

CHARACTERIZATION OF *ESCHERICHIA COLI* STRAINS ISOLATED FROM INFECTED PIGEONS IN ASSIUT PROVINCE

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ABSTRACT

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E. coli is a major pathogen of commercially produced poultry all over the world, causing colibacillosis and contributing high significantly to economic losses. In this study, isolation, serotyping, virulence factors and antimicrobial susceptibility test were characterized for avian pathogenic *E. coli* strains that isolated from tissues of the infected pigeons and from non-hatched pigeon eggs in Assiut Province. 124 samples (87 pigeons and 37 non-hatched pigeon eggs) were examined for *E. coli* infection. The samples include liver and kidney tissues of diseased and freshly dead pigeons plus yolk of 20 infertile eggs and yolk sac of dead-in-shell embryos of the non-hatched pigeon eggs. Twenty three bacterial isolates were identified, from which 19 isolates for *E. coli* and one isolate for each *Enterobacter agglomerans*, *Enterobacter cloacae*, *Hafnia alvei* and *Serratia marcescens* from tissues of infected pigeons while samples of pigeon eggs were found negative for *E. coli* isolation. Eight serogroups were identified among sixteen of pigeon *E. coli* isolates, however, 3 isolates were nontyped. The most common serogroup was O78 that identified in (21.05%) followed by serogroup O2:H6 (15.79%) among the *E. coli* isolates. Each serogroup of O1:H7, O128:H2 and O119: H4 were isolated by 10.53%, while each serogroup of O111:H4, O114:H21 and O44:H18 were represented with an incidence 5.26%. The characteristic virulence factors of hemolysis and Congo red binding activities of *E. coli* isolates were done. All *E. coli* isolates (100%) were found positive for the Congo red binding activity and (84.2%) of the isolates were positive for hemolytic activity. The *in vitro* antimicrobial sensitivity of the *E. coli* isolates were tested against 8 antimicrobial drugs used in pigeon treatment. Overall, the all *E. coli* isolates displayed resistance to neomycin at all (100%), the most strains were frequently highly resistance to trimethoprim-sulphamethoxazol followed by ampicillin, tetracycline, erythromycin and doxycycline. The isolates show high sensitivity to streptomycin (100%) and norfloxacin (89.5%). These findings suggest that multiple-antimicrobial-resistant of *E. coli* isolates are commonly present among infected pigeons that can be potentially transmitted from pigeons to humans. So the need for the introduction of surveillance programs to monitor antimicrobial resistance in pathogenic bacteria is recommended.

Key Words: *Escherichia Coli* – Colibacillosis – Pigeons – Serotyping.

INTRODUCTION

Out of 8600 of known species of birds, 289 species are of pigeon and these species of pigeons had been associated with human society both as a source of food and as cage birds from a long time (Dutta *et al.*, 2013). Pigeons are widely distributed in Egypt and considered as an important bird for many people especially in fest for hunting and racing, many farmers reared them in their houses and considered an essential food for rich people (Ibrahim, 2007).

Researchers from different countries have reported variable incidence of bacterial diseases in pigeons, among them *E. coli*, *Streptococci* and *Salmonella* infections are common in pigeons (Herdt *et al.*, 1994). *Escherichia coli* infection constitute one of the most important bacterial diseases affecting the poultry industry. Microbial flora of the pigeon gastrointestinal tract is characterized by occurrence of *E. coli* and *Enterococci*. *E. coli* is usually commensal but can also act as an opportune pathogen. Several factors are needed for *E. coli* to cause disease in pigeons, such as stress or adenoviral

