmonella goetebory*, *Salmonella kisii*) demonstrated at one week age, also (*Salmonella nigeria*, *Salmonella grampian*) were reported at two weeks age, (*Salmonella newport*, *Salmonella enteritidis*, *Salmonella noyo*, *Salmonella colindale*) were at three weeks age, at fourth weeks (*Salmonella seremban*, *Salmonella remo*, *Salmonella lindenburg*, *Salmonella kentucky*, *Salmonella enteritidis*, *Salmonella anatum*, *Salmonella virchow*, *Salmonella tamiland* and *Salmonella york* as shown in Table (4).

Biofilm formation of salmonella strains was detected using a glass tube test, where Salmonella strains were tested for biofilm formation on glass surface. The positive biofilm formation were produced rings at the liquid-air interface on the glass test tube walls or produced color staining at the bottom of the tube and the confirmation was done using *fim* H gene (Hojati *et al.,* 2015).

The percentage of 61.11% (22/36) of salmonella strains have the ability for biofilm producation, while 38.88 (14/36) have no ability for biofilm production Table (5), the positive biofilm formation was observed in *Salmonella Kentucky* (6/11) 54%, *Salmonella enteritidis* (2/4) 50% Seremban, *Salmonella norwich*, *Salmonella lindenburg*, *Salmonella virchow* (1/2) 50%, *Salmonella brancaster*, *Salmonella grampian*, *Salmonella belgam*, *Salmonella bardo*, *Salmonella york* while no biofilm formation in *Salmonella Kentucky* (5/11) 45%, *Salmonella* enteritidis (2/4)50%, *Salmonella goetebory*, *Salmonella anatum*, *Salmonella sekondi* П, *Salmonella lamberhurst*, *Salmonella virchow* (1/2) 50%, *Salmonella noyo*, *Salmonella timiland* Table (6).

The disk diffusion test revealed at the highest degree of resistance were observed with lomefloxacin (77.7%), tetracycline (61%), kanamcin (50%) and trimethoprime and levofloxacin (47.2%) and lowest resistance degree was observed with Ceftriaxone Table (7).

Antibiotic resistance was reported in both positive and negative biofilm formation in salmonella strains

(100%) and multidrug resistance was observed in the positive biofilm formation and negative biofilm formation.

Salmonella strains which had ability for biofilm was resistance to more than one antibiotics 17/22 (72%) and Salmonella strains which have not ability for biofilm were resistance to more than one antibiotics 10/14 (71%) Table (8),(9).

Table 4: prevalence of salmonella in broiler chickens.

Age of chicken samples	Number	Site of isolation	Signs/PM	Number of Positive	Type of isolated salmonella strain	Number of salmonella strains	Percentage of salmonella
1 day old	50	Yolk/ Liver	Apparently healthy	6/50	*Salmonella bardo*	1/6	12
					Salmonella norwich	1/6	
					Salmonella brancaster	1/6	
			Omphilites /perhepatitis percarditis		*Salmonella secondi II*	1/6	
					Salmonella lamberhurst	1/6	
					Salmonella belgdam	1/6	
One week	25	Internal organs	Diarrhea	5/25	*Salmonella kentucky*	2/5	20
					Salmonella enteritidis	1/5	
			Greenish liver / Percarditis		*Salmonella goetebory*	1/5	
					Salmonella kisii	1/5	
Two weeks	25	Ceacum	Diarrhea	2/25	*Salmonella nigeria*	1/2	8
			Typhilitis/ enlarged cecum		*Salmonella grampian*	1/2	
Three weeks	25	Ceacum	Diarrhea	4/25	*Salmonella newport*	1/4	16
					Salmonella enteritidis	1/4	
			Typhilitis/ enlarged cecum		*Salmonella noyo*	1/4	
					Salmonella colindale	1/4	
Four weeks	25	Internal organs	Diarrhea	5/25	*Salmonella seremban*	1/5	20
					Salmonella remo	1/5	
			Greenish liver / Percarditis		*Salmonella lindenburg*	1/5	
					Salmonella kentucky	1/5	
					Salmonella enteritidis	1/5	
	50	Cloacal swabs	Diarrhea	10/50	*Salmonella kentucky*	5/10	9
					Salmonella anatum	1/10	
					Salmonella enteritidis	1/10	
					Salmonella virchow	2/10	
	50	Brain	Diarrhea	5/50	*Salmonella kentucky*	3/5	20
			Nervous Signs Unable to walk		*Salmonella tamilandu*	1/5	
					Salmonella york	1/5	
Total	250			36/250			14.4

Table 5: Detection of biofilm formation by salmonella strains.

