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Phenotypic and genotypic characterization of avian pathogenic *E. coli* in airsacculitis of broiler chickens

Ahmed H. Abed[1*], Ahmed H. Orabi[2], Mounir M. Al-Safty[3], Hend A.A. Hashish[1], AlZahraa M.H. Meabed[4]

[1]*Bacteriology, Mycology and Immunology Department, Faculty of Veterinary Medicine, Beni-Suef University, Egypt.*
[2]*Microbiology Department, Faculty of Veterinary Medicine, Cairo University, Egypt.*
[3]*Central Laboratory for Evaluation of Veterinary Biologic, Egypt.*
[4]*Microbiology and Immunology Department, Faculty of Pharmacy, Nahda University, Egypt.*

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*Correspondence:

Corresponding author: Ahmed H. Abed
E-mail address: ahmed.moawad@vet.bsu.edu.eg

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ABSTRACT

Respiratory tract affections in poultry have significant economic importance worldwide. The current work focused on airsacculitis syndrome and phenotypic and genotypic characterization of *E. coli* associated with airsacculitis in broiler chickens. A total of 105 airsacculitis samples were collected aseptically from both diseased and freshly dead broiler chickens at different farms in El-Fayoum, Beni-Suef and El-Menia Governorates. The prevalence of airsacculitis in the examined broiler chickens was 43.9%. Bacteriological examination of the samples collected showed that a total of 94 *E. coli* isolates were recovered with a prevalence of 89.5%. All *E. coli* isolates were serogrouped into 10 O-serogroups ordered as follows, O91 (19.1%), O2 (16%), O127 (13.8%), both O18 & O26 (9.6%, for each), both O8 & O153 (7.4% for each), O78 (6.4%), and finally both O1& O44 (5.3% for each). The results of in-vitro antimicrobial susceptibility tests revealed that *E. coli* isolates mostly showed high resistances against β-lactams, gentamicin and sulfa antimicrobials meanwhile showed high sensitivities against fluoroquinolones, phenicols and tetracyclins. All isolates have been subjected to both combined disc diffusion (CDD) and cefinase tests for phenotypic detection of ESβLs production, which was confirmed in 50% and 61.7% of isolates, respectively. Haemolytic activity and biofilm formation were phenotypically detected in all *E. coli* isolates. PCR was conducted on 11 MDR isolates for determination of 5 resistance genes, *ampC*, bla_{CTX} and bla_{TEM}, *aad*B and sul1. The results revealed that all the tested *E. coli* isolates had *ampC*, bla_{CTX} and bla_{TEM}, while 90.1% and 27.3% of tested isolates had sul1 and *aad*B genes, respectively. Also, PCR was conducted to detect 5 virulence genes, *iss*, *iut*A, *pap*C, *fim*H and *hly*A. The results revealed that *iut*A and *fim*H genes were found in all the tested isolates, while *iss*, *pap*C and *hly*A genes were represented as follows, 90.1, 63.6 and 54.5%, respectively. It was concluded that *E. coli* is the most common bacterial isolates of airsacculitis that phenotypically has high antimicrobial resistance patterns, ESβLs production, haemolytic activity and biofilm production. All these phenotypic characters are supported by detection of their encoding genes. Such highly virulent and antimicrobial resistant strains are major health concerns as they could be transmitted to human.

Introduction

In Egypt, poultry sector is playing an essential role in the provision of animal protein, either meat or egg, to human (Radwan *et al*., 2021a). Moreover, it is one of the fastest growing industries playing a vital role in the national economy with investments reaching over 90 billion Egyptian pound. It provides manure for crops in addition to employment for approximately three million employees (Sabry, 2019). Therefore, occurrence of diseases represents a real threat to Egyptian poultry industry.

Poultry farms are susceptible to variable respiratory infections with different clinical manifestations that result in drastic effect on the poultry industry because of decreasing the produced protein as well as transmitting of zoonotic diseases (Contreras *et al*., 2007). Therefore, such infectious diseases cause high economic losses in poultry farms and it is considered one of the major problems in the poultry industry.

Airsacculitis, inflammation of the air sacs, is one of the respiratory manifestations caused by many bacterial and viral agents. Bacterial respiratory pathogens mostly colonize the respiratory tract following a primary viral infection (such as infectious bronchitis, avian influenza and Newcastle disease viruses), other bacterial pathogens (such as Mycoplasma gallisepticum (MG), and Mycoplasma synoviae (MS) or environmental factors, such as high ammonia levels (Abdelrahman, 2022).

Colibacillosis indicates localized or systemic infections caused by avian pathogenic *E. coli* (APEC) including many forms as colisepticemia, coligranuloma, CRD (airsacculitis), and other manifestations (Yue *et al*., 2018). APEC is considered one of the most important bacterial pathogens of major importance that can act as primary causes of the respiratory disease (Abed, 2007). On the other hand, respiratory colibacillosis may be caused by secondary infection with APEC. *E. coli* colonization in air sacs of chicken after viral or MG infections is considered an ideal example of secondary bacterial infection. In broiler chickens, *E. coli* causes significant economic losses in all ages as a result of reduction in growth, production, hatching rate and feed conversion efficiency in addition to high mortality, carcass condemnation during processing, and treatment costs (Abed, 2007).

Antimicrobial resistance (AMR) and emergence of multidrug resistance (MDR) in bacteria due to antibiotics misuse is one of the most important problems facing bacterial infections therapy. Food animals and their environments are involved as reservoirs for AMR bacterial pathogens as well as AMR genes that may be transferred to humans directly, by contact, or indirectly, via the food production chain (WHO, 2011). Mobile DNA elements (plasmids, transposons & integrons) are the main vectors in the AMR genes dissemination between bacterial species (Radwan *et al*., 2016).

Extended Spectrum β-Lactamases (ESβLs) in addition to plasmid-mediated *amp*C β-lactamases represent great risk in the bacterial infections therapy as they have necessary roles in the induction of AMR (Carmo *et al*., 2014). ESβLs are globally reported and have been related to successful enterobacterial clones with great epidemics (Zahar *et al*., 2009). ESβLs enzymes can hydrolyze different β-lactam antibiotics as well as the third and fourth cephalosporins generations and monobactams and can be inhibited by clavulanic acid (CA) (Poulou *et al*., 2014). ESβLs coding plasmids may also carry other AMR genes of other classes resulting in limiting the therapy options for ESβL producers and enhancing the ESβLs dissemination inter and intra-species (Zahar *et al*., 2009).

Therefore, phenotypic detection of ESβLs producing bacteria is necessary for epidemiological purposes as well as limitation of AMR mechanisms. The combined disc diffusion (CDD) test was recommended for

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phenotypic confirmation of ESβL production (CLSI, 2015) depending on determination of the growth-inhibition zones of cephalosporin's' discs (ceftazidime & cefotaxime) with or without CA. There are many produced groups of EsβLs, but the most frequent enzyme types detected in clinical Gram-negative isolates were TEM, CTX-M and SHV (Bush and Fisher, 2011).

For APEC pathogenicity, the existence of 8-13 virulence genes was necessary in highly pathogenic strains meanwhile 5-8 genes in intermediate pathogenic strains (Wang *et al*., 2016). The pathogenic strains firmly carried the virulence gene pattern of fimbriae adhesion genes (*fim*H, *fim*A), and pilus adhesion protein (*pap*C), iron uptake system (*iut*A) and temperature sensitive haemagglutinin (tsh) (El-Shazly *et al*., 2017). The present study was designed for the phenotypic and genotypic characterization of APEC associated with airsacculitis in broiler chickens.

Materials and methods

Ethical approval

Animal care was approved by the Institutional Animal Care and Use Committee of Beni-Suef University (BSU-IACUC), Egypt. The ethical approval number was BSU-IACUC-022-254.

Broiler chickens

A total of 239 of both diseased and freshly dead Hubbard and Ross broiler chickens (2-5 weeks age) from different farms in El-Fayoum, Beni-Suef and El-Menia Governorates were subjected to this study during the period from January to October 2023. These chickens were subjected to both clinical and/or postmortem examinations for detection of airsacculitis.

