APPLICATION OF POLYMERASE CHAIN REACTION (PCR) IN DIAGNOSIS OF SOME PATHOGENIC *E.COLI STRAINS* ISOLATED FROM NATURAL CASES OF COLIBACILLOSIS IN CHICKENS

NASHWA M. HELMY AND WAFAA M. M. HASSAN

Animal Health Research Institute, ARC, Dokki, Giza, Egypt

(Manuscript received 9 August 2008)

Abstract

Seventy- four and sixty *E.coli* isolates were recovered from the internal organs of colibacillosis infected broiler chickens, and cloacal contents of apparently healthy broiler chickens, respectively. The obtained *E.coli* isolates were biochemically identified and serologically serotyped to different 0, serogroups. Virulence testing of the obtained serotypes was done on one day old chicks. Antimicrobial susceptibility testing of the obtained strains was done against ten of the most commonly used antimicrobial drugs. Further attempts were made to characterize the obtained *E.coli* serogroups using polymerase chain reaction technique (PCR) of cvi colicine V immunity gene. The obtained results showed that:Six 0,serogroups (01, 02, 035, 078, 080 and 0157) were only isolated from the internal organs of the diseased broilers. Twelve 0, serogroups (044, 088, 091, 092,098, 0109, 0111, 0114, 0119, 0145, 0150 and 0158) were only isolated from cloacal contents of apparently healthy birds. Only one common 0' serogroups (073) was isolated from both healthy and diseased groups. Only , the isolates from colibacillosis cases were highly virulent, while , those from cloacal contents of healthy birds were non-virulent. Hundred percent of the obtained *E.coli* serogroups were susceptible for enrofloxacin, norofloxacin, ciprofloxacin and chloramphenicols. The PCR test was performed on one isolate of each of the 19 *E.coli* strains. A single band with the expected size of 760 bp was obtained with 17 strains (89.47 %). Only, two isolates from apparently healthy birds did not contain cvaC gene .

INTRODUCTION

Escherichia coli, as a main part of the normal microbes of birds, are normal commensal bacteria inhabiting the gastrointestinal tract of birds, and serve a useful function in the body by suppressing the harmful bacterial species and by synthesizing appreciable amounts of vitamins. However, avian pathogenic *E.coli* (APEC) are *E.coli* strains that can cause disease in birds of various ages including septicemia, chronic respiratory disease, vitellus infection, salpyngitis, peritonitis, chronic skin infections, osteomyelitis and swollen head syndrome (Dho-Moulin and Fair brother, 1999). Today, nevertheless, APEC represent *E.coli* strains causing invasive infection of poultry (chikens and turkeys), more especially of broilers, with the respiratory tract as portal of entry, septicemia, peritonitis, perihepatitis, air sacculitis and osteomyelitis (Barnes *et al.*, 2003).In general, lesions in which *E.coli* is the primary and often the secondary

agent, cause economic damage of the poultry industry due to lower corporal development, insufficient feed conversion, increasing mortality, higher cost with medicine and condemnation of carcasses (Rocha *et al.*,2008). Otherwise, some *E.coli* strains (0157: H7 and 0157: H7*) emerged as important food-born diseases including bloody diarrhea (hemorrhagic colitis) and hemolytic ureamic syndrome (Coia, 1998). Like most pathogenic *E.coli*, avian isolates cannot be distinguished biochemically from normal commensals inhabiting the gastrointestinal tract of birds (Anand *et al.*, 2006). In the last few decades, understanding of the genetic basis and molecular mechanisms of the bacterial virulence of *E.coli* has shed light on the mechanisms of pathogenesis associated with this diverse group of bacteria (Rocha *et al.*, 2008). Initial studies of APEC led to the conclusion that, certain serogroups, particularly, 01, 02 and 078 , were more commonly associated with colibacillosis, about half of the strains examined in different studies could not either be grouped into these classical serogroups or were untypeable (Ewers *et al.*, 2005). Therefore, serotyping cannot be recommended as the sole diagnostic tool for the identification of APEC. Especially in light of the fact that the designation of a serogroup does not reflect the virulence of the strain, previous investigations have indicated that the prevalence of various virulence genes among isolates from chicken with colibacillosis were useful markers for the detection and characterization of avian pathogenic *E.coli*, therefore, be used in the diagnosis of colisepticemia in poultry (Ewers, *et al.*, 2005). The virulence mechanisms of APEC have been continually studied and are believed to be multifactorial. The most frequently virulences mentioned are : adhesion capacity(pap and fel), colicin production (cva), aerobactin presence (iut), serum resistance (iss), hemagglutinin sensitive temperature (tsh), and the presence of certain capsular antigens (kps) (Parreira and Gyles , 2003). Considering the above points, attempts were made to classify and characterize 19 serotypes of *E.coli* using PCR of "cvi" colicin V immunity gene. In this study, we evaluated a polymerase chain reaction (PCR) technique to detect one of these virulence genes (cvaC) in order to establish a reliable diagnostic typing scheme for the identification of APEC , and moreover, to provide a useful epidemiologic tool for studying outbreaks of colibacillosis and infection chains in combination with other analytical assays.

