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Virulence of some Pathogenic Bacteria Isolated from Broiler Chicks up to Two Weeks of Age

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Abstract

Pathogenic bacteria causing diseases in broiler chicks are widely distributed. One hundred and fifty cases of broiler chick samples were obtained from 15 farms with complain of early mortalities. Out of them, 100 diseased and 50 freshly dead chicks aged 1 - 14 days were examined. Liver, heart blood, lung, yolk sac and thigh bones were collected . Bacteriological investigation in both diseased and freshly dead chicks revealed that the prevalence rate of *E. coli* was 70% with higher incidence in liver followed by yolk sac, *S. aureus* isolates represented as 18.5% mainly from thigh bone followed by liver, incidence of *Pseudomonas aeruginosa* was 20% mainly from yolk sac proceeded by liver, *Klebsiella pneumonia* incidences was 13.3% with high rate from liver. Prevalence of all previous isolated bacteria was higher in freshly dead than in diseased chicks. Serological identification of 68 isolates of *E. coli* (64.8%) were typed with 5 different serotypes as 15 (O119:H6), 8 (O1:H7), 10 (O146:H21), 20 (O78), 8 (O29) and 7 (O144) while 37 (35.2%) were untyped. *E. coli* antimicrobial resistance was performed with marked sensitivity reported with amoxicillin. PCR performed for detection of some virulence genes mainly *eaeA* and *iss* from *E. coli* isolates, and enterotoxin B (*SEB*) and enterotoxin D (*SED*) from *S. aureus* isolates with positivity 100% for each genes. This study indicated presence of some pathogenic bacteria in broiler chicks up to two weeks as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*e which cause diseases with a consequence of economic losses.

KEYWORDS
Antibiotic Resistance, Bacteria, Virulence Genes.

INTRODUCTION

One of the most frequent causes of death in chicks during the first week following hatching is bacterial infection (Pattison *et al*., 2008).

Escherichia coli is considered one of the important pathogens cause colibacillosis in broiler chicks leading to cost-effective losses with poor performance and high death rates (Hossain *et al*., 2023; Pais *et al*., 2023). Disorders include chronic respiratory sickness, omphalitis, synovitis, coligranulomatosis, salpingitis, perihepatitis, enteritis and colibacillosis (Ahmad *et al*., 2009), all ages of broiler chickens may suffer from colibacillosis, but youngers often have a more severe version of the disease. Clinical symptoms, polyserositis lesions, pathogen isolation, and pathogen identification are used to initially identify the condition (Waffa and Aqeel, 2023).

S. aureus is a widespread bacterium related with omphalitis, yolk sac and liver infections in first-week led to dead chicks and in-shell dead embryos (White *et al*., 2003). Infected chicks are weak, huddled together with watery diarrhea and opened umbilicus (Rai *et al*., 2003).

Salmonellosis is among the most prevalent infectious diseases for poultry (Selvaraj *et al*., 2010), that poultry is the main reservoirs with a great role in it's spread (EFSA, 2019). *Salmonella* spp. frequency and serovars in chicks vary by geographic location (Tan *et al*., 2022).

Pseudomonas aeruginosa is important poultry pathogen that causes nosocomial illnesses in chickens, as respiratory infections, septicemia and other forms especially in broiler result in economic loss by increasing mortality of newly hatched birds and mass death of embryos at later stage (Aml and Dena, 2020), led to severe t*iss*ue harm when invading blood effecting freshly hatched chicks with increased mortalities in chickens and embryos (Dinev *et al*., 2013).

Klebsiella pneumonia is a frequent infectious illness in chicks with severe economic effect (Aly *et al*., 2014).

