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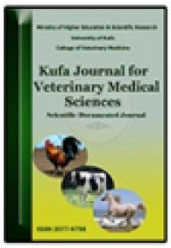

Antimicrobial resistance pattern and RAPD profile of *Salmonella* Ohio isolated from broiler farms in Al-Najaf and Al-Muthana provinces

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Abstract:

The present study aimed to investigate the molecular epidemiology and resistance patterns of S. Ohio isolates. All the Ohio isolates were resistant to more than three of the antibiotics, upto 10 antimicrobial agents. Overall, the highest proportions were found for resistance to the following agents: Tetracycline (100%), Nalidixic acid (94.7%), Doxycycline (94.7%), low level resistance to Ciprofloxacin (89.4%),Kanamycin (84.2%), Sulphafurazole (78.9%), and Co-Trimoxazole(52.6%).A total of 11 (DI = 0.958) antimicrobial resistance patterns were observed among 19 isolates of S. Ohio, with a MAR index value ranged between 0.2 to 0.43. The frequency of resistance to Te/Cip/Na/Dox was found in 17 (90%) of the 19 MDR S. Ohio isolates. Genotyping of S. Ohio isolates using 10-mer arbitrary primer, p1254 revealed five RAPD types, denoted by English letters from A to E yielding DI of 0.53, whereas RAPD analysis with primer OPA-4 discriminated only four patterns which assigned F to I (figure), yielding DI of 0.44. When the results of RAPD (with two primers) and antibiotic susceptibility tests are combined, 19 isolates of S. Ohio could be divided into 12 different profiles (DI=0.97). The finding of this study indicated that the broiler chicken can serve as a zoonotic reservoir of MDR bacteria which can be transmitted to human. This is an important implication for human health, because infections with MDR bacteria are difficult to treat and often requires expensive and long term therapy. The RAPD technique can be a useful tool for the analysis of S. Ohio strains. The method might be used as easy, faster, and cost-effective tool for molecular epidemiology research in each laboratory.

Key words: Salmonella Ohio, antimicrobial resistance, MAR index, RAPD-PCR.

نمط المقاومة للمضادات الجرثومية و هيئة الـRAPD لجراثيم السالمونيلا اوهايو المعزولة من مزارع دجاج اللحم في محافظة النجف الأشرف والمثنى

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الخلاصة:

تهدف الدراسة الحالية الى استقصاء الوبائية الجزيئية وأنماط المقاومة لجراثيم السالمونيلا اوهايو. أظهرت الدراسة إن جميع عزلات الأوهايو مقاومة لأكثر من ثلاثة من المضادات الحيوية المدروسة، وقد تصل الى لـ 10من المضادات الحيوية. عموماً ، فان أعلى نسب المقاومة كانت للتتراسايكلين (100%)، حامض الناليدكسك (94,78%)، الدوكسي سايكلين (94,78%) ،المستوى المنخفض من المقاومة للسايبروفلوكساسين (89.4%)،الكنامايسين (84,2%)، السلفا (78,9%)، و الكوترايموكسازول (52,6%). أظهرت عزلات *S.* Ohio (19 عزلة) احد عشر نمطاً مختلفاً من انماط المقاومة المتعددة للمضادات (معدل مؤشر التنوع DI=0,958). وقد تراوحت قيمة مؤشر المقاومة المتعددة (MAR) ما بين 0,2 – 0,43. كما لوحظ أن تكرار المقاومة المتعدة للمضادات (التتراسايكلين، سايبروفلوكساسين، حامض الناليدكسك، و الدوكسي سايكلين) قد تكرر في 17 (90%) عزلة من أصل 19 عزلة *S.* Ohio متعددة المقاومة.

