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CHARACTERIZATION OF *E. COLI* ASSOCIATED WITH HIGH MORTALITY IN POULTRY FLOCKS

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ABSTRACT

A total of 105 cases of broilers were collected from broiler flocks showing high mortality rates in Sharkia Governorate. *E. coli* was isolated from 84 (80%) cases. A number of 11 different serotypes were identified, of which O114:K90 was the most detected with 17.9% of the total isolates. While O125:K70, O55:K59, O111:K58 and O26:K60 were identified with the percentages of 14.3%, 14.3%, 10.7% and 10.7%, respectively. Five serotypes (O145:K –, O25:K11, O44:K74, O126:K71, O118:K –) had the same isolation percentage (3.6%). However, serotyping failed to determine 7.1% of the isolates. Studying antibiotic resistance pattern of *E.coli* isolates revealed that all the isolates demonstrated multidrug resistance pattern. The highest resistance rates were recorded against Doxycyclin (100%), while the resistance against Penicillin, Lincomycin, Nalidixic Acid, Tetracycline and Oxitetracycline was (96.4% each). The highest sensitivity rates were recorded to Colistin (89.3%), Ciprofloxacin (75%) and Gentamycin (50%). 380 bp of the island-associated gene *eaeA* was amplified by PCR for 14 representative isolates of the different serotypes to estimate their virulence. Quinolone resistance was detected by amplifying 403 bp of the *qepA* gene (Plasmid-encoded efflux pump gene), 516 bp, 469 bp, 417 bp and 113 bp of the *qnrA*, *qnrB*, *qnrS* and *aac(6')-Ib-cr* genes (Plasmid-mediated quinolone resistance genes). Amoxicillin resistance was detected by amplifying 516 bp of the *blaTEM* gene which encodes the β-lactamases. It was concluded that multiresistant virulent strains of *E. coli* are circulating in broiler flocks and are implicated in cases of high mortalities causing great economic losses.

Key words: *E. coli, Poultry flocks, plasmid-encode, efflux genes.*

INTRODUCTION

Most of the human extraintestinal *Escherichia coli* infections, including those involving antimicrobial resistant strains, are caused by members of a limited number of distinctive *E. coli* lineages, termed extraintestinal pathogenic *E. coli* (ExPEC), that has a special ability to cause disease at extraintestinal sites when they exit their usual reservoir in the host's intestinal tract. Multiple lines of evidence suggest that many of the ExPEC strains encountered in humans with urinary tract infection, sepsis, and other extraintestinal infections, especially the most extensively antimicrobial-resistant strains, may have a food animal source and may be transmitted to humans via the food supply (Manges and Johnson, 2012).

Pathologic lesions caused by ExPEC are reported for many farm animals, especially poultry, in which colibacillosis is responsible for huge losses within broiler chickens. As antimicrobials are commonly used for livestock production, infections due to antimicrobial-resistant ExPEC transferred from animals to humans could be even more difficult to treat. These findings, combined with the economic impact of ExPEC in the animal production industry demonstrate the need for adapted measures to limit the prevalence of ExPEC in animal reservoirs while reducing the use of antimicrobials as much as possible (Bélanger *et al.,* 2011). Antibiotic therapy helps in reducing both incidence and mortality associated with avian colibacillosis, unscrupulous use of antibiotics to prevent infections results in emergence of large numbers of drug resistant *E. coli* posing problems to control these infections (Sharada *et al.,* 2010).

Acquired antimicrobial resistance patterns were observed in *E. coli* isolates with predominant patterns being distributed widely across poultry types indicating a striking diversity of resistance patterns (Okoli *et al.,* 2005 and Persoons *et al.,* 2010). Xia *et al.* 2011 reported that Over 58% of *E. coli* isolates showed resistance to four or more antimicrobial agents, that indicates a need to better understand the role of certain meat types as potential sources of human ExPEC infection.

