Molecular Detection of Some Virulence Genes of *E. Coli* Isolated from Broiler Chickens and Ducks at Ismailia Governorate

Marwa, E. Hassan[*]; Mohamed, K. Moursi[**]; Rana, M. A. El-Fattah[**] and Mohamed, E. Enany[*]

[*]Bacteriology, Immunology and Mycology Department, Faculty of Veterinary Medicine, Suez Canal University. [**]Reference Laboratory for Veterinary Quality Control on Poultry Production, Animal Health Research Institute, Egypt.

Abstract

This study was conducted to investigate the existence of some virulence genes among *Escherichia coli* strains isolated from broiler chickens and ducks. A total of 252 samples (liver, heart blood and lung) were collected from diseased and freshly dead broiler chickens (192 organ from 64 bird) and ducks (60 organ from 20 bird) from different poultry farms at Ismailia Governorate, Egypt during period from May 2018 to July 2019. All samples were subjected to bacteriological examination for isolation and identification of *E. coli*. Eighteen *E. coli* isolates were subjected for detection of some virulence genes (*iss*, *iut*A, *pap*C, *tra*T, *eae*A and *vat*) by conventional polymerase chain reaction (cPCR). Out of 252 samples, 203 (80.5%) were positive for *E. coli*. Virulence genes were detected among 18 selected *E. coli* strains with variable percentage. All 18 *E. coli* isolates (100%) were *iss*, *iut*A and *vat* genes positive, 16 (88.8%) were *tra*T gene positive, 5 (27.7%) were *eae*A gene positive while, 2 (11%) were *pap*C gene positive.

Key words: Virulence Genes – *Escherichia coli* – Broiler Chickens – Ducks – cPCR.

Introduction

Escherichia coli is a part of the normal microflora in the poultry intestine, but certain strains, such as avian pathogenic *E. coli* (APEC), spread into various internal organs and cause avian colibacillosis ***(Oh et al., 2011).*** Colibacillosis affects multiple systems in the bird (respiratory, digestive and reproductive systems) resulting in many disease conditions end by death of the bird ***(Singh et al., 2011).*** The avian pathogenic *E. coli* (APEC) strains belonging to the extraintestinal pathogenic *E. coli* (ExPEC) groups and

causing colibacillosis in birds are reported to carry similar virulence attributes as human ExPEC. This suggests a possible route of ExPEC dissemination in the community through poultry and poultry products that may serve as a potential reservoir of ExPEC ***(Bergeron et al., 2012).***

Certainly, APEC like other pathogenic *E. coli*, acquired genes by horizontal transfer that encode virulence factors, which serve to distinguish APEC from commensal strains. These virulence genes may be clustered into chromosomal- or plasmid-located pathogenicity islands (PAIs) ***(Lisa et al., 2013 and Navarro-Garcia et al., 2019).*** The virulence factors, such as adhesins, invasins, toxins, iron acquisition systems (siderophores), and protectins, are involved in colonization, adhesion, invasion, and survival against host defenses ***(Janßen et al., 2001; Jeong et al., 2012 and Ghunaim et al., 2014).***

The objective of this study was molecular detection of some virulence genes of *E. coli* isolated from broiler chickens and ducks at Ismailia Governorate using conventional polymerase chain reaction (cPCR) as a rapid diagnosis for genotypic characterization.

Material and methods

1- Samples collection:

All samples were collected under aseptic condition and safety precautions ***(Vandemaele et al., 2002; Ahmed et al., 2009 and Farooq et al., 2009)***.

Liver, heart blood and lung were collected from each diseased and freshly dead broiler chickens and ducks using sterile scissor and forceps and under aseptic condition. The surface of each organ was seared by hot spatula and then sterile loopfuls were taken from liver, heart blood and lung through the seared portion then samples were subjected for bacteriological examination ***(Roshdy et al., 2012; Eid and Erfan 2013 and Abd El Tawab et al., 2016).***

2- Bacteriological examination:

Loopfuls taken from liver, heart blood and lung were inoculated in buffer peptone water and incubated at 37 ^{o}C for 18±2 hrs under aerobic condition. A loopful from each sample was separately streaked onto MacConkey's agar and eosin methylene blue agar (EMB) and incubated at 37 ^{o}C for 24 hrs. Suspected colonies were picked up, subjected to morphological and Gram staining reaction. Suspected isolates were confirmed by a series of biochemical identification according to ***Murray et al. (2003).***

3- Molecular characterization of *E. coli* isolates using conventional polymerase chain reaction (cPCR):

Eighteen *E. coli* isolates were subjected to cPCR for detection of 6 virulence genes (*iss*, *iut*A, *pap*C, *tra*T, *eae*A and *vat*). Six pairs of primers were supplied from **metabion (Germany) or Biobasic (Canada).** They have a specific sequence and amplify a specific product as shown in table (1).

3.1. Extraction of *E. coli* DNA: using QIAamp DNA Mini Kit instructions (Catalogue No. 51304).

3.2. Preparation of PCR Master Mix According to Emerald Amp GT PCR master mix (Takara) Code No. RR310A kit as shown in table (2).

3.3. Cycling conditions of the primers during cPCR: Temperature and time conditions of the primers during PCR are shown in table (3) according to specific authors and Emerald Amp GT PCR master mix (Takara) kit.

3.4. Agarose gel electrophoresis (Sambrook *et al.*, 1989). DNA Molecular weight marker: using Gene ruler 100 bp plus DNA ladder (cat. no. SM0243) supplied from Fermentas with size range: 100-1000 bp.

