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Phenotypic and Genotypic Characterization of Multi Drug Resistant E.coli Isolated from Chickens

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Abstract

This study was performed on 1284 chicken came from 77 poultry farms layers, broilers, grandparents, broiler breeder at Giza and Qalubia governorates for E. coli infection. Samples were taken from these chickens (liver, intestine and yolk sac) after clinical and post mortem examination for morphological changes. The result revealed that, E. coli was isolated from 1204 positive samples (93.7%) represented as 40 (100%) from grandparents, 360 (90%) from broiler breeder,100 (71.4%) from layers and 704 (100%) from broiler. By using polyvalent & monovalent anti sera, these isolates were characterized for 18 different serogroups, most common detected serogroups is O_{158}, O_{125}, O_{119}, O_{114} and O_{44} while there is 11 isolates are untypable. Antimicrobial susceptibility test by the disk diffusion method was performed in accordance with NCCLS (2008) for different 7 anti-microbial rugs (sulfamethazole, streptomycin, ciprofloxacin, enrofloxacin, tetracycline, trimethoprime and ampicillin). The results show that isolates were resistant to tetracycline and sensitive to ciprofloxacin. Eighty nine percent of isolates are multi drug resistant E. coli (resistant to 2 or more anti-microbial drug). In this study 20 isolates from total 73 E. coli isolates was examined for presence of resistance genes, the results revealed that: prevalence the gene aac(6′)-Ib-cr for ciprofloxacin and enrofloxacin resistance in 45% of isolates, the prevalence of the bla_{TEM} gene β- lactem resistance gene was detected in 80% of isolate**,** the prevalence of the tetA and sul1 genes tetracycline and sulfamethazole resistance genes was detected in 75% of isolates.

Keywords: Chickens, E.coli, Genotypic, Multi drug resistant, Phenotypic.

1. Introduction

E. coli is a member of the family Enterobacteriaceae, which may constitute a great hazard to poultry industry causing high mortality, loss of weight and reduction of egg production[3]. E. coli infection is one of the serious problems that cause a great threat to the profitability of birds' enterprises all over the word. Although E. coli is a normal inhabitant of the intestinal tract of birds, under the influence of predisposing factors, like inadequate and faulty ventilation, overcrowding, hunger, thirst, extremes of temperatures and low vitality[15]. E. coli strains classified by [19] into three major groups: commensal strains, intestinal pathogenic strains, and extra-intestinal pathogenic E. coli (ExPEC) strains. The species of E. coli are serologically divided into serogroups and serotypes on basis of their antigenic composition (somatic or O antigens for serogroups and flagella or H antigens for serotypes). Many strains express a third class of antigens (capsular or K antigens)[7]. Antimicrobial therapy is an important tool in reducing both the incidence and mortality associated with avian colibacillosis. However, resistance to existing antimicrobials is widespread and of concern to veterinarians [4]. This increasing resistance has received considerable national and international attention. Assessment of antimicrobial resistance of E. coli at molecular level is a useful tool for understanding the contribution of genetic elements responsible for developing and dissemination of resistance in bacteria [1]. The aim of the current work was designed to investigate the phenotypic and genotypic characters of multi drug resistant E. coli isolated from poultry.

2. Materials and methods

2.1 Sample collection

The examined birds submitted to the Reference Laboratory for Veterinary Quality Control on Poultry Production, Dokki, to be examined for the presence of E. coli infection. 1284 chicken were examined. The samples collected from different organs (intestine, yolk-sac and liver). The examined organs will collected aseptically to prevent cross contamination. This included the use of sterile sampling materials (bags, spatula) wearing disposable gloves. The collected samples were cultured within a time limit, which did not exceed 24 hours from collection.

2.2 Bacteriological examination

Bacterial culture was prepared within 24hr after collecting samples, from (liver, yolk sac and intestine) and a ready cultivated under complete aseptic condition onto MacConkey agar then E.M.B. (Ethylene methylene blue). The plates were incubated at 37^0C for 24-48 hours and then examined for the characteristic E. coli colonies. Indole, Methyl red, Voges- Proskauer and Simons citrate (IMVIC) tests were performed with the

colonies that showed growth characteristic of E.coli.

2.3 Serotyping of *E.coli* isolates

E.Coli isolates were serotyped by slide agglutination test according to [22] using standard e.coli antisera (SIFIN AND DENKA SEIKEN COMP) .