Different virulence-associated genes that play important roles individually or in combination in

adhesion, ferric transport system, hemolyzation, and toxin-production of APEC have been reported (Ewers *et al.,* 2004; Janben *et al.,* 2001; Johnson *et al.,* 2006; Mellata *et al.,* 2003 and Yaguchi *et al.,* 2007). *Eae*A gene encodes intimin, an outer membrane protein that is responsible for the attachment to the intestinal epithelial cells (Yu and Kaper, 1992). Intimin is a protein encoded by *eaeA* cromosomal gene and mediates adherence of attaching and effaching *E. coli* to the intestinal epithelial cell (Ghanbarpour and Oswald, 2010).

Resistance to β-lactam antimicrobial agents in *E. coli* is primarily mediated by β –lactamases (Livermore, 1995). These enzymes that are capable of hydrolyzing oxyimino L-lactams were isolated in the mid-1980s and are variants of the well-established TEM and SHV penicillinases (Thomson and Moland, 2000). Many different β -lactamases have been described (Bush *et al.,* 1995; Livermore, 1995 and Livermore, 1998). The classical TEM-1, TEM-2, and SHV-1 enzymes are the predominant plasmid-mediated β –lactamases of gram-negative rods (Briñas *et al.,* 2002).

Quinolone resistance in the Enterobacteriaceae is mostly mediated by point mutations in the quinolone resistance-determining regions (QRDR) of the target genes (*gyrA* and *gyrB*, which encode DNA *gyrase*, and *parC* and *parE*, which encode topoisomerase IV) (Fàbrega *et al.,* 2009; Cavaco *et al.,* 2009). Other resistance mechanisms include efflux pump mechanisms which is mediated by *QepA* genes (Yamane *et al.,* 2007), and more recently, Plasmid-mediated quinolone resistance encoded by the *qnr* genes which comprises a group of pentapeptide repeat proteins that protect bacteria against quinolones (Tran and Jacoby, 2002), and *aac(6')-Ib-cr* which is a modified aminoglycoside N-acetyltransferase that acetylates some fluoroquinolones, including Ciprofloxacin (Robicsek *et al.,* 2006).

Thus the aim of this study was to investigate and characterize multidrug resistant virulent *E.coli* isolated from broiler flocks suffered from high mortality rates.

MATERIALS and METHODS

Collection of samples:
A total of 315 samples from heart, liver and lung were collected under aseptic conditions from 105 freshly dead or diseased birds aged from 7 - 45 days and suffering from perihepatitis, pericarditis, pneumonia, airsacculitis, peritonitis, enteritis or yolk sac infection.

Isolation and Identification:
Tissue samples were inoculated on MacConkey agar (HIMEDIA) incubated at 37^{o}C for 24 hours, lactose fermenting colonies were subcultured on Eosin Methylene blue agar (HIMEDIA) incubated at 37^{o}C for 24 hours. Suspected *E. coli* colonies with metallic sheen were subjected to biochemical tests.

Biochemical identification:
Suspected *E. coli* colonies were tested biochemically by applying the following tests: (Oxidase, Catalase, Methyl Red, Vogues Proskaur, Indole, Citrate utilization, Nitrate reduction, Urease, TSI) according to (Kreig *et al.,* 1984).

Serotyping:
E. coli isolates were serotyped in Reference Laboratory for Veterinary Quality Control on Poultry Production using commercially available kits (Test Sera Enteroclon, Anti –Coli, SIFIN Berlin, Germany).

Antibiogram:
Antibiotic sensitivity was performed using Mueller Hinton Agar plates (HIMEDIA) using antibiotic discs of 20 commonly used antibiotics. Measuring the diameter of the inhibition zones produced was done according to (Bauer *et al.,* 1966).

DNA extraction:
DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 20 µl of proteinase K and 200 µl of lysis buffer at 56^{o}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer:
Primers used were supplied from Metabion (Germany) and are listed in Table (1).

PCR amplification:
Primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of template. The reactions were performed in a Biometra T3 thermal cycler.

Analysis of the PCR Products:
The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the PCR products were loaded in each gel slot. A 100 bp DNA Ladder (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra, Germany) and the data was analyzed through computer software.

RESULTS

Incidence rate of *E. coli* in broiler samples:
Bacteriological examination of lung, liver and heart samples from each bird case revealed 84 (80%) *E. coli* isolates out of the total 105 examined birds.