Table (1): *Oligonucleotide primers sequences used for amplification of virulence genes (iss, iutA, papC, eaeA, traT and vat):*

Target gene	Primer	Primer sequence (5'-3')	Gene description	Length of amplified product (bp)	Reference
iss	F	ATGTTATTTTCTGCCGCTCTG	Increased serum survival gene (outer membrane protein)	266 bp	Yaguchi *et al.*, 2007
	R	CTATTGTGAGCAATATACCC			
*iut*A	F	GGCTGGACATGGGAACTGG	Ferric aerobactin receptor (iron uptake transport)	300 bp	
	R	CGTCGGGAACGGGTAGAATCG			
*pap*C	F	TGATATCACGCAGTCAGTAGC	Pyelonephritis associated pili	501 bp	Wen-jie *et al.*, 2008
	R	CCGGCCATATTCACATAA			
*eae*A	F	ATGCTTAGTGCTGGTTTAGG	Attaching and effacing mechanism (intestinal gene)	248 bp	Bisi-Johnson *et al.*, 2011
	R	GCCTTCATCATTTCGCTTTC			
*tra*T	F	GATGGCTGAACCGTGGTTATG	Surface exclusion, serum survival (outer membrane protein)	307 bp	Kaipainen *et al.*, 2002
	R	CACACGGGTCTGGTATTTATGC			
vat	F	AACGGTTGGTGGCAACAATCC	Vacuolating auto-transporter toxin	420 bp	Boisen *et al.*, 2009
	R	AGCCCTGTAGAATGGCGAGTA			

Table (2): *Preparation of PCR master mix:*

Component	Volume/reaction
Emerald Amp GT PCR master mix (2x premix)	12.5 μl
PCR grade water	4.5 μl
Forward primer (20 pmol)	1 μl
Reverse primer (20 pmol)	1 µl
Template DNA	6 μl
Total	4 µl

Table (3): *Cycling conditions of the different primers during cPCR:*

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
***pap*C**	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
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 5 min.	94˚C 30 sec.	63˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
***tra*T**	94˚C 5 min.	94˚C 30 sec.	55˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
***eae*A**	94˚C 5 min.	94˚C 30 sec.	51˚C 30 sec.	72˚C 30 sec.	35	72˚C 7 min.
vat	94˚C 5 min.	94˚C 30 sec.	58˚C 40 sec.	72˚C 40 sec.	35	72˚C 10 min.

Results

1- Incidence of *E. coli* isolation:

The overall percentage of *E. coli* isolated from diseased and freshly dead broiler chickens and ducks was 80.5% (203/252). Out of 192 chicken samples 145 were positive for *E. coli* with the percentage of 75.5 %. While, 58 out of 60 duck samples were positive for *E. coli* with the percentage of 96.7%.

2- Bacteriological examination:

The results of bacteriological examination were presented in table (4).

3- Molecular characterization of *E. coli* isolates using conventional PCR (cPCR):

Figure (2), (3), (4), (5), (6), (7), (8) and table (5) showed the amplification and the detection of 6 virulence genes (*iss*, *iut*A, *pap*C, *tra*T, *eae*A and *vat*) in 18 *E. coli* isolates.

Table (4): *Prevalence of positive E. coli in different organs of diseased and freshly dead broiler chickens and ducks.*

Source	Organs	Number of examined samples	Positive samples		Negative samples	
			No.	%	No.	%
Broiler chicken	Liver	64	56	87.5	8	12.5
	Heart	64	53	82.8	11	17.2
	Lung	64	36	56.3	28	43.7
	Total	**192**	**145**	**75.5**	**47**	**24.5**
Duck	Liver	20	20	100	0	0
	Heart	20	20	100	0	0
	Lung	20	18	90	2	10
	Total	**60**	**58**	**96.7**	**2**	**3.3**

***The percentage was calculated according to the number of examined samples.**

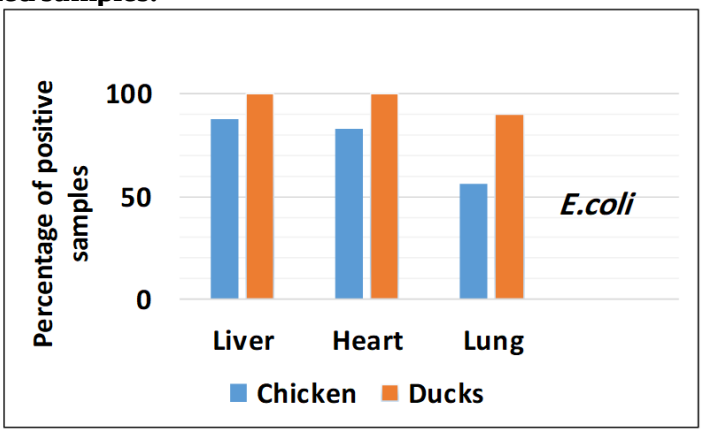

Fig. (1): Prevalence of positive *E. coli* in different organs of diseased and freshly dead broiler chickens and ducks.

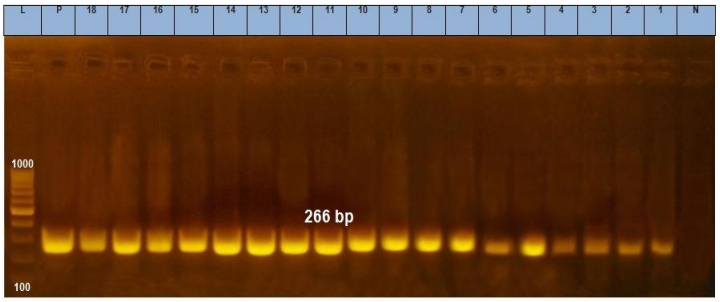

Fig. (2): Agarose gel electrophoresis of amplified *iss* gene PCR product (266 bp).

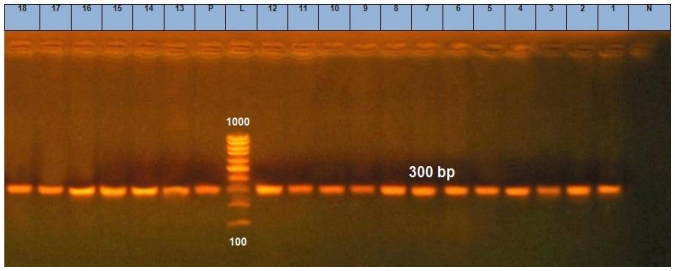

Fig. (3): Agarose gel electrophoresis of amplified *iut*A gene PCR product (300 bp).

