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Molecular Characterization and Antibiotyping of Some Local Isolates of Genus *Salmonella*

[1]N.A. El-Sebay, [1]H.M. Gebreel, [1]S.A. El-Zeedy and [2]A.A. Samy

[1]Veterinary Serum and Vaccine Research Institute (VSVRI), Egypt
[2]Department of Microbiology and Immunology, National Research Center, Dokki, Giza, Egypt

Abstract: Fourteen selected isolates of *Salmonella* were obtained from Kalubia and Giza governorates which isolated from different poultry spp. (chicken, chicken eggs, duck, duck eggs and turkeys). These isolates were characterized by phenotypic and genotypic methods. The serotyping revealed typical *Salmonella* serovars: *Salmonella rubislaw, Salmonella poona, Salmonella Typhimurium, Salmonella Virginia, Salmonella enteritidis, Salmonella montevideo* and *Salmonella sandiago.* The antibiotic sensitivity test revealed that 78.6%, 71.4%, 64.3%, 57% and 50% of the isolates were sensitive to (ciprofloxacin and sulphamethaxazone-trimethoprim), (norfloxacin and ofloxacin), gentamycin, ampicillin and chloramphenicol respectively. While 100%, 71.4% and 57% of the isolates were resistant to flucloxacillin, amoxicillin and tetracycline respectively. Whole cell protein analysis using SDS-PAGE of different *Salmonella* strains revealed about 18 protein bands ranged from 16-289.5 KDa but the differences were insufficient for differentiation between the isolates. *Salmonella* isolates showed a degree of variation in plasmid number from 1 plasmid up to 8 plasmids and molecular size from 2121 up to 22864 bp. Only one isolate of *S. rubislaw* have a plasmid of (22864 bp), while 71.4% of the isolates had plasmid of (16904 bp) that showed the highest incidence of antimicrobial resistance which may probably contributes for high resistance showed by these isolates. Also, different plasmids with variable molecular size from 2121 up to 15804 bp were observed in other *Salmonella* isolates.

Key words: *Salmonella* · SDS-PAGE · Plasmid analysis · Antibiotic resistance

INTRODUCTION

Salmonella infection in poultry is responsible for a variety of disease conditions such as: Pullorum disease caused by *Salmonella pullorum*, fowl typhoid caused by *Salmonella gallinarum* and paratyphoid infection caused by serotypes other than *Salmonella pullorum* and *Salmonella gallinarum* [1, 2]. The clinical disease caused by *Salmonella* is usually divided into four syndromes: gastroenteritis, enteric fever, septecaemia and asymptomatic infection or carrier state. Avian salmonellosis is an inclusive term designating a large group of acute and chronic diseases of poultry caused by one or more member of the genus *Salmonella* [3]. Poultry and poultry products are consistently identified as important source of *Salmonella* that cause human illness [4]. The molecular typing is considered as a very important epidemiological marker [5]. The plasmid profiling of Enterobacteria was the first geneotypic method used for strain separation. The plasmids ranging in size from 2 to 150 kb, but frequencies and size distributions vary between serovars according to *Shaberg et al*. [6]. Analysis of the whole cell protein patterns has been used extensively to the study of the differences among bacterial genera, species and strains [7]. Outer membrane protein (OMP) analysis has proved to be useful technique in the characterization of *Salmonella* [8, 9].

MATERIALS AND METHODS

Salmonella Isolates: Fourteen different *Salmonella* isolates were isolated from chicken, chicken eggs, duck, duck eggs and turkey.

Colonial Morphology [10]: A loopfull of the obtained samples was inoculated into selenite-F broth and incubated at 37°C for 16 hours then was streaked on to the surface of MacConkey's agar, *Salmonella-Shigella* agar, XLD and Hekton enteric agar and incubated at 37°C for 24 hours.

Corresponding Author: A.A. Samy, Department of Microbiology and Immunology, National Research Center, Dokki, Giza, Egypt.

Microscopical Examination [11]: The suspected *Salmonella* colonies were picked up, a film was made on glass slide and stained with Gram stain and examined microscopically.

Motility Test: Motility was assured by growing the bacteria into semi-solid agar.

Biochemical Examination: Biochemical examination were made for lactose negative isolates using the methods described by Cruickshank *et al*. [11] and Quinn *et al*. [12].

