Genetic Analysis of Antimicrobial Resistance Genes in *Salmonella* Isolated from Diseased Broilers in Egypt

Mona F. Shousha¹*, Aml M. Ragab² and Salwa M. Helmy³

¹Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafir El-Sheikh University, Kafir El-Sheikh 33516, Egypt
²Unit of Bacteriology, Animal Health Research Institute, Tanta Branch, Agriculture Research Center (ARC), Egypt

**ABSTRACT**

*Salmonella* spp. are known to be a major cause of foodborne infection; it primarily spreads from poultry to humans, significantly burdening public health, especially with the currently high rates of antimicrobial resistance and the emerging multidrug-resistant strains. As a result, this study determined the patterns of antibiotic resistance in *Salmonella* spp., which was isolated from sick broilers from different farms in Egypt. Then, we investigated the presence of extended-spectrum beta-lactamases and plasmid-mediated quinolone resistance genes in *Salmonella* isolates. First, 800 internal organs (heart, liver, intestine, and yolk sac) were collected from 200 infected broilers to genetically analyze their recovered *Salmonella* antimicrobial resistant genes. Ten isolates of *Salmonella* were recovered: two (20%) for each *S. enterica* serovar Grampian, *S. enterica* serovar Kentucky, and *S. enterica* serovar Blegdam and then one (10%) for each *S. enterica* serovar Hadar, *S. enterica* serovar Anatum, *S. enterica* serovar Kirkee, and *S. enterica* serovar Tranoroe in the serotypes of isolated biochemically identified *Salmonella*. As per the results of this study, *Salmonella* isolates demonstrated multidrug-resistant phenotypes, with the highest resistance being against ampicillin, cefotixin, cefpodoxime, and oxacillin (100%) and then against cefotaxime (80%), ceftazidime (70%), ciprofloxacin, ceftriaxone, and nalidixic acid (60%), including amoxicillin-clavulanic acid (50%). Furthermore, antimicrobial resistance genes, such as ESBL (*bla*₁<sub>TEM</sub>, *bla*₂<sub>TEM</sub>, and *bla*<sub>SHV</sub>), and quinolone resistance genes (*qnrA*, *qnrB*, and *qnrS*), and positive for the *bla*<sub>CMY-2</sub> were examined in these isolates. Results showed that although all isolates tested were found negative for *qnrA* and *qnrB* and positive for the *qnrS*, they were positive for the ESBL genes *bla*₁<sub>TEM</sub> and *bla*₂<sub>TEM</sub> and negative for *bla*<sub>SHV</sub>. In conclusion, the multidrug-resistant bacteria, *Salmonella*, demonstrated a high incidence in the diseased broiler chickens, with a possibility of human infection and treatment failure. Therefore, it is highly recommended that developing countries drastically reduce the overuse of antibiotics in poultry.