or herpesviral infection (Kimpe *et al.*, 2002 and Dutta *et al.*, 2013). *E. coli* organisms exist in nature as a number of strains that range from the most innocent to the most deadly. Some pathogenic strains of *E. coli* in the intestines may cause disease by their production of potent toxins that are absorbed through the intestinal wall into the blood stream, from which their far-reaching effects in many tissues. Other dangerous strains of *E. coli* are able to breach the intestinal wall, enter the blood stream where they multiply called ("septicaemia") and are distributed to a variety of tissues to produce signs of illness in some joints, brain and ovarian infections, etc. in pigeons are caused by these tissue-invasive strains of *E. coli* which can produce dead-in-shell embryos, sudden death in youngsters or old birds. If a pure culture of *E. coli* organisms is recovered from a variety of tissue organs from a freshly killed sick bird, there is a strong likelihood that they are the cause of that particular problem in the birds. Also, sick birds are vomiting, have mucoid diarrhea that has an odd odor, such findings are highly suggestive of a significant *E. coli* problem. *E. coli* can complicate other infectious diseases as secondary invaders. The pigeons may acquire infection from contaminated environment, feed and water or from other carrier birds. (Smith, 1998 and Gordon, 2010).

Many researchers isolated and serotyped different *E. coli* strains from diseased pigeons, there are distinct serotypical differences between the facultative *E. coli* and the ones that invade the tissue and cause infection, (Phangcho, 2001; Kimpe *et al.*, 2002; Ibrahim, 2007; Oboegbulem *et al.*, 2009; Farghaly and Mahmoud, 2011; Khudair, 2012 and Dutta *et al.*, 2013). Congo red and hemolytic activities for *E. coli* isolates were used to differentiate between pathogenic and non pathogenic strains *in vitro* at poultry farms, (Berkhoff and Vinal, 1985; Panigrahy and Yushin, 1990; Stebbins *et al.*, 1992; Raji *et al.*, 2003; Ahmad *et al.*, 2009; Ezz EL Deen *et al.*, 2010). The invasive *E. coli* strains frequently produce virulence factors such as the exotoxin α -hemolysin which causes hemolysis by forming pores in the erythrocyte membrane. (Cavalieri *et al.*, 1984; Bhakdi *et al.*, 1988 Johnson and Stell, 2000; Skals *et al.*, 2009 and Herlax *et al.*, 2010). Berkhoff and Vinal (1985) found a direct correlation between the ability of clinical isolates of *E. coli* to bind Congo red dye (CR) and their ability to cause septicemic infection in chickens, these preliminary findings suggest that the CR dye binding could be used as a phenotypic marker or virulence factor to distinguish between invasive and noninvasive isolates. Similar associations between virulence and Congo red binding have been shown for other bacteria (Surgalla and Beesley, 1969; Payne and Finklestein, 1977; Prpic *et al.*, 1983 and Harry and Yoder, 1989).

As a result of the lack of an efficient commercial vaccine, the control of colibacillosis mainly relies on the use of antimicrobial drugs. Recently, the prevalence of antimicrobial resistance has been increasing in major bacterial pathogens (Parry and Threlfall, 2008). Bacteria have developed strategies for survival within the host during an infection and one of these strategies is the resistance of isolates to the antimicrobial drugs. Antimicrobial resistance is a serious problem because it limits the therapeutic possibilities in the treatment of bacterial diseases in domestic animal species in general and poultry in particular (Williams and Heymann, 1998 and Nicole *et al.*, 2000). The number of multi-drug resistant *E. coli* are continuously increasing although various antimicrobial agents are being used (Hussain *et al.*, 1982). Uncontrolled use of antibiotics in medicine and animal husbandry for both treatment and prevention of bacterial diseases over the course of decades has fostered the selection of resistant bacteria (Tomasz, 1994 and Singer *et al.*, 2003). Antimicrobial-resistance *E. coli* occurred in diseased pigeons in various results as described by (Kimpe *et al.*, 2002; Ibrahim, 2007; Farghaly and Mahmoud, 2011 and Dutta *et al.*, 2013). The *E. coli* strains in particular had acquired resistance against the most commonly used antimicrobial in pigeons, (Kimpe *et al.*, 2002).

