Detection of Some resistance genes of Salmonella enterica subsp. Salamae and Salmonella enterica serotype Kentucky isolated from Turkey

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ABSTRACT

The aim of this study was to determine the serotyping and antimicrobial resistance of isolated Salmonella from the apparently healthy turkey. A total 150 of cloacal samples from apparently healthy turkey were screened bacteriologically for the occurrence of Salmonella. A total of 4% (6/150) of the Salmonella isolates were recovered. Serotyping revealed two different serotypes; Salmonella enterica subsp. Salamae (33.33%) and Salmonella enterica serotype Kentucky (66.67%). The isolated Salmonella were highly resistant to ampicillin, cefaclor, cefotaxime, cefazidime, amoxicillin/clavulanic acid (100%) followed by chloramphenicol and ciprofloxacin (83.3%) then gentamicin (66.67%) and azithromycin (33.3%). All isolates showed a high sensitivity for imipenem. All strains are multidrug-resistance (MDR). Polymerase chain reaction (PCR) was applied to Salmonella isolates to detect resistance genes. Antibacterial resistance genes blaTEM, blaOXA, floR, aadA and qnrA were detected in (100%), (9%), (100%), (100%) and (0%) of tested Salmonella respectively. A combination of serotype and phenotypic markers can be useful in studying genetic variation among Salmonella populations in turkey farms and delineating possible transmission pathways. In conclusion, apparently healthy turkeys could be a reservoir for Salmonella resistant to multiple antimicrobials and poses a serious public health threat.

1. INTRODUCTION

Antimicrobial resistance (AMR) is a global health threat, and as well as antimicrobial usage. AMR in animal production is one of its contributing sources. Poultry is one of the most widespread types of meat consumed worldwide (Nhung et al., 2017). Salmonella spp. and Escherichia coli are the two most important food-borne pathogens of public health impact transmitted to poultry meat worldwide (Adeyanju and Ishola 2014). The emergence and spread of resistant bacteria strain like Escherichia coli, salmonella from poultry products to consumers set humans at risk to new strains of bacteria that resist antibiotic treatment. Resistant bacteria inhibit antimicrobials by different mechanisms, as a synthesis of inactivating enzymes, alteration in configuration of the cell wall or ribosome and modification of membrane carrier systems (Apata et al., 2009). The development of antibiotic resistance is usually associated with genetic changes encoded by chromosomal and plasmid genes (Bennet et al., 2008).

Salmonella infection caused by a variety of Salmonella species and it is one of the most important bacterial diseases in poultry causing heavy economic losses through high mortality and decrease production (Haidar et al., 2004). Salmonella isolates from turkeys associated with high levels of antimicrobial resistance. Some studies indicating that, resistance is more frequent in Salmonella isolates from turkeys than in other livestock species. Therefore, Salmonella in turkeys and turkey meat have an impact of great public health significance (Poppe et al., 2005; Zhao et al., 2007). Salmonella spp. acquire antibiotic resistance by random chromosomal mutations, mutation of existing genes, and through mobile genetic elements, such as plasmids, transposons, and gene cassettes in integrons, which facilitates the acquisition and dissemination of resistance genes. The association of these integrons with plasmids that confer the extended-spectrum β-lactamase phenotype is an example (Fluit and Shmitz, 1999).

The present study was conducted to investigate the prevalence of Salmonella from apparently healthy turkey, the serotypes involved, the antimicrobial susceptibility patterns of Salmonella isolates and the detection of some resistance genes by PCR.

2. MATERIAL AND METHODS

2.1. Sample collection

A total of 150 cloacal samples collected from living apparently healthy turkeys (40 at 35 days old, 110 at 4 months old) from different farm in Gharbia Governorate using sterile swabs. Samples were collected under aseptic
condition as possible to prevent cross contamination in icebox and were then transferred to the laboratory.

