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Genotypic Characterization of Antibiotic Resistant Escherichia coli Isolates Recovered from Baladi Chicks in Fayoum Governorate

Samia, A.A. El-Kholi [a], Hatem, M.E.[b], El-Hariri, M. [c] Zaki, M.M.[d] and Abeer, A.E. Shehata[e]
a Directorate of Veterinary Medicine, Fayoum, Egypt
b Microbiology Department, Faculty of Veterinary Medicine, Cairo University.
c Microbiology Department, Faculty of Veterinary Medicine, Cairo University.
d Poultry Diseases, Faculty of Veterinary Medicine, Cairo University.
e Department of Bacteriology, Animal Health Research Institute, Fayoum Laboratory, Agricultural Research Center, Egypt.

Abstract

A total of 23 isolates of E. coli, recovered from Baladi chicks manifested respiratory signs associated with diarrhea and belonged to different O-serogroups was investigated for antimicrobial resistance and tested for their tetracycline and ciprofloxacin resistance genes. The result recovered that 91.3% of the isolates were resistant to tetracycline and 26.1% to ciprofloxacin (quinolone group). Ten out of 23 E. coli isolates tested for detection of antimicrobial resistance genes, 5 (50%) isolates were positive for tetA(A) gene, where 5 (50%) isolates were positive for tetA(B) gene. On the other hand all the E. coli isolates 10 (100%) were positive for gyrA gene, where only one isolate (10%) was positive for aac(6')-Ib-cr gene.

Keywords: Multiple drug resistant E. coli in poultry, Antimicrobial sensitivity test, PCR, Detection of the resistant genes of E. coli).

Introduction

Escherichia coli is one of the most common microorganisms, which affect both animals and humans worldwide by a wide spectrum of diseases ranging from opportunistic wound infection to severe systemic infections (Gyles and Fairbrother 2010). Avian colibacillosis refers to any localized or systemic infection caused entirely or partly by avian pathogenic Escherichia coli (APEC), including colisepticemia, coligranuloma (Hjarre's disease), air sac disease (chronic respiratory disease, CRD), coliform cellulitis (inflammatory process), swollen-head syndrome, coliform peritonitis, coliform salpingitis, coliform osteomyelitis/synovitis (turkey osteomyelitis complex), coliform panophthalmitis, and coliform omphalitis/yolk sac infection (Barnes 2000). It is the most frequently reported disease in surveys of poultry diseases or condemnations at processing and responsible for significant economic losses to the poultry industry (Yogaratnam 1995). Escherichia coli acquires antimicrobial resistance faster than other bacteria. Thus, changes in the resistance of this species may serve as a good indicator of resistance in potentially pathogenic bacteria (Von Baum and Marre 2005). The antimicrobial resistance profile is applied for detection of the relevant resistance genes (Rebeiro et al., 2011).

The aim of the present work was to study the genotying characterization of antibiotic resistant Escherichia coli isolates recovered from Fayoum Governorate in Egypt

Materials and Methods

Samples collection:

samples (157) from diseased chicks were collected from private farms, Baladi hatcheries and living poultry markets in Fayoum Governorate, Egypt during the period from September 2012 up to February 2013. Samples were from different organs (liver, spleen, lung, heart, yolk sac and gallbladder) of chicks that suffered from respiratory signs associated with diarrhea. The isolates belonged to different O-serogroups and showed resistance to one or more antibiotics.

Bacteriological examination conducted according to Cruickshank et al., 1975

Materials and methods used for confirmation of the biochemical characteristics and serogrouping of E. coli isolates (Poirel et al., 2005)

Antibacterial sensitivity discs (Oxoid):

A total of 13 different anti bacterial sensitivity discs (Oxoid) were used for confirmation of resistance of the isolates to one or more antibiotics: neomycin (N), gentamicin (GM), streptomycin (S), tetracycline (TE), doxycycline (DO), colistinsulphate (CL), chloramphenicol (C), (trimethoprim-sulphamethoxezole (SXT)., amoxciclin (AML),norofloxacin (NOR), enrofloxacin (ENR), ciprofloxicine (CIP) and

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erythromycin (E). The antibiotic susceptibility was determined according to the recommendations set by the Clinical and Laboratory Standards Institute **(Clinical and Laboratory Standards Institute, CLSI, 2007)** for the disk diffusion technique.The inhibition zones were measured and scored as sensitive, intermediate susceptibility or resistant according to the CLSI, 2007 recommendations.

