Study of Drug Resistance in *Salmonella* spp. Isolated from Native Eggs of Iran’s Southern Region

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Salmonellas are short bacteria, gram negative, some of them have caps, facultative aerobic and anaerobic have high resistance against physical and chemical factors. *Salmonella* causes bacillary white diarrhea in Avian. Its transference in Avian is vertical and horizontal. In human, it causes food poisoning too. The egg is the most important source of salmonella. Native eggs have been collected and transferred to the lab in order to study their contamination. These eggs were collected from areas around Shiraz (Iran). In this research the pattern of drug resistance of bacteria was studied. The egg shell is purified with Ethanol 70%, and then the contents of Fifty eggs are mixed in a dish and incubated with soab in Selenite-f. After 24 hour incubation at 37ºC the Selenite-f sample was transferred to salmonella spp and after incubation at 37ºC was analyzed in terms of suspected colonies to Salmonella. The suspected colonies were inoculated into lysine decarboxylase broth and TSI agar environment. The bacteria that had reactions were related to salmonella, and were analyzed by the PCR test, with special primer for salmonella spp, such as *S. enteritidis* and *S. typhimurium*. The results of this study indicated five samples of mixed eggs (at least 0/33% of eggs) were contaminated with salmonella of serotype *S. enteritidis*. Among the separated Salmonellas 85.9 % were resistant to Ampicillin, 14.5% to Tetracycline, and 42.9% to Kanamycin, but all of them were sensitive to Norfloxacin in this antibiotic resistance test. Excessive use of antibiotics and also having no information on sensitivity and bacterial resistance to antibiotics can make the antibiotic resistances more complex.

**Key words:** Salmonella spp., Salmonella enteritidis, Drug resistance.

*Salmonella* are a large group of negative Gram bacillus, 1 to 3 micron in length, and 0/5 to 0/8 micron in width, all of them have antigen H of the flagellum, and can move, except two serotypes of *Salmonella gallinarum* and *Salmonella pullorum* (Please check the entire document)¹⁻². *Salmonella* have high resistance to chemical agents, bile, also physical factors such as temperature and disinfectant material. According to the Tajbakhsh and Nazar, 1974 study, Salmonella Abortus ovis can survive more than 5 months in the soil and maintain its ability to ability to create disease. So far, more than 2,500 serotypes of Salmonella have been identified¹⁻⁴. In poultry, Salmonella can transmit horizontal and vertical and usually creates stable infection in contaminated flocks. Different serotypes of salmonella cause some diseases such as Pullorum diseasewhich is caused by *S.Salmonella pullorum*. Its transmission is usually vertical. Poultry Typhoid is caused by salmonella Gallinarum (review) which almost always transmitted horizontally and by bird faeces.
Paratyphoid is another disease with symptoms similar to Pullorum and is very important because it is a creating agent, which can be caused by Salmonella typhimurium, enteritidis and Salmonella Havana. However, these bacteria, especially Salmonella typhimurium and Salmonella enteritidis, do not inhabit a specific host, and they are the most important species involved in human salmonella contamination. Most research shows that salmonella enteritidis in most salmonellosis epidemics are involved with origin eggs. Infection of reproductive system by salmonella enteritidis can be transmitted to egg and contaminate the egg’s content. It seems that a major part of infection is upper site of Audact. Other serotype Paratyphoids include antibiotic-resistant serotypes, Salmonella typhimurium can also be replaced in the egg. Consumption of contaminated eggs is the most important source of Salmonella infections. A Salmonella bacterium does not have hard growth and after enrichment it can be separated from environment, clinical sample and nutrition. PCR technique provides precise and quick results. Regarding the importance of detection, because of salmonella contamination in the poultry breeding industry, public health and also increasing antibiotic resistance of salmonella bacteria, it is vital to detect salmonella contamination of local eggs. So the aim of this research is the study of drug resistance in Salmonella spp isolated from native eggs of the south of southern Iran.

MATERIALS AND METHODS

For isolation of salmonella bacteria, 1500 eggs were collected from the south of Iran and transferred to the lab. In the laboratory, the egg shells were cleaned and disinfected using ethanol 70%, calcareous shell was broken with sterilized scissors, and the contents of each 50 eggs was mixed in a sterilized glass dish. After 24 h incubation at 37°C the mixture was incubated and then or mixed with to selenite - F for enrichment. The mixture was incubated for 24 to 48 hours at 37°C. After removal of incubation on solid medium of salmonella-shigella it was incubated for 24 h at a temperature of 37 °C, Salmonella environment - Shigella agar for 24 hours incubated at 37°C. The suspected Salmonella colonies were then examined and in case of negative result, the incubation was continued for 24 hours. Salmonella that had no color after this time or suspected pale or yellow colonies with gray and black center were negative and deleted, then suspicious colonies were removed from salmonella-Shigella agar medium and were cultured Surface and deeply in TSI and LIA medium. Also, we provide a spread from the suspicious colonies which were stained with Gram method. If the rods Gram-negative bacterium was observed, a pure culture was provided and it was checked for existence of Salmonella.

Recognition of Salmonella serotype enteritidis and typhimurium by PCR test

For DNA extraction of the identified salmonella above, colony suspensions in sterile distilled water were prepared. The suspension was centrifuged for 5 min at 18°C at a speed of 14,000 rpm and bacterial genome was extracted from the precipitate by phenol-chloroform bacterial genome method (reference). Genome, by using specific primers of invasion gene, was used for PCR to detect Salmonella. To determine the serotype, the primers of Fimbria gene (sefA) for Salmonella enteritidis and primers of Virulence Fimbriae (pefA) for Salmonella typhimurium were used in PCR (Table 1). PCR products on agarose gel 1.5 percent electrophoresis and 100 bases per pair were used to determine the molecular weight of DNA fragments. Gel was stained with ethidium bromide solution, which has a Fleur Scans color. UV radiation device was used for observing bright bands under the ultraviolet light.