أظهر التنميط الجيني لعزلات *S.* Ohio باستخدام بادئ p1254، لتقنية الRAPD، وجود خمسة أنماط جينية أعطية حروف انكليزية من أ الى هـ وكانت قيمة مؤشر التنوع للتقنية (DI=0,53)، بينما تم الحصول على أربعة أنماط جينية (و إلى ي) فقط وبمؤشر تنوع DI=0,44 وذلك باستخدام بادئ (OPA-4). ارتفعت قيمة مؤشر التنوع إلى 0,97 حينما تم دمج نتائج تقنية ال RAPD (باستخدام البادئين معاً) ونتائج فحص المضادات الحيوية وبذلك فقد تنوعت سلالات ال *S.* Ohio إلى 12 صورة مختلفة. أشارت نتائج هذه الدراسة ان فروج اللحم يمكن إن يكون مضيف خازن للجراثيم المشتركة متعددة المقاومة والتي يمكن أن تنتقل للإنسان، وهذا يتضمن خطراً على صحة الإنسان لأن الإصابة بهذه الجراثيم تكون صعبة العلاج وغالبا ما تحتاج إلى فترة علاج طويلة ومكلفة. إن تقنية الـ RAPD يمكن استخدامها بشكل عملي ومفيد في تحليل سلالات الـ *S.* Ohio. و يمكن استخدامها لأجراء البحوث الوبائية داخل كل مختبر على حدة وذلك لرخصها وسهولتها.

Introduction:

Salmonellosis is an important public health problem in many countries and a frequent cause of gastroenteritis and zoonotic infections (1). Domestic animals have a role in the spread of infection between flocks and herds and as causes of human food poisoning (2). *Salmonella* spp. have frequently been found in broiler and their products (3) .

Infections caused by *Salmonella* spp. are increasing in many countries. During the last decades, the emergence of antimicrobial drug-resistant strains has been reported within different serotypes of *Salmonella enterica* (4). This emerging problem is due to the widespread use of antimicrobial agents in veterinary medicine and animal husbandry, and as growth promoters in livestock (5). For instance, multi-drug resistant (MDR) *Salmonella enterica* (*S. enterica*) serovar Typhimurium phage type DT104, resistant to ampicillin, chloramphenicol/florfenicol, streptomycin, sulfanomides, and tetracycline, has a global dissemination (6).

Quinolone and fluoroquinolone (norfloxacin, ciprofloxacin, and enrofloxacin) antimicrobials are classes of synthetic antimicrobial agents with an excellent activity against *E. coli* and other gram-negative bacteria used in human and veterinary medicine (7). A point mutation in gyrA between amino acids 67 and 106 (known as the quinolone resistance-determining region or QRDR) can confer nalidixic acid (a first-generation quinolone) resistance among isolates of *Salmonella* which usually accompanied by low-level resistance to ciprofloxacin. While high-level of Ciprofloxacin resistance is often a result of the sequential acquisition of mutations in a number of genes, namely *gyrA*, *parC* and *parE* and less frequently in *gyrB* (8).

Identification and genotype characterization of the bacterial isolates are essential for epidemiological surveillance and outbreak investigations. The primary method used for characterizing members of the genus *Salmonella* is serotyping (9). For further discrimination of isolates within the same serotype, phage typing is the primary sub-typing method. Unfortunately, phage typing frequently fails to discriminate between outbreak-related and unrelated isolates and usually applied by the reference laboratories (10).

Using DNA-related techniques, researchers now are able to better differentiate *Salmonella* isolates below the level of serotypes. These techniques include

plasmid profile, ribotyping, pulsed-field gel electrophoresis (PFGE), IS200 fingerprinting, PCR ribotyping, amplified fragment length polymorphism, MultiLocus Sequence Typing (MLST), and random amplified polymorphic DNA (RAPD) analysis (11). RAPD analysis is the most general procedure for the comparison of several isolated genomes over the course of a few days. This method is faster, relatively simple and more economical than other genomic typing methods (12).

The aim of the present study was asses the antibiotic susceptibility test and the genetic relatedness by RAPD analysis for discrimination of 19 strains of *S.* Ohio recovered from broiler farms in An Najaf Al-Ashraf and Al-Muthana governorates.