Collected samples

A total of 105 airsacculitis samples were collected aseptically from slaughtered diseased and freshly dead broiler chickens. All collected samples were assigned serial numbers, detailed information were recorded and then were transferred directly under chilled conditions to the laboratory of Bacteriology, Myology and Immunology Department, Faculty of Veterinary Medicine at Beni-Suef University, Egypt.

Bacteriological isolation and identification

The collected samples were aseptically cut into small pieces and inoculated into MacConkey's broth and then incubated aerobically at 37˚C for 24 hrs. Afterthat, a loopful from the inoculated broths were streaked onto and MacConkey's agar and incubated aerobically at 37˚C for 24-48 hrs.

The lactose fermenting (pink) colonies were picked up and inoculated onto eosin methylene blue agar medium and then incubated at 37˚C for 24-48 hrs. Colonies having characteristic greenish colour with metallic sheen were considered presumptively *E. coli* and confirmed morphologically and biochemically using the standard biochemical tests described by Collee *et al*. (1996) and Quinn *et al*. (2011) including oxidase, catalase, indole, methyl red (MR), Voges Proskauer (VP), citrate utilization, urease, nitrate reduction and H2S production on TSI medium and as well as sugar fermentation. Other non-biochemical tests including motility test in semisolid agar medium and haemolysis on blood agar were applied.

Further biochemical confirmation was applied using the Analytical Profile Index (API 20 E) system (BioMérieux, France) according to the manufacturer's instruction.

Serogrouping of E. coli isolates

E. coli isolates were serogrouped by slide agglutination test using standard polyvalent and monovalent *E. coli* antisera according to Quinn *et al*. (2011).

Antimicrobial susceptibility testing

All *E. coli* isolates were tested for their antimicrobial sensitivity to 14 different antimicrobial discs representing 7 antimicrobial classes using disc diffusion method on Muller Hinton agar that was conducted according to the technique described by Clinical and Laboratory Standards Institute (CLSI, 2021). The antimicrobial discs used were penicillin (P, 10µg), amoxycillin-clavulanic acid (AMC, 30µg), cefuroxime (CXM, 30µg), ceftazidime (CAZ, 30µg), cefotaxime sodium (CTX, 30µg), cefepime (FEP, 30µg), gentamicin (CN, 10µg), kanamycin (K, 30µg), amikacin (AK, 30µg), ciprofloxacin (CIP, 5µg), levofloxacin (LEV, 5µg), doxycycline HCl (Do, 30µg), florophenicol (FFC, 30µg), and sulphamethoxazol-trimethoprim (STX, 25µg) (Oxoid Ltd., Basing Stoke, UK).

Phenotypic detection of ESβLs producing isolates

Detection of ESβLs producing isolates using combined disc diffusion (CDD) test (CLSI, 2021)

ESβL production was tested with the CLSI confirmatory test using CDD test by using CXM, CAZ, CTX and FEP discs alone and alongside AMC disc. The CXM, CAZ, CTX and FEP discs were manually placed in the plate around AMC disc with 20 mm center to center then incubated at 37ºC overnight. The tested isolates were taken to produce ESβL when increasing the zone of growth-inhibition diameter around one or more of discs alongside AMC to at least 5 mm (i.e. ≥5 mm) against the agent's zone diameter when tested alone.

Detection of ESβLs producing isolates using Cefinase discs

Cefinase™ discs (BioMérieux, Marcy-l'Etoile, France) impregnated with nitrocefin were used in cefinase test for phenotypic rapid detection of ESβLs in different bacterial isolates according to the manufacturer's instruction.

Phenotypic detection of biofilm formation using microtiter plate assay (Stephanovic et al., 2000).

Overnight isolate cultures were diluted within 5 ml tryptic soy broth (TSB, Oxoid) supported using 0.5% w/v sodium chloride and subjected to incubation with shaking at 37◦C to OD of 0.8 at 620 nm. The cultures underwent dilution in the ratio of 1:40 with fresh NaCl/TSB and 150 µl were incorporated into all wells of a 96-well microtiter plate (HDPs existed from seeding in certain experiments). The para-film was used for sealing the plates, which were then incubated for 2 days at 37◦C. Active HDPs were used for treatment in TSB for 1 day or distilled water (DW) as the positive control. Crystal violet (CV) was utilized for biofilm staining. Shortly, after washing three times with DW, CV was used on the biofilms for 15 min. and removed, the biofilms were washed three-times using DW and the remainder of CV solubilized using 30% v/v acetic acid. The A620 nm was measured with a micro-plate reader (LabSystems, Multiskan EC). Average OD620 nm value of positive control was considered standard. Those values exceeding 0.2 were regarded as high producers of biofilm. Values that were less than 0.081 were classified into non- or low-biofilm producers. All assays were conducted in triplicate and redone three times.

The magnitude of biofilm production was ascertained through the formula: BF = AB/CW, where BF represents the formation of biofilm, AB represents the OD620 nm for stained attached bacteria whereas CW denotes the OD620 nm for stained control wells containing only bacteria-free medium (abiotic or unspecific factors).

For every assay, 16 wells for each strain were assessed, and the assays

were conducted in triplicate, this resulted in an overall of 48 wells for every tested control or strain.

Polymerase chain reaction (PCR) for E. coli isolates

PCR was conducted on 11 MDR *E. coli* isolates, representative for all the identified serogroups, for determination of 10 genes, 5 resistance encoding genes including resistance genes for β-lactams (*amp*C, bla_{CTX} and bla_{TEM}), aminoglycoside (*aad*B) and sulfonamide resistance (*sul*1), as well as 5 virulence encoding genes including genes encoding for increased serum survival protein (*iss*), iron uptake system (*iut*A), bacterial adhesion (*pap*C, Pilus adhesion and *fim*H, Type 1 fimbria adhesion) and α-haemolysin (*hly*A). The sequences and specificities of the primers (Metabion, Germany) as well as the size of the amplified products were summarized in Table 1.

Cycling conditions of the different primers in *E. coli* isolates during PCR were illustrated in Table 2.

Results

Prevalence of airsacculitis in the examined broiler chickens

The P.M. examination results of both slaughtered diseased and freshly dead Hubbard and Ross broiler chickens revealed that out of 239 examined chickens, 105 chickens showed airsacculitis with a prevalence of 43.9%.

Prevalence of E. coli isolates in diseased broiler chickens

Out of 105 airsacculitis sample, 94 *E. coli* isolates were recovered with a prevalence of 89.5%.

Serogrouping of E. coli isolates

Out of 94 *E. coli* isolates, 10 O-serogroups were obtained. The most prevalent serogroup was O91 which was represented in 18 isolates (19.1%) followed by the serogroups O2 (15 isolates, 16%), O127 (13 isolates, 13.8%), both O18 and O26 as 9 isolates (9.6%, for each), both O8 and O153 (7 isolates, 7.4% for each), and O78 (6 isolates, 6.4%). On the other hand, both O1 and O44 serogroups had the lowest prevalence, represented in 5 isolates (5.3%) for each.

In-vitro antimicrobial susceptibility test of E. coli isolates

The highest antimicrobial resistance of *E. coli* isolates was recorded against penicillin (89.4%) followed by sulfamethoxazole/trimethoprim (83%) and then gentamicin (78.7%). After that, ceftazidime, cefuroxime, cefotaxime sodium, cefepime and amoxicillin-clavulanic acid showed the following resistances, 71.3, 69.1, 68.1, 61.7 and 59.6%, respectively. Conversely, the highest sensitivity was recorded against levofloxacin as 78.7% followed by ciprofloxacin (76.6%), florophenciol (72.3%) and doxycycline HCl (70.2%). Then, amikacin and kanamycin showed sensitivity as 62.8 and 53.2%, respectively (Table 3).

Table 1. Primers sequences& amplified products for the different targeted genes for *E. coli* isolates.