2.4 Antimicrobial susceptibility test

Antimicrobial susceptibility test was assayed by the disc diffusion method NCCLS (2008). Multi-drug resistance (MDR) isolates are defined as that isolate resistance to two or more anti-microbial drug. The anti-microbial disc used were sulfamethazole, streptomycin, ciprofloxacin, enrofloxacin, tetracycline, trimethoprim and ampicillin. Susceptibility and resistance were determined according to the interpretation criteria to E. coli (ATCC No. 25922) established by Clinical Laboratory Standards Institute (CLSI) standard.

2.5 Conventional PCR technique for detection of anti-microbial resistance genes in isolated E.coli isolates

2.5.1 DNA extraction

DNA extraction was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH Catalogue no.51304). Oligonucleotide Primer. used were supplied from Metabion (Germany) are listed in Table (1) .2.5.2. PCR amplification: According to the instruction of the kit (Emerlad, Japan) primers were utilized in a 25- µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Emerland, Japan), 1 µl of each primer of 20 pmol concentrations, 4.5 µl of DEPC water, and 6 µl of template. The reaction was performed in a Biometra thermal cycler.

Table (1) Examined genes and its related Primers sequence concerning amplicon sizes and references

Target gene	Primers sequences	Amplicon size (bp)	Reference
aac(6′)-Ib-cr	CCCGCTTTCTCGTAGCA TTAGGCATCACTGCGTCTTC	113bp	Lunnet al., 2010
bla_{TEM}	ATCAGCAATAAACCAGC CCCCGAAGAACGTTTTC	516bp	Colom et al., 2003
tetA(A)	GGTTCACTCGAACGACGTCA CTGTCCGACAAGTTGCATGA	576 bp	Randall et al. 2004
sul1	CGGCGTGGGCTACCTGAACG GCCGATCGCGTGAAGTTCCG	433 bp	Ibekwe et al., 2011

2.5.2 Analysis of the PCR products

[25]: The products of PCR were separated by electrophoresis on 1-% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. GeneRuler 100 -bp Ladder (Thermo scientific), was used to determine the fragment size. The gel was photographed by a gel documentation system (Biometra BDA digital) and the data was analyzed through computer software.

3. Results

The results of the microscopical identification of the suspected isolates showed Gram negative, non sporulated, straight rods. Growth on EMB agar colonies showed metallic sheen and give characteristic pink colonies. The results of biochemical identification gave the biochemical characteristic. The incidence of E. coli recovered from A total number of 1284 chickens from different farms in Giza and Qalubia are showed in Table (2).

The serotyping of the isolated strains revealed 73 E. coli isolates recovered from different samples, 62 strains were identified serologically belonged to 18 different serogroups. The most commonly detected E. coli serogroups isolated from different organs of chickens were O_{158}, O_{125}, O_{91},O_{114},O_{119},O_{44}, while11 strains haven't been typed as shown in Table (3) From Table (4) it clears that the highest rate of resistance was shown against tetracycline group of Antibiotic, which was about 98.3% of isolate followed by 91.8%, 73.9%, 69.9%, 58.9% of isolates were resistant to sulfamethazole and ampicillin, streptomycin, trimethoprim and enrofloxacin, and ciprofloxacin respectively. The examination of the twenty isolates for most represented antibiotic resistant genes by PCR aac(6′)-Ib-cr, bla_{TEM}, tetA(A) and sul1genes revealed different percentage as shown in fig (1-4).

Table (2) Incidence of E.coli in different chicken flocks

Types of examined chickens	Number of examined farms	Incidence of isolation	
		No of positive farms	%
Grand parents	2	2	100
Broiler breeder	20	18	90
layers	7	5	71.4
broiler	48	48	100
total	77	73	93.7

Table (3) Different serotypes of isolated E.coli

The E. coli serotype	Number of positive isolates /73	Percentage of positive %
O158	13	17.8
O125	7	9.5
O91	5	6.8
O114	5	6.8
O119	3	4.1
O44	3	4.1
O27	2	2.7
O78	3	4.1
O25	3	4.1
O103	2	2.7
O26	2	2.7
O111	1	1.4
O127	1	1.4
O86	1	1.4
O169	1	1.4
O8	1	1.4
O63	1	1.4
O157	1	1.4
Un-typable	11	15
Total serotypes	62	84.9

Table (4) The percentage of antimicrobial resistance by disc diffusion method

Reaction of examined strains	Sulfamethazole	Strept.,	Tetra.,	Trime.,	Cipro.,	Enro.,	Ampi.,
Sensitivitg	6	19	1	22	30	22	6
Resistance	67	54	72	51	43	51	67
Percent of resistance	91.8%	73.9%	98.3%	69.9%	58.9%	69.9%	53%