Test	Number of positive biofilm formation	%	Number of negative biofilm formation	%
A.Phenotypic charaterization Tube agglutination test	22/36	61.11%	14/36	38.88
B.Polymerase chain reaction test	22/36	61.11%	14/36	38.88
1-*fim* H gene				

Number of salmonella (36)

Table 6: Percentage of isolated salmonella strain.

Salmonella Serotype	Antigenic structure	Total number	Numbers			
			Positive salmonella biofilm formation	%	Negative salmonella biofilm formation	%
Salmonella kentucky	O8,20,I,Z60	11	6	54%	5	45.4
Salmonella seremban	O9,12,I,1,5	1	1	100	0	0
Salmonella norwich	O6,8,e,h,1,6	1	1	100	0	0
Salmonella lindenburg	O6,8,I,1,2	1	1	100	0	0
Salmonella virchow	O6,7,14,r,1,2	2	1	50	1	50
Salmonella brancaster	O3, O10,e,n,x,1,7	1	1	100	0	0
Salmonella grampian	O6,7,r,l,w	1	1	100	0	0
*Salmonella sekondi*II	O1, O4, O12, O27,z_{29},-	1	0	0	1	100
Salmonella belgdam	O9,12,G,m,s,-	1	1	100	0	0
Salmonella york	O9,12;Z28;enZ15	1	1	100	0	0
Salmonella bardo	O8,e,h,1,2	1	1	100	0	0
Salmonella enteritidis	O1,9,12,g,m;-	4	2	50	2	50
Salmonella goetebory	O9,12,c,1,5	1	0	0	1	100
Salmonella anatum	O3,10;e,h,1,6	1	0	0	1	100
Salmonella lamberhurst	O3,10,e,h,e,n,z_{15}	1	0	100	1	100
Salmonella nigeria	O6,7,r,1,6	1	1	100	0	0
Salmonella colindale	O6,7,r,1,7	1	1	100	0	0
Salmonella noyo	O8,r,1,7	1	0	0	1	100
Salmonella kisii	O6,7;d;1,2	1	1	100	0	0
Salmonella newport	O6,8,20,e,h,1,2	1	1	100	0	0
Salmonella remo	O 1.4,12,27,r,1,7	1	1	100	0	0
Salmonella tamilandu	O 6,7,z_{41},z_{35}	1	0	0	1	0

Table 7: Antibiotic resistance profile for examined salmonella.

Antibiotic disk	Number of resistance antibiotic to isolated salmonella	%
CEPHEMS		
Ceftriaxone \ CRO_{30}	8⁄36	22.2
Aminoglycosides		
1-Gentamycin \ CN 10	11⁄36	30.5
2-Amikacin\ AK_{30}	13⁄36	36.1
3-Kanamycin \ K_{30}	18⁄36	50
4-Tobramycin \ TOB_{10}	11⁄36	30.5
Tetracyclines		
1-Tetracycline\ TE_{30}	22⁄36	61
2-Doxycycline \ DO_{30}	10⁄36	27.7
Fluoroquinoiones		
1-Ciprofloxacin \ CIP_5	15⁄36	41.6
2-Levofloxacin \ LEV_5	17⁄36	47.2
3-Lomefloxacin \ LOM_{10}	28⁄36	77.7
4-Ofloxacin \ OFX_5	15⁄36	41.6
5-Norfloxacin \ NOR_{10}	10⁄36	27.7
FOLATE Pathway inhibitors		
Trimethoprime \ TR_5	17⁄36	47.2

Table 8: Detection relation between antibiotic resistant and positive salmonella biofilm formation.