The overall purposes of this research are:

- 1- To identify the major serotypes of avian pathogenic *E. coli* prevailing in diseased pigeons.
- 2- To detect some virulence factors associated with pathogenic *E. coli* strains isolated from diseased pigeons.
- 3- To determine the level of antimicrobial susceptibility pattern in pathogenic *E. coli* strains isolated from diseased pigeons by disk diffusion antibiotic test method.

MATERIALS and METHODS

I - Samples:

A total number of 87 domesticated pigeons (freshly dead and diseased) of both sexes were obtained from 19 lofts located in various parts of Assiut province as in table 1. Samples were collected from pigeons suffering from sudden death and nonspecific clinical signs: (anorexia, depression, ruffled feathers, vomiting, diarrhoea, polyuria, fluid filled crops and respiratory and joint problems). Samples were collected from affected internal organs (liver and heart) plus 37 yolk or yolk sac samples from non hatched eggs (20 infertile eggs and 17 dead-in-shell embryos).

Table 1: Samples collected from the infected pigeons.

Pigeons	Adult	Squabs	Total
No. of diseased	8	60	68
No. of freshly dead	3	16	19
Total	11	76	87

II - Isolation and identification of the *E. coli***Isolates:**

All collected samples were inoculated in brain heart infusion broth (Oxiod) and incubated at 37°C for 24 hours. Loopfulls from inoculated broth were cultured onto MacConkey agar plates (Oxiod). After overnight incubation at 37°C, rose pink colonies were cultured onto selective culture in eosin methylene blue agar (Oxiod). Colonies with the characteristic metallic sheen of *E. coli* were inoculated onto nutrient agar slants. *E. coli* isolates were subjected to identification on the basis of their cultural and morphological characters. The standard biochemical confirmation of the strains was performed by conventional IMViC testes (Indole, Methyl red, Voges-Proskauer, and Citrate utilization), urease test, motility, triple sugar iron agar (TSI) inoculation and sorbitol, raffinose and cellobiose fermentation to confirm their identity as *E. coli* according to Quinn *et al.* (2004).

III - Serological identification of *E. coli*:

Pure cultures of each isolate were serotyped using standard references *E. coli* antisera (DENKA SEIKEN Co., Japan). They include 8 vials of polyvalent in addition to 43 vials of monovalent antisera and 5 H-sera. The isolates were serologically identified according to Kok *et al.* (1996) by using rapid diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) for diagnosis of the Enteropathogenic types.

IV - Virulence factors tests of *E. coli* isolates:

A)- Haemolytic activity (haemolysin) (Beutin *et al.*, 1989): *E. coli* isolates were inoculated onto blood agar containing 5% sheep blood for detection of

enterohaemolysis after 6 hours of incubation at 37°C. The colonies producing clear zones of hemolysis were recorded as hemolysin positive.

B)- Congo Red (CR) binding activity (Panigrahy and Yushin, 1990): The medium used for CR dye binding was tryptose agar with 0.2% galactose and 0.03% CR dye. The tested *E. coli* isolates were streaked onto CR agar plates and incubated at 37°C for 24 hours. The plates were further incubated at room temperature for additional 48 hours. The colonies were examined at 18, 24, 48 and 72 hours of incubation. The *E. coli* that produced red colonies between 18 and 72 hours of incubation were recorded as Congo Red positive and the ones that produced grayish-white colonies and remained so throughout the incubation period were recorded as Congo Red negative.

V- Antimicrobial susceptibility determination:

In-vitro antimicrobial susceptibility determination was tested by the single-disc diffusion method. Muller-Hinton agar (Oxoid, Basingstoke, UK) was prepared in a uniform thickness (4 mm) for testing of *E. coli* isolates. The *E. coli* strains were tested against 8 antimicrobial agents (Bioanalysis – Turkey), which represent the commonly used antimicrobials in pigeons: ampicillin (AM/10 µg), doxycycline (DO/30 µg), erythromycin (E/15 µg), neomycin (N/30 µg), norfloxacin (NOR/10 µg), streptomycin (S/10 µg), tetracycline (TE/30 µg) and trimethoprim-sulphamethoxazole (SXT/1.25µg /23.75 µg). The diameters of the zones of inhibition were interpreted by referring to the table which represents the NCCLS subcommittee's recommendation (NCCLS, 2002).

