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Antimicrobial Resistance and Integron Profiles of *Salmonella* Serovars and *Escherichia coli* Isolated from Broiler Chicken

[1]Fawzy R. El-Saedy, [2]Abeer A.E. Shehata and [3]Shaza Z. Mohamed

[1]Department of Bacteriology, Mycology and Immunology,
Faculty of Veterinary Medicine, Beni-Suef University, Beni-Suef, Egypt
[2]Department of Bacteriology, Animal Health Research Institute,
Fayoum Laboratory, Agricultural Research Center, Egypt
[3]Department of Bacteriology, Animal Health Research Institute,
Beni-Suef Laboratory, Agricultural Research Center, Egypt

Abstract: Bacterial species of family *Enterobacteriacae* could infect various body systems of broiler. 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 circulation 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.* Gueulatapee and *S*. Paratyphi A. (1/12, 8.33 each). The isolation of strict human serovar, *S*. Paratyphi is astonishing and giant hazard for human health. Antimicrobial susceptibility testing revealed the high prevalence of resistance against the most commonly used antimicrobial 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 exaggerated the antimicrobial resistance in most of the farms. Class 1 and 3 integrons were represented in both *Salmonella* spp. and APEC. Integron class 1 was the most prevalent followed by integron class 3. On the other hand, class 2 was detected only in *Salmonella* spp. and was the least one amongst them. Broiler could be a source of not bacterial species of zoonotic potential 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.

Key words: APEC · Broiler · Integron · *Salmonella* Paratyphi · Resistance

INTRODUCTION

Birds possess unique structure of the respiratory system with unidirectional air flow and long stay of air in the parabronchi to supply birds with the required amount of oxygen. This eerie structure could favor bacterial pathogenesis via colonization then adhesion and invasion. Avian pathogenic *Escherichia coli* (APEC) and *Salmonella* (*S*.) species get the benefit from this circumstance contributing to various disease conditions and result in great economic loses via mortalities, reduced growth rate and slaughter condemnation [1].

The prodigious diversity of the APEC and *Salmonella* serotypes stands as an obstacle in front of the effectiveness of the available vaccines against both infectious agents as they mostly protect against the homologous serotype. Poulterers use antimicrobial as a protective measure throughout the production cycle in order to prevent infection but it usually misses the mark and infection occur.

Chemotherapy is the prime aid to control bacterial infection in broiler chicken infected with APEC and *Salmonella* spp., but treatment failure becomes a usual upshot as a result of over use and misuse of

Corresponding Author: Abeer A.E. Shehata, Department of Bacteriology, Animal Health Research Institute Fayoum Laboratory, Agricultural Research Center, Egypt. E-mail: aae_shehata@yahoo.com.

antimicrobials in poultry industry that favor emergence and dissemination of Super Bugs [2] which of no doubt pose a potential threat to public health [3, 4].

Propagation of microbial resistance usually occurs via transmission of mobile genetic elements 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 contribute to the horizontal dissemination of antimicrobial resistance in the same bacterial species, different bacterial species, different hosts or different geographical areas via international travel or trade [5].

Surveillance of AMR and reveal its origins in agents of zoonotic bacteria would signify how to apprehend and control AMR [6]. This study aimed at analyzing the antimicrobial resistance amongst the APEC and *Salmonella* spp. isolated from chicken revealed respiratory manifestation against the commonly used antimicrobials in veterinary and/or human medicine with special reference to the presence of different classes of integrons.

MATERIALS 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 showing lesion of respiratory affections (heart, liver and lung) were targets for isolation of *E. coli* and *Salmonella* spp.

Isolation and Biochemical Identification: Isolation and identification of *E. coli* and *Salmonella* spp. were done in accordance to Collee *et al*. [7].

Serological Identification of *Salmonella* spp.: *Salmonella* serovars were documented according to Grimont and Weill [8].

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) [9]. 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.

Table 1: Oligonucleotide primers used for integrons detection in *E. coli* and *Salmonella* serovars

Gene		Primers sequences	Amplified amplicon (bp)	Reference
Int1	F	CCTCCCGCACGATGATC	280	[10]
	R	TCCACGCATCGTCAGGC		
Int2	F	TTATTGCTGGGATTAGGC	250	[11]
	R	ACGGCTACCCTCTGTTATC		
Int3	F	AGTGGGTGGCGAATGAGTG	484	
	R	TGTTCTTGTATCGGCAGGTG		

Detection of Integron Classes 1, 2 and 3: Integron class 1, 2 and 3 were detected in accordance to Bass *et al*. [10] and Goldstein *et al*. [11] amongst some selected APEC and *Salmonella* serovars represented the various antimicrobial susceptibility patterns. Table (1) reveals the target genes, primer sequences and the expected amplified amplicon size.