MATERIALS AND METHODS

Within the period from 2006 to 2007, colibacellosis suspected broilers belonging, to twenty - two farms in middle Egypt (out of these 4,7,8, and 3 were from Cairo, Giza, Kaliobiah and Fayoum, respectively) were bacteriologically examined for colibacellosis . Concurrently, cloacal contents of apparently healthy broilers from 18

farms (4,5,6, and 3 farms from Cairo, Giza, Kaliobiah and Fayoum governorates, respectively) were bacteriologically examind for presence of *E.coli*. Samples including splanchnic organs (liver , heart , liver and small intestine) from infected broilers were used for bacterial examination .

A loopfull was inoculated into nutrient broth and incubated at 37°C for 18-24 h. Subculture was made onto MacConkey agar plates and incubated at 37°C for 24 h. Suspected colonies were picked up and plated onto Eosin Methylene Blue (EMB) agar media. Colonies showing characteristic metallic sheen were expected to be *E.coli*. *Escherichia* coli isolates were stored in tryptone soya broth (Oxoid Hampshire, UK) with 5% glycerol at -70°C. Biochemical identification for isolated strains was carried out. The isolates were identified as *E.coli* if they were positive for lactose, adonitol, methyl-red and indole, and negative for H_2S and urease (Anand *et al.*, 2006).

0. Serotyping

The major somatic 0-antigens of *E.coli* was determined using specific antisera produced in rabbits by standard techniques (Edwards and Ewing, 1972). Somatic serogrouping was performed by the slide agglutination test. (Negeleka *et al.*, 2002).

Virulence test of the isolates in one day-old chicks

The virulence of the obtained 19 *E.coli* serotypes was tested by subcutaneous inoculation of one day-old chicks obtained from a local hatchery. Birds in groups of 10 were inoculated with 0.25 ml of the bacterial culture (approximately 5 x 10^3 CFU) and observed for mortality every 12 h for 5 days as described by Negeleka *et al.* (2002). Isolates that caused death of more than 50% of birds were consided virulent and those caused 10-50% or 0-10% dead birds were considered moderately virulent, or non-virulent, respectively. On necropsy of dead birds, swabs were taken from the splanchnic organs for reisolation of the infecting agent. Birds were handled according to the guidelines outlined in the guide to the care and use of experimental animals of Egyptian Council on Animal Care (Negeleka *et al.*, 2002).

Antibiotic sensitivity

Sensitivity of the recovered isolates to ten antimicrobial agents frequently used in local poultry farms was determined by the standard disk procedure of Bauer *et al.* (1966). Selection of disk concentrations and zone diameters interpretation were done as recommended by the manufacturers. The following antimicrobial agents and disk pontencies (µg) were used: amoxicillin (20 µg), enrofloxacin (5µg), gentamicin (10 µg), neomycin (30 µg), noroflaxacin (10µg), tetracycline (30µg), trimethoprim-

sulphamethexazole (1.25/ 23.75 µg), chloramphenicol (15 µg), ampicillin (20 µg) and ciprofloxacin (4 µg).

PCR

Recovered *E.coli* isolates were examined by the polymerase chain reaction (PCR) for the presence of gene responsible for colicin production (cvaC).

Extraction of DNA

The stock strains were cultured on LB agar at 37°C for 24 hours. Template DNA was prepared by placing a single colony into 40 ul of 1% triton X 100. This mixture was boiled for 10 min, then, centrifuged for 1 min at 10.000 rpm. The supernatant was stored as the DNA template at -20 °C prior to use (Yaguchi *et al.*, 2007).

