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Research Article

Molecular Characterization and Antimicrobial Resistance Gene of *E. coli* and *Salmonella* Kentucky Isolated from Turkeys in Egypt

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Abstract | This study aimed to determine the extent of possibility of apparently healthy turkeys being reservoirs for the pathogenic strains of *E. coli and Salmonella* kentucky. A total 150 cloacal swab samples from apparently healthy turkeys from Gharbia governorate, Egypt were investigated bacteriologically and biochemically. The overall prevalence of *E. coli* and *Salmonella* Kentucky *were 55 (36.67%) and 4 (2.7%) respectively.* The Congo red binding assay results revealed 10 pathogenic *E. coli* isolates out of *55 (18.18%).* The antibiotic sensitivity test revealed *E-coli and S.*kentucky isolates were 100% resistant to β-Lactames (ampicillin, amoxicillin/clavulanic, cefaclor and ceftazidime) while being 100% sensitive to Carbapenem (imipenem). *E-coli and S.*kentucky Isolates were 100% and 75% resistant to Phenicols (chloramphenicol), 60% and 75% resistant to Fluoroquinolone (ciprofloxacin), 50% and 50% resistant to Aminoglycoside (gentamicin) while being 10% and 25% resistant to Macrolides (azithromycin) respectively. Polymerase chain reaction (PCR) was applied on *E. coli* and *Salmonella* isolates to detect resistance gene (bla_{TEM}, bla_{OXA}, *flo*R, *aad*B *and qnr*A). All isolates were revealed to express these multi-drug resistant genes by (100%), (0%), (100%), (100%) and (0%) respectively. Our results concluded that turkeys could be a reservoir for resistant *E. coli* and *Salmonella* spp., resulting in economic and public health problems which require the development of strategies to reduce and control the development and spread of antimicrobial resistance especially in apparent health turkey flocks.

Keywords | *E. coli*, *Salmonella*, Turkey, Antibiotic, Resistance genes

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INTRODUCTION

Poultry is the most consumed type of meat worldwide. The overuse of antibiotics as therapeutics and growth promoters without precise supervision and control leads to the development of several aspects of antimicrobial resistance (Nhung et al., 2016).

Turkeys convey antimicrobial resistance to human which can be risky in consumption of retail meat. Primary monitoring finding also indicates that poultry meats contain resistant bacteria (Aslam, 2012). The developed resistant pathogens associated with diseases and the rising antibiotic resistant gene gathering in commensal bacteria is alarming. Therefore, more research is needed for understanding the prevalence and dynamics of antimicrobial resistance bacteria in poultry flocks (Chinivasagam et al., 2010). The irregular use of antimicrobials leads to selection of multi-resistant strains of *E. coli* and *salmonella* in poultry and plays important role in the transmission of antibiotic-resistant bacteria along the food chain to humans (Moawad et al., 2017; CDC, 2011). Turkey was the second highest category for foodborne outbreaks caused by meat, poultry, or their products between 1998 and 2010 in the

USA (CSPI, 2013). *E. coli* and *S.enterica* serovars kentucky are foodborne pathogens isolated from poultry and beef meat with the advent of antimicrobial resistance in Egypt (Moawed et al., 2017).

According to the National Antimicrobial Resistance Monitoring System (NARMS), *E. coli* and *Salmonella* exhibited resistance to more than 3 classes of antimicrobial among turkey. (NARMS, 2014). The use of a rapid molecular assay is considered as a useful tool for detection of antibiotic resistance in poultry production (El-adawy et al., 2012). Detection of resistance genes using PCR is highly specific and very ensitive method and less time consuming (Malkawi, 2003). This study aimed to elucidate the prevalence, serotyping, antimicrobial resistance and resistance-associated genes in *E. coli* and *S.* kentucky isolated from healthy turkey.

MATERIALS AND METHODS

Ethical approval

All samples were taken according to standard sample collection procedure without putting any stress on the bird. The current study was approved by the Ethical Committee for Medical Research at the faculty of veterinary sciences, Benha University and Animal Care Guidelines of the General Organization for Veterinary Services, Egypt.

Sample collection

A total of 150 cloacal samples were collected from living apparently healthy turkeys (40 of each at 35 days old and 110 of each at 4 months old) using sterile swabs. The samples were collected from different turkey farms located in different geographic areas of Gharbia governorate, Egypt. These samples were being transferred without delay to the laboratory in an ice box under complete aseptic condition to the laboratory for bacteriological examination.