Reference cultures used for quality assurance and PCR specificity

Escherichia coli,NCIMB-50034-ATCC-43894,was used as positive control and Salmonella Enteritidis, ATCC13076, was used as negative control.

DNA extraction and PCR amplification

DNAs of E. coli isolates were extracted by QIAamp® DNA Mini Kit (Cat. No. 51304 Qiagen). The preparation of PCR Master Mix for cPCR was carried out according to EmeraldAmp® MAX PCR Master Mix kit.

Oligonucleotide primers

Oligonucleotide primers sequences encoding for tetracycline and ciprofloxacin resistant genes:

tetA(A): 5'-GGTTCACTCGAACGACGTCA -3',**R:**5'-CTGTCCGACAAGTTGCATGA -3'(Randall et al., (2004)

tetA(B): 5'- CCTCAGCTTCTCAACGCGTG -3', **R:** 5'-GCACCTTGCTCATGACTCTT -3' Randall et al., (2004)

aac(6')-Ib-cr: 5'- CCCGCTTTCTCGTAGCA-3', **R:** 5'- TTAGGCATCACTGCGTCTTC -3'(Lunn et al., 2010)

gyrA: 5'-AAATCTGCCCGTGTCGTTGGT-3', **R:** 5'- GCCATACCTACTGCGATACC-3'(Fàbrega et al., 2009)

Results

Confirmation of E. coli isolates:

All recovered isolates showed the typical colony characteristics of E. coli, where they appeared as pink colonies on MacConkey's agar medium. All isolates showed the typical biochemical reactions. The serotyping confirmed the following serogroups: O27, O78, O153, O157 and O158.

It was clear that the highest rate of resistance was recorded against erythromycin and streptomycin was (100%). Moreover high resistance (more than 50%) pattern was observed against tetracycline and chlormaphenicol were (91.3% each), doxycycline (82.6%), amoxicillin and sulphamethaxzole+trimethoprim (78.3% each) and enrofloxacin (52.2%). Likewise Salmonella spp., E. coli showed reduced sensitivity against ciprofloxacine (34.8%). Additionally, E. coli isolates showed noticeable sensitivity (69.6%)against gentamicin when compared with old antimicrobial classes.

Table (1): Antimicrobial susceptibility profile of E. coli isolates recovered from Baladi

	Sensetive		Intermediate		Resistant	
	No.	%	No.	%	No.	%
Tetracycline	2	8.7	0	0.0	21	91.3
Doxycycline	2	8.7	2	8.7	19	82.6
Erythromycin	0	0.0	0	0.0	23	100.0
Amoxicillin	4	17.4	1	4.3	18	78.3
Sulphatrimethoprime +sulphadimethaxzole	3	13.0	2	8.7	18	78.3
Enrofloxacin	8	34.8	3	13.0	12	52.2
Norfloxacin	14	60.9	4	17.4	5	21.7
Neomycin	11	47.9	9	39.1	3	13.0
Chloramaphenicol	2	8.7	0	0.0	21	91.3
Ciprofloxacin*	8	34.8	9	39.1	6	26.1
Gentamicin	16	69.6	5	21.7	2	8.7
Colistinsulphate	23	100.0	0	0.0	0	0.0
Streptomycin	0	0.0	0	0.0	23	100.0