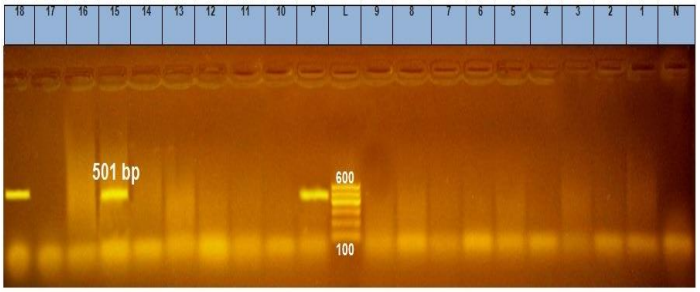

Fig. (4): Agarose gel electrophoresis of amplified *pap*C gene PCR product (501 bp).

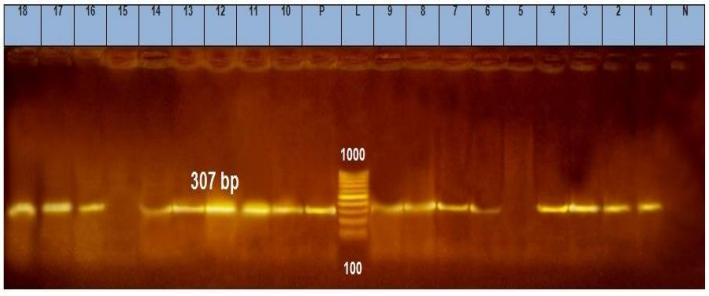

Fig. (5): Agarose gel electrophoresis of amplified *tra*T gene PCR product (307 bp).

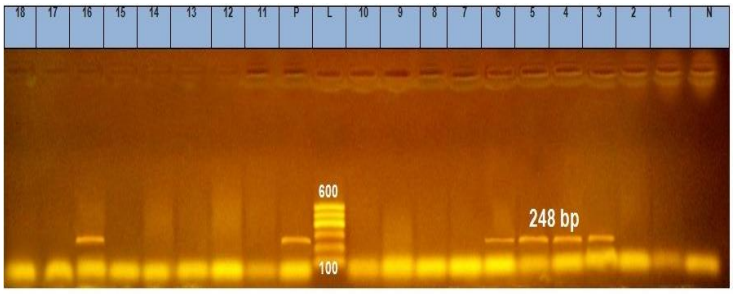

Fig. (6): Agarose gel electrophoresis of amplified *eae*A gene PCR product (248 bp).

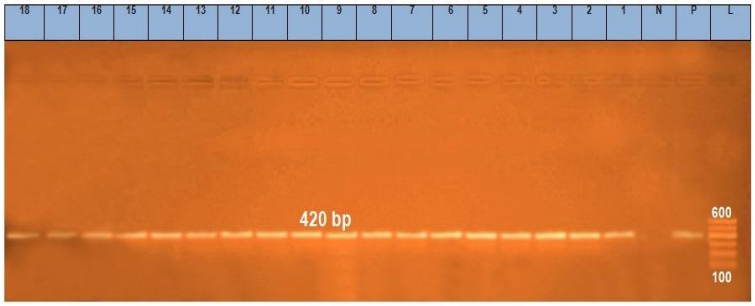

Fig. (7): Agarose gel electrophoresis of amplified *vat* gene PCR product (420 bp).

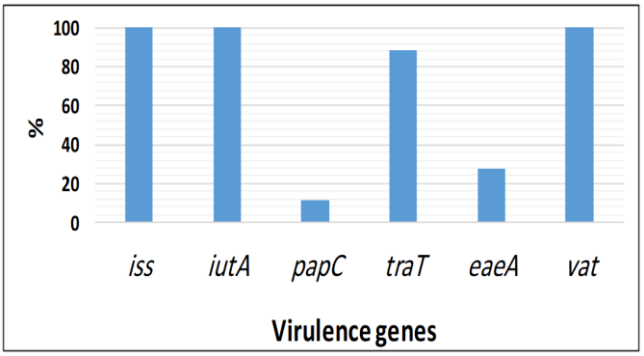

Fig. (8): Prevalence of 6 virulence genes (*iss*, *iut*A, *pap*C, *tra*T, *eae*A and *vat*) detected by cPCR among *E. coli* strains isolated from broiler chickens and ducks.

Table (5)*: Detection of virulence genes (iss, iutA, papC, traT, eaeA and vat) of 18 E. coli strains isolated from broiler chickens and ducks.*

Sources	Sample Code	Type of samples	No. of *E. coli*	Virulence genes					
				iss	*iut*A	*pap*C	*tra*T	*eae*A	*vat*
Broiler chickens	1	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	2	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	3	Heart	1	+ve	+ve	-ve	+ve	+ve	+ve
	4	Liver	1	+ve	+ve	-ve	+ve	+ve	+ve
	5	Liver	1	+ve	+ve	-ve	-ve	+ve	+ve
	6	Liver	1	+ve	+ve	-ve	+ve	+ve	+ve
	7	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	8	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	9	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	10	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	11	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
Ducks	12	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	13	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	14	Liver	1	+ve	+ve	-ve	+ve	-ve	+ve
	15	Liver	1	+ve	+ve	+ve	-ve	-ve	+ve
	16	Liver	1	+ve	+ve	-ve	+ve	+ve	+ve
	17	Heart	1	+ve	+ve	-ve	+ve	-ve	+ve
	18	Heart	1	+ve	+ve	+ve	+ve	-ve	+ve
Total			**18**	**18**	**18**	**2**	**16**	**5**	**18**
%			**100**	**100**	**100**	**11**	**88.8**	**27.7**	**100**

+ve: Positive **-ve: Negative**

Discussion

E. coli infection in poultry is usually related to high economic losses and this is because of high morbidity and mortality rates, decrease food conversion rate, body weight loss and decrease the egg production, condemnation of complete affected carcass or organs and the high cost of wide range of antibacterial agents used ***(Salama et al., 2007).***

The results of bacteriological examination showed that, the overall percentage was 80.5% (203/252) from diseased and freshly dead broiler chickens and ducks. These results were supported by ***Eid and Erfan (2013); Peer et al. (2013) and Mahmud et al. (2018)*** who recorded higher rates of *E. coli* isolation by 80%, 84% and 83.08%, respectively.