RESULTS**Table 2:** Represents the bacteria isolated from samples collected from 87 infected pigeons.

Identified bacterium	Number of isolates	% of isolated bacteria
<i>E. coli</i>	19	21.8 %
<i>Enterobacter agglomerans</i>	1	1.15 %
<i>Enterobacter cloacae</i>	1	1.15 %
<i>Hafnia alvei</i>	1	1.15 %
<i>Serratia marcescens</i>	1	1.15 %
Total	23	26.4 %

Samples of yolk of infertile eggs (20) and yolk sac from dead-in-shell embryos (17) of non-hatched pigeon eggs were found negative for *E. coli* isolation.

Table 3: Shows the incidence of *E. coli* strains isolated from squabs and adult pigeons.

Case	Squabs		Adults		Total	
	+ve <i>E. coli</i> / total	+ve %	+ve <i>E. coli</i> / total	+ve %	Total +ve <i>E. coli</i>	Total %
Diseased	9/60	15	2/ 8	25	11/68	16.2
Dead	6/16	37.5	2/ 3	66.7	8/19	42.1
Total	15/76	19.7	4/11	36.4	19/87	21.8

Table 4: Illustrate the results of serotyping *E. coli* strains ($n=19$) isolated from the infected pigeons.

O serogroups	No. of strains	Percentage of each serogroup	Strain characterization
O78	4	21.05	EPEC
O2 : H6	3	15.79	EPEC
O1 : H7	2	10.53	EPEC
O128 : H2	2	10.53	ETEC
O119 : H4	2	10.53	EPEC
O111 : H4	1	5.26	EHEC
O114 : H21	1	5.26	EPEC
O44 : H18	1	5.26	EPEC
Untyped	3	15.79	Untyped

n = Number of *E. coli* strains. EPEC = Enteropathogenic *E. coli*. ETEC = Enterotoxigenic *E. coli*. EHEC = Enterohemorrhagic *E. coli*.

Table 5: Represents the relationship between different serogroups and phenotypic virulence factors of 19 *E. coli* strains isolated from the infected pigeons.

Serogroups	Congo Red binding		α - Haemolytic activity	
	No. of +ve strains	(%)	No. of +ve strains	(%)
O78	4	100	3	75
O2 : H6	3	100	3	100
O1 : H7	2	100	1	50
O128 : H2	2	100	2	100
O119 : H4	2	100	2	100
O111 : H4	1	100	1	100
114 : H21	1	100	1	100
O44 : H18	1	100	1	100
Untyped	3	100	2	67
Total	19	(100 %)	16	(84.2%)

Table 6: Shows the antimicrobial susceptibility pattern among the 19 *E. coli* strains isolated from the infected pigeons.

Result		Antimicrobial agent							
		AMP	DO	E	N	NOR	S	STX	TE
S	<i>n</i>	3	5	4	0	17	19	2	4
	%	15.8	26.4	21.1	0	89.5	100	10.5	21.2
R	<i>n</i>	16	14	15	19	2	0	17	15
	%	84.2	73.6	78.9	100	10.5	0	89.5	78.9

S = sensitive, R = resistance, % = percentage and *n* = number of *E. coli* strains.

AMP = penicillin, DO = doxycycline, E = erythromycin, N = neomycine, NOR = norfloxacin, S = streptomycin, STX = trimethoprim-sulphamethoxazole and TE = tetracycline.

DISCUSSION

Housing system of pigeons under Egyptian conditions give the chance for pigeons to come into close contact with wild and domesticated bird that enabling direct transfer of the infectious agents to take place especially when kept out to doors. Also pigeon spreads of infectious agents through fecal contamination of drinking water sources, pastures and agricultural crops.