DNA amplification

Reaction mixture was composed of 5 ul of DNA template and 20 ul of a master mix in a tube. The master mix was composed of 13.9 ul of sterile distilled water, 2 ul of 10 x PCR buffer, 2 ul of mM $MgCl_2$, 1.6 ul of deoxynucleotidetriphosphate mix, 0.2 ul of 5 U/ul Taq polymerase and 1 ul of 20 pM of each primer (Rocha *et al.,* 2008). PCR was carried out in a thermocycler (model Perken Elmer 6900). The optimized cycle program of denaturation, annealing and extension temperature was as follows: initial denaturation at 94°C for 5 min, 35 cycles of 1 min at 94°C, 1 min at 63°C and 2 min at 72°C, and final polymerization at 72°C for 10 min. After amplification, a 5 ul of the reaction product was mixed with equal volume of gel loading buffer and subjected to electrophoresis on 1.5% agarose gel at 100 u for 30 min. Gel was stained with ethidium bromide and photographed on UV transilluminator (Yaguchi *et al.,* 2007).

Oligonucleotide primers

Two oligonucleotide primers forward and reverse were designated and synthesized. Each of 21 bp primer was constructed to target a 760 bp region of the colicicin V gene (Yaguchi *et al.,* 2007). The sequence of the two primers used in this study were listed in Table 1 .

Table 1. The sequences of oligonucleotide primers specific for APEC.

Primer	Sequences	PCR product
F	5'-CACACACAAACGGGAGCTGTT-3'	760 bp
R	5'-CTTCCCGCAGCATAGTTCCAT-3'	

RESULTS AND DISCUSSION

A total of 74 *E.coli* isolates were obtained from the splanchnic organs of colibacillosis expected broiler chickens belonging to 22 farms in Cairo, Giza, Kaliobiah and Fayuom Governorates in Egypt (Table 2). Sixty *E.coli* isolates were also obtained from the cloacal contents of apparently healthy broiler chickens under the same conditions and from the same localities. Morphological, cultural and biochemical identifications were enough to identify the obtained isolates of *E.coli*. A total of 134 *E.coli* isolates were recovered from splanchnic organs of affected broilers (74 isolates) and from cloacal contents of apparently healthy looking broilers (60 isolates).These conclusions agreed with those reported by Anand *et al.* (2006). Somatic serogroup showed that the *E.coli* isolates from colibacillosis suspected cases include 01, 02,035, 073, 078, 080 and 0157 serotypes, as well as some untypeable isolates . Out of these 74 isolates,44 (59.5%) were 01, 02, or 078 serotypes which agreed with the previous observations of Ewers *et al.* (2005) who cited that 01, 02 and 078 represented about 50% of the APEC. The obtained 0, serogroups included 01, 02, 035, 044, 073, 078, 080, 088, 091, 092,098, 0109, 0111, 0114, 0119, 0145, 0150, 0157, 0158. Two cases of the isolated APEC were serotyped as 0157 which is shiga toxin producing *E.coli*, and therefore, it is a highly pathogenic strain for humanbeing .Virulence testing of the obtained *E.coli* indicated the high viulence of the APEC isolated from the internal organs of the suspected cases of broiler to be colibacillosis , while, the *E.coli* isolates recovered from cloacal contents of apparently healthy broiler chickens were weak or nonvirulent (Table 3). These results supported the previous observations of Negeleka *et al* . (2002), Skyberg *et al.* (2003) and Ewers *et al.* (2005) .The virulence of one 0,serotype (073) isolated from internal organs of diseased broilers was against the non-virulent same 0.serotypes isolated from cloacal contents which may be attributed to the aquired gene or genes controlling virulence factors. Among the used antimicrobial drugs, enrofloxacin, norofloxacin, ciprofloxacin and chloramphenicol were 100% efficient against the obtained 19 serotypes of APEC (Table 4). Other antimicrobials were less efficient or even questionable against APEC. These may be attributed to common resistant factors against these antimicrobial drugs among the drug resistant APEC. These results were in harmony with the previous results obtained by Negeleka *et al.* (2002), and indicated the problem of drug resistance and resistant *E.coli* to a group of antimicrobial drugs.