E. coli have various strains as enterohaemorrahgic (EHEC), enteroinvasive (EIEC), enteropathogenic (EPEC), Shiga toxin-secreting (STEC), diarrhea-associated hemolytic (DHEC), enter aggregative (EAggEC), enterotoxigenic (ETEC) and cytolethal distending toxin recreating (CDTEC) (Butler, 2012). Different virulence-associated genes have great roles either separately or together in adhesion, hemolyzation, ferric transport system, and production of toxin of APEC (Yaguchi *et al*., 2007). A protein called intimin, which produced by the chromosomal gene (*eaeA*), regulates how *E. coli* attaches and detaches from intestinal epithelial cells (Ghanbarpour and Oswald, 2010). Since they can cause a range of infections and are typically very antibiotic resistant, extraint-

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estinal pathogenic *E. coli* (ExPEC) strains represent a severe and developing clinical *iss*ue. Many (ExPEC) strains have the ability to resist serum's bactericidal action and result in sepsis. The enhanced serum survival (*iss*) gene, which is strongly connected with complement resistance and lethality, is one important determinant for the onset of septicemia (Biran *et al*., 2021).

S. aureus is an important pathogen associated multiple infections, which were linked to the presence of some virulent surface proteins and production of some virulent enzymes and toxins (Rashid *et al*., 2021; Wafaa, 2021) .

This study aimed to determine the prevalence of several pathogenic bacteria, including *E. coli*, *S. aureus*, *Salmonella* spp., *Pseudomonas aeruginosa*, and *Klebsiella pneumonia*e, in broiler chicks up to two weeks of age. Next, it looked at the antibiotic resistance of *E. coli*. Additionally, some virulence genes of important isolates were determined by PCR.

MATERIALS AND METHODS

Ethical approval

The Animal Health Research Institute, Egypt's ethics committees' regulations were followed in terms of animal care and study protocols.

Sample collection

A total of 150 broiler chicks (100 diseased and 50 freshly dead) aged from 1 day up to 2 weeks from 15 farm at different localities in Bohera governorate with problem of early mortalities were collected during 2021-2022. Some chicks were clustered together and exhibited respiratory disorders, sleepiness, ruffled feathers, depression, diarrhoea. Samples (liver, heart blood, lung, unabsorbed yolk sac and thigh bone) were taken from each chick, transported under aseptic conditions for bacteriological assessment.

Bacterial isolation and identification

For isolation of E coli MacConkey and Eosin Methylene Blue (EMB) medium were used, biochemical tests (IMVIC) were performed (Quinn *et al*., 2002). Rapid antisera sets (DENKA SEIKEN Co., Japan) were used for serological determination of the isolates (Kok *et al.* , 1996).

S. aureus was isolated in accordance to ISO 6888-1 (2003), that all suspected colonies were subjected to further examination (coagulase test, catalase test and β hemolysis on blood agar). *Salmonella* was detected based on ISO 6579 – 1:2017/Amd.1: 2020 .

Pseudomonas aeruginosa was isolated by inoculating samples into nutrient broth tubes, which aerobically incubated at 37°C for 24 h. MacConkey agar and *Pseudomonas* agar medium plates, incubated aerobically for 24-48 h at 37 °C; pigment production and lactose fermentation were determined, distinctive colonies of *Pseudomonas aeruginosa* (large, irregular, translucent with a fruity-smelling, diffusible green pigment) were selected, the Gram staining was carried out, followed by biochemical identification (Quinn *et al*., 2002).

Klebsiella pneumonia was isolated by incubating samples aerobically in nutrient broth for 18 to 24 h at 37 °C, a loopful of inoculated broth was streaked onto MacConkey agar medium and incubated at 37°C for 24-48 h . Suspected colonies were subcultured onto XLD and EMB followed by biochemical tests (Trivedi *et al*., 2015).

Antimicrobial test

Agar disc diffusion method on Mueller Hinton Agar plates (HIMEDIA) and 10 widely used antibiotic discs were used at antimicrobial susceptibility testing (Hassan *et al*., 2017).

Molecular characterization of some virulence genes and bacterial toxins

DNA extraction for 10 identified *E. coli* and *S. aureus* isolates using QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with a slight modification from the manufacturer's instructions. Colonies suspension (200 µl) was incubated with proteinase K (10 µl) and lysis buffer (200 µl) at 56 °C for 10 min then absolute ethanol (200 µl) was mixed with lysate. Washing and centrifugation were made then nucleic acid was eluted by elution buffer (100 µl).

PCR amplification

EaeA, *Iss*, *SEB* and *SED* coding genes were identified using 12.5 µl reaction Emerald Amp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration (Metabion, Germany), 5.5 µl of water, and 5 µl of DNA template. Thermal profile were adjusted using thermocycler Applied Biosystems 2720 .