In the present study, bacteriological examination revealed isolation 23 bacterial isolates (26.4%) from internal organs (livers and hearts) of 87 infected pigeons, *E. coli* was the main causative agent of infection in diseased and freshly dead pigeons with an incidence 21.8% followed by *Enterobacter agglomerans*, *Enterobacter cloacae*, *Hafnia alvei* and *Serratia marcescens* (1.15% for each) as shown in table, 2. Also *E. coli* give higher incidence in freshly dead adult (66.7%) and squabs (37.5%) than diseased adult (25%) and squabs (15%), table 3. The incidence of *E. coli* infection was more high in dead and diseased adults of pigeons than that in dead and diseased squabs, it may attributed to the heavy infection of adults by round and tap worms that act as predisposing factor for secondary *E. coli* infection, this agree with Ibrahim, (2007) who isolated *E. coli* from livers of dead adult pigeons with higher incidence (28.57%) than in dead squabs (19.5%). The incidence of *E. coli* isolates according to the status of the examined pigeons and ages either squabs or adults, as it give higher incidence in freshly dead adults and squabs followed by diseased adults and squabs, the results in literatures obtained by (De Herdt *et al.*, 1993, Gonzalez *et al.*, 2004 and Hassan *et al.*, 2008) nearly similar to our results.

In table (4): Eight serotypes of pathogenic *E. coli* were detected in infected pigeons of Assiut Province. The most frequent serotype found was

O78 (21.05%), followed by O2:H6 (15.79%), O1:H7, O128:H2, O119:H4 (10.53% for each), O111:H4, O114:H21, O44:H18 (5.26% for each) while untyped strains were (15.79%). The all serotypes were EPEC except O128 and O111 serotypes were ETEC and EHEC respectively. The serotypes O78 and O2 were the most prevalent serotypes in this study, the same results were detected in diseased pigeons of Egypt by Farghaly and Mahmoud, (2011), also this agree with Dizva and Steven (2008), who mentioned that O2 and O78 are preferentially associated with colibacillosis outbreaks in poultry and represent 80% of disease cases worldwide. Also Particular serogroups such as O1, O2, and O78, especially the last two, were isolated frequently from birds with colibacillosis, (Sojka and Carnaghan, 1961; Dho-Moulin and Fairbrother, 1999 Blanco *et al.*, 1998; Gomis *et al.*, 2001 and Jeffrey *et al.*, 2002), these serotypes were also isolated from healthy birds or the birds' environment, (Blanco *et al.*, 1997 and Altekruze *et al.*, 2002). Most of *E. coli* serotypes identified in this study were reported to be the most prevalent and potential pathogenic for young and old poultry by Raji *et al.* (2003) and El-Jakee *et al.* (2012). Dutta *et al.* (2013) detected nine serotypes of pathogenic *E. coli* in pigeons, the serotype was O157 (9.89%) followed by O68, O121 (7.79%), O9, O75, O131 (5.49%), O2, O13, O22 (3.30%). There is evidence of *E. coli* serotypes O133, O50, O79, O21, O55, O2 and O125 were prevalent in Assam pigeons, Phangcho, (2001). Six serotypes were detected in Egyptian pigeons by Ibrahim (2007), the serotypes includes O8, O78, O86, O111, O157 and O166. From results of these investigations, variation in prevalence of *E. coli* serotypes was found in all serotypes in pigeons except for 2 serotypes O78 and O111 that detected in Egyptian pigeons by Ibrahim, (2007) and serotype O2 as reported by, Phangcho, (2001) and Dutta *et al.* (2013). The occurrence of a specific serotype and its role in disease production

depends upon the health status of the birds, climatic conditions, variation in feed and water supply along with geographical situation and management strategies, the variations are found in serotype prevalence from time to time and from region to region, (Ewers *et al.*, 2007 and Dutta *et al.*, 2013).