Primers		Primer Sequence 5′-3′		Amplified product	Reference
Virulence genes	*iss*	F	*ATGTTATTTTCTGCCGCTCTG*	266 bp	Yaguchi *et al.* (2007)
		R	*CTATTGTGAGCAATATACCC*		
	iutA	F	*GGCTGGACATGGGAACTGG*	300 bp	
		R	*CGTCGGGAACGGGTAGAATCG*		
	*pap*C	F	*TGATATCACGCAGTCAGTAGC*	501 bp	Wen-jie *et al.* (2008)
		R	*CCGGCCATATTCACATAA*		
	*fim*H	F	*TGCAGAACGGATAAGCCGTGG*	508 bp	Ghanbarpour and Salehi (2010)
		R	*GCAGTCACCTGCCCTCCGGTA*		
	*hly*A	F	*AACAAGGATAAGCACTGTTCTGGCT*	1177 bp	Piva *et al*. (2003)
		R	*ACCATATAAGCGGTCATTCCCGTCA*		
Resistance genes	*aad*B	F	*GAGCGAAATCTGCCGCTCTGG*	319 bp	Frana *et al*. (2001)
		R	*CTGTTACAACGGACTGGCCGC*		
	bla_{TEM}	F	*ATCAGCAATAAACCAGC*	516 bp	Colom *et al*. (2003)
		R	*CCCCGAAGAACGTTTTC*		
	*amp*C	F	*TTCTATCAAMACTGGCARCC*	550 bp	Srinivasan *et al*. (2005)
		R	*CCYTTTTATGTACCCAYGA*		
	*sul*1	F	*CGGCGTGGGCTACCTGAACG*	433 bp	Ibekwe *et al*. (2011)
		R	*GCCGATCGCGTGAAGTTCCG*		
	bla_{CTX}	F	*ATGTGCAGYACCAGTAARGTKATGGC*	593 bp	Archambault *et al*. (2006)
		R	*TGGGTRAARTARGTSACCAGAAYCAGCGG*		

Table 2. Cycling conditions of the different primers in *E. coli* isolates during PCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
iss	94˚C/5 min.	94˚C/30 sec.	54˚C/30 sec.	72˚C/30 sec.	35	72˚C/7 min.
*iut*A	94˚C/10 min.	94˚C/30 sec.	63˚C/30 sec.	72˚C/30 sec.	35	72˚C/7 min.
*pap*C	94˚C/5 min.	94˚C/30 sec.	50˚C/40 sec.	72˚C/40 sec.	35	72˚C/10 min.
*fim*H	95˚C/5 min.	94˚C/30 sec.	58˚C/40 sec.	72˚C/45 sec.	35	72C/7 min.
*aad*B	94˚C/5 min.	94˚C/30 sec.	58˚C/40 sec.	72˚C/40 sec.	35	72˚C/10 min.
bla_{SHV}	94˚C/5 min.	94˚C/30 sec	54˚C/40 sec	72˚C/40 sec	35	72˚C/10 min.
*amp*C	94˚C/5 min.	94˚C/30 sec	50˚C/40 sec	72˚C/40 sec	35	72˚C/10 min.
*sul*1	94˚C/5 min.	94˚C/ 30 sec.	60˚C/40 sec.	72˚C/45 sec.	35	72˚C/10 min.
bla_{CTX}	94˚C/5 min.	94˚C/45 sec	55˚C/45 sec.	72˚C/45 sec	35	72˚C/10 min.
*hly*A	94C/10 min.	94C/45 sec.	60C/40 sec.	72C/1 min.	35	72˚C/12 min.

Table 3. Antimicrobial susceptibility testing of *E. coli* isolates.

Class	Antimicrobial agent	Disc content (µg)	*E. coli* tested isolates (*n=94*)					
			R		I		S	
			No.	%	No.	%	No.	%
Penicillins	Penicillin	10	84	89.4	3	3.2	7	7.4
	Amoxycillin-Clavulanic Acid	30	56	59.6	6	6.4	32	34
Cephalosporins	Cefuroxime	30	65	69.1	7	7.4	22	23.4
	Cefepime	30	58	61.7	6	6.4	30	31.9
	Ceftazidime	30	67	71.3	6	6.4	21	22.3
	Cefotaxime Sodium	30	64	68.1	5	5.3	25	26.6
Aminoglycosides	Gentamicin	10	74	78.7	4	4.3	16	17
	Kanamycin	10	39	41.5	5	5.3	50	53.2
	Amikacin	30	28	29.8	7	7.4	59	62.8
Fluoroquinolones	Ciprofloxacin	5	18	19.1	4	4.3	72	76.6
	Levofloxacin	5	15	16	5	5.3	74	78.7
Tetracyclins	Doxycycline HCl	30	22	23.4	6	6.4	66	70.2
Phenicols	Florophenicol	30	22	23.4	4	4.3	68	72.3
Pot. Sulphonamides	Sulfamethoxazole/trimethoprim	25	78	83	2	2.1	14	14.9

%: was calculated according to the number of tested *E. coli* isolates (n=94).

Phenotypic detection of ESβLs in E. coli isolates

In the current work, *E. coli* isolates were mostly resistant to the tested extended spectrum β-lactams compounds. Therefore, all isolates (n=94) have been subjected to both combined disc diffusion (CDD) and cefinase tests for phenotypic detection of ESβLs production which revealed that ESβLs was confirmed in 47 isolates (50%) using CDD test while confirmed in 58 isolates (61.7%) using cefinase test.

Phenotypic detection of some virulence characteristics of E. coli isolates

Haemolytic activity on sheep blood agar

The results of haemolytic activity of *E. coli* isolates on sheep blood agar showed that all isolates (n=94) were haemolytic. Beta haemolysis was the most frequent type recorded in 83 isolates (88.3%), while alpha haemolysis was recorded in 11 isolates (11.7%).

Quantification of biofilm formation using microtiter plate assay.

The quantification of biofilm formation was measured at A620 nm using a microplate reader. The results of quantification of biofilm formation of *E. coli* isolates revealed that all *E. coli* isolates (n=94) showed values exceeding 0.2 and regarded as high biofilm producers. The average value of all *E. coli* isolates was 0.4336±0.0836.

Polymerase chain reaction (PCR) for the examined *E. coli* isolates.

Concerning the resistance encoding genes the results showed that all tested β-lactams genes, *amp*C, bla_{CTX} and bla_{TEM} were found in all the tested *E. coli* isolates (n=11, 100%) while 10 isolates (90.1%) harbored *sul*1 gene meanwhile *aad*B gene was found in 3 isolates only (27.3%).

On the other hand, the results of virulence encoding genes showed that *iut*A and *fim*H genes were the most prevalent and both were found in all the tested *E. coli* isolates (n=11, 100%), followed by *iss* gene which was found in 10 isolates (90.1%) then *pap*C gene, found in 7 isolates (63.6%). Finally, *hly*A gene was found in 6 isolates (54.5%).

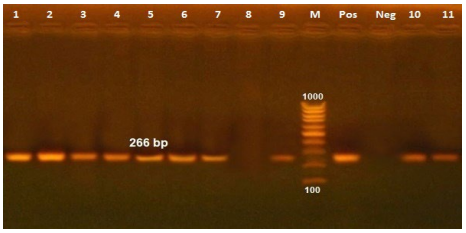

Fig. 1. PCR amplification of the 266 bp fragments of *iss* virulence gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

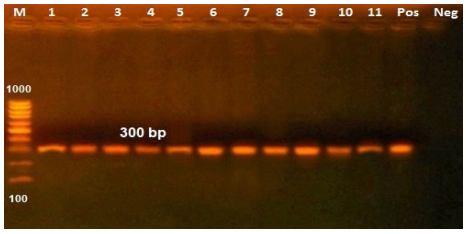

Fig. 2. PCR amplification of the 300 bp fragments of *iut*A virulence gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

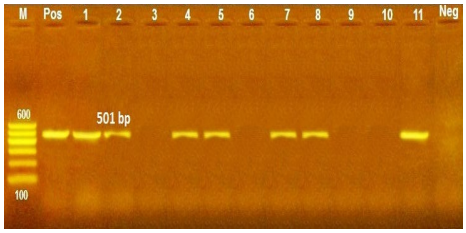

Fig. 3. PCR amplification of the 501 bp fragments of *pap*C virulence gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

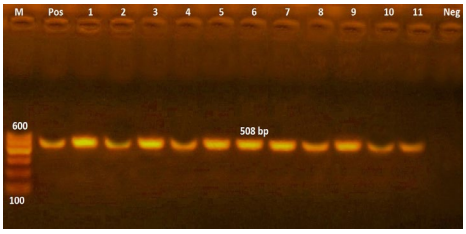

Fig. 4. PCR amplification of the 508 bp fragments of *fim*H virulence gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