Material And Methods :

Bacterial isolates and growth condition: this study was carried out with 19 Salmonella Ohio strains, 18 isolates were collected from broiler farms distributed in AL Najaf el-Ashraf governorate, and one isolate from Al-Muthana. The serotype Ohio strains and other serotypes (data not shown) were obtained from a total of 147 chicks submitted to the Avian Disease laboratory, Veterinary Hospital of An Najaf el-Ashraf Governorate during the period from September 2011 to April 2012. Cloacal swabs were collected using sterile disposable cotton swabs and immediately inoculated onto 10ml Selenite-F broth and incubated at 41.5°C± 0.5°C overnight (18-24 hours). Then, followed by subcultivation onto CHROMagar *Salmonella* agar (CHROMagar Company, Paris, France), and Hektoen Enteric (HE) agar. The plates will be incubated at 37°C for 18 to 24 hr. (13, 14).

Identification of suspected colonies: Suspected *Salmonella* colonies were identified by using and KBM002 HiMotility^{TM} Biochemical kit for *Salmonella*. All isolates were examined for positive agglutination with polyvalent O antisera by using HiSalmonella^{TM} Latex Test kit (15, 16).

Serotyping: All *Salmonella enterica* isolates were sent to Central Public Health Laboratory (CPHL) in Baghdad capital for serotying with O and H antisera.

Antimicrobial susceptibility testing: Antibiotic susceptibility test was performed by the standard disk diffusion method in Mueller-Hinton agar (Hi-Media) according to (17), and the results were interpreted in accordance to the criteria of the CLSI (18). The isolates were screened for resistance to 23 antibiotics listed in Table 3. All antibiotic discs were produced from Hi-Media Laboratories Privet. Ltd., Mumbai. Nalidixic acid-resistant and sensitive isolates will be subjected to minimum inhibitory concentration (MIC) for Ciprofloxcin by using HiComb MIC test strips procured Hi-media Laboratories Privet. Ltd., Mumbai. Reduced susceptibility (low-level resistance) to ciprofloxacin was defined as a MIC of ≥ 0.125 µg/ml and high-level resistance as a MIC of ≥4 µg/ml (16). MDR was defined as resistance to more than three antibiotics. Multiple antibiotic resistance (MAR) index was calculated according to method of (19). MAR index is defined as a/b where 'a' represents the number of antibiotics to which the particular isolate is resistant and 'b' the number of antibiotics to which the isolate is exposed. MAR index values higher than 0.2 are considered to have originated from high-risk sources where antibiotics are often used. MAR index values of less than or equal to 0.2 indicates a strain originated from sources where antibiotics are seldom or never used.

RAPD-PCR: The extraction of *Salmonella* Ohio genome was carried out as recommended by the manufacturer of Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid, USA). All *Salmonella* Ohio strains were genotyped by the RAPD PCR method, two primers were purchased from BioCorp, Canada, previously reported by (20) that provide good discriminatory power among *Salmonella* isolates were used,

namely, (i) primer P1254 (CCGCAGCCAA), and primer OPA-4 (AATCGGGCTG). PCR was conducted in a 25 µl volume containing 5µl of *S.* Ohio DNA, 100µM of primer, 12.5µl of 2×KAPA2G Robust HotStart ReadyMix (KAPABIOSYSTEMS, Cape Town, South Africa), and the volume was completed upto 25µl with PCR grade water.

Amplification conditions were performed as previously described by (21). All experiments were carried out at least twice to ensure the reproducibility of the results and only the reproducible and well-defined fragments were used to define amplicon profiles. PCR products were electrophoresed in 1.5% agarose. Gels were interpreted by visual comparison of banding patterns. Isolates differing by more than two bands were considered to represent distinct RAPD types (22).

The discrimination index (DI) (i.e., the probability that two unrelated strains obtained from the population would be placed in different typing groups) was calculated for 16 isolates, because isolates showing identical features and epidemiologically relatedness (collected from members of a single flock) were assigned to a single strain. The index was calculated by using Simpson's index of diversity. The formula used to define the diversity index or, better, Simpson's index of diversity D is:

$$D = 1 - \frac{1}{N(N-1)} \sum_{J=1}^{S} n_j(n_j - 1),$$

where N is the total number of strains in the sample population, S is the total number of types described, and nj is the number of strains belonging to the jth type (23).