Serological Identification of Salmonella: The isolates preliminary identified biochemically as *Salmonella* were subjected to serological identification according to Kauffman [13], using diagnostic polyvalent (O, H) and monovalent *Salmonella antisera* (Difco) for serological identification of *Salmonella*.

Sensitivity Test for *Salmonella* Isolates: The disk diffusion technique was adapted according to Finegold and Martin [14]. Using 10 antibacterial disks (Amoxicillin, Ampicillin, Chloramphenicol,Ciprofloxacin, Gentamycin, Norfloxacin, Ofloxacin, Flucloxacillin, Tetracyclin, Sulfamethoxazone-trimethoprim) "Oxoid". The degree of sensitivity to the antibacterial agents was determined according to NCCLS [15] and Koneman *et al*. [16].

Protein Profile Analysis by SDS-PAGE [17]: Using Prestained high range molecular weight protein marker (4-250 KDa - Invitrogen) electrophoresis was done using 10% separating gel and 5% stacking gel in denatured dissociating buffer system (SDS-PAGE) following the method of Sambrook *et al*. [17].

Plasmid Profile Analysis: Extraction of plasmid was performed by miniprep according to Sambrook *et al*. [17] and Towner and Cockayne [18]. The extracted plasmid was evaluated as visible bands being sized by Super coild DNA ladder marker (Invitrogen).

RESULTS

Morphological and Colonial Characteristics: The fourteen *Salmonella* isolates were identified as Gram-negative, motile and non sporulated bacilli. The colonial characters of *Salmonella* isolates on different media were pale non-lactose fermenting colonies on MacConkey agar medium, colorless colony with black center on *Salmonella-Shigilla* agar medium, blue-green colonies with black center on Hektoen enteric agar medium and red colonies with black center on XLD (xylose lysine deoxycholate) agar medium.

Biochemical Identification: All isolates were unable to ferment lactose, oxidase and indole production was negative. Meanwhile, catalase and lysine utilization were positive and the isolates were able to ferment arabinose and sorbitol.

Serological Identification of *Salmonella* Isolates: By using *Salmonella* polyvalent and monovalent antisera, the *Salmonella* strains were serotyped as shown in Table 1.

Antibiogram Sensitivity Test of *Salmonella*: Ten antimicrobial discs were used for sensitivity test on *Salmonella* isolates as shown in Tables 2, 3 and Fig. 1 illustrated that the isolates were sensitive to

Table 1: Serotyping of *Salmonella* isolates.

			Antigenic structure		
				Flagellar antigen (H)	
Serial no. of isolates	*Salmonella* serovars	Sero-group	Somatic antigen (O)	Phase I	Phase II
1	*Salmonella rubislaw*	F	11	r	e, n, x
2	*Salmonella rubislaw*	F	11	r	e, n, x, A
3	*Salmonella poona*	G1	13, 22	z	1, 6
4	*Salmonella poona*	G1	1, 13, 22	z	1, 6
5	*Salmonella poona*	G1	1, 13, 22	z	1, 6
6	*Salmonella Typhimurium*	B	1, 4, [5], 12	i	1, 2
7	*Salmonella Typhimurium*	B	1, 4, [5], 12	i	1, 2
8	*Salmonella virginia*	C3	8	d	1, 2
9	*Salmonella virginia*	C3	8	d	1, 2
10	*Salmonella enteritidis*	D1	1, 9, 12	g, m	-
11	*Salmonella enteritidis*	D1	1, 9, 12	g, m	-
12	*Salmonella montevideo*	C1	6, 7	g, m, s, [p]	-
13	*Salmonella montevideo*	C1	6, 7	g, m, s	-
14	*Salmonella sandiago*	B	4, [5], 12	e, h	e, h, z_{15}

Table 2: Susceptibility of *Salmonella* isolates to different antimicrobial agents