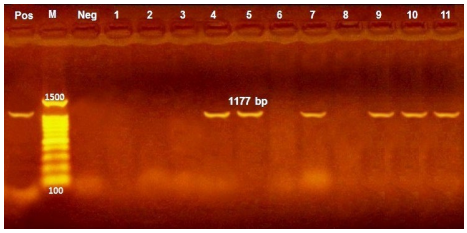

Fig. 5. PCR amplification of the 1177 bp fragments of hlyA virulence gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

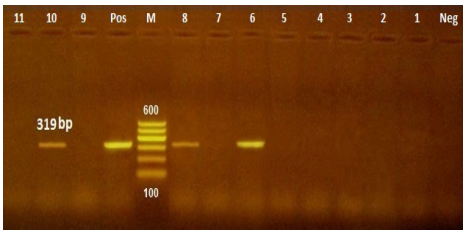

Fig. 6. PCR amplification of the 319 bp fragments of *aad*B resistance encoding gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

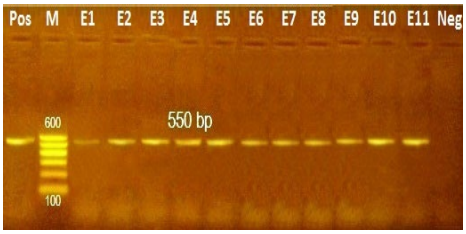

Fig. 7. PCR amplification of the 550 bp fragments of *amp*C resistance encoding gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* isolates Lanes (E1-E11).

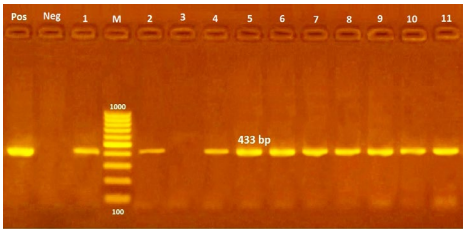

Fig. 8. PCR amplification of the 433 bp fragments of *sul*1 resistance encoding gene in 11 *E. coli* isolates showing positive amplicons migration with the molecular DNA size marker (M), positive control (Pos) (Field identified *E. coli* strain), negative control (Neg) and the tested *E. coli* Lanes (E1-E11).

Discussion

Respiratory diseases of chickens have significant economic importance worldwide. They are more complex syndromes that may have one or more etiologies (bacteria, fungi or viruses), in addition to stress factors exaggerating the disease. These pathogens are considered the most important causative agents infecting poultry resulting in high mortalities and variable morbidity and consequently great economic losses in poultry sectors. Food Standards Agency (FSA) reported that airsacculitis is one of seven P.M conditions that are of public health concern (MLCSL, 2013). Accordingly, great efforts should be paid to eliminate such pathogens (Abed, 2007).

The current work was focused on airsacculitis syndrome and phenotypic and genotypic characterization of APEC associated with airsacculitis in broiler chickens.

In this study, the prevalence of airsacculitis was 43.9%, which was supported by that obtained by Barnes *et al.* (2008) and Syuhada *et al.* (2014) who recorded airsacculitis as one of the primary lesions in CRD cases in broiler chickens. This prevalence was slightly higher than those recorded by Abed (2007), 32.3%, and Gomis *et al.* (2001), 34.6%.

Concerning results of *E. coli* isolation from airsacculitis samples revealed that the prevalence of *E. coli* isolates was 89.5%. This result was supported by that reported by Abed (2007), who found that *E. coli* was the most prevalent isolate from respiratory syndromes. Also, the obtained prevalence was nearly similar to previous studies by El-Sukhon *et al.* (2002), 88.2%, and Abd El-Aziz *et al.* (2007), 90%. Lower results were recorded by Yousseff *et al.* (2008), 68%, and Abed *et al.* (2022), 74.3%. Much lower prevalences were recorded by Gomis *et al.* (2001), 34.6%, Radwan *et al.* (2021a), 26.7%, and Radwan *et al.* (2022), 16.7%.

In the present work, the results of serogrouping of 94 *E. coli* isolates showed that all isolates were serogrouped into 10 O-serogroups. The obtained serogroups were ordered as follows, O91 (19.1%), O2 (16%), O127 (13.8%), both O18& O26 (9.6%, for each), both O8& O153 (7.4% for each), O78 (6.4%), and finally both O1& O44 (5.3% each). Such results were somewhat similar to that recorded by Abed (2007), who identified 6 serogroups ordered as follow, O78, O1, O2, O8, O25, O119 and Yousseff *et al.* (2008) who recorded 6 serogroups, O78, O1, O26, O2, O111and O157. Moreover, Eid *et al.* (2016) identified 11 serogroups, O1, O2, O26, O44, O55, O78, O111, O119, O125, O127 and O128, meanwhile Elshafae (2009) reported 5 serogroups, O78, O20, O125, O86 and O146. Radwan *et al.* (2018) obtained 7 O-serogroups, O125 (32%), O158 (24%), O55 (12%), O78 (10%), O1 (6%), O8 (6%), and O15 (4%). Furthermore, Abed *et al.* (2022) obtained 7 serogroups, O119 (30%), O78 (20%), O25 (20%), O55 (10%), O111 (10%), O1 and O26 (3.33%).

Antimicrobial therapy is one of the most essential tools for controlling infectious bacterial diseases, reducing their prevalence and mortality. Moreover, it could be used in rations as growth promoters at sub-therapeutic doses for maintaining both health and productivity of the birds limiting the high economic losses in the poultry sectors globally (Radwan *et al.*, 2021b). Therefore, in-vitro antimicrobial susceptibility tests are valuable in the selection of the suitable drug in prophylaxis programs and therapy. Also, it is very essential for the detection of MDR bacterial isolates which have public health concern.

In the present work, all *E. coli* (n=94) were tested for their antimicrobial sensitivity against 14 different antimicrobial agents. The results showed high resistance was mostly against β-lactams, gentamicin and sulfa antimicrobials. The highest resistance was recorded against penicillin (89.4%), sulfamethoxazole/trimethoprim (83%), gentamicin (78.7%), ceftazidime (71.3%), cefuroxime (69.1%), cefotaxime sodium (68.1%), cefepime and amoxicillin-clavulanic acid (59.6%). Conversely, high sensitivity was mostly recorded against fluoroquinolones, phenicols and tetracyclins. The highest sensitivity was observed against levofloxacin (78.7%), ciprofloxacin (76.6%), florophenciol (72.3%), doxycycline HCl (70.2%) and amikacin (62.8%). Nearly similar findings were recorded by Radwan *et al.*

(2016) who observed high resistance to β-lactams, gentamicin and sulfa antimicrobials and Radwan *et al*. (2018) who reported high resistance to sulfamethoxazole/trimethoprim and high sensitivity to enrofloxacin and ciprofloxacin. Also, Younis *et al*. (2017) reported high resistance of *E. coli* isolates to β-lactams and high susceptibility to fluoroquinolones and phenicols. Moreover, Radwan *et al*. (2014) and Abed *et al*. (2022) recorded high resistance to β-lactams and sulphamethoxazol/trimethoprim while showed high sensitivity to aminoglycosides. Meanwhile, Radwan *et al*. (2022) recorded high resistance to β-lactams and sulphamethoxazol/trimethoprim and ciprofloxacin.

Other previous studies showed high resistance of *E. coli* isolates against most of the tested antimicrobials including the same resistance patterns to β-lactams, gentamicin and sulfa antimicrobials which were recorded in the current study but also revealed high resistance to fluoroquinolones, phenicols and tetracyclins, which revealed high susceptibility in this study (Hassan *et al*., 2020b; Radwan *et al*., 2021a& b).

ESβLs were considered the main cause of β-lactam resistance in Gram-negative bacteria (Rawat and Nair 2010) and their production might be attributed to the widespread usage of cephalosporins and other β-lactams (Chaudhary and Aggarwal, 2004).

ESβL producing bacteria were defined as those evolve β-lactams resistance through β-lactamases enzymes that can inactivate or hydrolyze antimicrobials with an oxyimino side chain including cephalosporins (3rd& 4th generations) and monobactams as a result of mutations in the plasmid-mediated β-lactamases genes altering the enzyme configuration beside its active site to increase the hydrolytic affinity of the β-lactamase for β lactams antibiotics (Abd El Tawab *et al*., 2016) and they could be inhibited by β-lactamase inhibitors, as clavulanic acid or tazobactams (Bush and Fisher, 2011; Ghodousi *et al*., 2015), meanwhile they are unable to inactivate cephamycins and carbapenems. ESβL and carabepenemases producing bacteria are MDR, which presents a huge risk on health (Rahman *et al*. 2018), and they are more frequently produced by enterobacteriaceae members, especially *E. coli*, and other Gram negative bacteria (Meletis, 2016).