Results:

All the Ohio isolates were resistant to more than three of the antibiotics, including 7 isolates were resistant to 4 - 9 antimicrobial agents, 10 isolates were resistant to 7-9 agents and 2 isolates were resistant to 10 antimicrobials (table-1). Overall, the highest proportions of resistance were found for the following agents: tetracycline (100%), nalidixic acid (94.7%), doxycycline (94.7%), low level resistance to ciprofloxacin (89.4%), kanamycin (84.2%), sulphafurazole (78.9%), co-trimoxazole(52.6%), chlor-amphenicol (42.1%), colistin (15.78%), amoxicillin/clavulanic (15.78%), ampicillin (15.78%), streptomycin (15.78%), gatifloxacin (5.2%), and gentamicin (5.2%). None of the isolates was resistant to pipracillin-tazobactam, cephalothin, cefotaxime, ceftriaxone, ceftazidime, aztreonam, imipenem, tobramycin and amikacin.

A total of 11 (DI = 0.958) antimicrobial resistance patterns were observed among 19 isolates of *Salmonella* Ohio, two isolates of them exhibited resistance to ten antimicrobials as shown in **Table-2** with a MAR index value of 0.43. Ten isolates were resistant to seven to nine antibiotics with a MAR frequency of 0.3 - 0.39, and seven isolates were resistant to five to six antibiotics with a MAR frequency of 0.2 – 0.29. Furthermore, the frequency of resistance to TeCipNaDox were found in 17 (90%) of the 19 MDR *Salmonella* Ohio isolates.

Genotyping of *S.* Ohio isolates using 10-mer arbitrary primer, p1254 revealed five RAPD types, denoted by English letters from A to E (figure-1) yielding DI of 0.53. The RAPD patterns differed in the number of fragments (8-12) which ranged from 300-2000 bp in molecular weight. Thirteen (68%) isolates had pattern A, and the other 9(32%) isolates were distributed in 4 patterns (pattern B, P39A &P39C; pattern C, P40 &P50B; pattern D, P42; and pattern E, P16), whereas RAPD analysis with primer OPA-4 discriminated only four patterns which assigned F to I (figure-2), yielding DI of 0.44.

These profiles had 6 to 9 fragments ranging from 300-2000 bp in molecular

weight. Fifteen (79%) isolates had pattern F, and the other 4 (21%) isolates were distributed in 3 patterns (Table-2). Pattern G, was found among 2 (11%) isolates, while H, and I patterns had been seen among one (5%) isolate for each. Combination of RAPD patterns obtained using two different primers discriminated the isolates into 5 profiles (DI=0.53), profile AF was found in 13(68%) isolates (table-2).

When the results of RAPD (with two primers) and antibiotic susceptibility tests are combined, 19 isolates of *S.* Ohio could be divided into 12 different profiles (DI=0.97).

Table-1: Antimicrobial resistance of 31 *S. enterica* isolates from broiler flocks to 23 drug, during period from September 2011 to March 2012 in An Najaf el-Ashraf, Al-Qadyssia and Al-Muthana provinces.

Antimicrobials		***S.* Ohio (n =19)**
Ampicillin (AMP) 30µg		3 (15.78%)
Pipracillin-tazobactam (PIT) 100/10 µg		0
Amoxicillin/clavulanic (AMC) 20/10 µg		3 (15.78%))
Cephalothin (CEP) 30 µg		0
Cefotaxime (CTX) 30 µg		0
Ceftriaxone (CTR) 30 µg		0
Ceftazidime (CAZ) 30 µg		0
Aztreonam (AZ) 30 µg		0
Imipenem (IPM) 10 µg		0
Chloramphenicol (C) 30 µg		8 (42.1%)
Tetracycline (TE) 30 µg		19 (100)
Doxycycline (DOX) 30 µg		18 (94.7%)
Amikacin (AK) 10 µg		0
Gentamicin (GEN) 10 µg		1 (5.2%)
Tobramycin (TOB) 10 µg		0
Kanamycin (K) 30 µg		16 (84.2%)
Streptomycin (S) 10 µg		3 (15.78%))
Co-Trimoxazole (CoT) 1.25/23.75		10 (52.6%)
Sulphafurazole (SF) 300 µg		15 (78.9%)
Ciprofloxacin (CIN) 5 µg/LLR		17 (89.4%)
HLR		1 (5)
Gatifloxacin(GAT) 5 µg		1 (5)
Nalidixic acid (NA) 30 µg		18 (95)
Colistin (CL) 10 µg		3
Recapitulatory	4-6	7 (37)
	7-9	10 (53)
	10	2 (11)

Table-2: Distribution of *S.* Ohio isolates according to their Geographic area, Resistance Pattern, MAR indices and RAPD profile.