		Antibiotic sensitivity discs									
Strain No.	*Salmonella* serotype	AMC	AM	C	CIP	GN	NOR	OFX	FL	TE	SXT
1	*Salmonella rubislaw*	S	S	S	S	S	S	S	R	S	S
2	*Salmonella rubislaw*	R	R	R	R	R	R	R	R	R	R
3	*Salmonella poona*	R	R	R	S	R	S	S	R	R	S
4	*Salmonella poona*	R	R	R	R	R	R	R	R	R	R
5	*Salmonella poona*	R	R	R	R	R	R	R	R	R	R
6	*Salmonella Typhimurium*	R	R	S	S	R	S	S	R	R	S
7	*Salmonella Typhimurium*	R	R	S	S	S	S	S	R	R	S
8	*Salmonella virginia*	R	S	S	S	S	S	S	R	R	S
9	*Salmonella virginia*	S	S	S	S	S	S	S	R	S	S
10	*Salmonella enteritidis*	R	S	S	S	S	S	S	R	S	S
11	*Salmonella enteritidis*	S	S	R	S	S	S	S	R	R	S
12	*Salmonella montevideo*	S	S	R	S	S	S	S	R	S	S
13	*Salmonella montevideo*	R	S	R	S	S	R	R	R	S	S
14	*Salmonella sandiago*	R	S	S	S	S	S	S	R	R	S

R = resistant; S= sensitive; AMC= amoxicillin (30mg); AM= ampicillin (10mg); C= chloramphenicol (30mg); CIP= ciprofloxacin (5mg); GN= gentamycin (30mg); NOR= norfloxacin (10mg); OFX= ofloxacin (5mg); FL= flucloxacillin (5mg); TE= tetracycline (30mg); SXT= sulfamethaxone-trimethoprim (25mg)

Table 3: The distributional percentage of antibiotic sensitivity against the *Salmonella* isolates

	Resistant		Sensitive	
Antimicrobial drug	No. of strain	%	No. of strain	%
Amoxicillin	10	71.4	4	28.5
Ampicillin	6	42	8	57
Chloramphenicol	7	50	7	50
Ciprofloxacin	3	21.4	11	78.6
Gentamycin	5	35.7	9	64.3
Norfloxacin	4	28.5	10	71.4
Ofloxacin	4	28.5	10	71.4
Flucloxacillin	14	100	0	0
Tetracycline	8	57	6	42
Sulfamethaxone-Trimethoprim (25mg)	3	21.4	11	78.6

The percent was calculated in relation to the number of strains studied (14 strain).

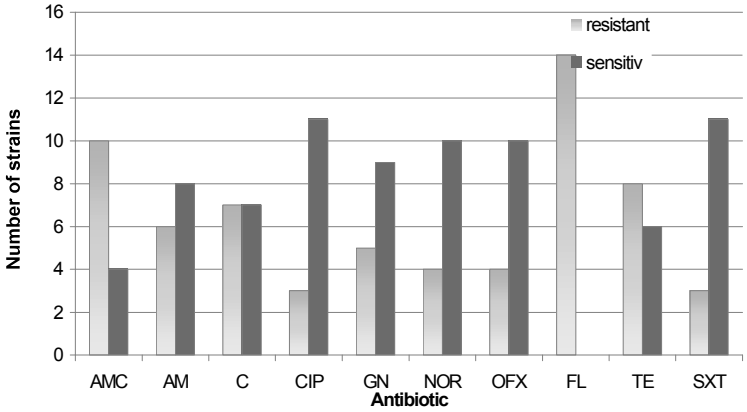

Fig. 1: Results of sensitivity test for *Salmonella* isolates using disk diffusion method.