Isolation and identification of bacteria

The detection and identification of *E. coli* and *S.* kentucky according to (Qunin et al., 2002) and ISO 6579 (2002). Sampling was carried out using sterile cotton swabs dipping in sterile 0.8% saline solution. For isolation of *E. coli*, 1 ml added to 9 ml MacConkey broth (Oxoid) and incubated at 37°C for up to 48 hours, while for *Salmonella* detection, cloacal swabs pre-enriched in buffered peptone water (Oxoid) at 37 ◦C for 18 hours. After overnight incubation, 0.1 mL of the incubated pre-enrichment was transferred to 10 mL of Rappaport-Vassilliadis enrichment broth (Oxoid) and incubated at 42°C ± 1°C for 24 hours. A loopful from Rappaport–Vassiliadis broth was streaked on xylose lysine deoxycholate (XLD) agar and Salmonella-Shigella agar (Oxoid) and from macConky broth on macConky agar plates. The pink (lactose fermenter) colonies were picked and cultured onto eosin methylene blue (EMB) agar (Oxoid, Manchester, UK). The inoculated plates were incubated aerobically at 37°C for 18–24h. Suspected colonies were stored onto semi-solid agar to be preserved at 4°C for further examination.

Vitro pathogenicity test of *E. coli* isolates.

The isolated *E. coli* were tested for the pathogenicity on Congo red (CR) dye binding assay described by (Berkhoff and Vinal, 1986). The Congo red medium (Sigma) was prepared by adding 0.03% of Congo red dye to the trypticase soya agar (TSA), the *E. coli* isolates were streaked onto the plates and plates were incubated at 37°C for 24-72 hours. Appearance of deep brick red coloured colonies after an incubation for 24, 48 and 72 hours indicated positive result, while pale or white colonies were considered as negative.

Serology

The obtained CR-positive *E. coli* isolates were serotyped using slide agglutination method using commercial antisera (SIFIN). Serological identification of *Salmonella* spp. based on somatic (O) and flagellar (H) antigens according to the Kauffmann–White typing scheme (Popoff et al., 2004). The serotyping was applied at the Serology Unit, Animal Health Research Institute, Dokki, Egypt.

Antimicrobials susceptibility testing

E. coli and *Salmonella* isolates were screened for their resistance to the following antibiotics (Oxoid): amoxicillin-clavulanic (AMC) 30µg, ampicillin 10µg (AMP), ceftzidime 30µg (CAZ), cefaclor 30µg (CEC), imipenem (IPM) 10µg, ciprofloxacin (CIP) 5 µg, gentamicin (CN) 10µg, chloramphenicol (C) 30µg and azithromycin (AZM) 15µg, according to (Koneman et al., 1997) and the degree of sensitivity was interpreted according (NCCLS, 2002, 2016).

Detection of resistant genes was determined by PCR

DNA was extracted from the isolated *E. coli* and *Salmonella* using QIAamp DNA mini kit. It was applied on 5 random isolates. PCR Master Mix and cycling conditions of the primers during PCR was prepared according to Emerald Amp GT PCR mastermix (Takara) kit. Oligonucleotide primers used in PCR have specific sequence and amplify a specific product (Table 1). DNA samples for uniplex PCR were amplified in a total of 25µl as follows: 12.5µl of Emerald Amp GT PCR mastermix, 1µl of each primer of 20 pmol concentrations, 4.5 µl of grade water and 6 µl of template DNA. The reaction was performed in a Biometra thermal cycler. Temperature and time conditions of the primers during PCR were applied. Aliquots of amplified PCR products were electrophoresed in 1.5 % agarose gel (ABgene) in 1x TBE buffer at room temperature. For gel analysis, 15 µl of PCR products were loaded in each gel slot.

Table 1: PCR primers used and amplicons size of antibiotic resistant genes.

Resistant genes	Primer Sequence (5'-3')	Amplicons size	Reference
*aad*B	F-GAGCGAAATCTGCCGCTCTGG	319 bp	Frana et al., 2001
	R-CTGTTACAACGGACTGGCCGC		
bla_{TEM}	F- ATCAGCAATAAACCAGC	516 bp	Colom et al., 2003
	R-CCCCGAAGAACGTTTTC		
bla_{OXA}	F-ATATCTCTACTGTTGCATCTCC	619 bp	
	R-AAACCCTTCAAACCATCC		
*qnr*A	F-ATTTCTCACGCCAGGATTTG	516 bp	Robicsek et al., 2006
	R-GATCGGCAAAGGTTAGGTCA		
*flo*R	F-TTTGGWCCGCTMTCRGAC	494 bp	Doublet et al., 2003
	R-SGAGAARAAGACGAAGAAG		

A 100 bp DNA ladder (QIAGEN Inc, Valencia, CA, USA) was used to determine the fragment sizes. The gel was photographed by a gel documentation system and the data was analyzed through computer software.