Antimicrobial Resistance

To determine the sensitivity of isolated salmonella to the antibacterial compounds, a disk agar diffusion method was used. First, colonies in Moler Hilton (Mueller Hinton) liquid environment reached to half Mcfoarlan, then from that, a culture was provided on the agar bis culture environment and simultaneously a disk containing antibiotic was placed (from the Padtan Teb Company on the culture environment). After 24 h incubation at 37°C, Salmonella growth inhibitions ring were determined. For antibiogram tests, four antibiotics disks containing: Ampicillin (10 ig), Tetracycline (30 ig), Kanamycin (30 ig) and Norfloxacin (10 ig) were used (Table 2). The result of salmonella isolation from eggs was estimated by the eggs contamination percentage. The results of
determining salmonella sensitivity to anti-bacterial effect on the base of the percentage were also estimated in 3 sensitive, semi-sensitive and resistant levels (based on the Padtan Teb is written differently company’s table from the number of salmonella samples) (using standard Kirby & Bauer technique).

RESULTS

To increase the isolation sensitivity of Salmonella, the enrichment step prior to inoculation of the selected medium was done. From the 30 mixed samples of eggs, 5 mixed samples in biochemical tests and PCR contaminated with Salmonella were Salmonella enteritidis (Figure 1). In the pattern of studying drug resistance of isolated salmonella, resistance rate was Ampicillin 85.9%, Kanamycin 42.9% and Tetracycline 14.5% respectively, while there was no observed resistant sample to Norfloxacin. Regarding the antibacterial drugs used, the most sensitive was observed for Norfloxacin (Table 2).

Row M: Indicator of molecular weight DNA; Row 1: positive control (standard strain of Salmonella enteritidis ATTC 13076) rows 5, 6, 7 are eggs contaminated with Salmonella enteritidis and rows of egg-free from salmonella enteritidis are 2, 3, 4. row 8 negative control.

DISCUSSION

According to the method of preparing samples for bacterial culture, it was shown that at least 0.33% of the eggs (one egg in each mixed sample) or up to 16.66% of eggs (all eggs in the mixture) were contaminated with salmonella. Salmonella contamination rate in Iran (Ahwaz) in native eggs was determined by using biochemical and serological tests and it was also determined that contamination rate was 1.66%\(^{22}\). In a study in the USA (Arkansas) salmonella contamination of content and shells of 1200 eggs were examined, just in 12 eggs, external contamination with Heidelberg salmonella was found, while all egg contents had no salmonella contamination\(^{23}\). In another study, the mass in the shell and content of egg were tested from more than 5700 eggs of 15 flocks that had been naturally contaminated with enteritidis salmonella. The contents of 32 eggs (6%) were infected\(^{9}\). Organisms inside the egg through direct transfer or egg shell contamination or through oviduct penetrate to the egg\(^{24}\). Native chickens are usually kept with domestic animals and their egg laying also takes place on the bed, so the contamination probability to bacteria with intestinal origin, such as salmonellosis, will increase; also, control programs and salmonella eradications are not performed. Therefore, to reduce the risk of salmonella, it is proposed that:

1 - Fully cooking eggs enough (70 ° C during 10 minutes) can remove Salmonella (25). 2- Teaching the dangers of salmonellosis to people and preventing methods of salmonella transmission to the food in the kitchen which is the most practical method to prevent salmonellosis caused by eggs\(^{5,26}\).

In patterns of studying drug resistance of isolated salmonella, resistance rate to the

<table>
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Fig. 1. Detection of Salmonella enteritidis, egg specimens native around Shiraz.
antibiotics were Ampicillin 85.9%, Kanamycin 42.9% and Tetracycline 14.5% respectively, while there was no observed resistant sample to Norfloxacin. Regarding the antibacterial drug used, the highest sensitivity was observed for Norfloxacin, which is a result of these antibiotics not being used in the area, so no resistance to it was found. Having no complete sensitivity to Tetracycline and Kanamycin can result in excessive use of these antibiotics in treatment, so resistance to these antibiotic is not unexpected. Ampicillin resistance may be due to the greater effect of these antibiotics on Gram- positive bacteria than Gram-negative bacteria²⁷. Graziani et al. examined antibiotic sensitivity of isolated Salmonella typhimurium from humans and animals, and also determined the resistance of salmonella (which was isolated from poultry) to Ampicillin (3.54%), Gentamicin (2.3%), Kanamycin (85%), chloramphenicol (5.24%), Tetracycline (52.1%) and ciprofloxacin (0%)¹⁵. Pan et al., 2009 studied Antibiotic resistance of Salmonella Antryka which showed approximately 39/95% percent isolated bacteria indicating high resistance, particularly against ampicillin (40.2%), Streptomycin (58%) and Tetracycline (58.9%). During the years 1999-1962, all isolated salmonella were sensitive to Norfloxacin, but during the years 2007-2000 low resistance (17/5) to Floxacin was seen¹⁶.

**CONCLUSIONS**

Nowadays, use of antibiotics in poultry is common. With regard to increasing antibiotic resistance, culture and antibiogram should be performed and prescription should be done with regard to antibiotic sensitivity of Microorganisms. Excessive use of antibiotics and also having no information on the sensitivity and bacterial resistance to antibiotics can make the antibiotic resistances more complex. According to this study, the most sensitive antibiotic for salmonella enteritis was Norfloxacin, and resistance to Ampicillin, Kanamyacin, and Tetracyclin is decreasing respectively.

**REFERENCES**