**INTRODUCTION**

Consumption of tainted food poses the risk of various foodborne diseases with the possibility of outbreaks, making food safety a global public health issue. The yearly cases of food poisoning are around 600 million (approximately 1 in 10 people worldwide) with 420,000 cases ending with death losing 33 million disability adjusted life according to a recent report from the WHO (WHO, 2020). Poultry and its products are major prevalent sources of non-typhoidal *Salmonella* infections in human (Eguale, 2018). *Salmonella* is one of the most prevalent bacteria that cause gastrointestinal illnesses in livestock and poultry. *Salmonella* infections are highly linked to the consumption of tainted poultry products (Cogan and Humphrey, 2003). Controlling *Salmonella* in poultry, on the other hand, is difficult; for broiler chickens, this has historically relied on a balance of farm biosecurity and antibiotic usage (Davies, 2005). Since the early 1960s, *Salmonella* isolates with clinically relevant antibiotic resistance have been documented as majority of the resistance was restricted to a single antibiotic (Bulling et al., 1973; Cherubin, 1981; Van leeuwen et al., 1979). However, since the mid-1970s, *Salmonella* isolates with MDR characteristics have been on the rise all across the world. Antimicrobial-resistant *Salmonella* has been found in foods of animal sources,
raising worries that treatment of human salmonellosis may be jeopardized because strains with antimicrobial resistance tend to be more frequently linked with severe illness than susceptible isolates (Helms et al., 2002; Varma et al., 2005). As antibiotic-resistant bacteria proliferate, curiosity in the genetics and resistance mechanisms that bacteria have developed to fend off antimicrobial drugs has increased (Ahmed and Shimamoto, 2012). Antibiotic misuse, abuse, and overuse have resulted in inefficiency and exacerbated the seriousness of this zoonotic disease (Cruchaga et al., 2001). The resistance to antimicrobial medications has risen over the past years creating a significant concern and challenge for public health professionals worldwide. However, the condition is much more severe in developing countries because strategies to prevent antimicrobial resistance are only of minor concern (Da Costa et al., 2013). Hence, such a high incidence of antimicrobial resistance in Salmonella spp. necessitates the determination of a resistance dissemination route, horizontally or vertically, in the evolution of MDR strains (Nemati and Ahmadi, 2020). However, as we gain a better understanding of the genome’s molecular fluidity, any attempt to combat bacteria results in more bacterial adaptation or evolution to occur in the new free ecological niche (Velge et al., 2005). Resistance molecular basis in Salmonella isolates from livestock and poultry worldwide have been identified in several investigations (Ahmed et al., 2009; Zhao et al., 2007). In different Salmonella serovars, studies have reported that the rapid improvement in resistance to extended-spectrum cephalosporin was related to the plasmid-mediated manufacturing of β-lactamase-producing bacteria (EFSA, 2008, 2009; Authority, 2018). TEM genes (blaTEM) and SHV genes (blaSHV) are the main genes involved for ESBL production (Habeeb et al., 2013). This ongoing evolution poses a serious threat to public health by causing bacterial infections treatment limitation (Sharma et al., 2013; WHO, 2013). Quinolone resistance genes mediated by plasmids have recently been discovered in several Enterobacteriaceae, and their incidence is increasing worldwide (Poirel et al., 2012). Although the PMQR genes expression only provides a limited amount of quinolone resistance, it can enable the additional chromosomal resistance mechanisms selection, resulting in the emergence of highly resistant quinolone-resistant bacteria (Strahilevitz et al., 2009; Tamang et al., 2011). Of particular concern is the recent plasmid-mediated quinolone resistance development in various parts of the world, which is encoded by a large number of qnr genes. Furthermore, both clinical and food isolates of Salmonella have recently sharply increased ciprofloxacin resistance (Lin et al., 2015). The relevant gene, qnr, was shown to be unique from other quinolone resistance genes previously identified (Tran et al., 2002). Therefore, in this study, we investigated how widespread the resistance genes for broad-spectrum beta-lactamase and quinolone antibiotics are in Salmonella isolates from diseased broilers.

**MATERIALS AND METHODS**

**Sampling**

A total of 800 internal organs (heart, liver, intestine, and yolk sac) were collected from 200 diseased broiler chickens from various farms in Egypt in a poultry lab. The broilers have clinical signs of salmonellosis as pasty vent, whitish diarrhea, roughed feather and poor general condition and their postmortem examination revealed bronze discoloration and enlargement of liver with necrotic foci and pericarditis with enlarged heart, peritonitis, perihepatitis, intestinal and caecal inflammation and unabsorbed yolk sac in young chicks. Sterile plastic bags were used to preserve the samples which were then transported in an icebox directly to the Animal Health Research Institute, Tanta branch.

**Isolation and identification of Salmonella (ISO 6579-1: 2017)**

The organs’ surface was scorched by hot spatula, then a sterilized loop was inserted through scorched part of the organ. All samples (liver, heart and yolk sac) were obtained aseptically and enriched in buffered peptone water for non-selective enrichment. Pre-enrichment is essential to allow the detection of low number of Salmonella or injured Salmonella. At room temperature, 10 ml of buffered peptone water were inoculated with 1 gm of the tested material using a 1/10 dilution (weight to volume). Then incubated at 37°C for 18 h. After that, all samples (Intestine, liver, heart and yolk sac) were inoculated into tubes containing Selenite F broth for inhibition of coliforms and certain other microbial species and thus, was beneficial in the restoration of Salmonella species. A tube containing 10 ml of selenite F broth and 1 cm of the pre-enrichment culture were combined, and they were incubated at 37 °C for 18 h. A 10 µl loop-full of selenite F broth was spread on the surface of xylose lysine deoxycholate (XLD) agar and incubated for 24 h at 37°C. By inoculating into triple sugar iron agar slopes, Salmonella-typical morphology in the form of doubtful colonies was verified biochemically. For upcoming research, the probable colonies were collected and preserved on semisolid agar.