RESULT

Prevalence of *E. coli* and *Salmonella* in samples

Out of 150 cloacal swab samples from apparent healthy turkey 55 *E. coli* (36.67 %) and 4 *Salmonella* (2.7%) were isolated and identified using bacteriological and biochemical methods. The Congo red binding assay results were positive in 10 *E. coli* isolates out of 55 (18.18%) indicating their pathogenicity.

Serological characterization of *E. coli* and *Salmonella* isolates

Ten *E. coli* isolates were serotyped as O86a, O119, O1, O27, O111 and O125 and four *Salmonella* isolates were typed as *Salmonella* kentucky.

Determination of antimicrobial susceptibility profiles

The antibiotic sensitivity test revealed *E-coli and S.*kentucky isolates were 100% resistant to β-Lactames (ampicillin, amoxicillin/clavulanic, cefaclor and ceftazidime) while being 100% sensitive to Carbapenem (imipenem). *E. coli and S.* kentucky Isolates were 100% and 75% resistant to Phenicols (chloramphenicol), 60% and 75% resistant to Fluoroquinolone (ciprofloxacin), 50% and 50% resistant to Aminoglycoside (gentamicin) while being 10% and 25% resistant to Macrolides (azithromycin) respectively.

Molecular detection of resistance-associated genes

Searching for the antibiotic resistant genes by PCR showed 3 genes (bla_{TEM}, *aad*B and, *flo*R*)* were expressed in all isolates of *E. coli* and *S.*kentucky. while bla_{OXA} and (*qnr*A)genes could not found in all isolates.

DISCUSSION

The incidence of *E. coli* in the present study was (36.67%). This result coordinated with (Slettemeas et al., 2019) who isolated *E. coli* from turkey with the percentage (50 %). In contrast, high level of *E. coli* contamination to turkey meat was obtained by (Davis et al., 2018) at the level (90.7%).

The CR-binding assay indicate that 10 isolates of 55 (18.18%) were positive indicating their pathogenicity due to their ability of invasion. A close result of 28.6% of virulent avian *E. coli* isolates exhibited CR-binding assay positive was reported by (Amer et al., 2015). This result disagrees with an investigation reported by (Yadaw et al., 2014) who detected (92.86%) of *E. coli* isolates have Congo red binding ability. The Congo Red binding assay considered a moderately stable, reproducible, and easily distinguishable phenotypic marker. The positive congo-red *E. coli* isolates serotyped as O1(3), O125(2), O119(2), O111(1), (O86a(1) and O27(1). These isolates nearly similar to investigation by (Circella et al., 2009) who detected O1, O86 from turkey but O111 detected by (Olsen et al., 2011).

Bacterial antimicrobial resistance is a global emerging problem of public health concern. In this study, antibiotic susceptibility testing of *E. coli* isolates isolates from turkeys showed (100%) resistance to three or more antibiotics. These findings suggest that there are greater antibiotic selective pressures in turkey production. This result is nearly similar to studies conducted by (Cunha et al., 2014) and (Hoepers et al., 2018) who detected 92% and 82% of the isolates being multi-drug resistant (MDR) respectively.

All *E. coli* isolates were (100%) resistant to ampicillin, cefaclor, ceftazidime, amoxicillin-clavulanic and chloramphenicol and 60% against gentamicin followed by 50% against ciprofloxacin. a finding nearly in agreement with several previous reports obtained by (Giovarnadi et al., 2013) for ampicillin (96%); (Abdallah et al., 2013) for amoxicillin/clavulanic acid (80%) and ciprofloxacin

(40%); (Jones et al., 2013) for ciprofloxacin (41.4%) in breeding flocks and (61.4%) in fattening flocks; (Khaitsa et al., 2008) for gentamicin (48%) and (Kaesbohrer et al., 2019) for ciprofloxacin (40%)..On other hand, our results disagreed with (Cunha et al., 2014); for cefotaxime and cefoxitin (10.2% and 5.7%) respectively; (Khaitsa et al., 2008) for ampicillin (22%); (Sheikh et al., 2012) for amoxicillin/clavulanic, chloramphenicol and ciprofloxacin (10.3%, 3.8 % and 0%) respectively; (Abdallah et al., 2013) for ceftazidime (32.5%); (Soufi et al., 2009) for gentamicin and ciprofloxacin(0%,8%) respectively; (Cunha et al., 2014) for gentamicin (19.5%); and (Slettemeas et al., 2019) for gentamicin and chloramphenicol (15.4%) and (10.3%) respectively.