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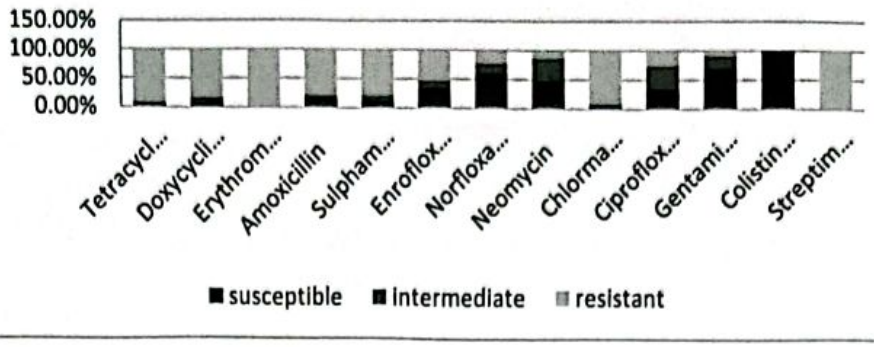
% was calculated according to the total number of isolates

Figure (1): Antimicrobial profile of *E. coli* isolates recoverd from Baladi chicks



Detection of tetracycline resistance genes

Ten out of 23 E. coli isolates were tested for detection of antimicrobial resistance gene, 5 (50%) isolates were positive for tetA(A), where 5 (50%) isolates were positive for tetA(B) gene **(Table 2, photo 2 and 3).**

Detection of ciprofloxacin resistance genes

All E. coli isolates 10 (100%) were positive for gyrA gene, where only one isolate (10%) was positive for aac (6')-Ib-cr gene **(Table 2 and photo 1).**

Table (2): Gene resistance prevalence of E. coli against tetracycline (tetA and tetB) and ciprofloxacin (aac(6)ib-cr):

	Resistance pattern	E. coli	
		Tested no.	Positive no. (%)
TetA(A)	Resistant	10	5 (50.0)
TetA(B)	Resistant	10	5 (50.0)
Aac(6)ib-cr	Resistant	5	1 (10.0)

% was calculated according to the total number of isolates

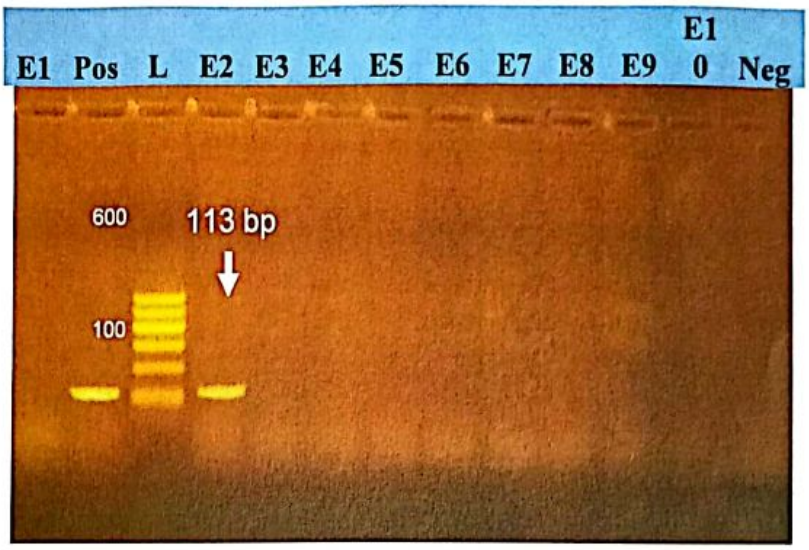


Photo (1):Agarose gel electrophoresis showing PCR results for detection of aac(6)ib-cr gene in E. coli that were not susceptible to ciprofloxacin. Lane E2 was positive for aac(6)ib-cr gene, Pos. is the control positive sample, Neg. is the control negative sample while the other lanes of E. coli (E1 and E3-E10) were negative. L: 600 pb ladder.