Electrophoresis of PCR products was done in 1.5% agarose gel along with 100 bp DNA ladder (Fermentas, Thermo, Germany) by loading each gel slot with 20 µl of the products and using gradients of 5V/cm in 1x TBE buffer at 37°C. Alpha Innotech, Biometra's gel documentation system used for gel photograph. The data were analyzed by a computer software (automatic image capture protein simple formerly cell bioscience, USA).

Table 1. Primers and thermal profiles used during the study.

Target gene	Primers sequences	Amplified segment (bp)	Primer Den.	Amplification (35 cycles)			Final Ext.	Reference
				Sec. den.	Ann.	Ext.		
eaeA	ATGCTTAGTGCTGGTTTAGG GCCTTCATCATTTCGCTTTC	248	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	BisiJohnson *et al*. (2011)
Iss	ATGTTATTTTCTGCCGCTCTG CTATTGTGAGCAATATACCC	266	94˚C 5 min	94˚C 30 sec.	54˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Yaguchi *et al*. (2007)
SEB	GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	94˚C 5 min.	. 94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	Mehrotra *et al*. (2000)
SED	CCAATAATAGGAGAAAATAAAAG ATTGGTATTTTTTTTCGTTC	278	94˚C 5 min.	94˚C 30 sec.	57˚C 30 sec.	72˚C 30 sec.	72˚C 7 min.	

Den: denaturation; Sec. den.: secondary denaturation; Ann.: annealing; Ext.: extension.

RESULTS

In this investigation, *E. coli* was detected in 70% (105/150) in both diseased and freshly dead chicks, *Staphylococcus* spp., in 36% (54/150), *Pseudomonas aeruginosa*, and *Klebsiella pneumonia* in 20% (30/150) and 13.3% (20/150) respectively, whereas *Salmonella* spp. was not isolated (Table 2). Prevalence of the isolated bacteria in are shown in Tables 3-7.

Serotyping of the 105 *E. coli* isolates revealed 68 (64.8%) typed isolates and the remaining 37 (35.2%) were untyped. The serotypes were 15 (O119:H6), 8 (O1:H7), 10 (O146:H21), 20 (O78), 8 (O29) and 7 (O144) *E. coli* with a percent of 14.3%, 7.6%, 9.5%, 19.1%, 7.6% and 6.7% respectively (Table 8).

Antimicrobial resistance was performed for 20 identified

Table 2. Prevalence of the isolated bacteria in diseased and freshly dead chicken.

Cases	Number	Bacterial isolates									
		E. coli		*Staphylococcus* spp.		*Salmonella* spp.		*Pseudomonas aeruginosa*		*Klebsiella pneumoniae*	
		Number	%	Number	%	Number	%	Number	%	Number	%
Diseased	100	67	67	34	34	0	0	18	18	12	12
Freshly dead	50	38	76	20	40	0	0	12	24	8	16
Total	150	105	70	54	36	0	0	30	20	20	13.3

%: percentage of examined cases

Table 3. Occurrence of the isolated bacteria in investigated organs and t*iss*ues of diseased chicken.

Isolate	Organs (100 case)										Total	
	Liver		Heart blood		Lung		yolk sac		Thigh bone			
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
E. coli	26	26	13	13	13	13	15	15	0	0	67	67
Staphylococcus spp.	4	4	8	8	7	7	8	8	7	7	34	34
Salmonella spp.	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	5	5	4	4	3	3	6	6	0	0	18	18
Klebsiella pneumoniae	4	4	3	3	2	2	3	3	0	0	12	12

Table 4. Occurrence of the isolated bacteria in freshly dead chicken.