Out of 192 chicken samples 145 were positive for bacterial isolation with the percentage of 75.5 % while, 58 out of 60 duck samples were positive with the percentage of 96.7%, Similar results were obtained by ***Abd El Tawab et al. (2015),*** who isolated *E. coli* with the same rate from broiler chickens with a percentage of 75%. Also ***Eid and Erfan (2013)*** recorded that, 84 (80%) out of 105 examined diseased and freshly dead cases were *E. coli* positive, in the same context, ***Sharada et al. (2008)*** reported that, *E. coli* were recovered 65 (76.47 %) out of 85 samples collected from different organs of broiler chickens. On the other hand, lower rates (38.7%, 44.61% , 36.20% and 35.74%,) were recorded by ***(Zhao et al., 2001; Sharada et al., 2010; Hasan et al., 2011 and Literak et al., 2013).***

Concerning isolation prevalence in various chicken organs revealed that, high recovery rate of isolation was recorded from liver 87.5% (56/64), followed by heart blood 82.8% (53/64) and lung 56.3% (36/64).The same result was obtained by ***Roshdy et al. (2012) and Abd El Tawab et al. (2016)*** who recorded high isolation rate from liver followed by heart blood then from lung. While, this result was agreed to some extent with ***Eid and Erfan (2013)*** who recorded high isolation rate from liver (57.14%), followed by lung (54.29%) and heart blood (37.14%). Also, ***Sarah et al. (2013)*** reported that out of 95 liver samples, 88 were positive for *E. coli* with a percentage of 92.6%, while this result was disagreed with ***Ola (2017),*** who found that 14% of *E. coli* isolates (28/200) were isolated from liver.

On the other hand, the isolation prevalence in of *E. coli* from ducks was (96.7%) which related to liver 100% (20/20),

heart 100% (20/20) and lung 90% (18/20). Such high prevalence of *E. coli* isolation from ducks was in agreement with ***Thu et al. (2019)*** who recorded that, 990 (99.6%) out of 994 examined duck samples were positive for *E. coli*. The highest rate of *E. coli* isolation was reported from liver (78.3%), followed by lung (71.8%). Simillar result was obtained by ***Singh et al. (2012) and Kissinga et al. (2018)*** who found that, the *E. coli* isolation rates from diseased ducks was 100% and 91%, respectively. While this result disagreed with ***Majumder et al. (2017)*** who reported a lower prevalence of *E. coli* obtained from ducks (43.33%).

Although many techniques can be used to identify virulence factors, the PCR still a powerful technique for detection of pathogens because of its rapidity, specificity and sensitivity. It is an effective procedure for generating large quantities of a specific DNA sequence *in vitro* ***(Holland et al., 2000).***

The *iss* gene encodes an outer membrane protein (OMP) that plays a role in serum resistance, protecting against the actions of the complement, and the presence of this gene in pathogenic avian strains has been shown to be highly significant ***(Pfaff-McDonough et al., 2000; Nolan et al., 2003 and McPeake et al., 2005).*** *Iss* gene is located on conjugated high molecular weight plasmids with virulence factors associated with the resistance to complement, bactericidal effects of serum, and antibiotics ***(Ewers et al., 2007).***

The results showed that, *iss* gene was detected in all 18 (100%) *E. coli* isolates. Similar results were recorded by ***Kwon et al. (2008), Jørgensen et al. (2019) and Oliveira et al. (2019)*** who mentioned that, *iss* gene was detected in 100% of the *E. coli* isolates. This high frequency reveals the importance of *iss* function that allows *E. coli* to evade host defenses, multiply and disseminate, thus promoting the development of the disease ***(López et al., 2017).***

The *iut*A gene is one of the 5 genes of the aerobactin operon. It encodes an outer membrane protein involved in the high-affinity binding of Fe^{+3} aerobactin and can be plasmid located ***(Johnson et al., 2006)*** or chromosomally encoded in some APEC strains ***(Schouler et al., 2012).***

In the present work, it was found that the prevalence rate of *iut*A gene was 100% of the tested *E. coli* isolates, the same prevalence rate of *iut*A gene was detected by ***Hussein et al. (2013), Chalmers et al. (2017)***

and Jørgensen et al. (2019) in 100%, 93.3% and 96.9% of the *E. coli* isolates, respectively. While, a lower prevalence rate recorded by ***Eftekharian et al. (2016)*** who detected *iut*A gene in 70% of *E. coli* isolates.

Adherence of bacteria to tissue surfaces is an important initial event in bacterial infections. In *E. coli*, P-fimbriae, which mediates bacterial colonization in the respiratory epithelium, is coded by the pyelonephritis-associated pili (pap) (*pap*C) gene. In addition to tissue adhesion, P-fimbriae protects *E. coli* from the antibacterial activity of neutrophils ***(Pourbakhsh et al., 1997).*** The *pap*C gene is localized to pathogenicity islands (PAIs) that are chromosomal in location ***(Janßen et al., 2001).***

The obtained results revealed that, the prevalence rate of *pap*C gene in *E. coli* isolates was (11%). Where, the cluster pap, consisting of 11 genes, including *pap*C ***(Leyton et al., 2011),*** *pap*C gene has been reported to be present at a low frequency in APEC isolates ***(Johnson et al., 2002).*** The obtained data was in agreement with ***Kown et al. (2008), Varga et al. (2018) and Tidiane et al. (2019),*** who reported *pap*C gene prevalence was 11%, 10.27% and 12.9% in *E. coli* isolates, respectively. While, a lower prevalence rate recorded by ***Oliveira et al. (2019)*** who detected *pap*C gene in 1.5% of *E. coli* isolates.

E. coli isolates were examined for detection of outer membrane protein serum resistance gene (*tra*T) and it was found that the virulence gene (*tra*T) was carried by 88.8% the examined *E. coli* strains. The same result detected by ***Kogovšek et al. (2019)*** who found *tra*T gene in 88.1% of *E. coli* isolates. While, **F*erreira et al. (2018)*** reported that, only 47.7% of *E. coli* isolates were positive for *tra*T gene.