Table 1: PCR primers used for amplification of virulence-associated genes and antibiotic resistant genes of E.coli.

Gene	Primers sequences	Amplified segment (bp)	Secondary denaturation	Annealing	Extension	Reference
eaeA	GACCCGGCACAAGCATAAGC	384	94˚C 45 sec.	54˚C 45 sec	72˚C 45 sec	Wen-jie JIN *et al*., 2008
	CCACCTGCAGCAACAAGAGG					
qepA	CGTGTTGCTGGAGTTCTTC	403	94˚C 45 sec.	50˚C 45 sec	72˚C 45 sec	Cattoir *et al*., 2008
	CTGCAGGTACTGCGTCATG					
qnrA	ATTTCTCACGCCAGGATTTG	516	94˚C 45 sec.	53˚C 45 sec	72˚C 45 sec	Robicsek *et al*., 2006
	GATCGGCAAAGGTTAGGTCA					
qnrB	GATCGTGAAAGCCAGAAAGG	469				
	ACGATGCCTGGTAGTTGTCC					
qnrS	ACGACATTCGTCAACTGCAA	417				
	TAAATTGGCACCCTGTAGGC					
aac(6')-Ib-cr	CCCGCTTTCTCGTAGCA	113	94˚C 30 sec.	52˚C 30 sec	72˚C 30 sec	Lunn *et al*., 2010
	TTAGGCATCACTGCGTCTTC					
blaTEM	ATCAGCAATAAACCAGC	516	94˚C 45 sec	54˚C 45 sec	72˚C 45 sec	Colom *et al*., 2003
	CCCCGAAGAACGTTTTC					

Table 2: Incidence of detected serotypes based on total number of *E. coli* isolates.

Serotype	Number of isolates	Incidence of serotypes
O 114:K 90	15	17.86%
O 125:K 70	12	14.29%
O 26:K 60	9	10.71%
O 44:K 74	3	3.57%
O 111:K 58	9	10.71%
O 55:K 59	12	14.29%
O126:K71	3	3.57%
O91:K-	6	7.14%
O 145:K -	3	3.57%
O118:K -	3	3.57%
O 25:K11	3	3.57%
Untypeable isolates	6	7.14%
Total	**84**	**100%**

In the present study, 84 *E. coli* isolates were differentiated into 3 different O groups: Poly 1, Poly 11 and Poly 111 of which 11 different serotypes were identified as follows, O 114:K 90 predominated with 17.86% of the total isolates, followed by O125:K 70 and O 55:K 59 with 14.29% each, O 111:K 58 and O 26:K 60 with 10.71% each. 7.14% of the isolates were untypeable using the commercially available kits, while all of the rest 5 identified serotypes had the same isolation rate 3.57% which were O 145:K -, O 25:K 11, O 44:K 74, O126:K71, O118:K -.

Table 3: The distribution of different serotypes in relation to organs of isolation.

Organ of isolation	Serogroups	Serotypes	Serotypes %	Isolation rate
Liver	Poly 1	9- O 114:K 90 9- O 125:K 70 3- O 26:K 60 3- O 44:K 74	90.91% (10)	57.14% (60)
	Poly 11	9- O 111:K 58 9- O 55:K 59 3- O126:K71 6- O91:K-		
	Poly 111	3- O 145:K - 3- O118:K -		
	Untypeable	3 –isolates		
Heart	Poly 1	12- O 114:K 90 3- O 125:K 70 6-O 26:K 60	63.64% (7)	37.14% (39)
	Poly 11	3- O 55:K 59		
	Poly 111	3- O145:K - 3-O25:K 11 3- O118:K -		
	Untypeable	6 – isolates		
Lung	Poly 1	9-O 114:K 90, 12- O125:K 70, 6-O 26:K 60, 3- O44:K 74,	72.73% (8)	54.29% (57)
	Poly 11	9- O 111:K 58 6- O 55:K 59		
	Poly 111	3- O 145:K - 3- O118:K -		
	Untypeable	6- isolates		

The maximum isolation percentage was from liver: 60 (57.14%), while there were 39 (37.14%) and 57 (54.29%) from heart and lung, respectively.