Isolate	Organs (**50** case)										Total	
	Liver		Heart blood		Lung		yolk sac		Thigh bone			
	Number	%	Number	%	Number	%	Number	%	Number	%	Number	%
E. coli	13	26	8	16	8	16	9	18	0	0	38	76
Staphylococcus spp.	3	6	5	10	4	8	6	12	2	4	20	40
Salmonella spp.	0	0	0	0	0	0	0	0	0	0	0	0
Pseudomonas aeruginosa	3	6	2	4	2	4	5	10	0	0	12	24
Klebsiella pneumoniae	3	6	2	4	1	2	2	4	0	0	8	16

Table 5. Percentage of Coagulase negative and positive *Staphylococci*

Cases	Number	Total Number of *Staphylococci*		Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
		Number	%	Number	%*	Number	%*
Diseased	100	34	34	6	17.6	28	82.4
Freshly dead	50	20	40	4	20	16	80
Total	150	54	36	10	18.5	44	81.5

%: percentage; %*: calculated from total Number of *Staphylococci*

Table 6. *S. aureus* isolated from diseased chicken (n.=100).

Organs	Total Number of *Staphylococci*		*S. aureus*		Coagulase negative *Staphylococci*	
	Number	%	Number	%*	Number	%*
Liver	4	4	1	25	3	75
Heart blood	8	8	1	12.5	7	87.5
Lung	7	7	1	14.3	6	85.7
yolk sac	8	8	1	12.5	7	87.5
Thigh bone	7	7	2	28.6	5	71.4
Total	34	34	6	17.6	28	82.4

%*: calculated from total Number of *Staphylococci* in each organ

E. coli isolates, the highest resistance was with Oxytetracycline (90%) followed by Erythromycin (85%) and Danofloxacin (85%). Resistance reported with Chloramphenicol was 80% and Lincomycin was 80%. Resistance for Flumequine and Neomycin were 75% and 70%, respectively. Other tested antibiotics has different levels of resistance (Table 9).

PCR analysis of 10 serotyped *E. coli* isolates was carried out for detection of *eaeA* gene and *iss* gene, the results revealed that all of examined isolates were positive with a percent of 100% for each examined gene (Table 10 and Figures 1 A and B).

Detection of enterotoxin B (SEB) and enterotoxin D (SED) by PCR for 10 isolates of *S. aureus* demonstrated that all the exam-

Table 7. *S. aureus* detected in freshly dead chicken (n.=50).

Organs	Total Number of *Staphylococci*		Coagulase positive *S. aureus*		Coagulase negative *Staphylococci*	
	Number	%	Number	%*	Number	%*
Liver	3	6	1	33.3	2	66.7
Heart blood	5	10	1	20	4	80
Lung	4	8	1	25	3	75
yolk sac	6	12	0	0	6	100
Thigh bone	2	4	1	50	1	50
Total	20	40	4	20	16	80

%*: calculated from total Number of *Staphylococci* in each organ

Table 8. Serological identification of *E. coli* isolates.

E. coli serotype	Isolates	
	Number	%
O119:H6	15	14.3
O1:H7	8	7.6
O146:H21	10	9.5
O78	20	19.1
O29	8	7.6
O144	7	6.7
Untypable	37	35.2
Total	105	100

Table 9. Antimicrobial susceptibility for *E. coli*.

Antimicrobial agents	Concentration. of disc	Symbol	**Isolates (n.=20)**			
			Sensitive		Resistant	
			Number	%	Number	**%**
Amoxicillin	25 ug	AMX	18	90	2	10
Neomycin	30 ug	O	6	30	14	70
Colistin sulfate	30 ug	CT	17	85	3	15
Chloramphenicol	30 ug	C	4	20	16	80
Danofloxacin	5 ug	D	3	15	17	85
Flumequine	2 ug	UB	5	25	15	75
Oxytetracycline	30 ug	OT	2	10	18	90
Lincomycin	2 ug	L	4	20	16	80
Enrofloxacin	5 ug	ENR	12	60	8	40
Erythromycin	15 ug	E	3	15	17	85

%: Percentage according to total examined isolates

Table 10. Incidence of virulence genes (*eaeA*) and (*iss*) of *E. coli* isolates.

Serotype	Number of tested isolates from each serotype	*eaeA* gene	*iss* gene
O119:H6	2	+	+
O1:H7	2	+	+
O146:H21	2	+	+
O78	2	+	+
O29	1	+	+
O144	1	+	+

ined isolates were positive with percent of 100% for each examined gene (Figures 1 C and D).