Intimin is a protein encoded by *eae*A, chromosomal gene and mediates adherence of attaching and effacing *E. coli* to the intestinal epithelial cell ***(Ghanbarpour and Oswald, 2010).*** Attaching and effacing was a term to describe an intestinal lesion (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 enterocyte; and "effacing" because of the localized disappearance of the brush border microvilli ***(Stordeur et al., 2000).***

The prevalence rate of *eae*A gene in the present study was (27.7%). Similar result was obtained by ***Kobayashi et al. (2009) and Eid et al. (2016)*** who reported that, *eae*A gene

prevalence rate was 25% and 33.3% of the *E. coli* strains, respectively. However, no *eae*A gene was detected among APEC isolates tested by ***Wen-jie et al. (2008)*** who supported the results by that obtained by ***Krause et al. (2005)*** who reported a lower detection rate (2.3%) of *eae*A gene in the tested *E. coli* isolates from fecal samples of healthy chicken. While, ***Zakeri (2014)*** recorded *eae*A gene in 48.1% (39/81) of *E. coli* isolates.

APEC secrete vacuolating autotransporter toxin (vat), encoded by (*vat*) gene ***(Mortezaei et al., 2013 and Huja et al., 2014)*** which has been shown to induce the formation of intracellular vacuoles with cytotoxic effects ***(López et al., 2017).*** According to the study findings, the vacuolating autotransporter toxin (*vat*) gene was present in 100% among tested 18 *E. coli* isolates. The same result recorded by ***Cunha et al. (2017)*** who detected prevalence rate of *vat* gene in 96% of *E. coli* isolates. While, a lower prevalence rate (89%) recorded by ***Kwon et al. (2008).***

In the present study, 100% of the *E. coli* isolates possessed at least 4 virulence-associated genes (from totally 6 virulence genes). These results agreed with ***Varga et al. (2018)*** who found that, the majority (94%) of *E. coli* isolates were had at least 3 virulence-associated genes.

Finally, it could be concluded that, *E. coli* is still as one of the most prevalent pathogenic agents in poultry and it is associated with a number of disease conditions. The results of this study indicated that the examined *E. coli* strains possess a characteristic set of virulence factors and some of these virulent factors may be of public health concern.

References

Abd El Tawab, A. A.; Ammar, A. M.; Nasef, S. A. and Reda, R. M. (2015): "Prevalence of *Escherichia coli* in diseased chickens with its antibiogram pattern." Benha Veterinary Medical Journal. 28(2): 224-230.

Abd El Tawab, A. A.; Mahmoud, H. B.; El-Hofy, F. I. and E. El-khayat, M. E. (2016): "Prevalence of *bla*TEM and *bla*SHV genes in genomic and plasmid DNA of ESBL producing *Escherichia coli* clinical isolates from chicken." Benha Veterinary Medical Journal. 31(1): 167-177.

Ahmed, A. M.; Shimabukuro, H. and Shimamoto, T. (2009): "Isolation and molecular characterization of multidrug-resistant strains of *Escherichia coli* and *salmonella* from retail

chicken meat in Japan." Journal of Food Science. 74(7): 405-410.

Bergeron, C. R.; Prussing, C.; Boerlin, P.; Daignault, D.; Dutil, L.; Reid-Smith, R. J.; Zhanel, G. G. and Manges, A. R. (2012): "Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in human, Canada." Emerging Infectious Diseases Journal. 18(3): 415-421.

Bisi-Johnson, M. A.; Obi, C. L.; Vasaikar, S. D.; Baba, K. A. and Hattori, T. (2011): "Molecular basis of virulence in clinical isolates of *Escherichia coli* and *Salmonella* species from a tertiary hospital in the Eastern Cape, South Africa." Gut Pathogens. 3(1): 9.

Boisen, N.; Ruiz-Perez, F.; Scheutz, F.; Krogfelt, K. A. and Nataro, J. P. (2009): "High prevalence of serine protease auto-transporter cytotoxins among strains of enteroaggregative *Escherichia coli*." The American Journal of Tropical Medicine and Hygiene. 80(2): 294-301.

Chalmers, G.; Cormier, A. C.; Nadeau, M.; Côté, G.; Reid-Smith, R. J. and Boerlin, P. (2017): "Determinants of virulence and of resistance to ceftiofur, gentamycin and spectinomycin in clinical *Escherichia coli* from broiler chickens in Québec, Canada." Veterinary Microbiology. 203: 149-157.

Cunha, M. P. V.; Saidenberg, A. B.; Moreno, A. M.; Ferreira, A. J. P.; Vieira, M. A. M.; Gomes, T. A. T. and Knöbl, T. (2017): "Pandemic extra-intestinal pathogenic *Escherichia coli* (ExPEC) clonal group O6-B2-ST73 as a cause of avian colibacillosis in Brazil." PLoS ONE. 12(6): e0178970.

Eftekharian, S.; Ghorbanpoor, M.; Shapouri, M. R. S. A.; Ghanbarpour, R.; Jafari, R. and Amani, A. (2016): "Frequency of selected virulence-associated genes in intestinal and extra-intestinal *Escherichia coli* isolates from chicken." Iranian Journal of Veterinary Medicine. 10(2): 91-96.

Eid, S. A. S. and Erfan, A. M. (2013): "Characterization of *E. coli* associated with high mortality in poultry flocks." Assiut Veterinary Medicine Journal. 59(139): 51-61.

Eid, H. I.; Algammal, A. M.; Nasef, S. A.; Elfeil, W. K. and Mansour, G. H. (2016): "Genetic variation among avian pathogenic *Escherichia coli* strains isolated from broiler chickens." Asian Journal of Animal and Veterinary Advances. 11(6): 350-356.

Ewers, C.; Li, G.; Wilking, H.; Kiessling, S.; Alt, K.; Antão, E. M.; Laturnus, C.; Diehl, I.; Glodde, S.; Homeier, T.; Böhnke, U.; Steinrück, H.; Philipp, H. C.; Wieler, L. H. (2007): "Avian pathogenic, uropathogenic and newborn meningitis causing *Escherichia coli*: How closely related are they?" International Journal of Medical Microbiology. 297(3): 163-176.

Farooq, S.; Hussain, I.; Mir, M. A.; Bhat, M. A. and Wani, S. A. (2009): "Isolation of atypical enteropathogenic *Escherichia coli* and Shiga toxin 1 and 2f-producing *Escherichia coli* from avian species in India." Letters in Applied Microbiology. 48(6): 692-697.

Ferreira, J. C.; Filho, R. A. C. P.; Kuaye, A. P. Y.; Andrade, L. N.; Chang, Y.; Darini, A. L. C. (2018): "Virulence potential of commensal multidrug resistant *Escherichia coli* isolated from poultry in Brazil." Infection, Genetics and Evolution. 65: 251-256.