	Salmonella strains	Antibiotic resistance	ABCs%
1	*Salmonella seremban* *	CRO_{30}, K_{30}, TE_{30}, DO_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	10/13(76.9)
2	*Salmonella kentucky* *	K_{30}, TE_{30}, DO_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	9/13(69.2)
3	*Salmonella norwich* *	AK_{30}, K_{30}, TE_{30}, LEV_5 , LOM_{10}	5/13(38.4)
4	*Salmonella kentucky* *	K_{30}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	8/13(61.5)
5	*Salmonella lindenburg* *	K_{30}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	8/13(61.5)
6	*Salmonella virchow* *	CN_{10}, K_{30}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	9/13(69.2)
7	*Salmonella brancaster**	AK_{30}, K_{30}, TOB_{10}, TE_{30}, LOM_{10}, TR_5	6/13(46.1)
8	*Salmonella* grampian *	CN_{10}, AK_{30} , TOB_{10}, TE_{30}, LOM_{10}, TR_5	6/13(46.1)
9	*Salmonella sekondi II* ^{N}	CRO_{30}, CN_{10}, AK_{30}, DO_{30}, LEV_5 , LOM_{10}	6/13(46.1)
10	*Salmonella kentucky* *	TOB_{10}, DO_{30}, CIP_5, LOM_{10}, OFX_5	5/13(38.4)
11	*Salmonella kentucky*^{N}	TOB_{10}, DO_{30}, CIP_5, LOM_{10}, OFX_5	5/13(38.4)
12	*Salmonella kentucky*^{N}	CRO_{30}, AK_{30}, K_{30}, LEV_5 , LOM_{10}	5/13(38.4)
13	*Salmonella belgdam* *	CN_{10},K_{30}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}	8/13(61.5)
14	*Salmonella york* *	CN_{10}, AK_{30},K_{30}, TOB_{10}, TE_{30}, CIP_5	6/13(46.1)
15	*Salmonella kentucky* *	K_{30}, TOB_{10}, TE_{30}, DO_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5	8/13(61.5)
16	*Salmonella bardo* *	CN_{10}, AK_{30},K_{30}, TOB_{10}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}	10/13(76.9)
17	*Salmonella kentucky* *	CRO_{30}, AK_{30}, LEV_5 , LOM_{10}, TR_5	5/13(38.4)
18	*Salmonella enteritidis**	CN_{10}, TOB_{10}, TE_{30}, DO_{30}, LOM_{10}, TR_5	6/13(46.1)
19	*Salmonella enteritidis* ^{N}	CN_{10}, TOB_{10}, TE_{30}, DO_{30}, LOM_{10}, TR_5	6/13(46.1)
20	*Salmonella kentucky*^{N}	CRO30,CN_{10}, AK_{30},K_{30}, TOB_{10}, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}	11/13(84.6)
21	*Salmonella enteritidis* ^{N}	CRO_{30}, LOM_{10}	2/13(15.3)
22	*Salmonella* goetebory^N	CRO_{30}, TE_{30}, LEV_5 , LOM_{10}, OFX_5 NOR_{10},, TR_5	7/13(53.8)
23	*Salmonella kentucky*^{N}	K_{30}, TE_{30}, DO_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5	7/13(53.8)
24	*Salmonella kentucky*^{N}	CRO_{30}, AK_{30}, K_{30}, LEV_5 , LOM_{10}	5/13(38.4)
25	*Salmonella* anatum ^{N}	AK_{30}, TE_{30}, DO_{30}, CIP_5, LOM_{10}	5/13(38.4)
26	*Salmonella lamberhurst*^{N}	AK_{30} , TE_{30}, TR_5	3/13(23)
27	*Salmonella virchow*^{N}	CN_{10},, TE_{30}, CIP_5 , LEV_5 , LOM_{10}, OFX_5 , NOR_{10}, TR_5	8/13(61.5)
28	*Salmonella kentucky* *	DO_{30}, CIP_5, LOM_{10}, OFX_5	4/13(30)
29	*Salmonella remo* *	CN_{10}, AK_{30}, LOM_{10}	3/13(23%)
30	*Salmonella* newport *	CN_{10}, K_{30}, TOB_{10}, TE_{30}	4/13(30%)
31	*Salmonella* nigeria *	AK_{30}, TOB_{10}, TR_5	3/13(23%)
32	*Salmonella enteritidis**	AK_{30} , TE_{30}, TR_5	3/13(23%)
33	*Salmonella colindale* *	AK_{30} , TR_5	2/13(15.3%)
34	*Salmonella noyo* ^{N}	AK_{30} , TR_5	2/13(15.3%)
35	S.Tamilandu ^{N}	LOM_{10}	1/13(7.69%)
36	*Salmonella kisii**	CN_{10}, K30, LOM_{10}	3/13(7.6%)

* Positive for biofilm (use glass tube and *fim* H gene)

^{N} Negative for biofilm formation (glass tube test and *fim* H gene) ″

Table 9: Relation between salmonella biofilm formation, Antimicrobial resistance and multidrug resistance.