Table 4: Antibiotic susceptibility pattern of the isolates.

Antibiotic group	SN	Chemotherapeutic agent	Symbol	Conc.of disc	Resistant	Sensitive
Penicillins	1	Penecillin	P	5 ug	96.43%	3.57%
	2	Amoxicillin	AMX	25 ug	92.86%	7.14%
	3	Ampicillin	AMP	10 ug	92.86%	7.14%
Nitrobenzene drevatives	4	Chloramphinicol	C	30 ug	82.14%	17.86%
Peptides	5	Colistin	CT	10 u	10.71%	89.29%
Macrolydes	6	Lincomycin	L	2 ug	96.43%	3.57%
	7	Erythromycin	E	15 ug	67.86%	32.14%
1st Generation Quinolones	8	Flumequine	UB	30 ug	92.86%	7.14%
	9	Nalidixic A	NA	30 ug	96.43%	3.57%
2nd Generation Quinolones	10	Ciprofloxacin	O	5 ug	25%	75%
	11	Danofloxacin	D	5 ug	89.26%	10.71%
	12	Enrofloxacin	ENR	10 ug	92.86%	7.14%
	13	Norfloxacin	NOR	10 ug	89.26%	10.71%
Aminoglycosides	14	Neomycin	N	30 ug	75%	25%
	15	Gentamycin	GM	10 ug	50%	50%
	16	Streptomycin	S	10 ug	92.86%	7.14%
Tetracyclines	17	Doxycycline	DFX	30 ug	100%	0%
	18	Tetracycline	TE	30 ug	96.43%	3.57%
	19	Oxytetracycline	OT	30 ug	96.43%	3.57%
Diaminopyrimidine	20	Trimethoprime	SXT	1.25 ug	82.14%	17.86%

The sensitivity and resistance pattern of *E. coli* isolates for various antibiotics were tested, it was observed that none of the used antibiotics was 100 % effective, on the other hand, multidrug resistance patterns have been recorded among all isolates. Out of the total tested 84 isolates, 6 (7.1%) were resistant to the 20 used antibiotics, 9 (10.7%) were resistant to 19 antibiotics, 15 (17.9%) were resistant to 18 antibiotics, 18 (21.4%) were resistant to 17 antibiotics, 12 (14.3%) were resistant to 16 antibiotics, 9 (10.7%) were resistant to 15 antibiotics, 9 (10.7%) were resistant to 14 antibiotics, 3 (3. 6%) were resistant to 13 antibiotics and 3 (3.6%) were resistant to 6 antibiotics.

Prevalence of virulence-associated genes and antibiotic resistance genes in *E. coli* are shown in Table (5), Fig. (1) and Fig. (2). Among 7 tested genes, the most prevalent gene was *blaTEM* gene (78.6%) and the least prevalent ones were *aac(6')-Ib-cr* and *qnrA* (21.4%).

Table 5: Individual PCR results of the different tested genes for the isolated serotypes.

Serotype	Quinolone resistance genes					Virulence	β–lactamase
	qepA	***qnrA***	***qnrB***	***qnrS***	***aac(6')-Ib-cr***	***eaeA***	***blaTEM***
O 114:K 90	+	-	-	+	-	+	+
O 145:K -	+	-	+	+	-	+	+
O 25:K 11	+	-	-	+	-	+	+
O125:K70	-	-	-	-	-	+	+
O 111:K 58	+	-	-	-	-	+	+
O 44:K 74	+	-	+	+	-	-	+
O 125:K 70	+	+	+	+	+	+	+
O126:K71	-	+	+	+	-	+	+
O91:K-	+	-	+	+	-	+	+
O 55:K 59	-	-	-	-	+	-	-
O 26:K60	-	-	-	-	+	-	-
O91:K-(x)	-	-	-	-	-	-	-
O118:K -	+	+	+	+	-	+	+
O 26:K 60	+	-	+	+	-	+	+
Pos / Total	9/14	3/14	7/14	9/14	3/14	10/14	11/14

