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# Prevalence and antibiotic sensitivity pattern of *Salmonella* isolated from eggs sold in commercial markets in Thanjavur, Tamil Nadu

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**Abstract**
Salmonellosis is a major public health problem globally. Poultry species acts as reservoir of this bacterium and most of the infections in humans results from the ingestion of contaminated eggs and poor hygienic practices followed by food handlers. Hence, the study was carried out with the objective to evaluate the prevalence of *Salmonella* in eggs from poultry and Japanese quail sold in local markets in Thanjavur district, Tamil Nadu. A total of 125 eggs were collected randomly for isolation of *Salmonella* in and around Thanjavur district. Briefly, the collected eggs were pre-enriched, enriched and streaked onto Xylose-Lysine Deoxycholate agar. The characteristic *Salmonella* colonies were subjected to biochemical tests, PCR and antibiotic susceptibility testing (ABST). A 20% prevalence of *Salmonella* was observed from poultry eggs, however, no *Salmonella* was recovered from eggs of Japanese Quails. The ABST results suggested that, all the isolates were sensitive to erythromycin, streptomycin, nalidixc acid, gentamicin, doxycycline, tetracycline and amikacin and resistant to methicillin and vancomycin. Results of the study suggest that *Salmonella* was detected in poultry eggs whereas; it was not detected in Japanese quail eggs in Thanjavur region of Tamil Nadu. Hence, effective salmonellosis control strategies should be followed to control the egg borne transmission. In addition, proper hygienic food safety practices and strict bio-safety measures while handling eggs and avoiding cross contamination needs to be employed for controlling salmonellosis.

**Keywords:** *Salmonella*, chicken eggs, Japanese quail eggs, prevalence, antibiotic susceptibility testing (ABST)

## Introduction
Salmonellosis is one the major food-borne and vertically transmitted bacterial diseases occurring worldwide. The poultry acts as the primary reservoir of *Salmonella* leading to contamination of poultry eggs [1, 2]. In India, *Salmonella* has been reported mainly from poultry, man, and foods of animal origin. Hens reared in villages can be infected through contact with domestic mammals and commercial poultry. In addition, since poultry acts as reservoir for *S. enteritidis*, they do not show any signs of infection. Consumption of raw or partially cooked eggs could lead to salmonellosis. In addition to being carrier, poultry species also acts as source of transmission of the organism to other animals and humans [3].

In recent times, public health concern due to consumption of poultry, meat and other foodstuffs contaminated with food-borne pathogens has become an important issue to increased incidence of antimicrobial-resistant bacteria associated with human illness [4, 5, 6]. Injudicious use of antimicrobial agents in poultry has resulted in emergence of antibiotic resistant strains of *Salmonella*. Infection with multidrug-resistant pathogens is of public health importance [7]. A high prevalence of antimicrobial resistant *Salmonella* in foods of animal origin has been reported earlier from India [8, 9].

In India, Government has taken steps to promote egg as a good source of protein and the consumption of egg has gone up considerably [10]. Various states of India are providing free eggs to rural school children as a part of mid-day meal scheme. Since the consumption has been increased, effective steps are needed to monitor the egg quality sold in local markets. Hence, the present study was undertaken to investigate the level of *Salmonella* contamination and its antibiotic susceptibility pattern in and around Thanjavur district of Tamil Nadu.

## Materials and Methods

### Sample collection

A total of 125 eggs (75 poultry and 50 Japanese quail eggs) were collected randomly in from different retail markets of Thanjavur, Tamil Nadu. Egg samples were collected in sterile bags and were directly subjected to microbiological analysis.

### Microbiological analysis

### Isolation of *Salmonella*

Isolation of *Salmonella* from eggs was carried out using method published earlier [10, 11]. Eggs collected from retail markets were first inoculated separately in 100 ml of buffered peptone water (Himedia, India) for pre enrichment for 24 h at 37 ˚C. After incubation, one ml of pre enrichment sample was cultured in 9 ml of selenite cysteine broth (Himedia, India) for enrichment for 24 h at 37 ˚C. The enriched cultures were streaked on *Salmonella* specific Xylose Lysine Dextrose (XLD) agar. Based on colony characteristics on specific medium, the isolates were characterized as presumptive *Salmonella* which were further characterized biochemically. Presumptive S*almonella* colonies were purified by sub-culturing in nutrient broth and further streaking on nutrient agar.

### Biochemical characterization

The presumptive *Salmonella* colonies isolated from eggs were subjected to Grams staining, catalase test, indole test, oxidase test, methyl red test, VP test, Simmon's citrate utilization tests and triple sugar iron utilization tests. Isolates showing Gram negative reaction, catalase positive, indole negative, methyl red positive, VP negative and citrate positive and produce yellow butt and red slant with hydrogen sulphide gas production on TSI medium were considered as *Salmonella* and the colonies were further subjected to Polymerase chain reaction (PCR).

### Polymerase chain reaction

DNA was extracted from the suspected *Salmonella* colonies by boiling method. Briefly 1.5 ml of the sample in broth was centrifuged at 10,000 rpm for 5 minutes. After centrifugation, the supernatant was discarded and the pellets were washed three times with sterile distilled water. After washing, pellets were reconstituted with 200 µl of sterile water and boiled in water bath at 100 °C for 10 minutes. The samples were centrifuged at 12,000 rpm for 5 minutes. The supernatant containing the DNA was used for DNA amplification with *Salmonella* specific primers [12, 13].