DISCUSSION

In the current study, prevalence of *E. coli* was 70% in diseased and freshly dead which nearly similar with Ahmed (2016) with incidence of 70.3%. Higher incidence was determined by Bisi-Johnson *et al.* (2006) and Eid and Erfan (2013) which were 92% and 80% respectively. A lower rates were noted by Zhao *et al.* (2001); Sharada *et al.* (2010); Hasan *et al.* (2011) and Literak *et al.* 2013) with incidence of 38.7%, 44.61%, 36.20% and 35.74%, respectively.

E. coli was prevalent in liver samples followed by unabsorbed yolk sac while both heart blood and lung samples were have the same percent in diseased and freshly dead, these findings disagree with Eid and Erfan (2013) as isolation rates from various organs with pathologic conditions was different as from the 105 examined organs from each type, 60 isolates (57.14%) were isolated from liver that showed perihepatitis, 57 isolates (54.29%) from lung showing pneumonia, and 39 isolates (37.14%) from heart with pericarditis.

Different *E. coli* serotypes were recovered in this study as O1:H7, O119:H6, O78, O29, O146:H21 and O144 which are similar to serotypes that reported previously by other authors (El- sayed *et al.*, 2015; Reem, 2015; Ashraf *et al.*, 2017). Our study showed the presence of untyped *E. coli* isolates which agree with Zhao *et al.* (2005) who found 60% of the avian *E. coli* isolates were untyped with the standard available antisera.

S. aureus was isolated in the present study at percentage of 18.5% in both diseased and freshly dead broiler chicks, to be lower than the isolation rate that recorded by Mamza *et al.* (2010) who identified *S. aureus* in 52.5% of examined chicks, also disagree with Suleiman *et al.* (2013) who detected *S. aureus* in 54% of chicken samples. Different rates were mentioned by Adeyeye and Adewale (2013); Abd El-Tawab *et al.* (2017) and Ali *et al.* (2017) with incidences 95%, 66% and 90%, respectively. In our findings, the highest prevalence of *S. aureus* was detected in thigh bone in both diseased and freshly dead broiler chicks (Szafraniec *et al.*, 2022). *S. aureus* was highly recovered from thigh bone followed by liver samples with an incidence of 28.6% and 25% respectively in diseased broiler chicks, and in freshly dead broiler chicks with prevalence 50% and 33.3% respectively, the same was reported by Abd El Tawwab *et al.* (2014).

Incidence of *Pseudomonas aeruginosa* in this study was 20% in both diseased and freshly dead broiler chicks which approximately similar with finding of Bakheet *et al.* (2017) and El-demerdash *et al.* (2020) with an incidence of 18.6% and 20% respectively. Lower rate eas reported by Mahmoud and Mousa (2000 and Abdel-Tawab *et al.* (2016) with incidence of 6.6% and 2.5% respectively, while Kurkure *et al.* (2001) and Shahat *et al.* (2019) reported higher incidences as 57% and 42% respectively. The obtained findings from unabsorbed yolk sacs were listed as the highest rates of *Pseudomonas aeruginosa* isolation, followed by liver, heart blood, and lung. However, Satish and Priti (2015) reported different findings, listing the liver, lung, air sacs, nose, and heart as the organs with the highest rates.

Incidence of *Klebsiella pneumonia*e in this investigation was 13.3% in total examined chicks which is close to incidence of 14% that determined by Abd El-Tawab *et al.* (2022), which was mainly detected in liver samples with an incidence 4% in diseased broiler chicks and 6% in freshly dead chicks, Marwa *et al.* (2018) recorded the higher prevalence of *Klebsiella pneuonmiae* in examined chicks was from lung followed by liver at percent 4% and 2%, respectively, which is disagree with that reported in the present study

