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ANTIMICROBIAL RESISTANCE AND INTEGRON PROFILES OF *SALMONELLA* SEROVARS AND *ESCHERICHIA COLI* ISOLATED FROM BROILER CHICKEN

By

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ABSTRACT

Bacterial species of family *Enterobacteriacae* could infect various body systems of broiler chicken. When reach the respiratory system they result in severe consequences. Out of 100 broiler farms manifested respiratory sings, avian pathogenic *Escherichia coli* (APEC) and *Salmonella* (*S.*) spp. were isolated from 57 (57%) and 12 (12%) respectively, while mixed infection was noted in 4%. Serotyping of *Salmonella* spp. revealed the presence of eight serotypes in the investigated farms, *S.* Blegdam, *S.* Cremieu *S.* Newport and *S.* Virchow (2/12, 16.67% each); *S.* Enteritidis, *S.* Ferruch, *S.* Gueuletapee and *S.* Paratyphi A. (1/12, 8.33% each). The isolation of strict human serovar, *S.* Paratyphi is astonishing and a terror for human health. Antimicrobial susceptibility testing revealed the high prevalence of resistance against the commonly used antimicrobials in poultry industry including, trimethoprim-sulfamethoxazole (82.46 and 66.67%), ciprofloxacin (77.19 and 75.00%), doxycycline (49.12 and 58.33%), cefotaxime (59.65 and 50.00%) and gentamicin (43.86 and 58.33%) for APEC and *Salmonella* spp. respectively. Multidrug resistance was reported in 75 and 96.49% in the recovered *Salmonella* serovars and *E. coli* respectively. Additionally, mixed infection increased the left behind treatment choices in some instance. Class 1 and 3 integrons were represented in both *Salmonella* spp. and APEC but integron class 1 prevailed integron class 3. On the other hand, class 2 was detected only in *Salmonella* spp. and was the least one amongst them. Broiler chicken could be a source for not only bacterial species of zoonotic significance but also for integrons, the mobile genetic elements that capture resistance genetic

cassettes and disseminate them intra and/or inter bacterial species. Subsequently, antimicrobial resistance returns the humanity to the pre-antibiotic era.

Keywords:

APEC, Broiler, Integron, *Salmonella* Paratyphi, Resistance.

INTRODUCTION

Birds possess unique respiratory system structure with unidirectional air flow and long stay of air in the parabronchi to supply birds with oxygen. This eerie structure could approve microbial pathogenesis via colonization then adhesion and invasion. Avian pathogenic *Escherichia coli* (APEC) and *Salmonella* (*S.*) species get the merit of this status quo contributing to sundry diseases leading to copious financial loses via mortalities, stunted growth and slaughter condemnation **(Lutful Kabir, 2010)**.

The prodigious diversity of the APEC and *Salmonella* serotypes stands as an obstacle in front of the efficacy of the available vaccines as they mostly do not protect against the heterologous serotypes infection **(Lynne *et al*., 2012; De Cort *et al*., 2014)**. Poulterers use antimicrobials as a preventive measure to safeguard flocks but it usually misses the mark and infection occurs.Chemotherapy is the prime aid to control bacterial infection in broiler chicken infected with APEC and *Salmonella* spp. Treatment failure becomes a usual upshot as a consequence of over use and misuse of antimicrobials in poultry industry that favor emergence and dissemination of Super Bugs **(Deng *et al*., 2015)** and that of no doubt pose a potential threat to public health **(Hammerum and Heuer, 2009; Centers for Disease Control and Prevention "CDC",2013)**.Propagation of microbial resistance occurs via transmission of mobile genetic elements likewise integrons carrying resistance genes between pathogens. Integrons are genetic elements capture genes by site-specific recombination encoding resistance to several antimicrobial classes. The mobilization of integrons via transposons and plasmids contributes to the horizontal dissemination of antimicrobial resistance in the same bacterial species, different bacterial species, different hosts or even different geographical areas via international travel and/or trade **(Du *et al*., 2005)**.

Surveillance of AMR and reveal its origins in zoonotic bacteria would signify how to apprehend and control AMR **(Boerlin *et al*., 2005)**. This study aimed at analyzing the antimicrobial resistance amongst the APEC and *Salmonella* spp. isolated from broiler chicken suffered respiratory manifestation against the commonly used antimicrobials in veterinary and/or human medicine with special reference to the presence of different classes of integrons.

MATERIAL AND METHODS

Samples:

Between September and December 2015, samples from 100 broiler farms were collected from Beni-Suef and Fayoum governorates. Samples from tissues and viscera showed lesion of respiratory affections (heart, liver, and lung) were targets for isolation of APEC and *Salmonella* spp.