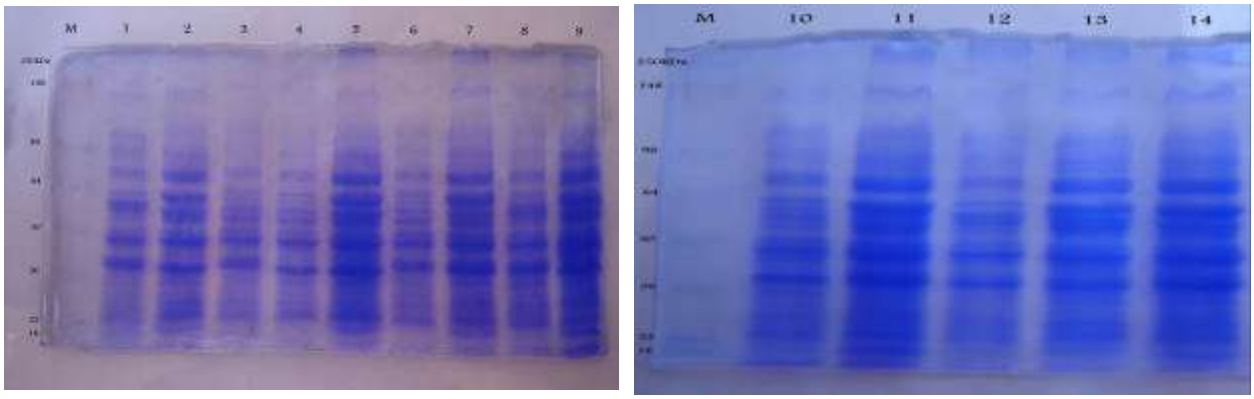

Photo 1,2: SDS-PAGE showing protein profiles of different *Salmonella* strains
M: Protein marker; **Lane 1 & 2:** *Salmonella rubislaw;* **Lane 3, 4, 5:** *S. Poona* **Lane 6,7:** *S. Typhimurium;* **Lane 8,9:** *S. viriginia;* **Lane 10,11:** *S. entretidis;* **Lane 12,13:** *S.montevideo*; **Lane 14:** *S. sandiago*.

Table 4: Protein profile of different *Salmonella* isolates

Lanes	Marker	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
r1		289.5	289.5	289.5	289.5	289.5	289.5	289.5
r2	250	--	--	--	--	--	--	--
r3	148	--	--	--	--	--	--	--
r4	--	140.3	140.3	140.3	140.3	140.3	140.3	140.3
r5	--	105.4	105.4	105.4	105.4	105.4	105.4	105.4
r6	--	--	--	--	--	--	--	--
r7	98	--	--	--	--	--	--	--
r8	--	88	88	88	88	88	88	88
r9	--	70	70	70	70	70	70	70
r10	64	--	--	--	--	--	--	--
r11	--	60	60	60	60		60	60
r12	--	57.9	57.9	57.9	57.9	57.9	57.9	57.9
r13	--	55	55	55	55	55	55	55
r14	--		52.4	52.4	52.4	52.4	52.4	52.4
r15	--	51.6	51.6	51.6	51.6	51.6	51.6	51.6
r16	50	--	--	--	--	--	--	--
r17	--	45.1	45.1	44.9	45.1	45.1	45.1	45.1
r18		43.3	43.3		43.3	43.3	43.3	43.3
r19	36	36.4	36.4	36.4	36.4	36.4	36.4	36.4
r20	--	--	--	33	33	33	--	--
r21	--	29	29	29	29	29	29	29
r22	--	24.1	24.1	24.1	24.1	24.1	24.1	24.1
r23	22	22	22	22	22	22	22	22
r24	16	16	16	16	16	16	16	16
Lanes	Marker	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
r1	--	289.5	289.5	289.5	289.5	289.5	289.5	289.5
r2	250	--	--	--	--	--	--	--
r3	148	--	--	--	--	--	--	--
r4	--	140.3	140.3	140.3	140.3	140.3	140.3	140.3
r5	--	105.4	105.4	105.4	105.4	105.4	105.4	105.4
r6	--	--	--	--	--	--	--	--
r7	98	--	--	--	--	--	--	--
r8	--	88	88	88	88	88	88	88
r9	--	70	70	70	70	70	70	70
r10	64	--	--	--	--	--	--	--

Table 4: Continued

Lanes	Marker	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Rows	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)	(mol.w.)
r11	--	60	60	60	60	60	60	60
r12	--	57.9	57.9	57.9	57.9	57.9	57.9	57.9
r13	--	--	--	55	55	55	55	55
r14	--	--	--	52.4	52.4	--	--	--
r15	--	51.6	51.6	51.6	51.6	52.4	52.4	51.9
r16	50	--	--	--	--	--	--	--
r17	--	45.1	45.1	45.1	45.1	45.1	45.1	45.1
r18	--	43.3	43.3	43.3	43.3	43.3	43.3	43.3
r19	36	36.4	36.4	36.4	36.4	36.4	36.4	36.4
r20	--	--	--	--	--	33	33	--
r21	--	29	29	29	29	29	29	29
r22	--	24.1	24.1	24.1	24.1	24.1	24.1	24.1
r23	22	22	22	22	22	22	22	22
r24	16	16	16	16	16	16	16	16

Lane 1, 2: *S.rubislaw;* Lane 3, 4, 5: *S.poona;* Lane 6, 7: *S. typhimurium;* **Lane 8, 9**: *S. virginia* **Lane 10, 11**: *S.enteritidis* **Lane 12, 13**: *S.montevideo;* **Lane 14**: *S. sandiago*, **mol.w**.: molecular weight.