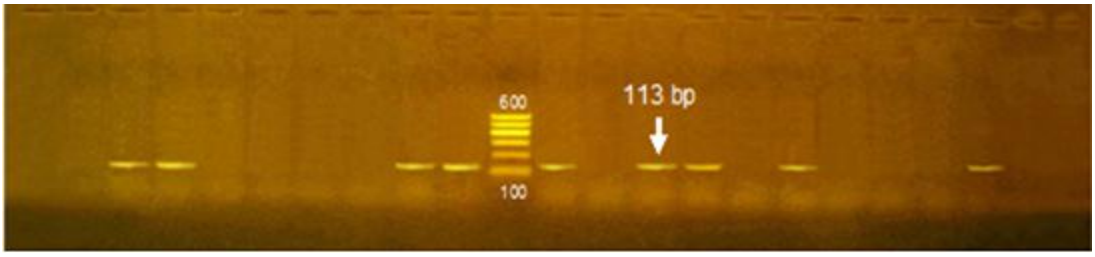

Fig (1) The prevalence of aac(6')-Ib-cr in 45% of isolates in isolates (2,6,8,9,11,12,13,17,18)

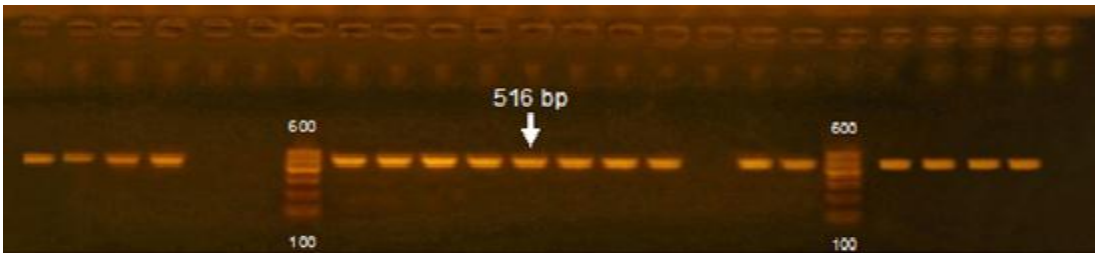

Fig (2) Show the prevalence of blaTEM gene in 17 (85%) of isolates (1,2,3,4,5,6,9,10,11,12,13,14,16,18,19&20)

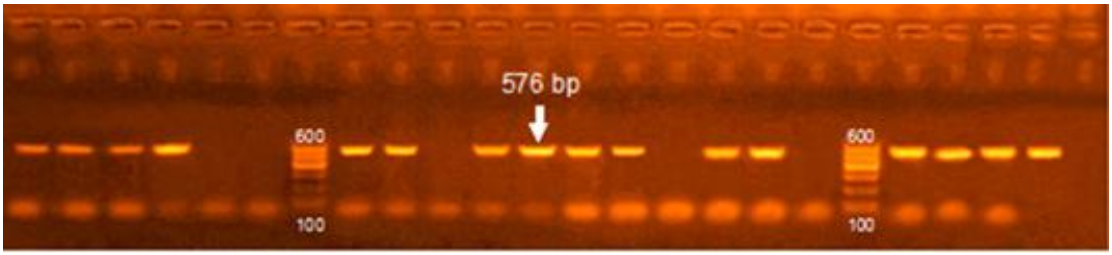

Fig (3) Show the prevalence of tetA GENE IN 15 (75%) OF ISOLATES IN ISOLATES (1,2,3,5,6,8,9,10,11,13,14,17,18,19,20)

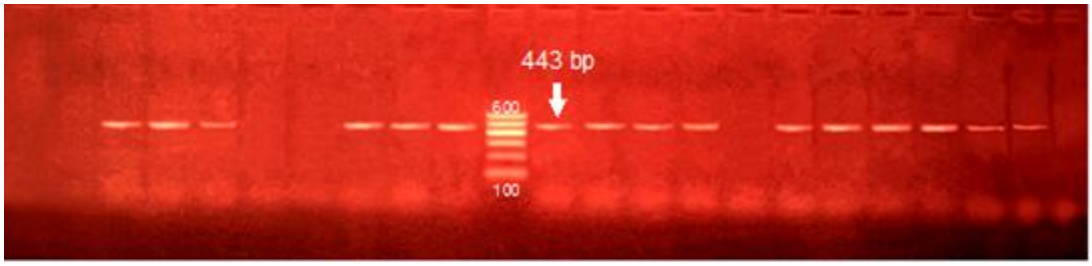

Fig (4) Show the prevalence of sull gene in 15 (75%) of isolates in isolates (1,2,3,4,5,6,8,9,10,11,12,13,16,17,18)

The highest prevalence appeared for the bla_{TEM} gene β- lactem resistance gene 16 (80%) followed by tetA (A) gene and the sul1 gene which was detected in 15 (75%) for each. And lastly aac (6′)-Ib-cr was 9 (45%).