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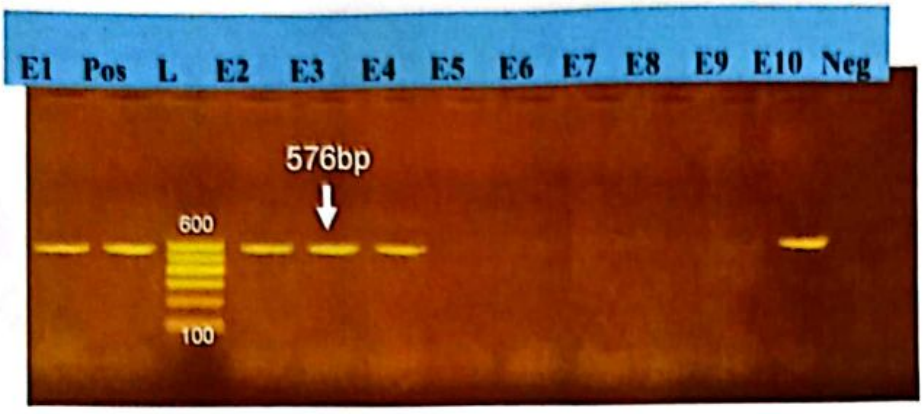

Photo (2):Agarose gel electrophoresis showing PCR results for detection of tetA(A) gene in E. coli that were not susceptible to tetracycline. Lanes E1, E2, E3,E4 and E10 were positive for tetA(A) gene, Pos. is the control positive sample, Neg. is the control negative sample while the other lanes of E. coli (E5-E9) were negative. L: 600 pb ladder.

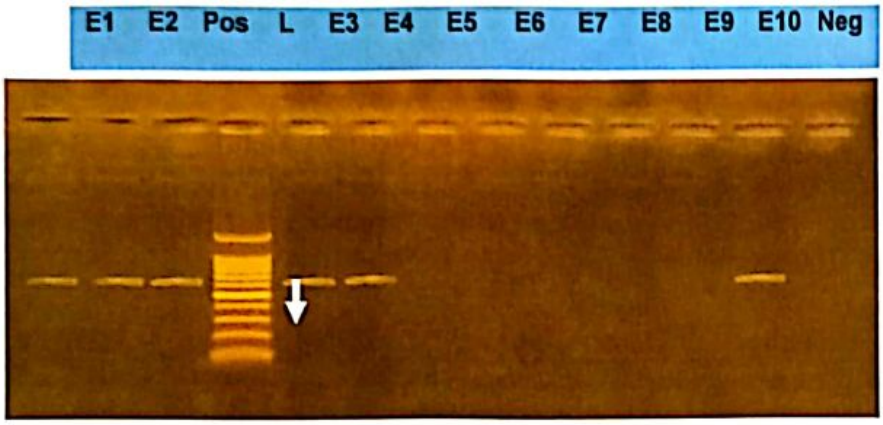

Photo (3):Agarose gel electrophoresis showing PCR results for detection of tetA(B) gene in E. coli that were not susceptible to tetracycline. Lanes E1, E2, E3, E4 and E10 were positive for tetA(B) gene, Pos. is the control positive sample, Neg. is the control negative sample while the other lanes of E. coli (E5-E9) were negative. L: 1500 pb ladder.

DISCUSSION

E coli is a normal inhabitant of the gastrointestinal tract of humans and animals; however, some strains are known to be pathogenic. These strains induce colibacillosis in chicken, which is an important cause of economic losses for the poultry industry (Amara et al., 1995).

Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis (Freed et al., 1993). Antibiotic usage is considered the most important factor promoting the emergence, selection and dissemination of antibiotic-resistant microorganisms in both veterinary and human medicine (Witte 1998).

The obtained results of antibiotic sensitivity for examined E. coli isolates as shown in Table (1) and figure (1) illustrate the antimicrobial susceptibility patterns of E. coli isolates from Baladi chicks against the most clinically used antibiotics in veterinary medicine. All the explored E. coli isolates were sensitive to colistin sulphate (100%). On the other hand 100% of the inspected E. coli isolates were resistant to erythromycin and streptomycin. Moreover high resistance (more than 50%) pattern were investigated to tetracycline and chlormaphenicol, (91.3% for each), doxycycline (82.6%), amoxicillin and sulphamethaxzole+trimethoprim (78.3% for each) and enrofloxacin (52.2%). Likewise E. coli showed reduced sensitivity against ciprofloxacine (34.8%) Additionally, gentamicin showed noticeable sensitivity (69.6%) against the explored E. coli isolates when compared with old antimicrobial classes.