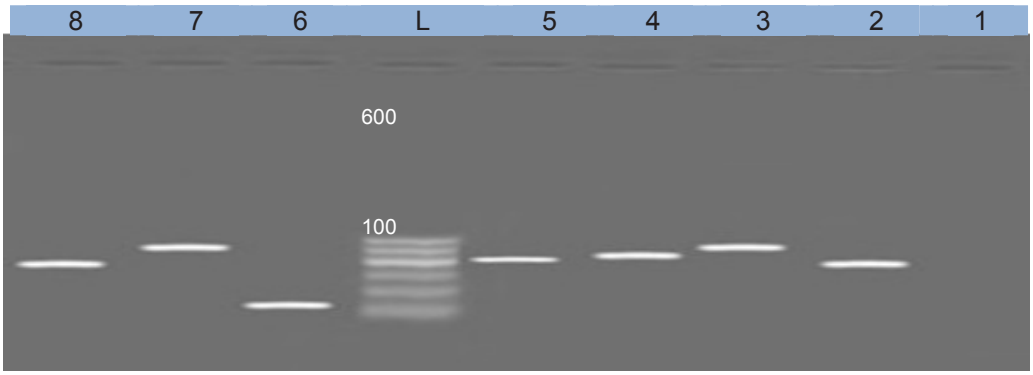
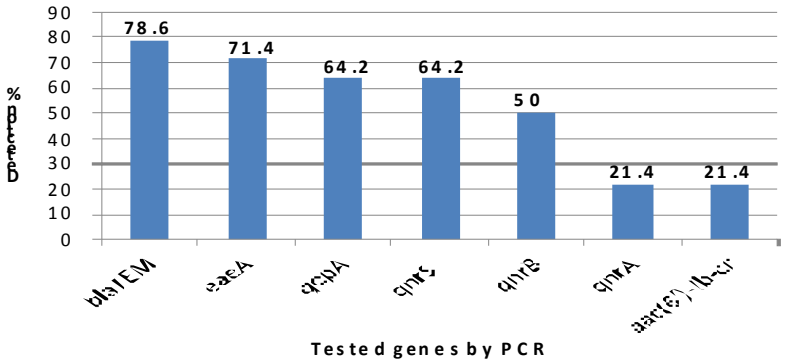


Fig. 1: PCR results for virulence-associated gene containing E. coli isolates and antibiotic resistant genes. L, 100 bp ladder (QIAGEN, Gmbh) (100-600 bp). 1-8 lanes were respectively: 1) Negative control, 2) *qepA* (403 bp), 3) *qnrA* (516 bp), 4) *qnrB* (469 bp), 5) *qnrS* (417 bp), 6) *aac(6')-Ib-cr* (113 bp), 7) *blaTEM* (516 bp) and 8) *eaeA* (384 bp).

Fig. 2 Detection percentages of the different tested genes by PCR.



DISCUSSION

In the present study, *E. coli* was recovered from 84 (80%) out of the total examined 105 freshly dead or diseased cases. A lower rate was recorded by (Zhao *et al.,* 2001; Sharada *et al.,* 2010; Hasan *et al.,* 2011 and Literak *et al.,* 2013) who isolated *E. coli* with the percentages of 38.7%, 44.61% , 36.20% and 35.74%, respectively. Higher rates were recorded by (Johnson *et al.,* 2005 and Lyhs *et al.,* 2012) who recovered *E. coli* in 92% and 94.5% of the tested samples, respectively.

Regarding isolation rates from different organs with pathologic conditions, the maximum rate was recorded in liver showing perihepatitis 60 (57.14%), followed by lung showing pneumonia 57 (54.29%) and the least percentage was recorded from heart (pericarditis) 39 (37.14%) out of the examined 105 organs from each type (liver, lung and heart). These findings are in accordance with (Sharada *et al.,* 2010) who recorded the highest percentage of isolation in their study from liver samples (44.61%) and the lowest percentage was from heart samples (16.92%). Isolation rates from liver, heart and lung was inaccordance with (Ghosh, 1988; Krishnamohan and Koteeswaran, 1994 and Sharada *et al.*, 2010) who reported the association of perihapatitis and pericarditis and stated that this association confirmed the high virulence of *E. coli* isolates causing the aforementioned pathologic conditions.