Designation of isolates	Geographic area	Resistance Pattern	MAR index value	RAPD profile with primer	
				P1254	OPA-4
P40	An Najaf, Al-hayderyia	TE, K, NA, DOX, CIP	0.22	C	G
P50B	An Najaf , Al-Barrakyia			C	G
P14B	An Najaf , Al-Barrakyia	TE, GEN, S, K, SF, DOX,	0.26	A	F
P15	An Najaf, Al-Manathera	TE,CL, NA, SF, DOX, CIP	0.26	A	F
P19	An Najaf, Ghan el-Rubaa			A	F
P38A	An Najaf , Al-Barrakyia	C, TE, K, NA, DOX, CIP	0.26	A	F
P49	An Najaf , Al-Barrakyia			A	F
P39A	An Najaf, Al-Hurria	TE, K, CoT, NA, SF, DOX, CIP	0.30	B	F
P39C	An Najaf, Al-Hurria			B	F
P48B	An Najaf, Al-Radhawyia			A	F
P48C	An Najaf, Al-Radhawyia			A	F
P36	An Najaf, Al-Mushekab	C, TE, K, CoT, NA, SF, DOX, CIP	0.35	A	F
P53A	An Najaf , Al-Barrakyia			A	F
P53B	An Najaf , Al-Barrakyia			A	F
P16	An Najaf , Al-Barrakyia	TE, S, K, CL, NA, SF, DOX, CIP	0.35	E	I
P42	Al-Muthana, Al-Sumawa	AMP, TE, K, AMC, GAT, NA, SF, CIP	0.35	D	H
P20	An Najaf, Al-Radhawyia	C, TE, K, CoT, CL, NA, SF, DOX, CIP	0.39	A	F
P38B	An Najaf , Al-Barrakyia	C, AMP, TE, S, COT, AMC, NA,SF, DOX, CIP	0.43	A	F
P48A	An Najaf, Al-Radhawyia	C, AMP, TE, K, COT, AMC, NA,SF, DOX, CIP	0.43	A	F