An important aspect of this study was to analyze resistance genes in *E. coli* isolates. In this study, the β-lactamase encoding gene bla_{TEM} conferring resistance to penicillins was detected in 100% of *E. coli* isolates but bla_{OXA} not detected. These results are close to that reported by (Randall et al., 2010) who detected bla_{TEM} gene of 60.9% rate and conflicts with the result of bla_{OXA} gene (52.1%). On other hand, bla_{TEM} gene was detected by (Sheikh et al., 2012) with low percentage (16.7%) while (Kaesbohrer et al., 2019) failed to detect bla_{TEM} in turkey. This variability in the presence of resistant genes between different localities could be attributed to the previous time of exposure to different types of antibiotics. This also can be attributed to antibiotics regimes implemented in these different locality that cause less or more extensive development of the resistant genes. In our study, the high sensitivity (100%) of *E. coli* and *salmonella* isolates to imipenem could be related to the absence of the bla_{OXA} gene in all isolates. Although only 60% of the isolates were resistant to ciprofloxacin, the *qnrA* gene could not be detected in any of the isolates. This may be explained by the presence of other resistant genes responsible for that part of resistance.This results is in accordance with that obtained by (Randall et al., 2010) and (Gosling et al., 2012). In this study, *flo*R gene associated with chloramphenicol resistance was detected in all isolates. This result was in agreement with the result reported by (Tadeese et al., 2018) who detected *flo*R with a percentage of 66.67%. Gene associated with aminoglycoside resistance (*aad*B gene) were identified in all gentamicin resistant *E. coli*. In contrast, *aad*B gene could not be identified by (Sheikh et al., 2012).

Salmonella infections considered as great danger to human and animal health. In the present study *Salmonella* spp. were isolated from turkeys with a percentage of 2.7 %. this result is little far from results obtained by (Rahimi, 2012), (Yeh et al., 2017) and (Osman et al., 2010) who isolated *Salmonella* 6.7%, 11.9% and 12.6% from turkey respectively. Our results are lower than that obtained by (Fakhr et al., 2006) who detected *Salmonella* in a rate of (40.5%). In the current study, the serotyping of the isolated *Salmonella* revealed all isolates being *Salmonella* Kentucky in turkeys. These results coincide with the finding of (Santos et al., 2007) who detected *S*. Kentucky in most of the isolates.

Among antibiogram, the result showed that all *Salmonella* isolates were resistant to ampicillin, cefaclor, ceftazidime, amoxicillin-clavulanic in 100%. The resistance to chloramphenicol and ciprofloxacin was 75%, in agreement with the finding of (Yeh et al., 2017) who reported 75.7% and 69.1% resistance against ampicillin and chloramphenicol respectively. This disagree with the finding by Beutlich et al. (2010) who reported low resistance against chloramphenicol; ciprofloxacin and amoxicillin/clavulanic acid to be 9%, 29% and 24% respectively. A high resistance against gentamicin (50%) reported in this study is in contrast to the finding of (Gad et al., 2018) and (Rahimi, 2012) who recorded maximum resistance gentamicin (100%). On the other hand, all *Salmonella* isolates in this study were sensitive to imipenem 100% followed by 75% to azithromycin in line with the finding of (Nisar et al., 2017) who reported also maximum sensitivity against azithromycin.

Moreover, in this study, all *Salmonella* isolates expressed bla_{TEM}, *aad*B and *flo*R (100%) while none of them expressed bla_{OXA}, *qnr*A. This result is in agreement with (Beutlich et al., 2010) who detected bla_{TEM}, *aad*B and bla_{OXA} by 100%; 98% and 0% respectively; (Yeh et al., 2017) who detected *flo*R with a percent (63.8%) and disagreed with (Yeh et al., 2017) who detected bla_{TEM} with a percentage of 42%.

CONCLUSION

In conclusion, the presence of MDR *Salmonella* kentucky and *E. coli* in apparent healthy turkey is alarming for the health concern. This mean that healthy turkey farms could be potential spots for development of MDR genes. These farms have the probability of playing a role in the dissemination of antimicrobial resistance among bacterial populations. Thus, considerable efforts need to be taken to precisely control antimicrobial resistance development in turkey and hence guard against human health concerns.

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AUTHORS CONTRIBUTION

Ashraf A. Abd El Tawab: shared in the study design,development of methodology and manuscript revision. Amira

Mohamed Rizk shared in collection of data, data analysis and interpretation in addition to writing and revision of the manuscript. Seham N. homouda shared in the study design, development of methodology. Emad E. El Mougy shared in the study design, and collection of samples. Alaa M. Gouda shared in samples collection, methodology and writing of the manuscript.

Conflict of interest

The authors declare that they have no conflict of interests. The authors declared that they did not have any funding source to support their study.

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