2.2. Bacterial isolation and identification of Salmonella
The isolation method was done according to ISO method (ISO, 2007). This method was based on the pre-enrichment method in buffered peptone water at 37 °C for 18 hours. After overnight incubation, 0.1 ml of the incubated pre-enrichment was transferred to 10 ml of Rappaport-Vassiliadis enrichment broth (Oxoid) and incubated at 42°C for 24 hours. After incubation, one loop of each selective enrichment broth was streaked onto xylose-lysine-deoxycholate agar (XLD) (Oxoid) and Salmonella-Shigella enrichment was transferred to 10 ml of Rappaport-Vassiliadis enrichment broth (Oxoid) and incubated at 42°C for 24 hours. After incubation, colonies were observed. The colony with a black center in XLD and blackish growth in SS agar were considered as presumptive Salmonella positive. The suspected colonies were picked up and kept in semi-solid agar for morphological, biochemical, and serological identification.

2.3. Identification of Bacteria
Suspected colonies were identified using standard microbiological identification techniques including motility test, indole, triple sugar iron test, H2S production test, citrate utilization test, voges-proskauer test, Hydrolysis of urea and Methyl-red test (Cheesbrough, 2000).

2.4. Serological typing of Salmonellae
The isolates that were identified biochemically as Salmonella were subjected to serological identification 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.

2.5. Antimicrobial Susceptibility Testing

| Antibiotic | Target resistance gene | Primer Sequence (5’→3’) | Amplicons size | Reference |
|------------|-------------------------|--------------------------|----------------|-----------|
| CN         | aadB                    | F-GAGGCAATCTGCAGCCTCTGG  | 319 bp         | Frutschi et al., (2001) |
|            |                         | R-CTGTGACAAAGACTGGCGCG   |                |           |
| AMP        | bladt                 | F-ATGCGAATAAAAACAGC      | 516 bp         | Coloma et al., (2003) |
|            |                        | R-CCTGAGAGAGGTTTTT      |                |           |
| AMP        | blattox               | F-ATATCTCTACTTCTGATCTCC  | 619 bp         | Robicek et al., (2006) |
|            |                        | R-AAACCCCTCAAAACCCATCC  |                |           |
| CIP        | qnrA                   | F-ATTTCCTACCGAGATGTTG   | 516 bp         | Dobleti et al., (2003) |
|            |                        | R-GATCGCAAAAGGTAGTTCGA  |                |           |
|            | blol                  | F-CTGCGGWCGCTMTCRGA     | 494 bp         | Doublet et al., (2003) |
|            |                        | R-SGAGAAAGAAGGAGAGAAG   |                |           |

PCR: Polymerase chain reaction, AMP=Ampicillin, CN=Gentamicin, CIP=Ciprofloxacin, C=Chloramphenicol

3. RESULTS

3.1. Salmonella isolation, identification and serogrouping.
From 150 cloacal samples, 6/150 (4%) Salmonella isolates were isolated. Four isolates belonged to the Salmonella enterica serotype Kentucky (66.67%) and two isolates to Salmonella enterica subsp. salamae (33.33%).

3.2. Antimicrobial susceptibility of the tested isolates:
Results of antibiotic sensitivity test showed that 100% of tested salmonella isolates exhibited resistance against ampicillin, cefaclor, ceftazidime, amoxicillin-clavulanic acid; 83.3 % for chloramphenicol and ciprofloxacin; 66.67% against gentamicin and 33.33 % against azithromycin. No resistance against imipenem detected.

3.3. Incidence of Antimicrobial Resistance Genes
The β-lactam resistance genes included bladt was detected (6/6) but Blattox was not detected in this study. Chloramphenicol resistance genes (floR) and gentamicin resistant gene (aadB) detected in all isolates of salmonella. Resistance gene of ciprofloxacin(qnrA) was failed for detection as shown in (Figure 1-3). Phenotypic resistance and resistance determinants found in Salmonella isolates were illustrated in table (2).
The incidence of Salmonella in the present study was (4%).

5. CONCLUSION

The current study revealed that the incidence of multidrug resistant Salmonella spp. in the cloacal swab samples of apparently healthy turkey flock could be a threat to public health. The results reinforce the need to develop monitoring strategies and to perform specific control procedure to reduce the use of antibiotics and consequently the development of antimicrobial resistance by misuse/over of antibiotic agents.
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