Ghanbarpour, R. and Oswald, E. (2010): "Phylogenetic distribution of virulence genes in *Escherichia coli* isolated from bovine mastitis in Iran." Research in Veterinary Science. 88(1): 6-10.

Ghunaim, H.; Abdu-Madi, M. A. and Kariyawasam, S. (2014): "Advances in vaccination against avian pathogenic *Escherichia coli* respiratory disease: Potentials and limitations." Veterinary Microbiology. 172(1-2): 13-22.

Hasan, B.; Faruque, R.; Drobni, M.; Waldenström, J.; Sadique, A.; Ahmed, K. U.; Islam, Z.; Parvez, M. B. ; Olsen, B. and Alam, M. (2011): "High prevalence of antibiotic resistance in pathogenic *Escherichia coli* from large- and small-scale poultry farms in Bangladesh." Avian Diseases. 55(4): 689-692.

Holland, J. L.; Louise, L.; Simor, A. E. and Louie, M. (2000): "PCR detection of *Escherichia coli* O157:H7 directly from stools: evaluation of commercial extraction methods for purifying fecal DNA." Journal of Clinical Microbiology. 38(11):4108-4113.

Huja, S.; Oren, Y.; Biran, D.; Meyer, S.; Dobrindt, U.; Bernhard, J.; Becher, D.; Hecker, M.; Sorek, R. and Ron, E. Z. (2014): " Fur is the master regulator of the extraintestinal pathogenic *Escherichia coli* response to serum." mBio. 5(4): e01460-14.

Hussein, A. H.; Ghanem, I. A.; Eid, A. A.; Ali, M. A.; Sherwood, J. S.; Li, G.; Nolan, L. K. and Logue, C. M.

(2013): "Molecular and phenotypic characterization of *Escherichia coli* isolated from broiler chicken flocks in Egypt." Avian Diseases. 57(3): 602-611.

Janßen, T.; Schwarz, C.; Preikschat, P.; Voss, M.; Philipp, H.C. and Wieler, L.H. (2001): "Virulence-associated genes in avian pathogenic *Escherichia coli* (APEC) isolated from internal organs of poultry having died from colibacillosis." International Journal of Medical Microbiology. 291(5): 371-378.

Jeong, Y.; Kim, T.; Kim, J. and Kwon, H. (2012): "Pathotyping avian pathogenic *Escherichia coli* strains in Korea." Journal of Veterinary Science. 13(2): 145-152.

Johnson, T. J.; Giddings, C. W.; Horne, S. M.; Gibbs, P. S. ; Wooley, R. E. ; Skyberg, J.; Olah, P.; Kercher, R.; Sherwood, J. S. ; Foley, S. L. and Nolan, L. K. (2002): "Location of increased serum survival gene and selected virulence traits on a conjugative R plasmid in an avian *Escherichia coli* isolate." Avian Diseases. 46(2): 342-352.

Johnson, T. J.; Johnson, S. J. and Nolan L. K. (2006): "Complete DNA sequence of a ColBM plasmid from avian pathogenic *Escherichia coli* suggests that it evolved from closely related ColV virulence plasmids." Journal of Bacteriology. 188(16): 5975-5983.

Jørgensen, S. L.; Stegger, M.; Kudirkiene, E.; Lilje, B.; Poulsen, L. L.; Ronco, T.; Dos Santos, T. P.; Kiil, K.; Bisgaard, M.; Pedersen, K.; Nolan, L. K.; Price, L. B.; Olsen, R. H.; Andersen, P. S. and Christensen, H. (2019): "Diversity and population overlap between avian and human *Escherichia coli* belonging to sequence type 95." mSphere. 4(1): e00333-18.

Kaipainen, T.; Pohjanvirta, T.; Shpigel, N. Y.; Shwimmer, A.; Pyörälä, S. and Pelkonen, S. (2002): "Virulence factors of *E. coli* isolated from bovine clinical mastitis." Veterinary Microbiology. 85(1): 37-46.

Kissinga, H. D.; Mwombeki, F.; Said, K.; Katakweba, A. A. S.; Nonga, H. E. and Muhairwa, A. P. (2018): "Antibiotic susceptibilities of indicator bacteria *Escherichia coli* and *Enterococci* spp. isolated from ducks in Morogoro Municipality, Tanzania." BMC Research Notes. 11(87): 1-6.

Kobayashi, H.; Kanazaki, M.; Hata, E. and Kubo, M. (2009): "Prevalence and characteristics of *eae*- and *stx*-positive strains of *Escherichia coli* from wild

birds in the immediate environment of Tokyo Bay." Applied and Environmental Microbiology. 75(1): 292-295.

Kogovšek, P.; Ambrožič-Avguštin, J.; Dovč, A.; Dreo, T.; Hristov, H.; Krapež, U.; Ravnikar, M.; Slavec, B.; Lotrič, M.; Žel, J. and Rojs, O. Z. (2019): "Loop-mediated isothermal amplification: rapid molecular detection of virulence genes associated with avian pathogenic *Escherichia coli* in poultry." Poultry Science. 98(3): 1500-1510.

Krause, G.; Zimmermann, S. and Beutin, L. (2005): "Investigation of domestic animals and pets as a reservoir for intimin-(*eae*) gene positive *Escherichia coli* types." Veterinary Microbiology. 106(1-2): 87-95.

Kwon, S. G.; Cha, S. Y.; Choi, E. J.; Kim, B.; Song, H. J. and Jang, H. K. (2008): "Epidemiological prevalence of avian pathogenic *Escherichia coli* differentiated multiplex PCR from commercial chickens and hatchery in Korea." Journal of Bacteriology and Virology. 38(4): 179-188.

Leyton, D. L.; Sevastsyanovich, Y. R.; Browning, D. F.; Rossiter, A. E.; Wells, T. J.; Fitzpatrick, R. E.; Overduin, M.; Cunningham, A. F. and Henderson, I. R. (2011): "Size and conformation limits to secretion of disulfide-bonded loops in autotransporter proteins." Journal of Biological Chemistry. 286(49): 42283-42291.