Isolation and Biochemical Identification:

Isolation and biochemical identification of APEC and *Salmonella* spp. were done in accordance to **Collee *et al.* (1996)**.

Serological Identification of *Salmonella* spp.:

Salmonella serotypes were acknowledged according antigenic formulas of **Grimont and Weill (2007)**.

Antimicrobial Susceptibility Testing:

The isolated APEC and *Salmonella* serovars were subjected to the antimicrobial susceptibility testing using the disk diffusion method recommended by the **Clinical and Laboratory Standards Institute "CLSI" (2013)**. All isolates were tested for susceptibility to aztreonam (ATM 30 µg), cefepime (FEP 30 µg), cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), cefoxitin (FOX 30 µg), ciprofloxacin (CIP 5 µg), colistin (CT 10 µg), doxycycline (DO 30 µg) and gentamicin (CN 10 µg) and trimethoprim-sulfamethoxazole (SXT 25 µg). All disks were purchased from Oxoid, UK. *E. coli* ATCC 25922 was used as a quality control strain.

Detection of Integron Classes 1, 2 and 3:

Integron classes 1, 2 and 3 were detected in accordance to **Bass *et al.* (1999) and Goldstein *et al.* (2001)** amongst some selected APEC and *Salmonella* serovars represented the various antimicrobial susceptibility patterns. Table (1) reveals the target genes, primers sequence and the expected amplified amplicon size.

Table (1): Primers used to detect integrons in APEC and *Salmonella* serovars.

Gene		Primers sequences	Amplified amplicon (bp)	Reference
*Int*1	F	CCTCCCGCACGATGATC	280	Bass *et al.* (1999)
	R	TCCACGCATCGTCAGGC		
*Int*2	F	TTATTGCTGGGATTAGGC	250	Goldstein *et al.* (2001)
	R	ACGGCTACCCTCTGTTATC		
*Int*3	F	AGTGGGTGGCGAATGAGTG	484	
	R	TGTTCTTGTATCGGCAGGTG		

RESULTS

Isolation and Biochemical Identification:

APEC and *Salmonella* spp. were isolated from 57 (57%) and 12 (12%) farms showed respiratory signs. Co-isolation of both bacterial species was noted in 4 (4%) farms.

Serological Identification of *Salmonella* spp.:

Serotyping of the 12 biochemically identified isolates as *Salmonella* spp. revealed the presence of eight serotypes in the investigated farms. *S*. Blegdam, *S*. Cremieu *S*. Newport and *S*. Virchow (2/12, 16.67% each); *S*. Enteritidis, *S*. Ferruch, *S*. Gueuletapee and *S*. Paratyphi A (1/12, 8.33% each).

Antimicrobial Susceptibility Profiles:

Antimicrobial Susceptibility Profile of E. coli:

Overviewing the results showed the critical situation of the antimicrobial resistance in APEC isolated from chicken in the present study as multi drug resistance (MDR) was reported in 55 (96.49%) out of 57 APEC isolates. The highest levels of resistance (82.46 and 77.19%) were detected against trimethoprim-sulfamethoxazole and ciprofloxacin respectively, followed by cefotaxime, doxycycline and gentamicin with resistance rates of 59.65, 49.12 and 43.86% in order. Growing non-susceptibility was noted against cefepime (19.30%) and colistin (15.79%) as (Table2) illustrates. Of note, all the isolates that were cefepime non-susceptible were also resistant to cefotaxime.

Table (2): Antimicrobial susceptibility profile of APEC isolated from broiler chicken revealed respiratory signs.

Antimicrobial agent	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Aztreonam	31	54.39	7	12.28	19	33.33
Cefepime	46	80.70	7	12.28	4	7.02
Cefotaxime	16	28.07	7	12.28	34	59.65
Ceftazidime	26	45.61	14	24.56	17	29.82
Cefoxitin	50	87.72	4	7.02	3	5.26
Colistin	48	84.21	0	0.00	9	15.79
Doxycycline	16	28.07	13	22.81	28	49.12
Trimethoprim-sulfamethoxazole	8	14.04	2	3.51	47	82.46
Gentamicin	23	40.35	9	15.79	25	43.86
Ciprofloxacin	7	12.28	6	10.53	44	77.19

%: calculated in relation to the 57 tested APEC isolates

Antimicrobial Susceptibility Profile of *Salmonella* spp.:

Ciprofloxacin showed the highest level of non-susceptibility -i.e. resistance and intermediate- (75%), follow by trimethoprim-sulfamethoxazole, doxycycline, gentamicin, ceftazidime and cefotaxime by 66.67, 58.33, 58.33, 50.00 and 50.00% respectively. Alike to APEC, growing resistance was recorded to colistin (25.00%). Two (16.67%) of the inspected isolates were non-susceptible to cefepime and they also resisted cefotaxime. MDR was noted in nine (75%) out of 12 inspected *Salmonella* serovars as (Table 3) divulges.