Table 5: Plasmid profile of different *Salmonella* isolates

Lanes	Marker	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6	Lane 7
Rows	(mol.S.)	(mol.S)	(mol.S)	(mol.S)	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)
r1	--	--	--	--	--	--	--	--
r2	--	22864	--	--	--	--	--	--
r3	--	--	--	16347	--	--	16904	16904
r4	16210	15544	--	15807	16904	16904	15675	--
r5	14174	--	--	--	--	--	13361	--
r6	12138	--	--	--	--	--	--	--
r7	10102	--	--	--	9322	9322	--	--
r8	8066	--	--	--	8330	8330	--	--
r9	7045	--	--	--	--	--	--	--
r10	6030	--	--	--	5750	5750	5425	--
r11	5012	--	4706	--	4683	4683		--
r12	3990	--	--	--	4312	4312		--
r13	--	--	--	3663	3299	3299	3207	--
r14	2972	--	3331	--	--	--	--	--
r15	--	--	2544	--	2531	2531	--	--
r16	2067	--	--	2121	--	--	--	2121
Lanes	Marker	Lane 8	Lane 9	Lane 10	Lane 11	Lane 12	Lane 13	Lane 14
Rows	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)	(mol.S.)
r1	--	--	--	--	--	--	--	--
r2	--	--	--	--	--	--	--	--
r3	--	16904	16904	16904	16904	16904	16904	16904
r4	16210	--	--	--	--	--	--	--
r5	14174	--	--	--	--	--	--	--
r6	12138	--	--	--	--	--	--	--
r7	10102	--	--	--	--	--	--	--
r8	8066	8602	--	--	--	--	--	--
r9	7045	7006	--	--	--	--	--	--
r10	6030	5201	--	--	--	--	--	--
r11	5012	4752	--	--	--	--	--	--
r12	3990	4049	--	--	--	--	--	--
r13	--	--	--	--	--	--	--	--
r14	2972	3176	--	--	--	--	--	--
r15		--	--	--	--	--	--	--
r16	2067	--	--	--	2132	--	--	--

Lane 1, 2: *S.rubislaw;* **Lane 3, 4, 5**: *S.poona;* **Lane 6, 7**: *S. typhimurium;* **Lane 8,9**: *S.virginia* **Lane 10, 11**: *S.enteritidis* **Lane 12, 13** : *S.montevideo;* **Lane 14** : *S.sandiago*, **mol.s**: molecular size.

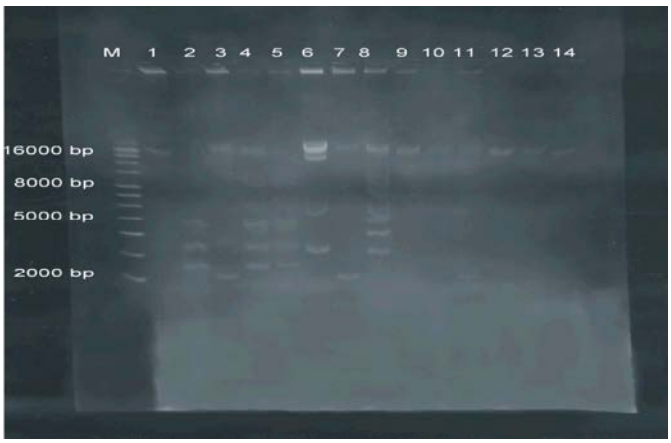

Photo 3: Agarose gel showing plasmid profiles of different *Salmonella* strains
M: Super coiled DNA marker; **Lane 1&2:** *Salmonella rubislaw*; **Lane 3, 4, 5:** *S. Poona*; **Lane 6,7:** *S. Typhimurium*; **Lane 8,9:** *S. viriginia;* **Lane 10,11:** *S. entretidis;* **Lane 12, 13:** *S. Montevideo*; **Lane 14:** *S. sandiago.*

(ciprofloxacin and sulfamethaxazone-trimethoprim), (Norfloxacin and Ofloxacin), gentamycin, ampicillin and finally chloramphenicol by percentage of 78.6%, 71.4%, 64.3%, 57% and 50% respectively. While, 100%, 71.4% and 57% of the isolates were resistant to flucloxacillin, amoxicillin and tetracyclin respectively.