Various biochemical tests such as oxidase reaction, urea hydrolysis test, triple sugar iron agar, indole reaction, methyl red test, reaction of Voges Proskauer, citrate utilization test, lysine decarboxylation test identification of Salmonella according to Quinn et al. (2002).
Serological typing of Salmonella

Using particular O and H agglutinating antisera, standard Salmonella isolates were further serotyped (USA, Difco, NJ, Franklin Lakes) in accordance with the Kauffmann White serotyping scheme (Grimont and Weill, 2007). Specifically, bacterial motility was detected following a previous study (Cruickshank et al., 1975). Then, Gram staining was used to microscopically identify suspected colonies under an oil immersion lens to observe the Gram-negative bacilli morphological traits (rod-shaped).

Antimicrobial susceptibility tests

Mueller-Hinton agar medium (Oxoid) is used according to the Clinical Laboratory Standard Institute (CLSI, 2011). According to the manufacturer’s instructions, the Mueller-Hinton agar was produced. Salmonella isolates were tested in vitro for quinolone resistance and extended-spectrum beta-lactamase. The following list of antibiotics in use: ampicillin (AMP), 30 µg; amoxicillin-clavulanic acid (AMC), 20/10 µg; cefotaxime (CTX), 30 µg; cefoxitin (FOX), 30 µg; cefpodoxime (CPD), 10 µg; ceftriaxone (CRO), 30 µg; ceftazidime (CAZ), 30 µg; ciprofloxacin (CIP), 5 µg; oxacillin (OXA), 30 µg and nalidixic acid (NAL), 30µg.

PCR screening for antimicrobial resistance genes in Salmonella

In our study, for DNA extraction from samples, we used the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with definite changes depending on the manufacturer’s instructions. Part of the sample suspension (200 µl) was treated with 10 µl of proteinase K and 200 µl of lysis buffer for 10 min at 56°C. 200 µl of 100 percent ethanol was then added to the lysate following incubation to be followed by sample washing and incubation based on the manufacturer’s instructions. Using a kit and 100 µl of elution buffer, the nucleic acid was eluted. This is an oligonucleotide primer. Metabion (Germany) contributed the primers, which are shown in (Table I). qnrA, qnrB, qnrS, blaTEM, blaSHV, and blaCMY-2 genes PCR amplification: To test the primers a 25 µl reaction that includes 12.5 µl of Emerald Amp Max PCR Master Mix (Takara, Japan), 5.5 µl of water, 1 µl of each forward and reverse primers at 20 pmol concentration, and 5 µl of DNA template. 2720 thermal cyclers were applied to proceed the reaction. 5V/cm gradients in 1x TBE buffer were used to separate the PCR products electrophoretically at room temperature on a 1.5% agarose gel (Applichem, Germany, GmbH). Each gel slot received 15 µl of the goods for analysis. For determining the fragment sizes, a gene ruler 100 bp ladder (Fermentas, Germany) was used. For gel photography, a gel documentation system (Alpha Innotech, Biometra) was used. Computer software was used to evaluate the data. Time conditions and temperature of the two primers during PCR are presented. S. enteritidis was used as positive control, while DEPC-treated pure water was used as negative control.

RESULTS

For all genes the 35 thermal cycles comprised each of primary denaturation at 94°C for 5 min, secondary denaturation at 94°C for 30 s, annealing at different temperatures (57°C for qnr A, 53°C for qnr B, 48°C for qnr S, 54°C for bla TEM and bla SHV, and 55°C for bla CMY-2) for 45 s, Extension at 72°C for 45 s and final extension at 72°C for 10 min.