Salmonella spp. were absent in all examined samples, which

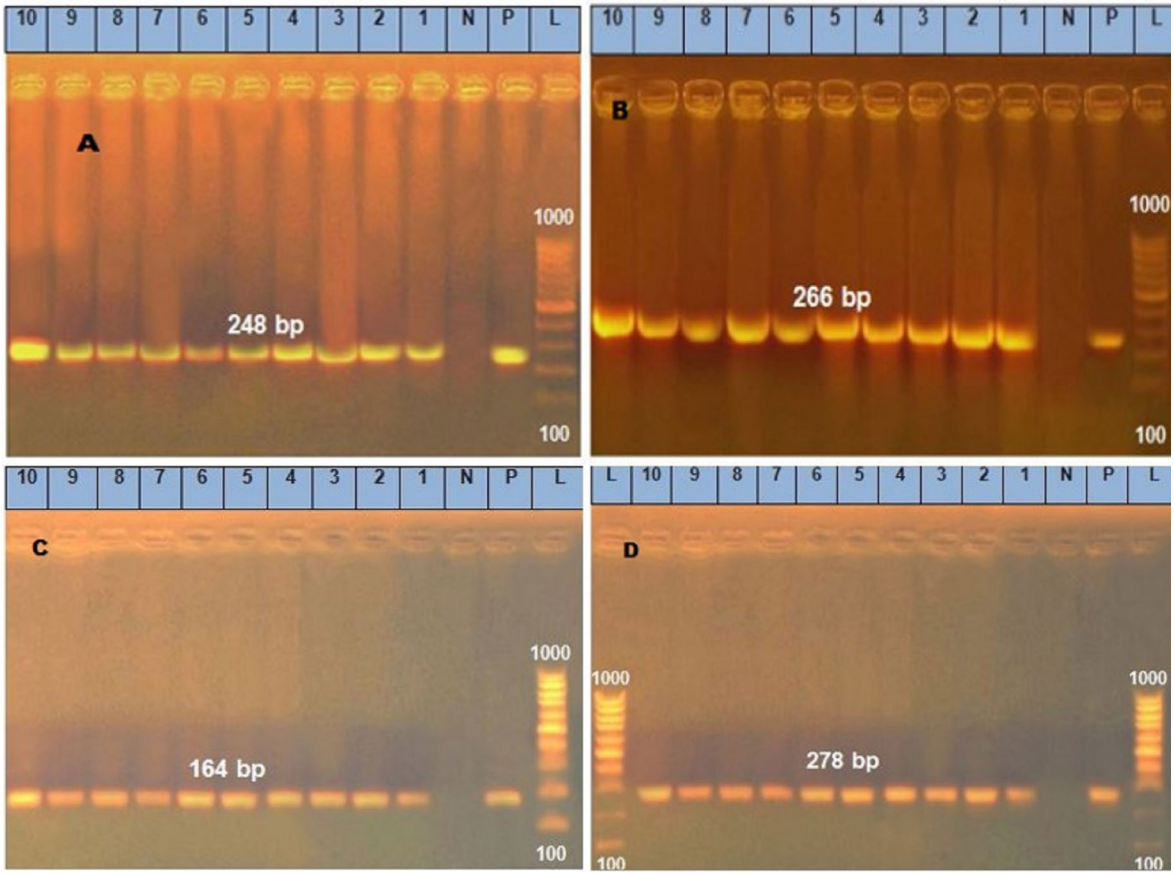

Fig. 1. Electrophoretic pattern of the investigated genes in *E. coli* and *S. aureus*.
Lane (L) :100 bp DNA ladder, Lane(P): Positive control, Lane (N): Negative control,
lanes from 1 to 10 Positive gene. A and B) *eaeA* and *iss* gene of *E. coli* , respectively. C and D) *S. aureus* enterotoxin B gene (*SEB*)and enterotoxin D gene (*SED*), respectively .

is disagree with Moe *et al*. (2017) who found that 97.9% of broiler chickens were positive cases, and also disagree Yang *et al*. (2011); Zdragas *et al*. (2012) and Trongjit *et al*. (2017) who detected *Salmonella* in a percentage of 52.2%, 40.5% and 41.7%, respectively.