β-lactamases enzymes genes are mainly carried on plasmids (Abd El Tawab *et al*. 2016), in addition to other mobile genetic elements (MGE), including integrons or transposons (Ghodousi *et al*., 2015). The most common enzymes among the ESβLs in clinical isolates were CTX-M (cefotaxime β-lactamases) (Pitout *et al*. 2005), Temoniera (TEM-1&2) (Bush and Fisher, 2011; Ghodousi *et al*., 2015; Abd El Tawab *et al*. 2016) and sulfhydryl variable (SHV-1) (Ghodousi *et al*., 2015; Abd El Tawab *et al*. 2016). Their encoding genes, ESβL genes, could be disseminated among different strains (Ghodousi *et al*., 2015).

CLSI recommended the combined disc diffusion (CDD) test as a phenotypic confirmatory test for detection of ESβL production (CLSI, 2015).

In the current work, all *E. coli* isolates have been subjected to both CDD and cefinase tests for phenotypic detection of ESβLs production and the results revealed that ESβLs was confirmed in 50% and 61.7% of isolates using CDD and cefinase tests, respectively. The obtained results are coincided with that recorded by Awad *et al*. (2016) who found that 58.6% of tested APEC isolates were ESβL producers. Moreover, these results are supported by Habeeb *et al*. (2013) who reported that the prevalence of ESβL in *E. coli* isolates had been significantly increased from 33.7% in 2005 to 60% in 2010. Lower prevalences of ESβL were recorded in Egypt by Bouchillon *et al*. (2004), 38.5%, and El-Shazly *et al*. (2017), 6%.

In the current study, some virulence characteristics such as haemolytic activity and biofilm formation were phenotypically detected in all *E. coli* isolates.

The results of haemolytic activity of *E. coli* isolates on sheep blood agar showed that all isolates were haemolytic, 88.3% of them were β-haemolytic while 11.7% were α-haemolytic.

Such results were reinforced by those obtained by Abd El Halim (2022) who recorded haemolytic activity in 84.6% and 100% of *E. coli*, and P. aeruginosa isolates, respectively, and they were mostly β-haemolytic. Regarding the results of *E. coli* haemolytic activity, such results are nearly similar to that recorded by Abd El-Halim (2020), 92.5%. Meanwhile, lower results were recorded by Yahia (2014), 41%, and Ali *et al*. (2019), 16.3%. Also, Henriques *et al*. (2014) recorded that few *E. coli* isolates were β-haemolytic while the majority was non-hemolytic.

In the current study, the results of the quantification of biofilm formation measured spectrophotometrically at A620 nm using a micro-plate reader showed that all *E. coli* were regarded as high biofilm producers with mean value of 0.4336±0.0836. Such results were nearly similar to that recorded by Hassan *et al*. (2011) who found that most of the tested APEC isolates were biofilm producers and recorded that 22.7, 41 and 36.3% of the tested isolates were strong, moderate and weak/or non-biofilm producers, respectively. They also added that the biofilm producing isolates showed AMR more than non-biofilm producing. Also, Wang *et al*. (2016) recorded that 85.6% of isolates were biofilm producers, arranged as 25.4, 31.3 and 28.9% for strong, intermediate and week biofilm producers, respectively, while 14.4% were non biofilm producers. Somewhat lower results were recorded by Saha *et al*. (2020), 81.7%, and Abed *et al*. (2022), 73%. Moori Bakhtiari *et al*. (2018) identified 16.6 and 53.3% of *E. coli* isolates as strong and intermediate biofilm producers, respectively. Lower results were obtained by several authors, Skyberg *et al*. (2007), 55.2%, Nascimento *et al*. (2014), 29.4%, Rodrigues *et al*. (2019), 55.8%, Nanda and Nayak (2020), 45.5%, Radwan *et al*. (2022), 50%, and Raheel *et al*. (2022), 43.7%.

In the present study, PCR was conducted on 11 MDR *E. coli* isolates for determination of 5 resistance encoding genes, 3 for β-lactams (*amp*C, bla_{CTX} and bla_{TEM}) in addition to 1 for aminoglycoside, gentamicin, (*aad*B) and sulfonamide (*sul*1). The results revealed that all the tested *E. coli* isolates (100%) had all β-lactams genes, *amp*C, bla_{CTX} and bla_{TEM}, while 90.1 and 27.3% of tested isolates had *sul*1 and *aad*B genes, respectively.

Collectively, the current findings were supported by that of Momtaz *et al*. (2012) who investigated some resistance genes, of them bla_{SHV}, $bla_{C\text{-}MY}$, *aad*A1, and aac3-IV, *sul*1, and found that all chickens *E. coli* isolates harbored one gene at least. Zhao (2013) recorded bla_{TEM} gene in all tested *E. coli* isolates and *aad*A1 gene in 45.5% of the isolates. Also, Abed *et al*. (2022) denoted that bla_{TEM} and *sul*1 genes were found in all tested isolates. On the other hand, Abd El Tawab *et al*. (2015) detected *aad*B gene in 26% of *E. coli* isolates.

Regarding the results of ESβL genes, *amp*C, bla_{CTX} and bla_{TEM}, which were recorded in all the tested isolates, such results run parallel to those of Li *et al*. (2016) who studied ESβL producing *E. coli* isolates and found that all isolates have at least one of the *bla* genes ($bla_{TEM\text{-}1}$, $bla_{CTX\text{-}M}$ and $bla_{SHV\text{-}5}$). Also, Abed *et al*. (2022) found that bla_{TEM} genes in all tested isolates. While Radwan *et al*. (2021b) recorded bla_{TEM} gene in all tested isolates while bla_{SHV} gene was only found in 28.6% of isolates. Furthermore, Qurani (2019) found that all the tested isolates harbored *amp*C and bla_{CTX} genes while 93.3% harbored bla_{SHV} gene. Lower prevalences were recorded by Blanco *et al*. (2009) who studied ESβL producing *E. coli* isolates and found that 57, 22, 10 and 7% of them harbored $bla_{CTX\text{-}M\text{-}14}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{SHV\text{-}12}$ and $bla_{CTX\text{-}M\text{-}32}$, respectively. Also, Simmons *et al*. (2016) studied the following β-lactamases genes, bla_{CMY2}, bla_{TEM}, bla_{ACT}, bla_{SHV} and $bla_{CTX\text{-}M\text{-}15}$ which were recorded as follows, 94.5, 24.4, 6.3, 4.7 and 3.2%, respectively. Moreover, Ma *et al*. (2018) recorded bla_{CMY} in 52.9% of isolates while bla_{CTX} was detected only in 17.6% of isolates.

Currently, there is no known complete relation between the virulence genes and the virulence where there is no single gene or specific genes combination is systematically associated with the pathogenic strains. PCR amplification of such virulence genes using specific primers was used for *E. coli* strains screening and constructing the virulence gene profiles. After that, the obtained data were used for confirming whether *E. coli* isolates have similar APEC virulence encoding genes or not (Radwan *et al*., 2014).

In the current study, PCR was conducted on 11 MDR *E. coli* isolates for determination of 5 virulence encoding genes, including genes encoding for increased serum survival protein (*iss*), iron uptake system (*iut*A),

bacterial adhesion (*pap*C, P-fimbriae and *fim*H, Type 1 fimbria adhesion) and α-haemolysin (*hly*A). The results revealed that *iut*A and *fim*H genes were found in all the tested isolates (100%), while by *iss*, *pap*C and *hly*A genes were represented as follows, 90.1, 63.6 and 54.5%, respectively.