SDS-PAGE Protein Profile Analysis: SDS-PAGE protein analysis of the isolated *Salmonella* revealed about 15 to 18 protein bands as shown in Photo 1, 2 and Table 4. The protein marker ranged from (4-250 KDa) and the protein bands of *Salmonella* strains ranged from (16-289.5 Kda).

Plasmid Profile Analysis: As shown in Photo 3 and Table 5, most of the isolates showed degree of variation in plasmid number, from 1 plasmid up to 8 plasmids and molecular size from 2121 bp up to 22864 bp corresponding to the size of super coiled DNA marker. Only one isolate of *Salmonella rubislaw* have plasmid of 22864 bp, while eleven isolates (*S.poona, S. typhimurium, S. virginia, S.enteritidis, S.montevideo* and *S. sandiago*) has plasmid of 16904 bp, three isolates (*S.rubislaw, S.poona* and *S. typhimurium*) carry plasmid from 15544 to 15807 bp. Meanwhile, variable bands with different molecular size from 2121 to 13361 bp were detected in different *Salmonella* isolates.

DISCUSSION

Fourteen selected strains of Salmonella spp. obtained from Kalubia and Giza governorates which isolated from different poultry spp. (chicken, chicken eggs, duck, duck eggs and turkeys) and these isolates were serotyped as *S.rubislaw* (2 isolates), *S.poona* (3 isolates), *S. typhimurium* (2 isolates), *S.virginia* (2 isolates), *S.enteritidis* (2 isolates), *S.montevideo* (2 isolates) and one isolate was types as *S.sandiago*. All isolates were characterized by phenotypic and genotypic methods. The addition of broad spectrum antibiotic to poultry feed has raised, the question of whether such sub-therapeutic doses may increasing resistant *Salmonella* strains which then can be transmitted to humans. Antimicrobial resistance genes are often located on transmissible plasmids in *Escherichia coli* and other normal flora antibiotic pressures can promote the transfer of these plasmids to other *E. coli* strains and to enteric pathogens such as *Salmonella* spp [19]. Antimicrobial resistance in pathogenic bacteria of animal and human origin is a major public health issue. Epidemiological and molecular methods have been used to suggest that antimicrobial use in animal agriculture and antimicrobial resistant bacteria from food animals can lead to antimicrobial resistant *Salmonella* infections in humans [20, 21].

Antimicrobial susceptibility patterns also have relatively poor discriminatory power, because antimicrobial resistance is under tremendous selective pressure in health care institutions and often is associated with mobile genetic elements (e.g., transposons and plasmids) [22]. Changes in antibiograms also may reflect spontaneous point mutations such as seen with fluoroquinolones. Thus, isolates that are epidemiologically related and otherwise genetically indistinguishable may manifest different antimicrobial susceptibilities due to acquisition of new genetic material over time or the loss of plasmids. Conversely, unrelated

isolates may have indistinguishable resistance profiles, which may represent acquisition of the same plasmid by multiple species [23]. The purpose of this study was to evaluate antimicrobial resistance profiles of *Salmonella* strains isolated from poultry origin. As shown in Table 3, 78.6%, 71.4%, 64.3%, 57% and 50% of the isolates were sensitive to (ciprofloxacin and sulfamethoxazone-trimethoprim),(norfloxacin and ofloxacin), gentamycin, ampicillin and chloramphenicol respectively. In this concern, *Chaslus-Dancla and Martel* [24], recommended that the fluoroquinolones are drug of choice for treatment of invasive *Salmonella* and some antibiotics namely enrofloxacin, danofloxacin and marbofloxacin which are also specifically approved for therapeutic veterinary use. Fluoroquinolone resistance was rarely found among *Salmonella* species until Heisig [25] who reported *S. typhimurium* serovars *conpenhagen* from cattle was highly resistant to ciprofloxacin. Thereafter, *Salmonella* species with high level of drug resistance have been continuously reported and the exact location and changes of genes associated with a mutation of these species have been searched [26].