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resistance was evoked against erythromycin and streptomycin followed by tetracycline, chloramphenicol, doxycycline, amoxicillin and finally sulphamethaxzole+trimethoprim.

These results comes in accordance to many studies that investigated E. coli has high resistance against oxytetracycline, trimethoprim+sulphamethoxazole, chloramphenicol and enrofloxacine and low resistance (less than 15%) to ampicillin, gentamicin and colistin (Amara et al., 1995), E. coli showed different resistance patterns against tetracycline (94%), followed by oxytetracycline (80%), chloramphenicol (58%), sulphamethoxazole-trimethoprim (50%), neomycin (48%), streptomycin (48%), enrofloxacin (44%) and ampicilline (28%) (Tabatabaei and Nasirian 2003), E. coli exhibited sensitivity to enrofloxacin, gentamicin, erythromycin and streptomycin (Abdelatif 2004), E. coli isolates showed different resistant patterns against tetracycline, streptomycin, chloramphenicol, amoxicillin, ciprofloxacin and sulfamethoxazole-trimethroprim Raji et al. (2007), E. coli isolates under test were resistant against tetracycline, amoxicillin, ampicillin and gentamicin Shah and Qureshi (2007),E. coli isolates were sensitive to enrofloxacin, colistin, amoxicillin, gentamicin and flurophenicol Galatanu et al. (2010). In contrast, most of the isolates were resistant to, tetracycline, streptomycin, neomycin and sulfamethoxazole-trimethroprim, E. coli isolates showed different resistance patterns to tetracycline (45.5%), trimethoprim-sulphamethoxazole (26.7%), streptomycin (20.8%), ciprofloxacin (12.9%), chlormaphenicol (8.9%), gentamicin (2%) (Badrul et al.2011), E. coli strains recovered showed different resistance patterns for neomycin , tetracycline, erythromycin, doxycycline and enrofloxacin (Fodor 2011), E. coli isolates were resistant against amoxicillin, streptomycin and trimethroprim while they were intermediate to oxytetracycline and sensitive to gentamicin (Abu Daud 2014), E. coli showed resistance against oxytetracycline (43.4%), sulfadimethaxzole-trimethoprim (39.6%), enrofloxacin (37.7%), chloramphenicol (20.75%), doxycycline (17.0%), ciprofloxacin (7.55%), and gentamicin (5.66%) Talebiyan et al. (2014). On the other hand this study results were differed in the investigation of many studies , whichrecorded that E. coli isolates have different sensitivity patterns in descending manner to norfloxacin (44.9%), gentamicin (37.1%), enrofloxacin (36.4%), chloramaphenicol (33.7%), amoxicillin (10.7%), doxycycline (10.7%), ampicillin (10.2%), sulpha and trimethoprim (9.1%), streptomycin (5.9%), oxytetracyclin (3.7%) (Iqbal et al., 2006), most of E. coli isolates were sensitive to chloramphenicol, cotrimoxazole and enrofloxacin and they were resistant against amoxicillin, erythromycin and tetracycline (Hussenia et al., 2008),E. coli isolates were sensitive to enrofloxacin, chloramphenicol, while all the isolates were 100% resistant against oxytetracycline (Yousseff et al., 2008), neomycin, gentamicin and trimethoprim were the most effective antibiotics for E. coli isolates (Mohamed 2009), the recovered E. coli were sensitive to chloramphenicol and gentamicin. Conversely, they were resistant against ciprofloxacin, norfloxacin, (Anyanwu et al. 2010), E. coli isolates were sensitive to chloramphenicol (Nasrin et al., 2012), E. coli isolates were sensitive to gentamicin and chloramaphenicol (Abadi et al., 2013), E. coli isolates were sensitive to ciprofloxacin, norfloxacin and chloramphenicol (Debasish and Samal 2013), E. coli isolates exhibited sensitivity to ciprofloxacin, norfloxacin and chloramphenicol. While most of the E. coli isolates were resistant against oxytetracycline, co-trimoxazole, amoxicillin and gentamicin (Peer et al., 2013).