Previous investigations have indicated that the distribution of various virulence factors are useful markers for the detection and characterization of APEC and used as diagnostic aids of colisepticemia in poultry (Ewers *et al.*, 2005). In this study, we

assessed the utility of PCR for rapid concurrent detection of one virulence-associated factor in APEC by using a oligonucleotide primer to amplify colicin V plasmid gene (cvaC) . Out of the 19 obtained serogroups, 17 (89.5%) were positive for cvaC gene and gave a single band with the expected size of 760 bp (Fig.1) . This result agreed with that of McPeake *et al* . (2005) who found it to be 99.1% , while, other authors obtained lower percentages as Blanco *et al.* (1997) who reported 22%, and Rodriguez-Siek *et al.* (2005) who cited 66.8%. In colicin producing samples, the genetic determiners and the protein that accompany them are located in plasmids which are called Col factors (Luria and Suit , 1987). Colicin V was found in virulent bacteria involved in extra-intestinal infections affecting humans and animals, and it inhibits the growth of similar or closely related bacterial strains interfering with the potential of membrane formation (Yang and Konsky, 1984).

From the above mentioned results, it was concluded that the PCR used in this study may have diagnostic value for the rapid cost and time-effective concurrent detection of virulence-associated factors in APEC. PCR could also supplement epidemiological studies to investigate infection chains, providing valuable information about the pathways within and in between poultry flocks. The PCR test was performed on all the 19 *E.coli* strains. Of the isolates obtained from apparently healthy birds, 2 isolates (*E.coli* 0158 and *E.coli* 0150) did not contain cvaC gene. These results suggest that, although these isolates were obtained from healthy birds, they may be able to cause disease if introduced into a vulnerable host. (Skyberg *et al.*, 2003).

Table 2. Isolation and somatic 0 serogrouping of *E.coli* from colibacillosis infected and apparently healthy broiler chickens.

H.C of farms	No of farms	Locality	No of *E.coli* isolates	0.Serotyping
Infected broilers farms	4	Cairo	15	01(5/15), 02(4/15), 035 (1/15), 0157 (1/15) and untypeable (4/15).
	7	Giza	22	01 (4/22), 073 (3/22), 078 (7/22) and untypeable (8/22)
	8	Kal	30	01 (7/30), 02 (6/30), 073 (2/30), 078 (8/30) and untypeable (7/30).
	3	Fay	7	02 (1/7), 078 (2/7), 080 (1/7), 0157 (1/7) and untypeable (2/7).
Total	22	Mid. Egypt	74	01 (16/74), 02 (11/74), 035 (1/74), 073 (5/74), 078 (17/74), 080 (1/74), 0157 (2/74) and untypeable (21/74)
Apparently healthy broiler farms	4	Cairo	13	044 (2/13), 088 (1/13), 091 (2/13), 0109(2/13) and untypeable (6/13)
	5	Giza	16	073 (3/16), 0111 (1/16), 0114 (2/16), 0119 (2/16) and untypeable (8/16)
	6	Kal	18	092 (2/18), 0109 (3/18), 0111 (2/18), 0126 (2/18), 0158 (3/18) and untypeable (6/18)
	3	Fay	13	0126 (2/13), 0145 (2/13), 0150 (1/13), 158 (3/13) and untypeable (5/13)
Total	18	Mid. Egypt	60	044 (2/60), 073 (3/116), 088 (1/60), 091 (2/60), 092 (2/60), 098 (5/60), 109 (4/60), 0111 (3/60), 0114 (2/60), 0119 (2/60), 145 (2/60), 0150 (1/60), 0158 (3/60) and untypealbe (25/60)

Kal = Kaluobiah - Fay = Fayoum

H.C. Farm : Healthy conditions of birds in the farm .

Table 3. Virulence of the obtained *E.coli* from diseased and apparently healthy broiler chickens.