Inability to detect *E. coli* from non-hatched eggs can be interpreted by Harry and Hemsley, (1965), they reported that, pathogenic coliform are more frequent in the gut of newly hatched chicks than in egg from which they hatched plus low environmental contamination of pigeon eggs in comparison with other birds.

Two virulent tests which included the uptake of Congo-red dye, and hemolytic activity tests were performed on 19 avian *E. coli* isolates from diseased and freshly dead pigeons in table (5). The all isolates were found positive for the Congo red binding activity (100%). A direct correlation was found between the ability of clinical isolates of pathogenic *E. coli* to bind Congo red dye and their ability to cause infection in pigeons, this results were the same that obtained by Berkhoff and Vinal (1985) in *E. coli* septicemic infection in chickens, also Ezz EL Deen *et al.* (2010) recovered that, 100% for *E. coli*, isolated from diseased chickens in Egypt, were have Congo red bind activity. The hemolysis, as another virulence factor, was carried on all *E. coli* isolates and was found that, (84.2%) of the *E. coli* isolates were positive for α - hemolytic activity. The previous studies have shown that hemolytic activities of APEC isolates correlated to the virulence of avian *E. coli*. McNamee *et al.* (1998); Moon *et al.*, (2006) and Ezz EL Deen *et al.* (2010) detected hemolytic activity of *E. coli* in diseased chickens with percentages of 86.4%, 90% and 100% respectively. It is remarkable that *E. coli* isolates, isolated from diseased pigeons, were holding pathogenic characteristics like Congo red binding and hemolysis activities.

Table (6) show the *in-vitro* antimicrobial susceptibility pattern of pathogenic *E. coli* strains isolated from internal organs of the examined infected pigeons that revealed variable results in susceptibility and zones of inhibition to the different antimicrobial drugs which commonly used in pigeon treatments. Neomycin was not effective at all for all *E. coli* strains, the most strains were frequently highly resistance to trimethoprim-sulphamethoxazol (89.5%) followed by ampicillin (84.2%), tetracycline (78.9%), erythromycin (78.9%) and doxycycline (73.6%). In this aspect is in agreement with results of Hassan *et al.* (2008) who detected that the most *E. coli* strains of pigeons were resistant to amoxicillin, sulphamethoxazol, neomycin, oxytetracycline and trimethoprim. The results revealed high sensitivity of the isolates to

streptomycin (100%) and norfloxacin (89.5%). The same record reported by Hassan *et al.* (2008) and Dutta *et al.* (2013) for the most examined antimicrobial drugs but differ in streptomycin result that show (61.64%) resistance. Antimicrobial resistant *E. coli* strains in domestic pigeons were studied by Sato *et al.* (1978) in Japan and Kimpe *et al.* (2002) in Belgium, high levels of resistance were found among *E. coli* isolates, while resistance to tetracycline (detected in 50% of the isolates) was the most prevalent. Another study in domestic pigeons in Japan was conducted by Ishiguro *et al.* (1978), the isolated *E. coli* strains were resistant to tetracycline, streptomycin, sulfonamides and quinolones, they concluded that the high antimicrobial resistance occurrence may reflect the abusive use of antimicrobial substances in society. The authors conclude that the feeding habits of the pigeons and close contact with humans in the urban environment could enable them to become contaminated with medically important bacteria or residual antimicrobials and chemicals, because they may rely on garbage or trash as food sources. *E. coli* strains show acquired resistance against the most commonly used antimicrobials in pigeon treatments. All results show high increase in antimicrobial resistance of pathogenic *E. coli* in pigeons. So surveillance programs may be introduced to monitor antimicrobial resistance of pathogenic *E. coli* in pigeons.