	Negative biofilm formation salmonella	Positive biofilm formation salmonella
Biofilm formation	14/36 (38.88%)	22/36 (61.11%)
Antimicrobial resistance	14/14 (100%)	22/22(100%)
Multidrug resistance	10/14(71%)	17/22(72%)
Severity in clinical signs and Postmortem	1-High degree of mortality and morbidity in farm infected with salmonella. 2-Signs of depression and diarrhea 3-Omphilitis in young chicks 4-Perhepatitis, pericarditis. 5-Inflammation in brain	1-High degree of mortality and morbidity in farm infected with salmonella 2- Signs of depression and diarrhea. 3- Omphilitis young chicks 4- Perhepatitis, pericarditis. 5- Inflammation in brain

Multidrug resistance: resistance for more than 3 antibiotic groups

DISCUSSION

Some salmonella strains have ability for biofilm production 61.11% (22/36) and the others have n't 38.88% (14/36), also antimicrobial resistance was observed in both positive and negative biofilm formation (100%), and resulted in that there is no relation between biofilm formation and antimicrobial resistance and multidrug resistance. Also both positive and negative biofilm formation were showed same degree of mortality and morbidity, Signs of depression, diarrhea, Omphilitis in young chicks, perhepatitis, pericarditis, Oophritis, redness in brain. The obtained results were agree with (Wang *et al*., 2013) who reported that no significant correlation between antimicrobial resistance and biofilm production as well as agree with (Ghasemmahdi *et al*., 2015) who demonstrated that all *Salmonella* typhimurium isolates showed a high multiple antibiotic resistant with low biofilm formation capabilities which proposed low association between biofilm formation and antibiotic resistance of a major food important pathogen. As well as the results were agree with (Apellanis *et al.*, 2017) who reported that no relationship was found between biofilm production and antimicrobial resistance in *Salmonella* enteritidis strains. While the present results were disagree with (Costerton *et al*., 1999, Hall-Stoodley *et al*., 2004) that demonstrated that biofilms were important factors in antimicrobial resistance, and play a key role in the pathogenesis of many bacterial infections. Bacteria with biofilms are inherently protected from their surrounding environment and often exhibit increased resistance to host defense and antimicrobial agents, making these infections difficult or impossible to eradicate. (Arciola *et al*., 2001, Costerton *et al*., 2003 and Szomolay *et al*., 2005) demonstrated that bacteria with biofilms may have an increased resistance to antimicrobials, ambient pressure and the host immune system, also disagree with Gong *et al.* (2013), reported that the proportion of biofilm-positive *Salmonella* pullorum isolates increased over time. The antimicrobial resistance rates of positive isolates were higher than those of negative isolates. The proportion of multidrug resistance for positive and negative biofilm formation isolates was no significant different.

In conclusion, no correlation between biofilm production and multidrug resistant in examined isolates.

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الكشف عن تكوين البيوفيلم ومقاومة المضادات الحيوية للسالمونيلا في دجاج التسمين

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٢٥٠ عينة من دجاج التسمين من قطعان مختلفة تم فحصها لعزل ميكروب السالمونيلا وكانت نسبتها ١٤.٤% وتم عزل عترات مختلفة منها سالمونيلا باردو وسالمونيلا نوروش وسالمونيلا برانكاستر وسالمونيلا سكوندي وسالمونيلا كيسي وسالمونيلا لامبرهيرتس وسالمونيلا بلجام وسالمونيلا كنتاكي وسالمونيلا انترتديس وسالمونيلا جوتبوري وسالمونيلا نيجرا وسالمونيا جرامبين وسالمونيلا نيوبورت وسالمونيلا نوياو وسالمونيلا كولندال وسالمونيا سيرمبان وسالمونيلا رمو وسالمونيلا لندنبرج وسالمونيلا اناتم وسالمونيلا فيرشو وسالمونيلا تاميلند وسالمونيا يورك. كما تم عمل اختبار حساسية ووجد نسب مقاومات مختلفة للمضادات الحيوية وكانت اعلي نسبة مقاومة ضد المضاد الحيوي الليموفلوكساسين ٧٧.٧% والتتراسيكلين٦١% والكاناميسين ٥٠% وكلا من الترايميسوبريم والليفوفلوكساسين ٤٧.٢%. كما تم عمل اختبار لجميع العترات لتكوين البيوفيلم باستخدام اختبار الانبوبة الزجاجي وتم التاكيد عليها بالكشف عن الفيم جين ووجد ان نسبة تكوين البيوفيلم كانت ٦١.١١% . كما وجد ان العترات الايجابية والسلبية لتكوين البيوفيلم كلاهما مساوي لمقاومة المضادات الحيوية بنسبة ١٠٠% كما انه وجد ان لايوجد فرق معنوي ايضا بينهما في تعددية المقاومة للمضادات الحيوية وذلك يوضح عدم وجود علاقة بين تكوين البيوفيلم والمقاومة للمضادات الحيوية.