Source of *E.coli*	O.serotypes	Groups of day old chicks	Post inoculation mortality		Pathological finding		Virulance	Reisolation
			0-<3D	3-5D	0-<3D	3-5D		
Isolates from splanchenic organs of coli bacallosis chickens	01	Group 1	6/10	2/4	Septicemia	Septicemia + peritonitis + vitellus infection	H.V	8/8
	02	Group 2	7/10	1/3			H.V	8/8
	035	Group 3	5/10	2/5			H.V	7/7
	073	Group 4	4/10	2/6			H.V	6/6
	078	Group 5	8/10	1/2			H.V	9/9
	080	Group 6	3/10	3/7			H.V	6/6
	0157	Group 7	8/10	2/2			H.V	10/10
Isolates from cloacal contents of apparently healthy broiler chickens.	044	Group 8	1/10	0/9	Non specific	Non specific	Non virulent or weak virulent	No recovery for inoculated serotypes from internal organs
	073	Group 9	0/10	0/10				
	088	Group 10	0/10	1/10				
	091	Group 11	0/10	0/10				
	092	Group 12	0/10	0/10				
	098	Group 13	0/10	0/10				
	0109	Group 14	1/10	1/9				
	0111	Group 15	0/10	1/10				
	0114	Group 16	0/10	0/10				
	0119	Group 17	0/10	1/10				
	0145	Group 18	1/10	1/9				
	0150	Group 19	0/10	1/10				
	0158	Group 20	0/10	0/10				

Groups of ten , one day old chicks each received 0.25 ml contain $5x10^3$ CFU .

Negative control chicks each received 0.25 ml of sterile normal saline .

Table 4. Antimicrobial sensitivity test of the obtaind *E.coli* serogroup.

O Serotype	Amoxicillin	Enorofloxacin	Gentamycin	Norofloxacin	Tetracyclin	Trimethoprim-Sulphamethoxazol	ChloramphenicoL	Ampicillin	Ciprofloxacin	Neomycin
O1	R	+++	++	+++	R	++	+++	R	+++	++
O2	R	+++	++	+++	R	+	++	R	+++	R
O35	+	++	+	+++	R	R	+++	+	+++	+
O44	R	+++	++	+++	+	++	+++	R	++	++
O73	+	+++	++	+++	R	++	+++	+	+++	++
O78	R	+++	+	+++	R	+	++	R	+++	++
O80	+	+++	R	+++	+	+	++	+	+++	R
O88	R	+++	++	+++	+	R	+++	R	+++	++
O91	R	+++	++	+++	R	+	++	+	++	R
O92	R	+++	+	+++	+	R	+++	R	++	R
O109	R	+++	R	+++	R	+	+++	R	+++	+
O111	+	+++	+	+++	++	++	++	R	+++	++
O114	R	+++	++	+++	++	++	+++	++	+++	++
O119	R	+++	++	+++	+	++	+++	+	+++	+
O145	R	+++	+	+++	R	R	+++	R	+++	++
O150	R	+++	+	+++	R	R	++	+	+++	++
O157	R	+++	R	+++	R	+	+++	+	+++	R
O158	R	+++	+	+++	R	+	+++	R	+++	R
O98	+	+++	+	+++	+	+	++	+	+++	+

+++ : Highly sensitive ++ : Moderately sensitive + : Low sensitive R : Resistance

