Molecular detection of Quinolone Resistant-Salmonella isolates from poultry farms in Al- kut, IRAQ.

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Abstract

The current study included collecting cloae samples from poultry within Wasit Governorate's farms. It aimed to isolate salmonella from broiler chickens and to determine the pattern of resistance to antimicrobial drug besides to detect of Quinolone Resistance genes. The results showed isolation of (3) types of Salmonella (s. gallinarum s.typhimurium, s.enteritidis). The highest percentage of resistance isolates to tested antibiotics (nalidixic acid, ciprofloxacin ofloxacin, enrofloxacin, levofloxacin) depending on disc diffusion test, were as follow (81%), (78.26) and (91.66%) respectively. The remaining small percentage of the isolates were sensitive to all quinolones. On the other hand. The results of the molecular diagnostics that were conducted found that all antibiotic-resistant strains contain qnr B and iaa (6) ib gene.

Keywords: Salmonella, Quinolone Resistance, qnr genes

1. Introduction

Poultry farms are considered one of the profitable sources of income among farmers for providing poultry products, which are an important source for meeting the daily human needs of protein[1]. However, there are some obstacles that may prevent or affect the progress in poultry production, foremost among which are biosecurity measures and infectious diseases, such as multi-drug resistant salmonellosis which is a global problem that causes millions of diseases and annual deaths and huge economic losses, due to the high mortality rate that reaches 90% [2, 3]. One evidence of continuous enteropathy by Salmonella spp. in certain birds was the presence of this Bacterial species in a smear taken from uninfected chickens cloaca [4]. On the other hand, its Appearing in dropping of poultry and Ventilation of bird scraps may serve as a means of spreading pathogens to other birds and contaminating agricultural environments, putting birds at risk [5].

The antibiotic resistance of these strains, particularly quinolone, has recently become a challenge and a threat because It will affect directly on growth rate and increased death between infected chickens, or may be cause transmission of salmonella to humans as well as the risk of food poisoning in humans [6]. So this work based on molecular detection for the most common genes related with quinolone resistance within the detected region.

2. Materials and Methods
2.1 Sampling and identification of bacteria

A bout of 200 cloaca samples were getting in antiseptic bags from different poultry farms distributed in Wasit city, afterward in ice box have been sended to lab.

Isolation and Serotyping of isolates

Culturing of gathered samples on 1% pepton broth, selenite F broth, MacConkey, Salmonella shigella agar, and/or XLD medium followed with incubation step. picking up of Suspicious colonies have been, holding them as stock also in slant agar for more biochemical confirmation tests concerning salmonella[7].

Susceptibility test

To evaluate salmonella susceptibility a group of widely used-antimicrobial listed below, disk diffusion test depended, with assistance of CLSI guidelines to interpret inhibition zones diameters forms around each antibiotic disk.

Disk diffusion assay on Mueller-Hinton agar (Oxoid, UK) was employed to evaluate the susceptibility of Salmonella isolates to a group of commonly used antibiotics (the same origin). The guidelines of CLSI were followed to perform the Modified Kirby-Bauer method and to interpret the inhibition zones diameters around antibiotic disks [8]. Antimicrobial agents included 11 antimicrobials (i.e., amoxicillin/clavulanic acid, nalidixic acid, ampicillin, doxycycline, gentamicin, trimethoprim/sulfamethoxazole, chloramphenicol, ciprofloxacin, meropenem, and ceftriaxone). Escherichia coli ATTC 25922 as control strain has been used for all susceptibility tests[9].