In the present study, 100% of the examined isolates were resistant to flucloxacillin, while 71.4% and 57% were resistant to amoxicillin and tetracycline, respectively. These results agree with Murugkar *et al.* [27], who found that the most *Salmonella* isolates were resistant to doxycycline (61.05%), ampicillin (51.57%), amoxicillin (45.26%), tetracycline (44.21%), nitrofurantoin (15.79%), trimethoprim (9.5%) and gentamycin (6.3%). They also agree with El-Zeedy *et al*. [28], who found that the highest number of *Salmonella* isolates showed resistance against amoxicillin and tetracycline followed by nitrofurantoin, chloramphenicol, nalidixic acid, sulfamethoxazone-trimethoprim and gentamycin, while the least resistance rates were detected against cepheridin, norfloxacin and ciprofloxacin. On the other hand, Bounar-Kechih *et al*. [22] concluded that fifty-three percent of the isolated *Salmonella* strains were resistant to at least one antibiotic, among which 15.09% were multiresistant, the most frequently observed resistance was to quinolones (58.49%). Also, Hassanin *et al*. [29] suggested that the *Salmonella* isolates showed high susceptibility to fluoroquinolone group of antibiotics followed by cephridin and all the examined serovars showed multiresistance to more than one of the tested antibiotics. Ahmed and Shimamoto [30] found that the examined *Salmonella* isolates displayed multidrug resistance phenotypes, particularly against ampicillin, streptomycin, spectinomycin, kanamycin, tetracycline, chloramphenicol and trimethoprim/sulfamethoxazole. Multidrug resistant *Salmonella* serovars cause severe and septicaemic salmonellosis more frequently than those that are not resistant [31, 32]. SDS-PAGE protein analysis of whole cell protein patterns has been used extensively to the study of the differences among bacterial genera, species and strains [7]. Outer membrane protein analysis has proved to be useful technique in the characterization of *Salmonella* [8, 9]. In this study the protein profile of the isolated *Salmonella* strains from different poultry revealed about 18 protein bands ranged from (16-289.5 Kda) as shown in Photos 1&2 and Table 4. The differences were insufficient for reliable differentiation between the isolates. The (29 KDa) protein band was the common antigen and this agree with the result of Soad [33], while there were protein bands of 88, 70, 57.9, 51.6, 45.1, 36.4, 24.1, 22, 16 KDa which agree with the results of Ochea-Reparaz *et al.* [9] who found bands of flagellin 53 KDa and 45.1 KDa, porins 35-36 KDa, OmpA 34 KDa and Omp 22.1 KDa. Nese *et al*. [34], found that *Salmonella typhimurium* isolates obtained OMPs with molecular size 70 KDa and this agree with this study which revealed that the band of OMP= 70 KDa present. Also, the results agree with Jaradat and Zawistowski [35] who mentioned that, the two major proteins (24 and 35 KDa) were identified and the latter protein was present only in *Salmonella*. The molecular weight of bands in this study is agreement with those obtained by El-Zeedy *et al*. [28], who mentioned that different protein bands (67, 57, 43, 36, 33, 29, 22 KDa) were present in all the isolates. The band of 33 KDa only present in two strains (*Salmonella poona* and *Salmonella montevideo*).

Animal to human transmission of *Salmonella* strains is sometimes difficult to document with traditional epidemiological tools such as serotyping and biotyping. Many of the *Salmonella* serotypes are so commonly isolated (e.g., *S. typhimurium*) that epidemiologists need other means of subdividing the strains. The term clone has been used to define apparently identical strains originating from a single source. Plasmid profile analysis has been found to be one of the best methods for the routine identification of bacteria originating from the same clone [36]. Plasmid profile analysis has been used as a rapid, easy method and has shown some success in the discrimination of several *Salmonella* strains [37, 38]. Also, plasmid fingerprinting was the first molecular method to be used as a bacterial typing tool and played an important role in studies of zoonotic aspects of salmonellosis as well as *Salmonella* biology [2].

Most of the isolates showed a degree of variation in plasmid number from 1 plasmid up to 8 plasmids and molecular size from 2121 bp up to 22864 bp. As shown in