Regarding the recorded prevalence of *iut*A gene (100%) such result was supported by many previous studies detecting the same prevalence (Trampel *et al*., 2007; Abd El Tawab *et al*., 2014; Radwan *et al*., 2021a). Furthermore, Ngeleka *et al*. (1996) reported that all *E. coli* isolates expressed 2-5 iron-regulated outer membrane proteins and had DNA sequences symmetrical to *iut*/iuc. Lower prevalences of *iut*A gene were recorded in many previous studies; Rocha *et al*. (2003), 45.9%, Moon *et al*. (2006), 50%, Won *et al*. (2009), 50%, Radwan *et al*. (2014), 5%, Abd El-Latif (2016), 70%, Radwan *et al*., (2016), 78.6%, and Hassan *et al*. (2020a), 40%.

Collectively, the obtained results were supported with those of Dai *et al*. (2010), who found that *iut*A, *fim*H, and *iss* were the most predominant virulence genes while *hly*A and *pap*C genes were less frequent, and Raheel *et al*. (2022), who detected *fim*H, *pap*C and *iss*, genes that in all tested *E. coli* isolates. While Kassé *et al*. (2016) reported that *fim*H and *hly* genes were the most predominant (89 and 87%, respectively) while *pap*C gene was the least (9%). Meanwhile, Paixão *et al*. (2016) investigated *iss*, *fim*H and *pap*C genes and recorded that *iss* gene was the most frequent. Also, Abed *et al*. (2022) recorded that *fim*H, *pap*C and *hly* genes were detected as 80, 60 and 20%, respectively. Moreover, Kwon *et al*. (2008) recorded *iss* and *pap*C genes as 100% and 11% of the tested isolates, respectively while Mohamed *et al*. (2014) detected the same genes as 72% and 44.4%, respectively. Meanwhile, Bicudo *et al*. (2019) recorded *fim*H and *hly*A genes as 91% and 17%, respectively.

Conclusion

Airsacculitis syndrome is a global economically important problem affecting broiler chickens resulting in high morbidity and mortality. *E. coli* is the most common bacterial etiologies of airsacculitis. *E. coli* isolates have high resistance patterns against most of the tested antimicrobials, especially against β-lactams, gentamicin and sulfa antimicrobials. Conversely, they have high sensitivities against fluoroquinolones and phenicols in addition to tetracyclins. ESβLs present in 50% and 61.7% of *E. coli* isolates using CDD and cefinase tests, respectively. All *E. coli* isolates are haemolytic high biofilm producers. All the tested *E. coli* isolates harbor β-lactams resistance genes, *amp*C, bla_{CTX} and bla_{TEM}, while 90.1% and 27.3% has *sul*1 and *aad*B genes, respectively. Moreover, all isolates harbor *iut*A and *fim*H virulence genes, while *iss*, *pap*C and *hly*A genes represent as follow, 90.1%, 63.6% and 54.5%, respectively.

Conflict of interest

The authors have no conflict of interest to declare.

References

Abd El Halim, S.A., 2022. Phenotypic and genotypic characterization of some bacterial pathogens associated with subclinical mastitis in sheep and goats. Ph.D. Thesis, (Microbiology), Fac. Vet. Med. Beni-Suef Univ. Egypt.

Abd El Tawab, A.A., Ammar, A.M., Nasef, S.A., Reda, R.M., 2015. Antibacterial resistance and resistance gene detriments of *E. coli* isolated from chicken. Benha Vet. Med. J. 28, 231-240.

Abd El Tawab, A.A., Maarouf, A.A.A., Abd El Al, S.A., El Hofy, F.I., El Mougy, E.E.A., 2014. Detection of some virulence genes of avian pathogenic *E. coli* by polymerase chain reaction. Benha Vet. Med.J. 26, 159-176.

Abd El Tawab, A.A., Mahmoud, H.B., El-Hofy, F.I., El-khayat, M.E., 2016. Prevalence of bla_{TEM} and bla_{SHV} genes in genomic and plasmid DNA of ESβL producing *Escherichia coli* clinical isolates from chicken. BVMJ 31, 167-177.

Abd El-Aziz, E.E., Hassan, A.M., Badr, J.M.,, 2007. In-vitro efficacy of some antimicrobials on the *E. coli* and Mycoplasma isolates from cases of chronic respiratory disease in broiler in Egypt. Zag. Vet. J. 35, 40-49.

Abd El-Halim, M.W., 2020. Genotypic characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. M. V. Sc. Thesis, (Microbiology), Fac. Vet. Med. Beni-Suef Univ. Egypt.

Abd El-Latif, M.A.A., 2016. Phenotypic and genotypic characterization of bacterial pathogens associated with cellulitis in chickens. M. V. Sc. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Abdelrahman, A.A., 2022. Investigation on the causes of respiratory affections in broilers associated with high mortalities. M. V. Sc. Thesis, (Poultry Diseases), Fac. Vet. Med. Beni-Suef Univ. Egypt.

Abed, A.H., 2007. A contribution towards the bacterial pathogens associated with respiratory problems in broiler chickens. M. V. Sc. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.

Abed, A.H., Orabi, A., Hassan, W., Gaber, G., 2022. Resistance and virulence genes constellation associated with biofilm forming avian pathogenic *E. coli* recovered from broiler chickens. J. Egypt. Vet. Med. Assoc. 82, 189-208.

Ali, A., El-Mawgoud A.I.A., Dahshan, A.M., EL-Sawah A.A., Nasef, S.A., Ibrahim, M., 2019. Virulence gene constellations associated with lethality in avian pathogenic *E. coli* recovered from broiler chickens. Adv. Anim. Vet. Sci. 7, 1076-1108.

Archambault, M., Petrov, P., Hendriksen, R.S., Asseva, G., Bangtrakulnonth, A., Hasman, H., Aarestrup, F.M., 2006. Molecular characterization and occurrence of extended-spectrum beta-lactamase resistance genes among *Salmonella enterica* serovar Corvallis from Thailand, Bulgaria, and Denmark. Microb. Drug Resist. 12,192-198.

Awad, A., Arafat, N., Elhadidy, M., 2016. Genetic elements associated with antimicrobial resistance among avian pathogenic *Escherichia coli*. Annals. Clin. Microbiol. Atimi. 15, 59.

Barnes, H.J., Nolan, L.K., Vaillancourt, J.P., 2008. Colibacillosis, p691-732 In Saif YM, Fadly AM, Glisson JR, McDougald LR, Nolan LK, Swayne DE, editors, Diseases of poultry,12th ed, Blackwell Publishing, Ames, IA.

Bicudo, L.C., Oba, E., Bicudo, S.D., Leite, D.S., Siqueira, A.K., Monobe, M.M.S., Nogueira, M., Pantoja, J.C.F., Listoni, F.J.P., Ribeiro, M.G., 2019. Virulence factors and phylogenetic group profile of uterine *Escherichia coli* in early postpartum of high-producing dairy cows. Anim. Prod. Sci. 59, 1898-1905. doi.org.10.1071/AN17729.

Blanco, M., Alonso, M.P., Nicolas-Chanoine, M.H., Dahbi, G., Mora, A., Blanco, J.E., Puentes, B., 2009. Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain. dissemination of clone O25b: H4-ST131 producing CTX-M-15. J. Antim. Chemother. 63, 1135-1141.

Bouchillon, S.K., Johnson, B.M., Hoban, D.J., Johnson, J.L., Dowzicky, M.J., Wu, D.H., Visalli, M.A., Bradford, P.A., 2004. Determining incidence of extended spectrum beta-lactamase producing Enterobacteriaceae, vancomycin-resistant Enterococcus faecium and methicillin-resistant Staphylococcus aureus in 38 centres from 17 countries: The PEARLS study 2001-2002. Int. J. Antimicrob. Agents 24, 119-124.

Bush, K., Fisher, J.F., 2011. Epidemiological expansion, structural studies, and clinical challenges of new β-lactamases from Gram-negative bacteria. Annu. Rev. Microbiol. 65, 455-478. https://doi.org/10.1146/annurev-micro-090110-102911

Carmo, L.P., Neilsen, L.R., da Costa, P.M., Alban, L., 2014. Exposure assessment of extended- spectrum beta-lactamases *amp*C beta-lactamases producing *E. coli* in meat in Denmark. Infect. Ecol. Epidmiol. 4, 22924. https://doi.org/10.3402/iee.v4.22924

Chaudhary, U., Aggarwal, R., 2004. Extended Spectrum β-Lactamases-An Emerging threat to Clinical therapeutics. Ind. J. Med. Microbiol. 22, 75-80.