Lisa, K. N.; Barnes, J.; Jean-Pierre, V.; Tahseen, A. A. and Catherine, M. L. (2013): "Colibacillosis." In: Diseases of Poultry, 13th Ed. Swayne, David E. (Editor in chief) ISBN: 978-0-470-95899-5.

Literak, I.; Reitschmied, T.; Bujnáková, D.; Dolejska, M.; Cizek, A.; Bardon, J.; Pokludova, L.; Alexa, P.; Halova, D. and Jamborova, I. (2013): "Broilers as a source of quinolone resistant and extraintestinal pathogenic *Escherichia coli* in the Czech Republic." Microbial Drug Resistance. 19(1): 57-63.

López, V. H. M.; Serrano, I. Q.; Delgado, P. D. P. M.; Rodríguez, L. E. V.; Olague-Marchán, M.; Rodríguez, S. H. S.; Luna, M. A. L.; de la Torre A. F. and Santoyo, R. M. R. (2017): "Genes of virulence and phylogenetic group in isolates of avian pathogenic *Escherichia coli*." Archives of Medicine. 9(6): 1-5.

Mahmud, S.; Nazmul, K. H. M.; Nazir, H. and Tanvir, R. M. (2018): "Prevalence and

molecular detection of fluoroquinolone-resistant genes (*qnr*A and *qnr*S) in *Escherichia coli* isolated from healthy broiler chickens." Veterinary World. 11(12): 1720-1724.

Majumder, S.; Akter, M. M.; Islam, M. M.; Hussain, K.; Das, S.; Hasan, I.; Nazir, N. H. and Rahman, M. (2017): "Prevalence, isolation and detection of virulent gene in *Escherichia coli* from duck." British Journal of Medicine and Medical Research. 20(2): 1-8.

McPeake, S. J. W.; Smyth, J. A. and Ball, H. J. (2005): "Characterisation of avian pathogenic *Escherichia coli* (APEC) associated with colisepticaemia compared to faecal isolates from healthy birds." Veterinary Microbiology. 110(3-4): 245-253.

Mortezaei, N.; Singh, B.; Bullitt, E.; Uhlin, B. E. and Andersson, M. (2013): "P-fimbriae in the presence of anti-PapA antibodies: new insight of antibodies action against pathogens." Scientific Reports. 3(3393): 1-9.

Murray, P. R.; Baron, E. J. O.; Pfaller, M. A.; Jorgensen, J. H. and Yolken, R. H. (2003): "Manual of clinical microbiology." 8th Ed., Vol.1, ASM press, Washington D. C.

Navarro-Garcia, F.; Ruiz-Perez, F.; Cataldi, A. and Larzabal, M. (2019): "Type VI secretion system in pathogenic *Escherichia coli*: structure, role in virulence and acquisition." Frontiers in Microbiology. 10(1965): 1-17.

Nolan, L. K.; Horne, S. M.; Giddings, C. W.; Foley, S. L.; Johnson, T. J.; Lynne, A. M. and Skyberg, J. (2003): "Resistance to serum complement, *iss* and virulence of avian *Escherichia coli*." Veterinary Research Communications. 27: 101-110.

Oh, J. Y.; Kang, M. S.; Kim, J. M.; An, B. K.; Song, E. A.; Kim, J. Y.; Shin, E. G.; Kim, M. J.; Kwon, J. H. and Kwon, Y. K. (2011): "Characterization of *Escherichia coli* isolates from laying hens with colibacillosis on 2 commercial egg-producing farms in Korea." Poultry Science. 90(9): 1948-1954.

Ola, A. I. M. (2017): "Bacteriological and molecular studies on bacteria causing omphalitis in chicks." Ph. D. Thesis, Veterinary Medical Science, Benha University.

Oliveira, E. S.; Cardozo, M. V.; Borzi, M. M.; Borges, C. A.; Guastalli, E. A. L. and Avila, F. A. (2019): "Highly pathogenic and multidrug resistant avian pathogenic *Escherichia coli* in free-range

chickens from Brazil." Brazilian Journal of Poultry Science. 21(1): 1-8.

Peer, F. U.; Ansari, M. M.; Gani, I. A. and Willayat, M. M. (2013): "Serotyping and antibiotic sensitivity patterns of *Escherichia coli* isolates obtained from broiler chicks in Kashmir Valley, India." Advances in Animal and Veterinary Sciences. 1(2): 75-76.

Pfaff-McDonough, S. J.; Horne, S. M.; Giddings, C. W.; Ebert, J. O.; Doetkott, C.; Smith, M. H. and Nolan, L. K. (2000): "Complement resistance-related traits among *Escherichia coli* isolates from apparently healthy birds and birds with colibacillosis." Avian Diseases. 44(1): 23-33.

Pourbakhsh, S. A.; Dho-Moulin, M.; Bree, A.; Desautels, C.; Martineau-Doize, B. and Fairbrother, J. M. (1997): "Localization of the *in vivo* expression of P and F1 fimbriae in chickens experimentally inoculated with pathogenic *Escherichia coli*." Microbial Pathogenesis. 22(6): 331-341

Roshdy, H.; Abd El-Aziz, S. and Refai, M. (2012): "Incidence of *Escherichia coli* in chickens and ducks in different governorates in Egypt." 1st Conference of Animal Health Research Institute Association. 420-426.

Salama, S. S.; Kheder, A. A.; Elebiary, E. A. and Taha, M. M. (2007): "Molecular strategies for the differentiation and identification of local *Escherichia coli* isolated from chicken: I. Characterization of protein profile." BS. Veterinary Medicine Journal. 17(1): 25-28.

Sambrook, J.; Fritscgh, E. F. and Mentiates, T. (1989): "Molecular cloning." A laboratory manual, 2nd Ed., Cold spring Harbor Laboratory press, New York.

Sarah, A. Y.; Ammar, A. M. and Ahmed, D. A. (2013): "Serological and molecular typing of avian pathogenic *E. coli* originating from outbreaks of colibacillosis in chicken flocks." International Journal of Science and Research (IJSR). 4(2): 2082-2088.

Schouler, C.; Schaeffer, B.; Brée, A.; Mora, A.; Dahbi, G. and Biet, F. (2012): "Diagnostic strategy for identifying avian pathogenic *Escherichia coli* based on four patterns of virulence genes." Journal of Clinical Microbiology. 50(5): 1673-1678.