Table I. Oligonucleotide primer sequences for detecting resistant Salmonella genes.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Nucleotide sequence 5’→3’</th>
<th>Amplified product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnrA</td>
<td>GATAAAGTTTTTTCAGCAAGAGG ATCCGATCGGCAAAAGGTGA</td>
<td>543 bp</td>
<td>Cambau et al., 2006</td>
</tr>
<tr>
<td>qnrB</td>
<td>ATGACGCCATTACCTTATAAAC ATCGCAATGTTGAAAGTTT</td>
<td>562 bp</td>
<td>Azeez et al., 2018</td>
</tr>
<tr>
<td>qnrS</td>
<td>ATGGAAACCTACTGATACAA ATCCGCAATGTTGAAAGTTT</td>
<td>491 bp</td>
<td>Le Thi Minh Vien et al., 2009</td>
</tr>
<tr>
<td>blaTEM</td>
<td>ATCGCAATAAACCAGC ATCTCGACTACTAAGT</td>
<td>516 bp</td>
<td>Colom et al., 2003</td>
</tr>
<tr>
<td>blaSHV</td>
<td>AGGATTGACCTGCTTTTGA ATTACGCTAGCTTG</td>
<td>392 bp</td>
<td></td>
</tr>
<tr>
<td>CIT (blaCMY-2)</td>
<td>TGGCCAAGAATCCTGACCGCTTCTG</td>
<td>462 bp</td>
<td>Pérez-Pérez and Hanson, 2002</td>
</tr>
</tbody>
</table>
Prevalence of Salmonella

All suspected colonies (pink with black centers) were identified on the XLD media, including a typical colony on the Salmonella–Shigella agar (colorless with or without black center). Specifically, Gram-negative nonspore-forming rods were observed on Gram-stained colonies. Then, motility test revealed that the Salmonella isolates were extremely motile. Furthermore, biochemical analysis revealed that while all isolates were nonlactose fermenting with a negative oxidase reaction, most isolates produced hydrogen sulfide and were positive for methyl red and citrate and negative for Voges–Proskauer, indole, and urease hydrolysis tests. Nevertheless, the total percentage of Salmonella species identified by biochemical tests was 10%, resulting in 80/800 Salmonella isolates from the investigated organs (24/200 isolates from the liver, 32/200 isolates from the yolk sac, 8/200 isolates from the heart, and 16/200 isolates from the intestine) (Table II).

Table II. Prevalence of Salmonella isolated from diseased broiler chickens.

<table>
<thead>
<tr>
<th>Examined organs in 200 broiler chickens</th>
<th>Positive Salmonella</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>Liver</td>
<td>24</td>
</tr>
<tr>
<td>Intestine</td>
<td>16</td>
</tr>
<tr>
<td>Heart</td>
<td>8</td>
</tr>
<tr>
<td>Yolk sac</td>
<td>32</td>
</tr>
<tr>
<td>Total (800)</td>
<td>80</td>
</tr>
</tbody>
</table>

Note: The (%) rate of each number is obtained by dividing the number by the total number of samples.

Serotyping of isolated Salmonella

The isolates were two for each Salmonella enterica serovar Grampian, Salmonella enterica serovar Kentucky, and Salmonella enterica serovar Blegdam and then one for each Salmonella enterica serovar Hadar, Salmonella enterica serovar Anatum, Salmonella enterica serovar Kirkke, and Salmonella enterica serovar Tranoroa.

Antimicrobial susceptibilities of different Salmonella isolate serotypes

Ten isolated Salmonella serovars were tested for their resistance to ESBL and quinolone. Results showed that 100% of the isolates were resistant to ampicillin, cefpodoxime, cefoxitin, and oxacillin, while 80% were found to be resistant to cefotaxime; 70% to ceftazidime; 60% to ciprofloxacin, cephraxone, and nalidixic acid; and 50% to amoxicillin–clavulanic acid.

Incidence of PMQR and β-lactamase-encoding genes in Salmonella isolated from diseased broilers

Plasmids are known to mediate quinolone resistance genes. At 543 and 562 bp, while all isolates tested negative for qnrA and qnrB, respectively, they all tested positive for qnrS (at 491 bp). Meanwhile, although all isolates tested positive for ESBL, blaTEM (516 bp) and blaqvi (392 bp) genes, they were negative for blacMY (462 bp) (Supplementary Fig. 1).