4. Discussion

Colibacillosis is an economically important disease, which is prevalent throughout the world [16]. E.coli has been implicated in a variety of diseases in poultry such as colisepticaemia, coligranuloma, air sacculitis, peritonitis, pericarditis, omphalitis and oophoritis, accounting for about 5-50 % mortality in poultry flocks [26] and perihepatitis, cellulitis, air sacculitis, or swollen head syndrome [10,6] in this study the incidence of E.coli in broiler and broiler breeder and grandparents is higher than in layers, these results agree with [21] who stated that the percentages of resistant E.coli were significantly higher in turkeys and broilers than in the laying-hen population.

In this study the incidence of E. coli was 93.7%, Also higher incidence 81.46% was reported by [27] and an incidence of 88.2% was mentioned by [12] . On the other side, lower incidence 34.3% was reported by [28]. The variations in the prevalence rates of E. coli in cases of diarrhea and septicemia may be due to the difference in the pathogenicity, virulence of the strains, the severity of the cases and the immunological status of the host.

The species of E. coli are serologically divided in serogroups and serotypes on basis of their antigenic composition (somatic or O antigens for serogroups and flagella or H antigens for serotypes) [13] . Many strains express a third class of antigens (capsular or K antigens) [7]. More than 1000 E. coli serotypes have been reported but only small percentages have been implicated in poultry diseases [9].

In this study 62 strains can be identified serologically. They belonged to 18 different serogroups. The most commonly detected E. coli serogroups isolated from different organs of chickens were O_{158} (16.4%), O_{125} (9.5%), O_{91}(6.8%), O_{114} (6.8%), O_{119} (4.1%), O_{44} (4.1%), while11 strains were Not typed. These results go hand to hand with the previous [20]; [5,14] that reported that serogroups O_{44}, O_{158}, O_{114} and O_{91} were traditionally associated with colibacillosis in poultry.

In this study, the antibiogram was carried out against different E. coli serotypes using 7 different antibiotics. The results revealed that, about 89% of the isolates are multi-resistant as they resist at least 3 antibiotics, this resistance pattern, the so called multiple antimicrobial resistance (> or =3 antimicrobials) of E.coli recovered from poultry was reported by [8,11,24,18].

These finding agreed with that reported by [4,9] who attributed the development of drug resistance to frequent usage of drugs in veterinary practices at suboptimal concentrations or may be due to usage of antibiotics to control the infectious diseases [2].

In our study the isolates show high resistance to tetracycline by using disc diffusion about 93.8%. However, PCR test revealed lower percentage of 75 % of isolates carry the resistance gene (tetA). the results agree with [17] that studied the actual frequency of antimicrobial resistance in fecal Escherichia coli (E. coli) isolated from chicken at the phenotype level and to determine the genetic background for the two major resistance phenotypes (streptomycin and tetracycline). One hundred and nine E. coli isolates were higher resistant to ampicillin (68.8%) streptomycin (60.6%), ciprofloxacin (65.1%), and tetracycline (96.3%). Resistant gene tetA was amplified while the tetA 22 was 20.2%. Also they insured that the significant differences could be observed between isolates not only at the phenotype level but also at the genotype level.

The resistance to ciprofloxacin antibiotics was about 58.9% by using disc diffusion. On other hand the PCR test results for the aac (6′)-Ib-cr gene was (45%) for ciprofloxacin and enrofloxacin resistance (Fig1). The obtained results during this study was confident with some results achieved by [23] who showed high rates of resistance to quinolones from different parts of the world. In China, for example, more than 50% of the clinical strains of E. coli isolated during 1997-1999 were resistant to ciprofloxacin. While [22] observed that 198 avian E. coli isolates from Shandong, China were resistant to enrofloxacin 99%, ciprofloxacin100%, norfloxacin 100%, amoxicillin/clavulanic

acid87.4%, ampicillin 99.5%, gentamicin 97% and amikacin 27.8%. .

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