Bacterial DNA Extraction

The bacterial genetic material was extracted from 72 species (previously diagnosed as Salmonella using DNA Kit (BIONER, KOREA) According to company instruction, the extracted DNA was identified and qualified using the nano-drop, and stored at 20 ° C. The extracted DNA have been introduce to pcr to amplify (PMQR) including qnrA, qnrB, and qnrS as well as aac (6) -Ib genes. The primers used in this study and the conditions explained in Table (1) and (2) respectively[10].

| Table (1): Primers used for detection of qnrA, qnrB, qnrS and their characteristics. |
|-----------------|-----------------|-----------------|-----------------|
| Primer symbol  | Primer sequence | Amplicon size(bp) |
| qnrA      | F: ATT TCT CAC GCC AGG ATT TG  |
|           | R: GAT CCG CAA AGG TTA GGT CA  |
|           | 516                           |
| qnrB      | F: GTT GCC GAA AAA ATT GAC AGA A  |
|           | R: ACT CCG AAT TGG TCA GAT CG  |
|           | 526                           |
| qnrS      | F: ACG ACA TTC GTG AAC TGC AA  |
|           | R: TTA ATT GGC ACC CTG TAG GC  |
|           | 417                           |
| -Ib aac(6) | F: TGA CTT TG CAG TAT CTT ATG  |
|           | R: TTAG CC AT CAG CTG GTT C    |
|           | 508                           |

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3. Results and Discussion

The rising existence of antimicrobial Resistant-Salmonella poses a real and threatening public health problem.

Of the 200 samples, only 72 (46%) sample out of 200 were distinguished as salmonella spp. based on its colony shape and biochemistry tests. The most prominent breed detected were s. gallinarum 37 (59.78%) followed by s. typhimurium 23 (40.22%) and twelve were (2.17%) s. enteritidis (percentage explained in schema 1). That came in line with [11, 12] included that most isolates are the same as recorded here. Regarding Findings of susceptibility testing, Nalidixic acid resistance and and low ciprofloxacin response have been reported among thirty (66.6%) of Salmonella gallinarums examined.

While, eighteen of inspected Salmonella typhimurium had been non-sensitive to all tested antibiotics. Whilst, 11 of the third variants did not respond to drugs.

Table (2): Thermo-cycler programming.

| PCR steps       | Temperature | Time | Cycle no. |
|-----------------|-------------|------|-----------|
| Initial Denaturation | 94          | 10:00| 1         |
| Denaturation    | 94          | 1:00 | 30        |
| Annealing       | 52          | 1:00 | 30        |
| Elongation      | 72          | 1:00 | 30        |
| Final Extension | 72          | 7:00 | 1         |

Figure (1): Percentage of detected strains of salmonella in poultry.
Fifty-nine strains that were reported as being resistant to antibiotics underwent to PCR to investigate for genes related with anti-biotic resistance. However, out of 30 of the tested s.gallinarum resistant to both nalidixic acids and ciprofloxacin were positive to all previous four genes. aac (6’)-Ib and Qnr b genes were recorded in both resistant s.typhimurium and s. enteritidis isolates as presented in table (3) and fig (2) below.

**Table (3): results of susceptibility and molecular tests.**

| Salmonella serovar | No. of resistant isolates | Detected gene | |
|--------------------|---------------------------|---------------|
|                    |                           | qnra | qnr b | qnrs | aac(6)-Ib |
| s. gallinarum      | 30/37                     | +    | +    | +    | +          |
| s.typhimurium      | 18/23                     | -    | +    | -    | +          |
| s.enteritidis      | 11/12                     | -    | +    | -    | +          |

In light of the results obtained, the study found that all antibiotic-resistant species contain a qnr B gene and iaa(6)ib gene. Thus, We can explain this because both genes possibly responsible for the observed increase in poultry salmonella resistance in recent years to the treatments used. Contrast to this current study, a study conducted by [13, 14] which qnrB was confirmed. Although its determinants give a decreased level of quinolone resistance spontaneously. As well, A growing acquisition of resistance mutations and resistance in susceptible strains.

In the Iranian research, it was reached that (25.8%) of 85 clinical isolates positive to qnra gene, while qnr b and qnrs were identified in (1.17%) among them [15]. increased resistance to quinolone were associate with existence of qnr gene and as a result, it is considered an insufficient treatment when used for
therapeutic purposes. Given that the qnr-carrying plasmids have a large and universal distribution, care must be noticed when using quinolones to treat diseases [16,17].

4. References

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