E. coli can cause a Number of diseases and aid in the spread of antibiotic resistance (Rasheed *et al*., 2014). The highest sensitivity of the examined *E. coli* isolates was reported with Amoxicillin (90%) followed by Colistin sulphate (85%), which completely differ from results that reported by Eid and Erfan (2013) with 92.86% resistance rate against Amoxicillin but agree with a percentage of 89.29% sensitive to Colistin. These findings corroborated those of Galani *et al*. (2008), who found important Colistin potency against isolated *E. coli* and also agreed with Al-Saati *et al*. (2009) and Sharada *et al*. (2010) with sensitivity rates ofn94.00% and 95% respectively. Ashraf *et al*. (2015) found that, the most potent antibiotics against *E. coli* were Gentamycin, Doxycycline, Norfloxacin, and Chloramphenicol. Resistance to Colistin sulphate were 15% which is nearly similar to a percentage of 13.2% that was reported by Khong *et al*. (2022). Resistance percentages to Lincomycin in this study were 80%, partially similar levels were observed by Salehi and Farashi (2006); Ionica (2011) and Eid and Erfan (2013) who reported 100%, 80.95% and 96.43%, resistance percentages, respectively. Lower rates were found by Al-Saati *et al*. (2009) and Sharada *et al*. (2010) which were 5% and 39.50%, respectively. Infections with antimicrobial-resistant bacteria led to treatment difficulties and increase in mortality (Munita and Arias, 2016). Although incidence and death of avian colibacillosis are decreased by antibiotic therapy, incorrect antibiotic use results in the creation of high Numbers of drug-resistant *E. coli*, making it challenging to manage these disorders (Sharada *et al*., 2010).

Even though *E. coli* is frequently found as a commensal bacteria, certain strains can cause extraintestinal or intestinal disease when they pick up virulence factors from plasmids or other mobile genetic elements through horizontal gene transfer (De Oliveira *et al*., 2020). The identification of the (*eaeA*) gene was connected to the intestinal lesion (AE lesion) brought on by particular strains of *E. coli*. "Attaching" refers to the close association of the bacteria with the enterocyte's exposed cytoplasm membrane while the localized removal of the brush border microvilli is known as "effacing" (Stordeur *et al*., 2000).

Suardana *et al*. (2011) and El-Jakee *et al*. (2012), detected the (*eaeA*) gene in 95% and 95.9% of the studied *E. coli* isolates, respectively, to be compatible with the obtained findings while moderate rate as 71.4% of *eaeA* gene detection was recorded by Eid and Erfan (2013) followed by Ashraf *et al*. (2015) with detection rate (50%). Hamza *et al*. (2016) reported incidence as 33.3% positive for *eaeA* gene from different *E. coli* serotypes isolated from broiler chicks, also this gene was detected in a lower prevalence rate (25%) in the wild birds and suggested that most of the *E. coli* strains are probably related to atypical EPEC strains (Kobayashi *et al*., 2009). However, Wen-jie *et al*. (2008) supported the results of Krause *et al*. (2005) who reported a low detection rate (2.3%) of *eaeA* gene in the examined *E. coli* isolates from faecal samples of healthy chickens.

Detection of *iss* gene of *E. coli* isolates in our finding was high (100%), which agree with Ibrahim *et al*. (2019) with incidence of 93.3% during genes study from sick chickens but disagree with Toshiyuki and Hiroichi (2022) as *iss* gene represented 88% of *E. coli* isolates from layer hens . A lower incidence of 33.8% was reported by Khong *et al*. (2022) while Ewers *et al*. (2004) and D*iss*anayake *et al*. (2014) determined the prevalence rate of *iss* gene as 82.7% and 80.5%, respectively, of avian pathogenic *E. coli* (APEC) isolated from infected birds .

In contrast to our findings, which revealed that *SEB* and *SED* were detected in 100% of the tested isolates, Islam *et al*. (2019) discovered that more than 74% of *S. aureus* isolates had enterotoxin B genes (*SEB*) and enterotoxin D genes (*SED*) with distribution percentages of 11%, and 13%. Also, Wafaa (2021) reported that many *S. aureus* toxin gene groups as enterotoxin B (*SEB*) and enterotoxin D (*SED*) were found in broiler chicks .

CONCLUSION

The present study reported high incidence of some bacterial infections in broiler chicks up to two weeks of age with marked occurrence of some virulence genes for *E. coli* isolates mainly for *eaeA* and *iss* genes and also in case of *S. aureus* when examined for the detection of enterotoxin B (*SEB*) and enterotoxin D (*SED*) genes, so this study suggest a future research to detect other virulence genes together with relation to incidence of marked signs in broilers at this age.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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