Table (3): Antimicrobial susceptibility pattern of *Salmonella* serovars isolated from broiler chicken with respiratory signs

Antimicrobial agent	Susceptible		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Aztreonam	8	66.67	0	0.00	4	33.33
Cefepime	10	83.33	1	8.33	1	8.33
Cefotaxime	6	50.00	2	16.67	4	33.33
Ceftazidime	6	50.00	4	33.33	2	16.67
Cefoxitin	9	75.00	3	25.00	0	0.00
Colistin	9	75.00	0	0.00	3	25.00
Doxycycline	5	41.67	2	16.67	5	41.67
Trimethoprim-sulfamethoxazole	4	33.33	0	0.00	8	66.67
Gentamicin	5	41.67	4	33.33	3	25.00
Ciprofloxacin	3	25.00	2	16.67	7	58.33

%: calculated in relation to the 12 tested *Salmonella* isolates.

Antimicrobial Susceptibility of both APEC and *Salmonella* spp. Isolated from the same Broiler Chicken Farm:

Mixed infection of APEC and *Salmonella* spp. was noted in four farms. It resulted in increasing the burden of the antimicrobial resistance and reducing the treatment choices in two farms. Additionally, APEC and *Salmonella* spp. isolated from the same farms (F3 and F4) showed the same antimicrobial susceptibility profile except for colistin in F3 (Table 4).

Table (4): Antimicrobial non-susceptibility pattern of both APEC and *Salmonella* spp. isolated from the same broiler chicken.

	F1		F2		F3		F4	
Aztreonam	**N**	**S**	**S**	**S**	**S**	**S**	**N**	**N**
Cefepime	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Cefotaxime	**N**	**S**	**N**	**S**	**N**	**N**	**I**	**I**
Ceftazidime	**N**	**S**	**I**	**S**	**I**	**I**	**N**	**I**
Cefoxitin	**S**	**S**	**S**	**S**	**S**	**S**	**S**	**S**
Colistin	**S**	**S**	**S**	**S**	**S**	**N**	**S**	**S**
Doxycycline	**N**	**S**	**N**	**S**	**N**	**I**	**I**	**N**
Trimethoprim-sulfamethoxazole	**N**	**S**	**N**	**N**	**N**	**N**	**N**	**N**
Gentamicin	**S**	**S**	**S**	**S**	**S**	**S**	**N**	**N**
Ciprofloxacin	**S**	**N**	**S**	**N**	**N**	**N**	**N**	**N**
AMR classes of each isolate alone	**5**	**1**	**3**	**2**	**4**	**5**	**6**	**6**
AMR classes of both isolates	**6**		**5**		**5**		**6**	

S: susceptible N: non-susceptible

Detection of integron classes:

***Integron Classes in APEC*:**

Out of the inspected 13 APEC isolates, *int*1 was detected in nine (69.23%) and it was detected either alone (four isolates) or combined with *int*3 (five isolates). On the other hand, *int*2 was not detected in the APEC isolates.

***Integron Classes in Salmonella spp.*:**

The three integron classes were reported in five *Salmonella* isolates with 41.67% overall prevalence. In the 12 tested isolates, *int*1 and *int*3 were more prevalent (4/12, 33.33% each) than *int*2 (2/12, 16.67%). Different combination was also reported, *int*1, *int*2 and *int*3 (one isolate), *int*1 and *int*3 (two isolates), *int*2 and *int*3 (one isolate) and *int*1 alone (one isolate).

Table (5): Integron classes in APEC and *Salmonella* spp. Isolated from broiler chicken revealed respiratory signs.