In the present study, serotyping of the isolated *E. coli* revealed 11 different serotypes which were identified as follows, O 114:K 90 predominates with 17.86% of the total isolates, O 125:K 70 and O 55:K 59 with 14.29% each, O 111:K 58 and O 26:K 60 with 10.71%, while 7.14% of isolates were untypeable using the commercially available kits, while all of the identified serotypes were detected with 3.57% isolation rate which were O 145:K $^{-}$, O 25:K 11, O 44:K 74, O126:K71, O118:K $^{-}$. These results agreed with those of (Rosario *et al*., 2004 and Oh *et al*., 2012) who failed to identify the serogroup of 15% and 50% out of their tested isolates, respectively. Saif *et al.* 2003 stated that there are variations according to the geographic region and that some pathogenic isolates do not belong to known serotypes or are untypeable. They also reported that only 15% of the strains belonged to the serogroups O1, O2, O35, O36, and O78 that have been associated previously with avian colibacillosis were isolated from diseased birds, suggesting that this might signal the emergence of new pathogenic serotypes. Zhao *et al.,* 2005 reported that the majority of the avian *E. coli* isolates (60%) were non-typeable with the standard available antisera.

The Results for Antibiotic sensitivity showed that all the isolates demonstrated multidrug resistance against most of the 20 types of antibiotics used. This agreed with (Yang *et al.,* 2004*;* Johnson *et al.,* 2005*;* Wang *et al.,* 2010*;* Hasan *et al.,* 2011 and Jiang *et al*., 2009) who recorded that multiple-antimicrobial resistant *E. coli* isolates, including fluoroquinolone-resistant variants are commonly present among diseased chickens. Our results for multidrug resistant isolates also agreed with that of (Tricia *et al.,* 2006) who stated that avian *E. coli* isolates that were tetracycline resistant are more likely to become resistant to additional antimicrobial agents as kanamycine and nalidixic acid.

The study recorded maximum resistance against tetracyclines; as resistance against doxycyclin was 100% followed by tetracycline, oxytetracyclin which were both 96.43%. This results agreed with that of (Zhang *et al.,* 2012-b; Ionica, 2011 and Jiang *et al*.,

2009) who recorded resistance to doxycyclin and tetracycline (70.12%, 84.76%), (95.6%, 93.4%) and (97,62%, 90,48%), respectively. Lower rates were observed against tetracyclines by (Guerra *et al*., 2003; Hasan *et al*., 2011 and Xia *et al*., 2011) which were (15-30%), (45.5%) and (67.0%), respectively.

The present study recorded resistance rates against penicillin (96.43%), and 92.86% for ampicillin, and amoxicillin. These findings agreed with those of (Yang *et al.,* 2004; Li *et al*., 2007 and Jiang *et al*., 2009*)* who reported resistance to ampicillin by the percentages of 79%, 99.5% and 83%, respectively. lower resistance rates were recorded to ampicillin by (Guerra *et al.,* 2003; Sharada *et al.,* 2010 and Hasan *et al*., 2011) who recorded the resistance with the percentages of 25.7%, 27.69% and 15-30%, respectively.

The present study revealed that 96.43% of the *E.coli* isolates were resistant to nalidixic acid. This finding disagreed with those of (Guerra *et al*., 2003; Johnson *et al.,* 2003 and Amy *et al*., 2010) who recorded resistance rates of (11%), (37%) and (41.5%), respectively, almost similar results were recorded by (Yang *et al*., 2004 and Salehi and Farashi, 2006) (100%) both.

The study showed resistance percentages to lincomycin of 96.43%. Almost similar resistance were detected by (Salehi and Farashi, 2006 and Ionica, 2011) who reported 100% and 80.95% resistance percentages, respectively. lower rates were detected by (Al-Saati *et al.,* 2009 and Sharada *et al*., 2010) (5% and 39.50%, respectively).