photo 3, Table (5), only one isolate of *Salmonella rubislaw* has plasmid of (22864 bp), while eleven isolates (71.5%) (*S. poona*, *S. typhimurium*, *S.virginia*, *S.enteritidis*, *S.montevideo* and *S.sandiago*) has plasmid of 16904 bp. Three isolates (*S.rubislaw*, *S. poona* and *S. typhimurium*) carry plasmid from 15544 to 15807 bp. Meanwhile, variable bands with different molecular size from 2121 to 13361 bp were detected in different *Salmonella* isolates. These findings go hand in hand with the findings of Hassanin *et al*. [29], who mentioned that, plasmid analysis of all serotypes of different *Salmonella* strains carry different numbers of plasmids with variable molecular weights ranged from 759 to 21722 bp and also agreed with Olsen [39], who reported that strains of *Salmonella* often carry plasmids ranging in size from 2 to 150 kbp but frequencies and size distributions vary between serovars. Bounar-Kechih *et al*. [22], concluded the incompatibility groups of plasmids belong to the F1me and Com1 classes and the molecular weight of the plasmid DNA was greater than 100 kb from all examined *Salmonella* isolates. The results in this study also agree with Suh and Song [40], who mentioned that a band of (15 kbp) size was detected in all *Salmonella* isolates and the band sizes smaller than (15 kbp) were found only in isolates from chickens. Nakamura *et al*. [41], concluded that when *S. typhimurium* strains isolated from animals reared in limited areas exhibit identical or similar plasmid patterns, they are derived from the same source and that when these strains isolated in a limited area exhibit quite a different plasmid pattern, these strains are derived from independent sources. *S. typhimurium* strains contained 4 plasmids of 45, 16, 6 and 3.5 MDa [42], while, Holmberg *et al*. [43] recorded that *S. typhimurium* carry several plasmids of (140 to 2 MDa= 210 to 3 kbp) but another strains have no plasmids. Majority of *S. typhimurium* strains carry plasmid of (60 MDa= 90 kbp) and other strains carry small plasmid of (5-1 MDa= 7.5-1.5 kbp) [44]. Other *Salmonella* strains carry plasmids from (3 to 38.5 MDa = 4.5 to 57.7 kbp) [45]. *S. enteritidis* strains contained plasmids of 42, 58, 20, 17, 16, 12, 10, 8, 6, 4.8, 2.6 and 1.4 MDa in different combinations [42]. Tekeli *et al*. [46] showed the different plasmids from (2.5-100 kbp) in different *S.enteritidis* strain. Meanwhile, Bakshi *et al*. [47] studied the plasmid profile of 24 strains of *S*.enteritidis, most of the strains contained only a single plasmid of 55 kbp, additional plasmids of 23.2 kbp and 8.7 kbp were seen in one of the strains and another strains carried only two plasmids of 23-2 kbp and 8.7 kbp but four strains did not carry any plasmids. Also, plasmid profile of 10 multiple drug resistant *Salmonella* serovars from different origin (poultry, cattle and human) was performed, 8 serovars were harboring plasmid with molecular weight ranged from (16-31.5 kbp), while the plasmid of two serovars could not be detected [48]. Few recently published articles involve the use of plasmid profiling as a stand-alone procedure. Although this and other studies show that plasmid profiling is not the most sensitive method, the technique does hold significant advantages, particularly the short time in which the procedure can be performed. The rapidity, combined with the relative simplicity of the procedure and basic apparatus required, makes it adequate as an initial procedure that may be used by laboratories which are less able to perform more complicated methods. The inherent mobility of the plasmid DNA suggests instability of the characteristic under scrutiny. This is a limitation which must be recognized in epidemiological research and has brought into question which can be regarded as suitable plasmid profile for analysis [49].

Several investigators reported that resistance to different antimicrobial agents was mediated by large plasmid [50, 51]. In the present investigation, the largest plasmid of (16904 bp) was detected in (71.4%) of the present isolates that showed the highest incidence of antimicrobial resistance. This agrees with Samy [52], who concluded that the plasmid of (16732 bp) showed the highest antibiotic resistant pattern for the tested *Salmonella Typhimurium* isolates (30%). The large plasmid conferred resistance to gentamycin plus tetracycline to gentamycin alone or amoxicillin alone [53]. The presence of large number of plasmids affected on the resistant to antibiotic and this appear in more than one strain of *Salmonella* isolates in this study (*S.rubislaw, S. typhimurium, S.viriginia* and *S.enteritidis*) and this is in agreement with those obtained by Samy [52],who mentioned that if the number of plasmids increase, the resistant to antibiotic increase also. In contrast, Liebana *et al.* [54] concluded that plasmid profiling might not be an adequate way of identifying clones, especially over long periods. Also, Maslow *et al.* [55] suggested that numerous plasmids must be present and regard the presence of a single plasmid was insufficient as representative of a clone. Results from his study showed that plasmid profiling alone might not be sufficient for accurate identifying clones.

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