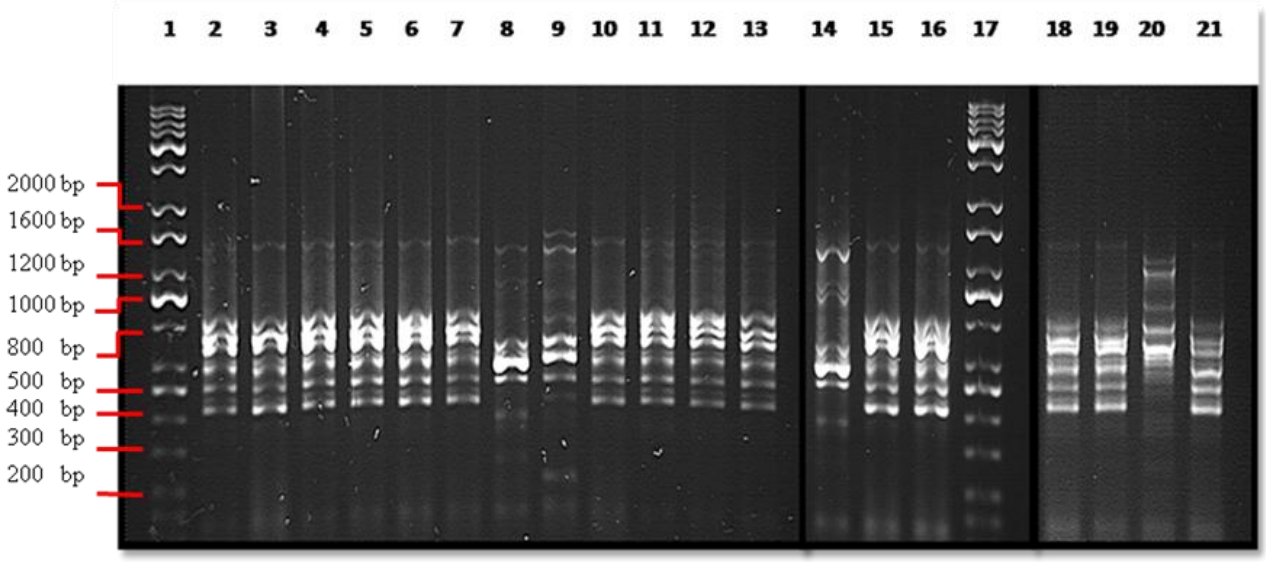

Figure 2 – RAPD patterns of Salmonella Ohio isolates with primer OPA – 4: Lanes 1 & 17: KAPA Universal DNA Ladder; Lane 2: P20 (pattern I); Lane 3:P36 (pattern I); Lane 4: P38A (pattern I); Lane 5: P38B (pattern I); Lane 6: P39A (pattern I); Lane 7:P39C (pattern I); Lane 8: P40 (pattern II); Lane 9: P42 (pattern IV); Lane 10: P48A(pattern I); Lane 11: P48B(pattern I); Lane 12: P48C (pattern I); Lane 13: P49 (pattern I); Lane 14: P50B (pattern II); Lane 15: P53A (pattern I); Lane 26: P53B (pattern I); Lane 18: P14B (pattern I); Lane 19: P15 (pattern I); Lane20: P16 (pattern III); Lane 21:P19 (pattern I).

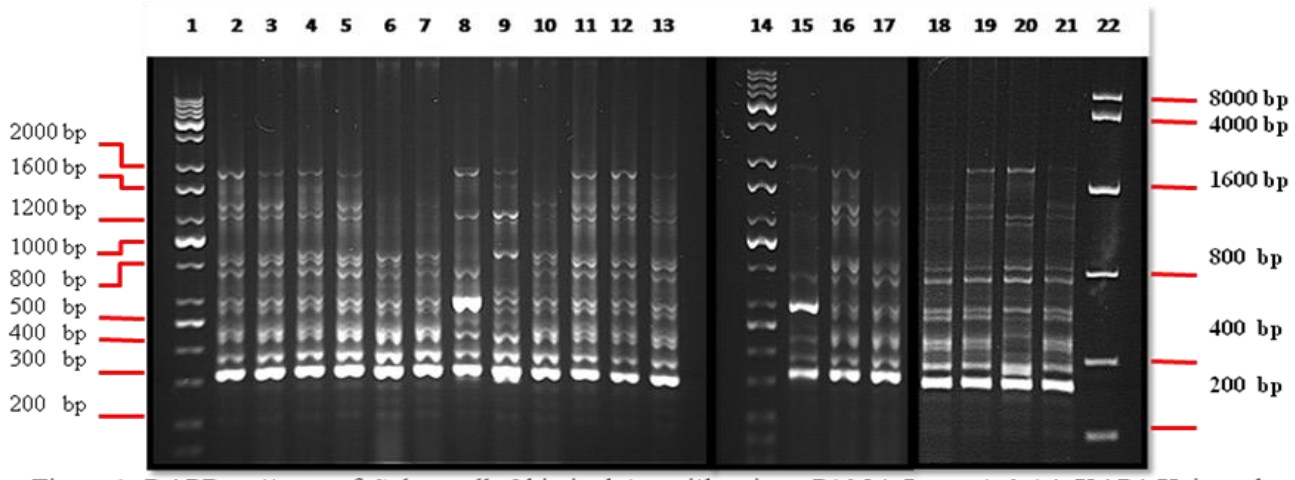

Figure 1- RAPD patterns of *Salmonella* Ohio isolates with primer P1254: Lanes 1 & 14: KAPA Universal DNA Ladder; Lane 2:P20(pattern I); Lane 3:P36(pattern I); Lane 4: P38A(pattern I); Lane5: P38B(pattern I); Lane6: P39A(pattern II); Lane 7:P39C(pattern II); Lane 8: P40 (pattern III); Lane 9: P42(pattern V); Lane 10: P48A(pattern I); Lane 11: P48B(pattern I); Lane 12: P48C(pattern I); Lane 13: P49(pattern I); Lane 15 P50B (pattern III); Lane 16: P53A (pattern I); Lane 17: P53B (pattern I); Lane 18: P14B (pattern I); Lane 19: P15(pattern I); Lane 20: P16(pattern IV); Lane 21:P19(pattern I); Lane 22:KAPA Express DNA Ladder (8000, 4000, 1600, 800, 400, 200 bp).