Clinical and Laboratory Standards Institute (CLSI), 2015. Performance standards for antimicrobial susceptibility testing. Twenty fifth informational supplement update. CLSI document M 100-S25. Clinical and Laboratory Standards Institute. Wayne, PA.

Clinical and Laboratory Standards Institute (CLSI), 2021. Performance Standards for Antimicrobial Susceptibility Testing. 31st Ed. CLSI supplement M100. Wayne, PA, 2021.

Collee, J.G., Fraser, A.G., Marmion, B.P., Simmons, A., 1996. Practical Medical Microbiology. 14th Ed.

Colom, K., Pèrez, J., Alonso, R., Fernández-Aranguiz, A., Lariño, E., Cisterna, R., 2003. Simple and reliable multiplex PCR assay for detection of bla_{TEM}, bla_{SHV} and $bla_{OXA.1}$ genes in *Enterobacteriaceae*. FEMS Microbiol. Lett. 223, 147-151.

Contreras, A., Sierra, D., Sanchez, A., Corrales, J.C., Marcoc, P.C., Paape, M.J., Gonzalo, C., 2007. Mastitis in small ruminants. Small Rum. Res. 68, 145-153.

Dai, J., Wang, S., Guerlebeck, D., Laturnus, C.,Guenther, S., Shi, Z., Lu, C., Ewers, C., *et al*. 2010. Suppression subtractive hybridization identifies an autotransporter adhesin gene of *E. coli* IMT5155 specifically associated with avian pathogenic *Escherichia coli* (APEC). BMC Microbiol. 10, 236.

Eid, H.I., Algammal, A.M., Nasef, S.A., Elfeil, W.K., Mansour, G.H., 2016. Genetic variation among avian pathogenic *E. coli* strains
isolated from broiler chickens. Asian J. Anim. Vet. Adv. 11, 350- 356.

Elshafae, S.M.A., 2009. Pathological studies on natural and experimental *Escherichia coli* infections among poultry farms in Kaluobia Governorate. M.V.Sc. Thesis (Pathology), Fact. Vet. Med., Banha Univ., Egypt.

El-Shazly, D.A., Nasef, S.A., Mahmoud, F.F. and Jonas, D. 2017. Expanded spectrum β–lactamase producing *Escherichia coli* isolated from chickens with colibacillosis in Egypt. Poult. Sci. 96, 2375-2384.

El-Sukhon, S.N., Musa, A., Al-Attar, M., 2002. Studies on the bacterial etiology of air sacculitis of broilers in northen and middle Jordan with special reference to *Escherichia coli*, *Ornithobacterium rhinotracheale* and *Bordetella avium*. Avian Dis. 46, 605-612.

Frana, T.S., Carlson, S. A., Griffith, R.W., 2001. Relative distribution and conservation of genes encoding aminoglycoside-modifying enzymes in *Salmonella enterica* serotype Typhimurium phage type DT104. Appl. Environ. Microbiol. 67, 445-448.

Ghanbarpour, R., Salehi, M., 2010. Determination of adhesin encoding genes in *E. coli* isolates from omphalitis of chicks. Am. J. Anim. Vet. Sci. 5, 91-96.

Ghodousi, A., Bonura, C., Dinoto, A.M., Mammina, C., 2015. Extended-spectrum ß-lactamase, *Amp*C-Producing, and fluoroquinolone-resistant *Escherichia coli* in retail broiler chicken meat, Italy. Foodborne Pathog. Dis. 12, 619-625.

Gomis, S.M., Riddell, C., Potter, A.A., Allan, B.J., 2001. Phenotypic and genotypic characterization of virulence factors of *Escherichia coli* isolated from broiler chickens with simultaneous occurrence of cellulitis and other colibacillosis lesions. Can. J. Vet. Res. 65, 1-6.

Habeeb, M.A., Sarwar, Y., Ali, A., Salman, M., 2013. Rapid emergence of ESβL producers in *E. coli* causing urinary and wound infections in Pakistan. Pak. J. Med. Sci. 29, 540.

Hassan, A., Usman, J., Kaleem, F., Omair, M., Khalid, A., Iqbal, M.,
2011. Evaluation of different detection methods of biofilm formation in the clinical isolates. Braz. Infec. Dis., 15, 305-311.

Hassan, W.H., Abd El-Latif, M.A.A., Abed, A.H., 2020a. Bacteriological and molecular studies on *E. coli* isolated from broiler chickens. Assiut Vet. Med. J. 66, 34-47.

Hassan, W.H., Abd El-Latif, M.A.A., Abed, A.H., 2020b. Bacteriological studies on ascites in broiler chickens. Assiut Vet. Med. J. 66, 24-33.

Henriques, S., Silva, E., Lemsaddek, A., Lopes-da-Costa, L., Mateus, L., 2014. Genotypic and phenotypic comparison of *Escherichia coli* from uterine infections with different outcomes: Clinical metritis in the cow and pyometra in the bitch. Vet. Microbiol. 170, 109-116. https://doi:10.1016/j.vetmic.2014.01.021

Ibekwe, A.M., Murinda, S.E., Graves, A.K., 2011. Genetic diversity and antimicrobial resistance of *Escherichia coli* from human and animal sources uncovers multiple resistances from human sources. PLoS ONE 6, e20819.

Kassé, F.N., Fairbrother, J.M., Dubuc, J., 2016. Relationship between *Escherichia coli* virulence factors and postpartum metritis in dairy cows. J. Dairy Sci. 99, 4656–4667. doi:10.3168/jds.2015-10094.

Kwon, S.G., Cha, S.Y., Choi, E.J., Kim, B., Song, H.J., Jang, H.K., 2008. Epidemiological prevalence of avian pathogenic *E. coli* differentiated by multiplex PCR from commercial chickens and hatchery in Korea. J. Bacteriol. Virol. 38, 179-188.

Li, S., Zhao, M., Liu, J., Zhou, Y., Miao, Z., 2016. Prevalence and antibiotic resistance profiles of extended-spectrum β-lactamase–producing *Escherichia coli* isolated from healthy broilers in Shandong Province, China. J. Food Protection 79, 1169-1173.

Ma, Z., Ginn, A., Kang, M., Galvão, K.N., Jeong, K.C., 2018. Genomic and virulence characterization of intrauterine pathogenic *Escherichia coli* with multi-drug resistance isolated from cow uteri with metritis. Front. Microbiol. 9, 1-12. https://doi:10.3389/fmicb.2018.03137

Meat and Livestock Commercial Services Ltd (MLCSL), 2013. An Evaluation of Food Chain Information (FCI) and Collection and Communication of Inspection Results (CCIR). FSA project