DISCUSSION

Salmonella is known to be a major zoonotic pathogen, with poultry serving as one of its primary hosts. Therefore, infections with Salmonella are a significant hazard to the poultry farming sector in developing countries (Li et al., 2018). In this study, we demonstrated that the yolk sac had the highest rate of Salmonella isolates (16%), followed by the liver (12%). However, this rate differed from that previously reported (El-Mohsen et al., 2022), which observed that Salmonella was more prevalent in the liver by 13.3% than in the yolk sac by 9.3%. Also Menghistu et al. (2011) found the prevalence of Salmonella was 2.7% (7/260) from 220 poultry tissue samples and 40 egg samples and the highest number of Salmonella isolates came from liver and intestine. The findings of our study also differ from yet another study by Eguale (2018), which observed a Salmonella prevalence rate of 4.7%. Finally, 14% of the understudied samples were Salmonella positive in the study by El-Tawab et al. (2019). Alternatively, results of serotyping matched those by Rady et al. (2020). They reported S. kentucky as the most common serotype of the Salmonella isolates and with Zhang et al. (2018) who found S. Kentucky as one of the most dominant serotypes in chicken samples by (12.6%). Whereas Ammar et al. (2016) disagreed with these findings because their study isolated Salmonella enterica serovar Kentucky in 12.5% of Salmonella isolates, alongside other serotypes Salmonella enterica serovar Enteritidis (56.25%) and Salmonella enterica serovar Typhimurium (18.75%). Additionally, Salmonella isolates in our study showed different antimicrobial resistance results, similar to a previous study by El-Tawab et al. (2019). While they detected that 89% of Salmonella species were cefotaxime-resistant, Rady et al. (2020) detected that many isolates were resistant to both ampicillin (90%) and nalidixic acid (88%). Nevertheless, nalidixic acid and ampicillin had the highest antibiotic resistance against Salmonella isolates within the chicken production chain, whereas ciprofloxacin was linked to low resistance levels (Castro-Vargas et al., 2020). This could partially agree with Yildirim et al. (2011) who found that all isolates of Salmonella spp., exhibited resistance to ampicillin, oxacillin and cefotaxime were evident 97%,
85.2% and 2.9%, respectively. Also Waghamare et al. (2018) mentioned that *Salmonella* isolates were resistant to ampicillin, ciprofloxacin and cefotaxime by 21.43%, 19.05% and 14.19%, respectively. While Singh et al. (2013) reported that all *Salmonella* isolates were sensitive to ampicillin. Our study finding the resistance to amoxicillin-clavulanic acid by 50% and this higher than Khan et al. (2021) who found it by 2.4%. Our results for antimicrobial resistance were different from Yang et al. (2013) who found the resistance to ampicillin by 45.6%, nalidixic acid by 75.8%, ciprofloxacin by 12.1%, ceftriaxone by 6.0% and cefoxitin 4.0%. Regarding these findings, the careful use of antibacterial medicines in clinical, veterinary, and agricultural contexts is strongly suggested to preserve antibiotic efficacy and prevent the development of cross-resistance. Quinolones are widely used in veterinary medicine to treat *Salmonella* infections over the world Mehdi et al. (2018). This work looked for ESBL and PMQR genes in *Salmonella* isolates from infected broiler chickens. According to global studies, there has been an alarming increase in beta-lactam antibiotic resistance. In this study, we have showed that although *Salmonella* strains were negative for *qnrA* and *qnrB* they were positive for the *qnrS* in all isolates of this investigation partially agreeing with the study by Dembéle et al. (2020), who could not identify *qnrA* and *qnrS* in any *Salmonella* strain. These findings highlight the low incidence of *qnr* among *Salmonella* isolates. However, Soliman et al. (2017) found the plasmid-mediated quinolone-resistance gene *qnrA1*. Furthermore, we observed that *Salmonella* isolates were more fluoroquinolone-resistant, as evidenced by PCR for *qnrS*, PMQR genes, revealing that 100% of the samples tested positive for *qnrS*. This outcome was greater than what had previously been reported by Abo-Remela et al. (2015) who were able to identify that 18% were positive for *qnrS*. Furthermore, another study by Zhao et al. (2017) discovered that while *qnrA* and *qnrB* had a high incidence, *qnrS* had a low incidence. But in 2020 (Zhao et al., 2020) reported *qnrB* with low incidence (6/67, 9.0%). However Yang et al. (2013) could identify *qnrA*, *qnrB* and *qnrS* genes by (46.6%), (12.7%), (19.5%) respectively. Besides, although Dembéle et al. (2020) could not identify *bla* _TEM_ and *bla* _SHV_ in ESBL, dominant beta-lactamase genes detected in our investigation were similar to the previously reported data by Eguale et al. (2017). While Ramatla et al. (2022) could find high levels of β-lactamase encoding genes *bla* _TEM_ in their *Salmonella* isolates. Also Zhao et al. (2021) found the majority of isolates harbored the *bla* _TEM_ gene (74.4%). Shahada et al. (2010) also could identify the wild-type *bla* _TEM_ gene that mediated resistance to ampicillin. Soliman et al. (2017) also found *bla* _TEM_ in *Salmonella* isolates and Zhao et al. (2020) found *bla* _TEM_ in all *Salmonella* isolates (100%). Rady et al. (2020) found that all isolates were positive ESBLs genes but were negative for *bla* _CMY_ gene. While Ahmed and Shimamoto (2012) could identify *bla* _CMY_ in one isolate of *Salmonella enterica* serovar Enteritidis only. Moreover, Adel et al. (2013) could identify the wild-type *qnrA* and *qnrB* genes, including *bla* _SHV_2, *bla* _CMY_2 (AmpC type), and *bla* _TEM_ in the *Salmonella* isolates. Sabry et al. (2020) found 16 of *Salmonella* isolates were ESBL-producing with the majority carrying *bla* _SHV_ and *bla* _TEM_ genes and 4 ESBL-negative isolates carried *bla* _CMY_.

