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

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#### Keywords

- Resistance genes
- Salmonella
- Serotype
- Turkey

#### ARTICLE INFO

**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, ceftazidime, 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 *bla*TEM*, *bla*OXA*, *floR*, *aad*6, *qnr*A and *erm*A were detected in (100%), (9%), (100%), (100%) and (0%) of tested Salmonella respectively. A combination of genomic and phenotypic markers can be useful in studying genetic variation among Salmonella populations in turkey farms and determining 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 in 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 b-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 agar (SS); (Oxoid) at 37 °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, voge–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

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Target resistance gene</th>
<th>Primer Sequence (f’-r’)</th>
<th>Amplicons size</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CN</td>
<td>aadB</td>
<td>F-GAGGCAATTCGCGCTTGG</td>
<td>319 bp</td>
<td>Franze et al., (2001)</td>
</tr>
<tr>
<td>AMP</td>
<td>blaTEM</td>
<td>F- ATCACGAATTAAACACGG</td>
<td>516 bp</td>
<td>Colloms et al., (2003)</td>
</tr>
<tr>
<td>AMP</td>
<td>blaOXA</td>
<td>F-ATATCTTCATGTGTCATG</td>
<td>619 bp</td>
<td>Robicsek et al., (2006)</td>
</tr>
</tbody>
</table>

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 blaTEM was detected (6/6) but blaOXA 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%).

These results very close to the results were obtained by Yeh et al. (2017) who isolated 11.9% from a turkey farm. Conversely, this result is lower than that obtained by Fakhr et al. (2006), who detected salmonella by (40.5%). Salmonella isolates were serotyped using poly and monoclonal “O” and “H” antisera and the result of this study revealed that 2 different serogroups were identified as Salmonella enterica subsp. Salamae (33.33%) and Salmonella enterica serotype Kentucky (66.67%) from turkeys. These results coincide with El Allaoui et al., (2017), who detected Salmonella enterica serotype Kentucky as the most prevalent serotype; Santos et al., (2007), who reported that Salmonella enterica serotype Kentucky was the most prevalent serotype.

Multidrug-resistant (MDR) due to Salmonella is known as a major public health problem around the world and there is increased use of antibiotics in human and animal settings (Hsu et al., 2013).

In the present study all isolated strains were resistant to at least four or more of the used antibiotics Among antibiogram, all isolated salmonella were resistant to ampicillin, cefaclor, ceftazidime, amoxicillin-clavulanic with 100% followed by chloramphenicol and ciprofloxacin with 83.3% then gentamicin with 66.67% and azithromycin with 33.33%. Meanwhile, 100% of tested Salmonella isolate showed sensitivity against imipenem. Similar results were obtained by Beutlich et al. (2010) for ampicillin (82%) and gentamicin (78%); Yeh et al. (2017) for chloramphenicol (69.1%); Gad et al. (2018) for amoxicillin-clavulanic (96%) and cephalothin (81%). Conversely, these results disagreed with Yeh et al. (2017) for ciprofloxacin (0.8%) with Santos et al. (2007) for ampicillin, Fakhr et al. (2006) for gentamicin and Nisar et al. (2017) for ciprofloxacin and azithromycin(0%) for each. The expanded use of antibiotics as supplements for growth promotion and prophylaxis and has advanced the selection of antimicrobial-resistant Salmonella strains at the farm during poultry production. Since salmonellosis is primarily transmitted through food, especially food of animal origin, the presence of antimicrobial resistant Salmonella in raw meat products has important public health hazard especially in developing countries, where there is widespread and uncontrolled use of antibiotics (Hart et al., 1998).

PCR has emerged as a highly sensitive and specific method for identifying pathogens (Lim et al., 2004). In this study, none of the examined samples harbored blaCTX, qnrA while blaTEM, aadB and floR detected in all isolates. This result agreed with Beutlich et al., (2010), who detected blaTEM, aadB and blaCTX by 100%, 98% and 0% respectively. Similar results were conducted by Yeh et al., (2017), who detected floR gene and blaTEM with 63.8% and 42% respectively.

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 subsequently the development of antimicrobial resistance by misuse /over of antibiotic agents.

Table 2 Phenotypic resistance and resistance determinants found in Salmonella isolates in this study

<table>
<thead>
<tr>
<th>Salmonella isolates</th>
<th>Sample no.</th>
<th>Resistance phenotype</th>
<th>Resistance genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Salmonella</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
<tr>
<td>2 enterica serotype Kentucky</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
<tr>
<td>3 Salmonella</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
<tr>
<td>4 enterica subsp. Salamae</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
<tr>
<td>5 S. Enterica subsp. Salamae</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
<tr>
<td>6 Salmonella</td>
<td>AMP, CTZ, CEC, AMC, CIP, C, CN, AZM</td>
<td>blastr, floR, aadB</td>
<td></td>
</tr>
</tbody>
</table>

AMC-amoxicillin-clavulanic acid, AMP ampicillin, AZM Azithromycin, CEC Cefaclor C12, ceftazidime, CRO chloramphenicol, CIP Ciprofloxacin, CN Gentamicin

4. DISCUSSION

The incidence of Salmonella in the present study was (4%).
6. REFERENCES