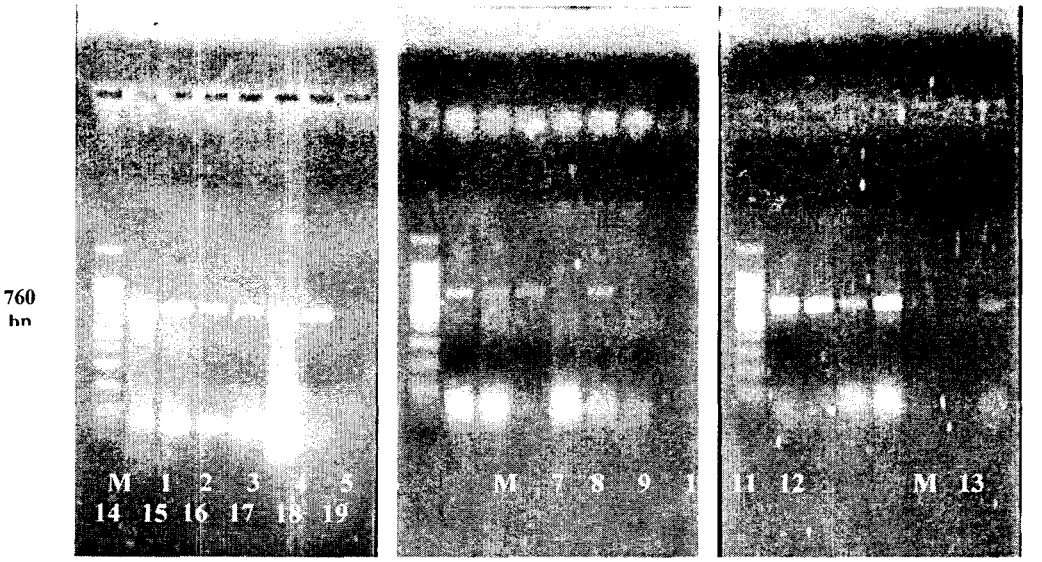

Fig.1. Agarose gel electrophoresis of PCR-amplified *cvaC* gene fragments from different *E.coli* strains isolated from apparently healthy and diseased broiler chickens . The figure shows a single band at 670-bp DNA fragment. Lane M:100-bp marker. Lane 1:*E.coli* 01.Lane 2: 0157. Lane 3: 078. Lane 4 : 02. Lane 5: 080. Lane 6: 035. Lane 7: 092. Lane 8: 044. Lane 9: 0145. Lane 10: 0109. Lane 11: 0111.Lane 12: 0114. Lane 13: 088. Lane 14: 091. Lane
15: 098. Lane 16: 0119. Lane 17: 0158. Lane 18: 0150. Lane 19: 073.

REFERENCES

1. Anand, S.K., Shadama, F. G. G. Joshi and J. H. Purohit. 2006. Molecular characterization of *E.coli* using PCR single strand conformation polymorphism analysis of 165 rRNA gene. Indian J. Poul. Sci., 41 (1): 1-5.
2. Barnes, H. J., J. P. Vallancourt and W. B. Gross. 2003. Colibacillosis in: Saif, Disease of poultry IIth ed. Iowa state university press Ames, Iowa: 270-278.
3. Bauer, A. W., W. Kirby, J. Sherris and M. Turek. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Patho., 45: 493-496.
4. Blanco, J. E., M. Blanco, A. Mora and J. Blanco. 1997. Production of toxins (enterotoxins, verotoxins, and neurotoxins) and colicins by *Escherechia coli* strains isolated from septicemic and healthy chickens: relationship with in vivo pathogenecity. J. Clin. Microbiol., 35: 2953-2957.
5. Coia, J. E. 1998. Clinical, microbiological and epidemiological aspects of *E.coli* 0157 infection. FEMS. Immunol. Med. Microbiol., 20: 1-9.
6. Dho-Moulin and J. M. Fairbother. 1999. Avian pathogenic *E.coli.* (APEC), Vet. Res., 30: 299-316.
7. Edwards, P. R. and W. H. Ewing. 1972. Identification of Enterobacteriaceae. Burgess. Publ. Co., Mineapolis, MN.
8. Ewers, T., J. Traute, K. Sabine, C. P. Hans and H. W. Lothar. 2005. Rapid detection of virulence-associated genes in avian pathogenic *Escherichia coli* by multiplex polymerase chain reaction. Avi. Dis. 49: 269-273.
9. Gilson, L., Mahanty and R. Kolter. 1987. Four plasmid genes are required for colicin V synthesis , export , and immunity . J. Bacteriol., 169 : 2466-2470 .
10. Luria, S. E. and J. L. Suit. 1987. Colicine and Col plasmids, p. 1615-1624. In: Neidhardt F.C. (ed.), *Escherichia coli* and *Salmonella typhimurium*: cellular and molecular biology. Vol. 2. American Society of Microbiology, Wahsington, D.C.
11. McPeake, S. J., J. A. Smyth and H. J. Ball. 2005. Characterization of avian pathogenic *Escherichia coli* (APEC) associated with colisepticaemia compared to faecal isolates from health birds. Vet. microb., 110: 245-253.
12. Negeleka, M., L. Brereton, G. Brown and J. M Fairbother. 2002. Pathotypes of avian *E.coli* as related to tsh-,pap-,pil-,and inc-DNA sequences, and antibiotic sensitivity of isolates from internal tissues and the cloacae of broilers. Avi. Dis., 46: 143-152.

13. Parreira, V. R. and C. L. Gyles. 2003. A novel pathogenicity island integrated adjacent to the th1W tRNA gene of avian pathogenic *Escherichia coli* encodes a vaculating autotransporter to xin. Infect. immune., 71: 5087-5096.