In this study as shown in Table (2) and photo (1,2 and 3) illustrate the gene resistance prevalence of E. coli recovered from Baladi chicks manifested respiratory signs associated with diarrhea against tetracycline (tetA and tetB) and ciprofloxacin (aac(6)ib-cr). Five out of ten E. coli isolates (50%) showed resistance behavior against tetracycline disk were positive for tetA and tetB gene. On the other hand, one out of ten E. coli (10%) showed resistance behavior against ciprofloxacin disk was positive for aac(6)ib-cr gene. The result agreed with Some authors detected tet(A) with the percent of (66%) and tet(B) with the percent of (42%) (Guerra et al., 2003). While others found that 21.4 and 42.9% gave positive results for tetA and tetB, respectively (Diarrassouba et al., 2007), tetA and/or tetB in the E. coli strains were detected (Kim et al., 2007),11.7% aac(6')-Ib-cr of E. coli isolates were recorded (Huang et al., 2009), positive rates of tetA and tetB with a percent of 57.93% and 38.41% (Zhang et al., 2012) and aac(6')-Ib-cr (36.04%) was the most frequently identified gene in all E. coli isolates (Xie et al., 2014).

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Conclusion

Ten out of 23 E. coli isolates tested for detection of antimicrobial gene resistance, 5 (50%) isolates were positive for TetA(A), where 5 (50%) isolates were positive forTetA(B) gene. On the other hand all the tested E. coli isolates 10 (100%) were positive for gyrA gene, where only one isolates (10%) were positive for aac (6')-Ib-cr gene.

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الملخص العربي

الخصائص الجينية للمضادات الحيوية المقاومة لعزلات الميكروب القولوني المعزول من الكتاكيت البلدي بمحافظة الفيوم

سامية علي أحمد مصطفي الخولي[1]د محمود عصام حاتم-د محمود الحريري-د محمد محمود زكي-د عبير أحمد السيد شحاتة

مديرية الطب البيطري بالفيوم[1]-قسم الميكروبيولوجي[2]-قسم الدواجن[3]-كلية الطب البيطري-جامعة القاهرة-معهد بحوث صحة الحيوان بالفيوم[4]

تم عزل عدد 23 معزولة ايشيريشيا كولاي من كتاكيت بلدي (الكبد-الطحال-الرئة-القلب-الحوصلة المرارية وكيس المح لكل كتكوت) عليها أعراض تنفسية مصحوبة بإسهالات من معامل تفريخ ومزارع خاصة ومن أسواق بيع طيور حيه بمحافظة الفيوم في الفترة من سبتمبر 2012 إلي فبراير 2013 وقد تم إجراء اختبار حساسية الميكروب للمضادات الحيوية باستخدام 13 نوع من أقراص المضادات الحيوية واشارت النتائج ان 91.3% من المعزولات مقاوم لمجموعة التتراسيكلين و26.1% من المعزولات مقاوم لمجموعة الكوينيلون (السيبروفلوكساسين و بإجراء اختبار البلمرة المتسلسل للكشف عن الجينات المسئولة عن المقاومة لمجموعة التتراسيكلين علي عدد 10 معزولات أظهرت النتائج إيجابية الكشف عن جين TetA(A) عدد خمس معزولات بنسبة 50% وايجابية الكشف عن جين TetA(B) عدد خمس معزولات بنسبة 50% وعلي الجانب الاخر تم الكشف عن الجينات المسئولة عن المقاومة لمجموعة الكوينيلون (السيبروفلوكساسين) وقد أظهرت النتائج ايجابية العشر معزولات لجين gyrA بنسبة 100% وعدد عزلة واحدة إيجابية لجين aac (6')-Ib-cr.

الممسوحة ضوئيا بـ CamScanner