Discussion:

Several serotypes of *S. enterica* infect poultry which may being a source of infection to human, conversely, a poultry producer suffers losses due to *Salmonella* infection of the flock including loss of birds and production time (24). Even though *S.*Ohio is not included within the twenty most common *Salmonella* serotypes described by WHO Global Salm-Surv among human salmonellosis (25), it is well characterized as a causative agent of clinical salmonellosis in the principality of Asurias, Spain (26).

The overuse of antimicrobials in different fields including therapeutics, prophylactics, or as growth promoters, created a selective pressure for occurrence of antimicrobial resistance among bacterial pathogens and endogenous microflora (27). The application of a single drug may results not only in direct selection of the corresponding resistance but also in development of cross-resistance (the resistance to several structurally-related antimicrobials) and co-resistance (the resistance to several structurally-unrelated antimicrobials), via specific resistances mechanisms (5).

Consequently, this study found that all Ohio isolates displayed multi-drug resistance to more than three antimicrobial agents up to ten. Maripandi and Al-Salamah have reported similar observations (28). While, other studies have been indicated lower (46.5% and 65.4%) levels of multi-drug resistance in *Salmonella* isolates recovered from broilers (29, 30).

Overall, high resistance levels were observed against tetracycline (100%), doxycycline (94.78%), kanamycin (84.2%), sulphafurazole (78.9%), co-trimoxazole (52.6%) and chloramphenicol (42.1%). Currently sulphonamides and tetracyclines are widely used in broiler-chicken flocks in numerous countries worldwide (31), including Iraq, which might be related to the emergence of resistance to such antimicrobials &/or cross and co-resistance phenomenon. Several researchers have been recorded resistance levels potentially compatible with present results. In Senegal, they were found that the most often resistance observed to ampicillin, trimethoprim, co-trimoxazole, tetracycline, and sulphonamides in *Salmonella* strains isolated from broiler carcasses (Bada-Alambedji et al., 2006). In Japan, Ishihara *et al.* were reported high resistance rates observed against oxytetracycline (83.8%), dihydrostreptomycin (79.3%), kanamycin (41.0%), and Trimethoprim Sulfa (37.8%) among *Salmonella* recovered from broilers (32).

The data from present study showed that 95% of *S.* Ohio isolates were resistant to nalidixic acid. Moreover, we found 89.4% of isolates was resistant to ciprofloxacin and 5% were highly resistant. Relatively, lower level (50%) of resistance to nalidixic acid resistance was reported in *Salmonell*a isolated from poultry in Spain (33), also, Cheong *et al.* (34) recorded similar frequency of nalidixic acid resistance among broiler-chicken NTS in Korea. Rad *et al.* reported that 40% of *Salmonella* and 90% of *E. coli* strains isolated from animal origin (involving poultry) were found to be resistant to nalidixic acid (35), while fifty-six percent of *E.coli* strains were resistant to ciprofloxacin. In a study by Li *et al.*, they found high resistance to enrofloxacin (83%), and ciprofloxacin, (81%) among *E.coli* isolates recovered from chickens with colibacillosis in Henan province, China (36). However, Murray *et al.* have been found of the 1,254 *Salmonella* isolates received in 2003 which isolated from human, veterinary, and environmental origin, against a panel of 14 antibiotics, including nalidixic acid and ciprofloxacin, 267 (21.3%) isolates were nalidixic acid resistant, and 260 (97.4%) of which had reduced susceptibility to ciprofloxacin (8). Resistance to nalidixic acid (a first-generation quinolone) in isolates of *Salmonella* is regarded as an indicator of decreased susceptibility to ciprofloxacin (37, 38).