14. Rocha, A. C. G. P., L. S. R. Silvio, A. V. L. Carlos, F. S. Guilherme, L. S. M. Hamilton, O. S. S. Felipe, B. M. Lucas and T. P. S. Carlos. 2008. Genes associated with pathogenicity of avian *Escherichia coli* (APEC) isolated from respiratory cases of poultry. Pesq. Vet. Bras., 28 (3): 183-186.

15. Rodriguez-Siek, K.E., C. W. Giddings, C. Doetkott, T. J. Johnson, M. K. Fakhr and L. K. Nolan. 2005. Comparison of *Escherichia coli* isolates implicated in human urinary tract infection and avian colibacillosis. Microbiology, 151: 2097-2110.

16. Seong Moon, G. I., W. J. Kim and W. S. Shin. 2004. Optimization of rapid detection of *E.coli* 0157: H7 and *Listeria monomcytogenes* by PCR and application to field test. J. food protection, 67 8): 1634-1640.

17. Skyberg , J. A., M. H. Shelly, W. G. Catherine, E. W. Richard, S. G. Penelope and K. N. Lisa. 2003. Characterizing avian *E.coli* isolates with multiplex polymerase chain reaction. Avi. Dis., 47: 1441-1447.

18. Timothy, J. J., W. G. Catherine, H. Shelley, S. G. Penelope, E. W. Richared, S. Jerod, O. Pam, K. Ronda, S. S. Julie, L. F. Steven and K. N. Lisa. 2002. Location of increased serum survival gene and selected virulence traits on a conjugative R plasmid in an avian *E.coli*, isolates. Avi. Dis., 46: 342-352.

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

20. Yang, C. C. and J. Konisky. 1984. Colicin V treated *Escherichia coli* does not generate membrane potential J. Bact.,158:757-759.

تطبيق أختبار البلمرة المتسلسل PCR لبعض الميكروبات العصوية القولونية الممرضة المعزولة من حالات إصابة طبيعية بالعصويات القولونية فى الدجاج

نشوى محمد حلمى ، وفاء محمد محمد حسن

معهد بحوث صحة الحيوان – مركز البحوث الزراعية – دقى – جيزة

تم عزل أربع و سبعين و كذلك ستين عترة معزولة من الميكروبات العصوية القولونية وذلك من الأعضاء الحشوية لصيصان التسمين المحتمل إصابتها بالعصويات القولونية أو من محتويات المجمع لصيصان تسمين سليمة ظاهريًا.

تم تصنيف المعزولات بيوكيميائيًا و كذا تصنيفها سيرولوجيًا إلى عدة عترات مختلفة. تم إختبار ضراوة العترات التى تم الحصول عليها و ذلك بإستخدام صيصان عمر يوم.

و كذلك تم إختبار حساسية العترات التى تم الحصول عليها و ذلك مقابل عشر مضادات حيوية شائعة الإستخدام. علاوة على ذلك تم محاولة لتمييز عترات الميكروبات المعزولة بإستخدام تقنية PCR لتمييز جين cvi – C .

أوضحت النتائج التى تم الحصول عليها عزل ست ٦ عترات (01,02,035,078,080,0157) من الحالات المرضية فقط بينما تم عزل إثنتا عشرة عترة (0158 044,088,091,098,0109,0111,0114,0119,0145,0150,0157,) من محتويات المجمع لطيور سليمة ظاهرياً. هذا و قد تم عزل عترة واحدة (073) من طيور مريضة و أخرى سليمة ظاهريًا.

أظهرت الدراسة أن المعزولات التى تم الحصول عليها من الحالات المصابة بالعصوى القولونى كانت شديدة الضراوة بينما وجد أن المعزولات من محتويات المجمع لطيور سليمة ظاهريًا غير ضارية.

تبين ظهر كذلك أن جميع المعزولات (كانت حساسة (100%) لكل من الأنروفلوكساسين، النورفلوكساسين، السيبروفلوكساسين، و الكلورامفينكول.

تم عمل PCR على تسعة عشرة عترة تم الحصول عليها؛ يتم الحصول على شريطالحجم المتوقع 760 bp وذلك بفحص سبعة عشرة عترة من العترات بنسبة (89.47%) بينما وجدت عترتان معزولتان من طيور سليمة ظاهريًا لا تحتوى على ال cviC جين.