**CONCLUSION**

*Salmonella* serovars obtained from diseased broilers have a high resistance rate to quinolones and β-lactams. Accordingly, this study has detected quinolone-resistant and ESBL-producing Enterobacteriacaes in rather significant numbers. Furthermore, high frequencies of *qnrS*, *bla* _TEM_ and *bla* _SHV_ were observed in all isolates. Thus, identifying quinolone-resistant and ESBL-producing Enterobacteriacaes is critical for effective therapy and infection management. Hence, proper use of these antibiotics will restrict the propagation of resistance genes while reserving their use for therapeutic purposes.

**Supplementary material**

There is supplementary material associated with this article. Access the material online at: https://dx.doi.org/10.17582/journal.pjz/20220802110804

**Statement of conflict of interest**

The authors have declared no conflict of interest.

**REFERENCES**


El-Mohsen, A., and El-Sherry, S., 2022. Serological and antibacterial characteristics of *Salmonella* isolates from chickens in Assiut., Egypt. *Benha...
Online First Article


Li, Q., Wang, X., Xia, J., Yuan, Y., Yin, C., Xu, L., Li, Y.

Le Thi Minh Vien, S.B., Le Thi Phuong Thao, L.T.,

Helms, M., Vastrup, P., Gerner-Smidt, P. and


Microorganisms, **10**: 313. https://doi.org/10.3390/microorganisms10020313


Genetic Analysis of Antimicrobial Resistance Genes in *Salmonella* Isolated


Supplementary Material

Genetic Analysis of Antimicrobial Resistance Genes in *Salmonella* Isolated from Diseased Broilers in Egypt

Mona F. Shousha¹*, Aml M. Ragab² and Salwa M. Helmy¹

¹Department of Bacteriology, Mycology and Immunology, Faculty of Veterinary Medicine, Kafr El-Sheikh University, Kafr El-Sheikh 33516, Egypt
²Unit of Bacteriology, Animal Health Research Institute, Tanta Branch, Agriculture Research Center (ARC), Egypt

Supplementary Fig. 1. Agarose gel electrophoretic PCR pattern for detecting *qnrA*, *qnrB, qnrS*, *bla*₂₅₄, *bla*₅₁₉ and *bla*₅₄₅ at 543 bp, 562 bp, 491 bp, 516 bp, 392 bp and 462 bp respectively. L: Ladder from 100 bp to 1000 bp P: Positive control N: Negative control: field isolate tested and confirmed by PCR to be negative for the related genes. Lane 1 to 10: Negative amplification of *qnrA, qnrB* and *bla*₅₄₅ and Positive amplification of *qnrS, bla*₂₅₄ and *bla*₅₁₉.

* Corresponding author: mona_shousha95@yahoo.com

Copyright 2023 by the authors. Licensee Zoological Society of Pakistan. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).