The highest sensitivity rate detected in our study was to colistin 89.29%. This result agreed with that of (Galani *et al*., 2008) who reported that colistin exhibited excellent activity against *Escherichia coli* isolates, and also agreed with (Al-Saati *et al*., 2009; Sharada *et al*., 2010 and Ionica, 2011) who observed 94.00%, 95% and 61.90% sensitivity rates, respectively.

The study showed high sensitivity rate to ciprofloxacin (75%). This finding agreed with those of (Bogaard *et al*., 2001; Al-Saati *et al.,* 2009; Hasan *et al*., 2011 and Lyhs *et al*., 2012) who recorded 90%, 95%, 87.1% and 100% sensitivity rates, respectively. Our results of sensitivity to ciprofloxacin disagreed with those of (Yang *et al*., 2004; Li, *et al*., 2007 and Wang *et al*., 2010) who detected high resistance rates (79%, 81% and 87%, respectively).

It was also reported that 50% sensitivity rate was found to gentamycin. Similar resistance rates were recorded by (Mahmood and Reza, 2010; Sharada *et al*., 2010 and Zhang *et al.,* 2012-a) who reported 54.5%, 60% and 60%, respectively. Lower rate (31%) were detected by (Zhao *et al*., 2005). Higher sensitivity rates were detected by (Salehi and Farashi, 2006; Hasan *et al*., 2011 and Ionica, 2011) (100%,98% and 90.48%, respectively).

The results of Antibiotic susceptibility of our study are invariance with some studies and inaccordance with others, indicating that antibiotic suscebtibility pattern varies with different isolates, time and development of multiple drug resistant *E.coli*. as reported by (Holmberg *et al.,* 1984 and Sharada *et al*., 2010).

Attaching and effaching was a term to describe an intestinal lession (AE lesion) caused by specific strains of *E. coli*. 'Attaching' because of the intimate attachment of the bacteria to the exposed cytoplasm membrane of the enyterocyte; and 'effaching' because of the localized disapperance of the brush border microvilli (Stordeur *et al.,* 2000). This was represented in our results by the high incidence rate (71.4%) of *eaeA* gene detection that was recorded, as it was detected by PCR in 10 isolates out of the 14 tested isolates. The high incidence rate of *eaeA* gene detection was recorded by many authors as (Suardana *et al*., 2011 and El-Jakee *et al*., 2012) who detected *eaeA* gene in 95% and 95.9% of the tested *E. coli* O157:H7 isolates, respectively. The *eaeA* gene was detected also in a lower prevalence rate (25%) in the wild birds and it was suggested that most of the *E. coli* strains are probably related to atypical EPEC strains found in humans as reported by (Kobayashi *et al*., 2009). However, no *eaeA* gene was detected among 216 APEC isolates tested by (Wen-jie *et al*., 2008) who supporeted the results by that obtained by (Krause *et al*., 2005) who reported a low detection rate (2.3%) of *eaeA* gene in the tested *E. coli isolates* from fecal samples of healthy chickens.

PCR detected 11 positive isolates for *blaTEM* out of the 14 tested isolates (78.6%). This results agreed with the results of the antibiogram test in which all of the PCR tested isolates were resistant to Penicillins (Penicillin, Amoxicillin and Ampicillin). The high incidence of *blaTEM* detection in *E. coli* was previously recorded by many authors as (Colom *et al*., 2003) who detected *blaTEM* gene in 45 out of 51 Amoxicillin-clavulanate resistant *E. coli* isolates with 88.2%. The high *blaTEM* gene prevalence was recorded by (Brinas *et al*., 2002) who detected the gene in 83% of 124 Ampicillin resistant *E. coli* isolates including food isolates of chicken origin. A close *blaTEM* detection percentage to our results was detected also by (Yuan *et al.,* 2009) who detected this gene in 79.3% of the chinese *E. coli* isolates that were studied for Extended-spectrum b-lactamase (ESBL) production.