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 species was noted in 4 (4%) farms.

Serological Identification of *Salmonella* spp.: Serotyping of the recovered 12 isolates belonged to *Salmonella* spp. revealed the circulation 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. Gueulatapee* and *S. Paratyphi* A (1/12, each).

Antimicrobial Susceptibility Profile

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 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 59.65, 49.12 and 43.86% in order. Growing non-susceptibility was reported against cefepime (19.30%) and colistin (15.79%) as table (2) illustrates. Of note, all the isolates that were cefepime non-susceptible were also resistant to cefotaxime.

Table 2: Antimicrobial susceptibility pattern of APEC recovered from broiler chicken with respiratory manifestations

	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

Table 3: Antimicrobial susceptibility pattern of *Salmonella* serovars recovered from broiler chicken with respiratory manifestations

	Susceptible		Intermediate		Resistant	
Antimicrobial	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 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. Similar to APEC, growing resistance was recorded towards 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.

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 in two out of them. 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).

Detection of Integron Classes:

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

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

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

Table 5: Ingeron classes were detected in APEC and *Salmonella* spp. isolated from broiler chicken suffered respiratory manifestation

	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)	13 (100)	2 (13.67)	10 (83.33)
Integron class 3	5 (38.46)	8 (61.54)	4 (33.33)	8 (66.67)

DISCUSSION

In the present work, a total of 100 broiler farms with respiratory manifestations were investigated bacteriologically for the isolation of APEC and *Salmonella* spp. during the period from September and December 2015. The prevalence of APEC and *Salmonella* spp. were 57 and 12% respectively. The APEC prevalence is closely matched to 52% prevalence reported by Roy *et al*. [12] while he reported higher prevalence of *Salmonella* spp. (30%). On the other hand, in Egypt, Ossman *et al*. [13] reported closely matched *Salmonella* spp. prevalence (12.6%) and higher prevalence (22.7%) was noted by Helmy [14]. Regarding APEC variable prevalence was reported by scholars; higher prevalence (66.67%) was noted by Abd El-Fatah *et al*. [15] and lower prevalence (41.67%) was noted by Moawad [16]. The variation of the prevalence of the APEC and *Salmonella* spp. amongst different studies of the broiler chicken could be attributed to the source of chick, biosecurity measures of the farm and management system.

Serotyping of *Salmonella* spp. revealed the circulation 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 [4]. *S*. Newport, *S*. Enteritidis and *S*. Virchow were previously isolated from diseased chicken [13, 14]. *S*. Blegdam was isolated from chicken in Lebanon [17], while *S*. Cremieu was isolated from chicken in China [18]. *S*. Ferruch and *S*. Gueuletapee were previously isolated from retail chicken [19, 20]. 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 [21]. This finding could solve the query behind the increase in partyphoid fever cases in Japanese travellers returning from Cambodia in 2013 [22].

Antimicrobial susceptibility testing of the APEC and *Salmonella* spp. revealed perilous situation of the colibacillosis and salmonellosis antimicrobial treatment options, since high rates of resistance were reported against the commonly used antimicrobial classes in the field of veterinary medicine (Tables 2 and 3) 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. The availability and the affordability of the traditional antimicrobials favor their routine unwise use in Egypt and then the progressive upsurge in the antimicrobial resistance rate [23] which also could explain the high rate of MDR observed in APEC (96.49%) and *Salmonella* spp. (75%). Even though the unused of cefepime in veterinary medicine, growing rate of non-susceptible pattern was noted in both APEC (19.67%) and *Salmonella* spp. (16.67%) and all of them resisted cefotaxime so it could explained by the presence of ESBLs that hydrolyze both third (cefotaxime) and fourth generation cephalosporins (cefepime) [24].

Overviewing the effect of mixed infection on the antimicrobial treatment approach, Table (4) illustrates its negative influence by increasing the left behind options of treatment in two farms by summation influence. 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 transfer of these mobile genetic elements between the two bacterial species [25].

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 concede with the previous report of Labbate *et al*. [26] who stated that class 1 integrons prevails the Gram-negative bacterial species. Additionally, Awad *et al*. [27] 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 *int1* and *int2* in *Salmonella* spp. isolated from Egypt [28], 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 *int3* 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 [26].

Isolation of the strict human *S.* Paratyphi A from broiler is an alarm for potential transmission of this serovar to humans through retail chicken to cause typhoid fever. 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 consider a potential risk to capture resistance genetic cassettes and then disseminate them intra and/or inter bacterial species.

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