FS145002 report.
Meletis, G., 2016. Carbapenem resistance: Overview of the problem and future perspectives. Therap. Adv. Infec. Dis. 3, 15-21.
Mohamed, M.A., Shehata, M.A., Rafeek, E., 2014. Virulence genes content and antimicrobial resistance in *Escherichia coli* from broiler chickens. Vet. Med. Inter. 2014, 6. http://dx.doi.org/10.1155/2014/195189
Momtaz, H., Rahimi, E., Moshkelani S., 2012. Molecular detection of antimicrobial resistance genes in *E. coli* isolated from slaughtered commercial chickens in Iran. Veterinarni Medicina 57, 193-197.
Moon, B.M., Won, G.Y., Choi, Y.Y, Jin, J.K., Oh, I.J., Park, J.H., Eo, S.K., Lee, J.H., 2006. Isolation and of characteristics of avian pathogenic *Escherichia coli* from birds associated with colibacillosis. Chulalongkorn Uni. Fac. Vet. Sc. pp, 26-29.
Moori Bakhtiari, N., Gooraninezhad, S., Karami, M., 2018. Biofilm-producing ability of bovine extra intestinal pathogenic *Escherichia coli* and its correlation with attachment factors. Jundishapur J. Hlth. Sci. 10, e77130. https://doi.org.10.5812/jjhs.77130
Nanda, A., Nayak, S., 2020. Virulence factors and drug resistance of gastrointestinal *Escherichia coli* isolated from different animals. Ind. J. Anim. Hlth. 59, 159-168.
Nascimento, H.H., Silva, L.E., Souza, R.T., Silva, N.P., Scaletsky,
I.C., 2014. Phenotypic and genotypic characteristics associated with
biofilm formation in clinical isolates of atypical enteropathogenic *Escherichia coli* (aEPEC) strains. BMC Microbiol. 14, 1-7.
Ngeleka, M., Kwaga, J.K.P., White, D.J., Whittam, T.S., Riddell, C., Goodhope, R., Potter, A.A., Allan, B.B., 1996. *Escherichia coli* cellulitis in broilers: Clonal relationships among strains and analysis of virulence-associated factors of isolates from diseased birds. Infect. Immun. 64, 3118-3126.
Paixão, A.C., Ferreira, A.C., Fontes, M., Themudo, P., Albuquerque, T., Soares, M.C., Corrêa de Sá, M.I., 2016. Detection of virulence-associated genes in pathogenic and commensal avian *Escherichia coli* isolates. Poult. Sci. 95, 1646-1652.
Pitout, J.D.D., Nordmann, P., Laupland, K.B., Poirel, L., 2005. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESβLs) in the community. J. Antimicrob. Chemother. 56, 52-59.
Piva, I.C., Pereira, A.L., Ferraz, L.R., Silva, R.S.N., Vieira, A.C., Blanco, J.E., Blanco, M., Blanco, J., Giugliano, L.G., 2003. Virulence markers of enteroaggregative *Escherichia coli* isolated from children and adults with diarrhea in Brasília, Brazil. J. Clin. Microbiol. 41, 1827-1832.
Poulou, A., Grivakou, E., Vrioni, G., Koumaki, V., Pittaras, T, Pournaras, S., Tsakris, A., 2014. Modified CLSI Extended spectrum β-Lactamase (ESβL) confirmatory test for phenotypic detection of ESBLs among Enterobacteriaceae producing various β. Lactamases. J. Clinc. Microbio. 52, 1483-1489. https://doi.org/10.1128/JCM.03361-13
Quinn, P.J., Markey, B.K., Leonard, F.C., Hartigan, P., Fanning, S., Fitzpatrick, E., 2011. Veterinary Microbiology and Microbial Disease, 2nd Ed., John Wiley & Sons: Hoboken, NJ, USA, 2011, p. 893. ISBN 14051582399781405158237
Qurani, R.O., 2019. Phenotypic and genotypic characterization of Trypsin producing *Escherichia coli* isolated from broiler chickens. Ph. D. Thesis (Microbiology), Fact. Vet. Med., Beni-Suef Univ., Egypt.
Radwan, I.A., Abd El-Halim, M.W., Abed, A.H., 2021a. Molecular characterization of antimicrobial resistant *Escherichia coli* isolated from broiler chickens. J. Vet. Med. Res. 27, 128-142. https://doi.org/10.21608/JVMR.2020.31870.1009
Radwan, I.A., Abed, A.H., Abd Allah, M.M., Abd El-Latif, M.A.A., 2018. Bacterial pathogens associated with cellulitis in chickens. J. Vet. Med. Res. 25, 68-79.
Radwan, I. A., Abed, A.H., Abd Al-Wanis, S.A., Abd El-Aziz, G.G., El-Shemy. A., 2016. Antibacterial effect of cinnamon and oreganium oils on multidrug resistant *Escherichia coli* and Salmonellae isolated from broiler chickens. J. Egy. Vet. Med, Assoc. 76,169-186.
Radwan, I.A., Hassan, H.S., Abd-Alwanis, S.A., Yahia, M.A., 2014. Frequency of some virulence associated genes among multidrug-resistant *Escherichia coli* isolated from septicemic broiler chicken. Inter. J. Adv. Res. 2, 867-874.
Radwan, I.A., Moustafa, M.M.M., Abdel-Wahab, S.H., Ali, A., Abed, A.H., 2022. Effect of essential oils on biological criteria of Gram-negative bacterial pathogens isolated from diseased broiler chickens. Inter. J. Vet. Sci. 11, 59-67. https://doi.org/10.47278/journal.ijvs/2021.078
Radwan, I.A., Shany, S.A.S., Amin, S.S.E., Abed, A.H., 2021b. Inhibitory effects of carvacrol on blaTEM and *exo*S genes expression in ESβL producing Pseudomonas aeruginosa isolated from kidney lesions of broiler chickens. Adv. Anim. Vet. Sci. 9, 1408-1415. https://doi.org/10.17582/journal.aavs/2021/9.9.1408.1415
Raheel, I., Orabi, A., Erfan, A., Raslan, M.A., Wahab, S.H.A.E., Mohamed, E.A.A., 2022. Intestinal tract of broiler chickens as a reservoir of potentially pathogenic curli producing ESβL *Escherichia coli*. Inter. J. Vet. Sci. 11, 498-503. https://doi.org/10.47278/journal.ijvs/2021.124
Rahman, S.U., Ali, T., Ali, I., Khan, N. A. Han, B., Gao, J., 2018. The growing genetic and functional diversity of extended spectrum Beta-Lactamases. Biomed. Res. Int. 2018, 9519718.
Rawat, D., Nair, D., 2010. Extended-spectrum beta-lactamases in Gram Negative Bacteria. J. Glob. Infect. Dis. 2, 263-274.
Rocha, C.L., Coburn, J., Rucks, E.A., Olson, J.C., 2003. Characterization of Pseudomonas aeruginosa exoenzyme S as a bifunctional enzyme in J774A.1 macrophages. Infect. Immun. 71, 5296-5305. https://doi.org/10.1128/IAI.71.9.5296-5305.2003
Rodrigues, S.V., Laviniki, V., Borges, K.A., Furian, T.Q., Moraes, H.L., Nascimento, V.P., Salle, C.T., 2019. Biofilm formation by avian pathogenic *Escherichia coli* is not related to in-vivo pathogenicity. Current Microbiol. 76, 194-199
Sabry, M., 2019. Current situaton of poultry industry in Egypt. In: EVPA, Salaheldein, H. (Ed.), Porto Marina, Egypt.
Saha, O., Hoque, M.N., Islam, O.K., Rahaman, M., Sultana, M., Hossain, M.A., 2020. Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. Microorganisms 8, 11352.
Simmons, K., Islam, M.R., Rempel, H., Block, G., Topp, E., Diarra, M.S., 2016. Antimicrobial resistance of *Escherichia fergusonii* isolated from broiler chickens. J. Food Protection 79, 929-938.
Skyberg, J.A., Siek, K.E., Doetkott, C., Nolan, L.K., 2007. Biofilm formation by avian *Escherichia coli* in relation to media, source and phylogeny. J. Appl. Microbiol. 102, 548-554.
Srinivasan, V., Nam, H.M., Nguyen, L.T., Tamilselvam, B., Murinda, S.E., Oliver, S.P., 2005. Prevalence of antimicrobial resistance genes in Listeria monocytogenes isolated from dairy farms. Foodborne Pathogens & Disease 2, 201-211.
Stephanovic, S., Vu-kovic, D., Dakic, I., Savic, B., Svabic-Vlahovic, M., 2000. A Modified microtiter-plate test for quantification of staphylococcal biofilm formation. J. Microbiol. Methods 40, 175-179.
Trampel, D.W., Wannemuehler, Y., Nolan, L.K., 2007. Characterization of *Escherichia coli* isolates from peritonitis lesions in commercial laying hens. Avian Dis. 51, 840-844.
Wang, X., Wei, L., Wang, B., Zhang, R., Liu, C. Bi, D., Chen, H., Tan, C., 2016. Complete genome sequence and characterization of avian pathogenic *Escherichia coli* field isolate ACN001. Stand Genomic Sci. 11, 13.
Wen-jie, J, Zhi-ming, Z, Yong-zhi, Z, Ai-jian, Q, Hong-xia, S, Yue-long, L, Jiao, W., Qian-qian, W., 2008. Distribution of virulence-associated genes of avian pathogenic *Escherichia coli* isolates in China. Agricultural Sciences in China 7, 1511-1515.
Won, G., Moon, B., Oh, I., Matsuda, K., Chaudhari, A.A., Hur, J., Eo, S., Yu, I., Lee, Y., Lee, Y., Kim, B., Lee, J.H., 2009. Profiles of virulence-associated of avian pathogenic *Escherichia coli* isolates from chickens with colibacillosis. Poult. Sci. 46, 260-266.