Integron classes	APEC 13 isolates		*Salmonella* spp. 12 isolates	
	Positive No. (%)	Negative No. (%)	Positive No. (%)	Negative No. (%)
Integron class 1	9 (69.23)	4 (30.77)	4 (33.33)	8 (66.67)
Integron class 2	0 (0.00)	13 (100)	2 (13.67)	10 (83.33)
Integron class 3	5 (38.46)	8 (61.54)	4 (33.33)	8 (66.67)

%: calculated in relation to the examined number of each bacterial species

DISCUSSION

APEC and *Salmonella* spp. were isolated in the rates of 57 and 12% respectively amongst 100 broiler chicken farms presented respiratory signs. The APEC prevalence nearly matched to 52% prevalence reported by **Roy *et al.* (2012)** while they reported higher prevalence of *Salmonella* spp. (30%). On the other hand, in Egypt, **Osman *et al.* (2010)** reported closely matched *Salmonella* spp. prevalence (12.6%) and higher prevalence (22.7%) was noted by **Helmy (2015)**. Regarding APEC variable prevalence was reported by scholars; higher prevalence (66.67%) was noted by **Abd El-Fatah *et al.* (2011)** and lower prevalence (41.67%) was noted by **Moawad (2007)**. The variation in the prevalence of the APEC and *Salmonella* spp. amongst different studies of the broiler chicken could be attributed to the chick sources and the farms' biosafety and biosecurity policies **(Lahellec *et al.*, 1986; Mo *et al.*, 2016)**.

Serotyping of *Salmonella* spp. revealed the presence of eight *Salmonella* serovars including *S.* Blegdam, *S.* Cremieu *S.* Newport and *S.* Virchow (two each); *S.* Enteritidis, *S.* Ferruch, *S.* Gueuletapee and *S.* Paratyphi A (one each) and all (except *S.* Gueuletapee) are of potential zoonotic risk **(CDC, 2013)**. *S.* Newport, *S.* Enteritidis and *S.* Virchow were previously isolated from diseased chicken in Egypt **(Osman *et al.*, 2010; Helmy, 2015)**. *S.* Blegdam was isolated from chicken in Lebanon **(Dalloul, 1995)**, while *S.* Cremieu was isolated from chicken in China **(Lai *et al.*, 2014)**. *S.* Ferruch and *S.* Gueuletapee were previously isolated from retail chicken in China and Egypt by **Wu *et al.*, 2013 and Hassan *et al.*, 2016**

respectively. The most critical and astonishing finding of the present study is the isolation of *S*. Paratyphi A from chicken for the first time to the best of our knowledge that is previously known as strict human pathogen **(Crump *et al*., 2004)**. Subsequently, it could reach human via food chain and increase the rate of paratyphoid fever.

APEC and *Salmonella* spp. high rates of resistance revealed perilous situation of their cure probability (Tables 2 and 3). The highest resistance rates were to trimethoprim-sulfamethoxazole (82.46 and 66.67%), ciprofloxacin (77.19 and 75.00%), doxycycline (49.12 and 58.33%), cefotaxime (59.65 and 50.00%) and gentamicin (43.86 and 58.33%) for APEC and *Salmonella* spp. respectively. The availability and the affordability of the traditional antimicrobials favor their routine unwise use in Egypt and then the upsurge in the antimicrobial resistance rate **(Van *et al*., 2012)**. It also could explain the high rate of MDR observed in APEC (96.49%) and *Salmonella* spp. (75%) isolated from diseased broiler chicken. Cefepime non-susceptibility was noted in APEC (19.67%) and *Salmonella* spp. (16.67%) despite its non-use in veterinary practices. All cefepime non-susceptible isolates also resisted cefotaxime and it could be explained by the presence of ESBLs that hydrolyze both third (cefotaxime) and fourth (cefepime) generation cephalosporin **(Bradford, 2001)**. APEC and *Salmonella* spp. mixed infection was of negative impact on farms by increasing the left behind treatment options by influence summation (Table 4). It was obvious that APEC and *Salmonella* spp. isolated from the same farms in two cases revealed nearly similar antimicrobial resistance profile. Gene responsible for these resistance phenotypes (aztreonam, cefotaxime, ceftazidime, doxycycline, trimethoprim-sulfamethoxazole, gentamicin and ciprofloxacin) are carried on mobile genetic elements so this similarity could be a result of these mobile genetic elements transmission between the two bacterial species **(Poppe *et al*., 2005)**.Integrons profiling of 13 selected isolates of APEC for the three integrons classes revealed the presence of *int*1 and *int*3 but not *int*2. *Int*1 was more prevalent (9/13, 69.23%) than *int*3 (5/13, 38.46%) and these results conceded with the previous report of **Labbate *et al.* (2009)** who stated that class 1 integrons prevails the Gram-negative bacterial species. In Egypt, **Awad *et al.* (2016)** detected integron class 1 (29.3%) and class 2 (3.4%) but not class 3. Regarding *Salmonella* spp., amongst the 12 explored isolates the three integron classes were represented. But, *int*1 and *int*3 were more prevalent (4/12, 33.33% each) than *int*2 (2/12, 16.67%). In a previous study explored the prevalence of *int*1 and *int*2 in *Salmonella* spp. isolated from Egypt **(Ahmed *et al*., 2009)**, the *int*1 was nearly in line (39.1%),