On the other hand, the high rates of nalidixic acid and ciprofloxacin resistance among broiler isolates would be related to the widespread use of enrofloxacin in commercial farms, and contributing to the risk of creating highly resistant zoonotic agents (35). Therefore, such resistance is an alarming sign because fluoroquinolones are the most commonly used antimicrobial agents for the treatment of invasive salmonellosis in human and failure of therapy has been reported in patients with nalidixic acid-resistant *Salmonella* infections (39).

The drug resistance pattern of *Salmonella* reflects the spectrum of antimicrobial agents usage in the veterinary practice (40). This can interpret that all isolates were susceptible to pipracillin-tazobactam, cephalothin, cefotaxime, ceftriaxone, ceftazidime, aztreonam, imipenem, tobramycin and amikacin, which may be due to rarest usage of such drugs in poultry farms.

A great diversity of resistant patterns (11 patterns in 19 isolated strains) with DI of 0.95 were achieved by application of antibiotic susceptibility test as a phenotypic method, 17 (90%) of isolates exhibited multiple resistance to TeCipNaDox and 13 (68%) of isolates were further resistant to SF (table-3). Besides, all the *Salmonella* Ohio exhibited multiple antibiotic resistance (MAR) with frequencies ranged between 0.22 to 0.43. MAR index values higher than 0.2 were considered to have originated from high-risk sources where antibiotics are often used. Similar results have been reported among poultry litter isolates of *Salmonella*, *Shigella* and *E.coli* (41).

Increased MDR strains have been reported in *Salmonella* in the Saudi Arabia (42), the UK (43), the USA (44) and elsewhere in the world. MDR isolates may be colonized in the human intestinal tract (due to consumption of poultry meat or through direct contact) and the genes coding for antibiotic resistance can be transferred to the bacteria of natural microflora or pathogenic bacteria (41).

By the RAPD genotyping, performed with primer p1254, the *S.* Ohio strains were subdivided into 5 (A-E) profiles, yielding good discriminatory index of 0.53. In contrary, 26) when combined data from 3 arbitrary primers (S, OPB-6, and OPB-17) used for genotyping of 50 strains of *S.* Ohio, 5 profiles were observed with D.I. of 0.22. The present study showed higher discriminatory power of RAPD analysis of *S.* Ohio strains which may be due to; first, oligonucleotide primer used is capable of recognizing DNA polymorphisms among isolates; second, the using of commercially available optimized PCR readymix kit which contains a novel hotStart DNA polymerase, engineered for robust PCR in a proprietary reaction buffer, dNTPs (0.2 mM of each dNTP at 1X), $MgCl_2$ (2 mM at 1X) formulated for the amplification of diverse amplicons.

Considerably, it has been postulated that RAPD analysis may have good discriminatory power for the differentiation of *Salmonella* strains (45, 46), and some investigators have reported that RAPD analysis has greater discriminatory power than PFGE for the differentiation of *Salmonella* serovar Enteritidis strains (47). The present results showed that RAPD-PCR analysis performed with p1254 and OPA-4 primers under well-defined conditions proposed herein, can yield reproducible results that discriminate true polymorphisms beyond serotype.

With regard to reproducibility, a weakness frequently reported for RAPD analysis (48), it should be pointed out the following: although the band patterns for a particular strain may include one or more bands that differ in intensity between assays, each strain was always classified within the same RAPD group; and it has been shown to be a very useful tool for epidemiological purposes (26). In respect to the discriminatory power and ease of application, the combination of RAPD

analysis (genotypic) and antibiotic susceptibility pattern (phenotypic) were resulted in increased discriminatory index up to 0.97.

Conclusively, the present study emphasizes that the broiler chicken can serve as a zoonotic reservoir of MDR bacteria which can be transmitted to human. This is an important implication for human health, infections with MDR bacteria are difficult to treat and often requires expensive and long term therapy. The RAPD technique can be a useful tool for the analysis of S. Ohio. Although the establishment of an international library based on RAPD analysis of the strains is not possible due to low reproducibility between different laboratories, the method might be used as cost-effective tool for molecular epidemiology research in each laboratory.

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