Quinolones are synthetic compounds that have been used extensively for treatment of a variety of infectious diseases. (Hooper, 2001). This wide spread

use has been followed by increasing bacterial resistance (Tran and Jacoby, 2002). *QepA* is a plasmid-encoded efflux pump that significantly affects susceptibility to Norfloxacin (Yamane *et al*., 2007). This was reported in our results, as all of the strains that showed positive amplification of the *qepA* gene showed resistance to Norofloxacin by the antibiotic sensitivity test. As shown in Table (6) and Fig. (2), the detection percentages of the *qepA, qnrA, qnrB, qnrS and aac(6')-Ib-cr* genes were 64.2%, 21.4%, 50%, 64.2% and 21.4%, respectively. The percentages was much higher than most of the previously reported results by the researchers worked at this field as (Chen *et al*., 2012) who detected each of the *qepA, qnrB, qnrS* in 1.3% of the isolates and detected *aac(6')-Ib-cr* gene in 3.1% of the isolates, but the *qnrA* gene was not detected in any of the 384 chicken isolates. Also, the *qnrS* gene, but not *qnrA, qnrB,* and *qepA* genes were detected in 6/300 chicken isolates (2%) from 30 chicken farms in Taiwan (KUO *et al*., 2009). Only *qnrA* gene was found in 5.3% of *E. coli* isolates, while *qnrB* and *qnrS* were not detected when (MÜSTAK *et al*., 2012) tested 94 chicken *E. coli* isolates for the three mentioned genes. However, some researchers reported high incidence of the quinolones resistant genes as (Wang *et al*., 2008) who detected *qnrA* in 72.7% of the isolates and detected each of the *qnrB* and *qnrS* genes in 45.4% of the 14 *E. coli* isolates tested. However, the high incidence of the detected genes in our results and in the previously mentioned researcher results may be due to the low number of the tested isolates.

Our study reported the high incidence of virulent multidrug resistant E. coli isolates among diseased broiler flocks, this is in accordance with (Okoli *et al.,* 2005; Persoons *et al.,* 2010 and Xia *et al.,* 2011).

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عزل وتوصيف ميكروب الاشيرشيا كولاى المصاحب لحالات النفوق المرتفع في قطعان الدجاج.

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تم جمع عينات من ١٠٥ حالة دجاج تسمين تعانى من ارتفاع الوفيات بمحافظة الشرقية. تم عزل الأشريشيا كولاي من ٨٤ حالة (٨٠%). تم عزل ١١ نمط مصلى مختلف وكان أكثرها هو O114:K90 بنسبة ١٧.٨٦% من المعزولات. كما تم عزل الأنماط 125:K70 و O55:K59 و O111:K58 و O26:K60 بنسب ١٤.٢٩% و ١٤.٢٩% و ١٠.٧١% و ١٠.٧١% على الترتيب. كما تم عزل ٥ أنماط مصلية مختلفة بنفس النسبة ٣.٥٧% وهذه الأصناف هي – O145:K و O25:K11 و O44:K74 و O126:K71 و – O118:K. وكانت نسبة المعزولات الغير مصنفة هي ٧.١٤%. دراسة حالات مقاومة المضادات الحيوية للمعزولات أظهرت تعدد مقاومتها للمضادات الحيوية. تم تسجيل أعلى نسبة مقاومة للدوكسيسيكلين (١٠٠%)، بينما كانت نسب المقاومة للبنسلين والليفوميسين وحمض النالديكسيك والتتراسيكلين والأوكسيتتراسيكلين ٩٦.٤% لكل منهم. بينما كانت أعلى نسبة للحساسية ٨٩.٣% للكولستين و ٧٥% للسيبروفلوكساسين و٥٠% للجينتاميسين. تم فحص بعض المعزولات لجين eaeA المسئول عن الاتصال بواسطة اختبار تفاعل انزيم البلمرة لتحديد ضراوة المعزولات. كما تم الكشف عن الجينات المسئولة عن المقاومة للكينولونات مثل جين qnrA و qnrB و qnrS و qepA و *aac(6')-Ib-cr* . كما تم تحديد المقاومة للأموكسيسيللين بالكشف عن جين blaTEM المسئول عن انزيمات β-lactamases.