while the *int*2 (8.7%) was lower than our results. To the best of our knowledge, it is the first time to detect *int*3 in both APEC and *Salmonella* spp. in Egypt. The existence of integrons in APEC and *Salmonella* spp. consider a potential risk to capture resistance genetic cassettes and then disseminate them intra and/or inter bacterial species **(Labbate *et al*., 2009)**.

Isolation of the strict human *S.* Paratyphi A from broiler considers a terror as retail chicken could be a source for paratyphoid fever in human. Additionally, the risk that animals and humans face by the intensity of antimicrobial resistance and mixed infection exaggerate the situation. The existence of integrons in APEC and *Salmonella* spp. with the detection of the class 3 integron for the first time in Egypt considers a potential risk to capture resistance genetic cassettes and then disseminate them intra and/or inter bacterial species.

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صور المقاومة البكتيرية والإنتيجرونات لعترات السالمونيلا والإيشريشيا كولاى المعزولة من دجاج التسمين

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3.قسم البكتريولوجى - معهد بحوث صحة الصحة الحيوانية بالفيوم

المستخلص العربى

إن الفصائل البكتيرية لعائلة اللإنتيروبكتريسى يمكنها إصابة مختلف أجهزة جسم دجاج التسمين وعندما تصيب الجهاز التنفسى فإنها تؤدى على تبعيات خطيرة. فبدارسة 100 مزرعة من دجاج التسمين بها إعراض تنفسيها فإن الإيشريشيا كولاى وفصيل السالمونيلا تم عزلهم بنسب 57 و 12% على التوالى كما وجد إصابة 4% من المزارع المفحوصة بكلا نوعى البكتيريا. وبالتصنيف السيرولوجى لمعزولات السالمونيلا فتأكد تواجد ثمانية أنواع من العترات المختلفة منها *S.* Blegdam, *S.* Cremieu *S.* Newport and *S.* Virchow (بنسبة 16.67% لكل منها على حدى) وكذلك عترات Enteritidis, *S.* Ferruch, S. Gueuletapee and S. Paratyphi A. (بنسبة 8.33% لكل منها على حدا). ويعتبر عزل Paratyphi A والمعروف بأنها عترة خاصة بالإنسان وضع مرعب لصحة الإنسان. وبفحص كلاً من عترات الإيشريشيا كولاى والسالمونيلا للإختبار الحساسية للمضادات البكتيرية وجد إرتفاع فى نسبة المقاومة ضد معظم المضادات البكتيرية المستخدمة فى مجال صناعة الدواجن ومنها الترايميثوبريم (82.46، 66.67%)، السيبروفلوكساسين (77.19، 75%)، الدوكسيسيلكين(49.12، 58.33%)، السيفوتاكسيم (59.65، 50%) والجينتاميسين (43.86، 58.33%) لعترات الإشيريشيا كولاى والسالمونيلا على التوالى. كما وجد أن نسبة المقاومة المتعددة للمضادات البكتيرية كانت 75 و96.49% فى عترات السالمونيلا والإشيريشيا كولاى على التوالى. كما أن الإصابة بفصيلى السالمونيلا والإيشريشيا كولى زاد من عدد المضادات البكتيرية الغير فعالة فى بعض الحالات. وبفحص بعض عترات السالمونيلا والإيشريشا كولاى لوجود الإنتيجرونات (1، 2، 3)، وجد كلاً من النوعين 1و 3 من الإنتيجرونات فى كلا الفصيلين وكان نسبة تواجد النوع إنتيجرون 1 أعلى من إنتجرون 3. وعلى الجانب الأخر وجد إنتيجرون 2 فى فصيل السالمونيلا فقط وكان أقلهم. وقد يكون دجاج التسمين ليس مصدراً فقط للبكتيريا المسببة للأمراض المشتركة ولكنها أيضاً قد تكون مصدر للإنتيجرونات والتى هى عناصر جينية متنقلة لها القدرة على الإمساك بجينات المقاومة الدوائية ونشرها ما بين البكتيريا من نفس الفصيل أو بين الفصائل البكتيرية المختلفة ومن ثم تعود المقاومة الدوائية بالبشرية إلى عصر ما قبل إكتشاف المضادات الحيوية.