CONCLUSION

Different serotypes of pathogenic *E. coli* were isolated from diseased pigeons but it can not isolated from non-hatched pigeon eggs in Assiut Province. Serotypes O78 and O2 were found the most common serotypes in pigeons. All *E. coli* serotypes isolated from pigeons were reported between previously detected serotypes of avian pathogenic *E. coli* that were found pathogenic to young and adult avian species. Congo red binding and hemolysin activities can be used as factors of virulence for pathogenic *E. coli* in pigeons. The pathogenic *E. coli* isolated from pigeons have multiple antimicrobial resistance to the common used drugs.

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توصيف عترات الميكروب القولوني المعزولة من الحمام المصاب في محافظة أسيوط

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الميكروب القولوني هو ممرض رئيسي في الدواجن المنتجة تجاريا في جميع أنحاء العالم مما يسهم بشكل كبير في ارتفاع الخسائر الاقتصادية. في هذه الدراسة تم توصيف عزلات الميكروب القولوني الممرض المعزولة من أنسجة الحمام المصاب ومن بيض الحمام الغير فاقس في محافظة أسيوط بالتصنيف السيرولوجي وبعض عوامل الضراوة واختبار الحساسية للمضادات الميكروبية. تم فحص ١٢٤ عينة (٨٧ حمام و ٣٧ بيض حمام غير فاقس) لعزل الميكروب القولوني منها. شملت العينات أنسجة الكبد والقلب في الحمام المريض والنافق حديثا بالإضافة الى المح من ٢٠ بيضة غير مخصبة و ١٧ من كيس المح للأجنة النافقة من بيض الحمام غير الفاقس. وقد تم عزل ٢٣ من العزلات البكتيرية وبتعريفها حدد منها ١٩ عزلة للميكروب القولوني وعزلة واحدة لكل من الأمعائية أجلوميرانس ، الأمعائية المذرقية ، الهافنية النخرية والسرانية الذابلة في أنسجة الحمام المصاب، بينما كانت عينات البيض سالبة لعزل الميكروب القولوني. وقد تم تحديد ثمانية مجموعات مصلية بين ستة عشر عزلة من الميكروب القولوني في الحمام ، بينما لم تحدد المجموعة المصلية لثلاثة من عزلات الميكروب القولوني. كانت المجموعة المصلية O78 هي الأكثر شيوعا بنسبة ٢١.٥٪ ، تلتها المجموعة O2:H6 بنسبة ١٥.٧٩٪ من معزولات الميكروب القولوني في الحمام المصاب. وتم عزل المجموعات المصلية O114:H21 ، O111:H4 ، O119:H4 ، O128:H2 ، O1:H7 ، O44:H18 بنسبة ٥.٢٦٪ لكل مجموعة. تم اجراء اختبار عوامل الضراوة الخاصة بقدرة عزلات الميكروب القولوني على تحلل الدم وارتباطه بصبغة الكونجو الحمراء. تبين أن كل معزولات الميكروب القولوني ايجابية الارتباط لصبغة الكونجو الحمراء بنسبة ١٠٠٪ و ايجابية لتحلل الدم بنسبة ٨٤.٢٪. تم اختبار حساسية معزولات الميكروب القولوني ضد ٨ من المضادات الميكروبية المستخدمة في علاج الحمام في المختبر. عموما كل معزولات الميكروب القولوني كانت مقاومة للنيومايسين بنسبة ١٠٠٪. وكانت معظم العزلات شديدة المقاومة للتراي ميثوبريم - سلفاميسوكسازول ، يليها الأميسيلين ، التتراسيكلين ، الاريثروميسين والدوكسيسيكلين. أظهرت العزلات حساسية عالية للاستربتومايسين ١٠٠٪ ونورفلوكساسين ٨٩.٥٪. هذه النتائج تشير إلى أن الميكروب القولوني المعزول من الحمام مقاوم للعديد من المضادات الميكروبية والتي يحتمل أن تنتقل من الحمام إلى البشر. لذلك يجب إدخال برامج رقابية للتحكم في مقاومة البكتريا المسببة للأمراض للمضادات الميكروبية.