Sharada, R.; Ruban, S. W. and Thiyageeswaran, M. (2008): "Antibiotic resistance pattern of *Escherichia coli*

isolated from poultry in Bangalore." The Internet Journal of Microbiology. 7(1): 1-5.

Sharada, R.; Ruban, S. W. and Thiyageeswaran, M. (2010): "Isolation, characterization and antibiotic resistance pattern of *Escherichia coli* isolated from poultry." American-Eurasian Journal of Scientific Research. 5(1): 18-22.

Singh, A.; Khan, M. S. R.; Saha, S.; Hassan, J. and Roy, U. (2012): "Isolation and detection of antibiotic sensitivity pattern of *Escherichia coli* from ducks in Bangladesh and Nepal." Microbes and Health. 1(1): 6-8.

Singh, S. D.; Tiwari, R. and Dhama, K. (2011): "Predisposing effect of *Escherichia coli* to *Mycoplasma gallisepticum* infection in layer chicken." Shanlax International Journal of Veterinary Science. 1(1): 1-3.

Stordeur, P.; China, B.; Charlier, G.; Roels, S. and Mainil, J. (2000): "Clinical signs, reproduction of attaching/effacing lesions and enterocyte invasion after oral inoculation of an O118 enterohaemorrhagic *Escherichia coli* in neonatal calves." Microbes and Infection. 2(1): 17-24.

Thu, H. T. V.; Anh, D. T. L. and Dong, L. V. (2019): "*Escherichia coli* infection in ducks in the Mekong Delta: Bacterial isolation, serogroup distribution and antibiotic resistance." Can Tho University Journal of Science. 11(1): 24-29.

Tidiane, K.; Daniel, S. N.; Karamoko, Q.; Abou, Q.; Moussa, S.; Ouattara, G. A. and Adama, C. (2019): "Antibiotic resistance and prevalence of virulence and quinolone resistance genes in *Escherichia coli* strains of avian origin isolated from semi-industrial farms in Cote d'Ivoire." International Journal of Advances in Scientific Research and Engineering (IJASRE). 5(6): 127-134.

Vandemaele, F.; Assadzadeh, A.; Derijcke, J.; **Vereecken, M.** and **Goddeeris, B. M. (2002):** "Avian pathogenic *Escherichia coli* (APEC)." Journal of Veterinary Medicine. 127(19): 582-588.

Varga, C.; Brash, M. L.; Slavic, D.; Boerlin, P.; Ouckama, R.; Weis, A.; Petrik, M.; Philippe, C.; Barham, M. and Guerin, M. T. (2018): "Evaluating virulence-associated genes and antimicrobial resistance of avian pathogenic *Escherichia coli* isolates from broiler and broiler breeder chickens in Ontario,

Canada." Avian Diseases. 62(3): 291-299.

Wen-jie, J.; Zhi-ming, Z.; Yong-zhi, Z.; Ai-jian, Q.; Hong-xia, S.; Yue-long, L.; Jiao, W. and Qian-qian W. (2008): "Distribution of virulence-associated genes of avian pathogenic *Escherichia coli* isolates in China." Agricultural Sciences in China. 7(12): 1511-1515.

Yaguchi, K.; Ogitani, T.; Osawa, R.; Kawano, M.; Kokumai, N.; Kaneshige, T.; Noro, T.; Masubuchi, K. and Shimizu, Y. (2007): "Virulence factors of avian pathogenic *Escherichia coli* strains isolated from chickens with colisepticemia in Japan." Avian Diseases. 51(3): 656-662.

Zakeri, A. (2014): "Detection of the *eae*A gene in *Escherichia coli* isolated from broiler chickens by polymerase chain reaction." Iranian Journal of Applied Animal Science. 4(2): 417-420.

Zhao, C.; Ge, B.; De Villena, J.; Sudler, R.; Yeh, E.; Zhao, S.; White, D. G.; Wagner, D. and Meng, J. (2001): "Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* serovars in retail chicken, turkey, pork and beef from the Greater Washington, D.C., area." Applied and Environmental Microbiology. 67(12): 5431-5436.

التحديد الجزيئي لبعض جينات الضراوة للإيشيرشيا كولاي المعزولة من بداري التسمين والبط بمحافظة الإسماعيلية

*** مروة السيد حسن ، ** محمد كمال مرسى ، ** رنا محمد عبد الفتاح و * محمد السيد عنانى**

* قسم البكتريا والمناعة والفطريات. كلية الطب البيطرى. جامعة قناة السويس.

**المعمل المرجعى للرقابة البيطرية على الإنتاج الداجنى. معهد بحوث صحة الحيوان.

الملخص العربى

أجريت هذه الدراسة للكشف عن مدى تواجد بعض جينات الضراوة فى عترات الميكروب القولونى المعزولة من بداري التسمين والبط. تم تجميع عدد 252 عينة من الاعضاء المختلفة (الكبد، دم القلب و الرئة) من بداري التسمين المصاب (192 عينة من 64 طائر) ومن البط المصاب (60 عينة من 20 طائر) من مزارع الدواجن المختلفة في محافظة الاسماعيلية وذلك خلال الفترة من مايو 2018 حتي يوليو 2019. و قد تم اجراء الفحص البكتريولوجي لجميع العينات لعزل وتصنيف الميكروب القولوني. تم استخدام اختبارتفاعل انزيم البلمرة المتسلسل لعدد 18 عترة من الميكروب القولوني المعزولة من بداري التسمين و البط للكشف عن وجود جينات الضراوة وهي كالآتي (*iss* ، *iut*A ، *pap*C ، *tra*T ، *eae*A و *vat*). وقد أظهرت النتائج أن النسبة العامة لعزل ميكروب الإيشيرشيا كولاي كانت 80.5 % (بواقع 252/203). وقد وجد أن جميع المعزولات (بنسبة 100%) كانت إيجابية لجين *iss* و جين *iut*A و جين *vat* ، وأن 88.8 % (18/16) من المعزولات كانت إيجابية لجين *tra*T ، وأن 27.7 % (18/5) من المعزولات كانت إيجابية لجين *eae*A ، بينما 11 % (18/2) من المعزولات كانت إيجابية لجين *pap*C.