The PCR amplification against *invA* gene (284 bp) of *Salmonella* containing forward primer (5' GTG AAA TTA TCG CCA CGT TCG GG 3') and backward primer (5' TCA TCG CAC CGT CAA AGG 3') is carried out in 25 µl reaction mixture consisting of 12.5 µl of PCR master mix, 1 µl of each primer, 1 µl of DNA and 9.5 µl of molecular grade water. Amplification was conducted in master-gradient thermocycler (Eppendorf). The cycling conditions for PCR were initial denaturation 94 ˚C of 1 min, followed by 35 cycles of denaturation for 1 min at 94 ˚C, annealing for 30 seconds at 64 ˚C and extension for 30 sec at 72 ˚C and final extension for 7 min at 72 ˚C. The amplified DNA products from *Salmonella* specific PCR products were analyzed on electrophoresis with 1.2% agarose gel and visualized by UV illumination.

### Antimicrobial susceptibility pattern of isolated *Salmonella*

All the *Salmonella* isolates in this study were tested for antimicrobial susceptibility using disc diffusion methods [10, 14]. The agents used in this study were procured from Himedia and include Methicillin (5 mcg), Vancomycin (30 mcg), Erythromycin (15 mcg), Streptomycin (10 mcg), Nalidixc acid (30 mcg), Gentamicin (120 mcg), Doxycycline (30 mcg), Tetracycline (30 mcg), Amikacin and Amoxycilin-clavulinic acid (20 and 10 mcg), Chlormphenicol (30 mcg), Trimethoprim (5 mcg). Pure cultures of *Salmonella* were enriched in brain heart infusion broth at 37 ˚C for 6–8 h. The *Salmonella* cultures were streaked on Mueller Hinton agar plates (Himedia, India) using a sterile cotton swab and the antibiotic discs were dispensed using a disc dispenser (Himedia, India) with sufficient space in between each disc to avoid overlapping. The agar plates were incubated at 37 ˚C for 16–18 h and the zones of inhibition for each antibiotics were measured.

## Results and Discussion

Food-borne salmonellosis is still a major public health problem in human and the significance of *Salmonella* spp. as causes of human and animal disease has increased in the recent years. Moreover, increased globalization and trade between countries, importing countries demand *Salmonella* free poultry and poultry products [15]. Since the poultry eggs and their products are common cause of infection, this study was undertaken to find out the status of salmonellosis. A total of 75 chicken eggs and 50 Japanese quail eggs collected from retail markets in Thanjavur district were processed for isolation and characterization of *Salmonella* by selective enrichment and culture technique**.** *Salmonella* isolates were obtained by selective plating on XLD from enriched Selenite broth samples. *Salmonella* specific red colonies with black center on XLD (figure 1) were selected for biochemical characterization. All the biochemically confirmed isolated were tested for PCR assay targeting *inv*A gene of *Salmonella* for further confirmation. This assay relied on amplification of 284 bp product. Based on Gram staining (Gram negative rod, figure 2), IMViC reaction (-/+/-/+, figure 3), TSI utilization (alkaline slant, acidic but with $H_2S$ production, figure 3) and amplification of *inv*A gene by PCR reaction (figure 4), a 20% prevalence of *Salmonella* was observed from poultry eggs, however, no *Salmonella* was isolated from eggs of Japanese quails (figure 5). Egg contaminated with *Salmonella* can occur due to egg contact with faecal material, insects, feed, during transportation, storage and handling. These factors can cause variation in the prevalence of *Salmonella* in eggs [16].

In recent times, emergence of antibiotic resistant strains has been increasing and become public health problem. Antibiotic resistance in *Salmonella* is also increasing. Hence, studying of antimicrobial resistance among microorganisms is essential for monitoring the status of resistance pattern within the study area. The antimicrobial susceptibility tests in the present study revealed that all the isolates were sensitive to streptomycin, gentamicin, doxycycline and tetracycline. All the isolates were resistant to methicillin and vancomycin whereas 20% of the isolates are resistant to erythromycin, amikacin, nalidixic acid, amoxicillin-clavulinic acid and trimethoprim. *Salmonella* develops resistance to antibiotics because of injudicious use of antibiotics for longer period or use of antibiotics at suboptimal doses during treatment. The results of the current study showed that *Salmonella* isolates were resistant to more than one antibiotics.

Prevalence of *Salmonella* and its antibiogram in chicken eggs, meat and other sources in India and other countries has been reported by several researchers. Previously conducted study [17] had more than 50% of duck eggs contaminated with *Salmonella* and were resistance to tetracycline, oxytetracycline and nalidixic acid among *Salmonella* isolated from eggs of commercial layer hens. Another study [18] reported overall prevalence of 14% among samples collected from diarrhoeic livestock and humans with *Salmonella* isolates belong to 5 serotypes, *S*. Typhimurium, *S*. Enteritidis, *S*. Gallinarum and *S*. Paratyphi B and S in north eastern part of India. A previously published paper from our laboratory in Thanjavur has investigated the presence of *Salmonella* in chicken and slaughtering place and presence of 33% in chicken and 60% in water used in slaughtering place was also observed [11].


**Fig 1:** Growth of Salmonella on XLD agar with characteristics red colonies with black centre

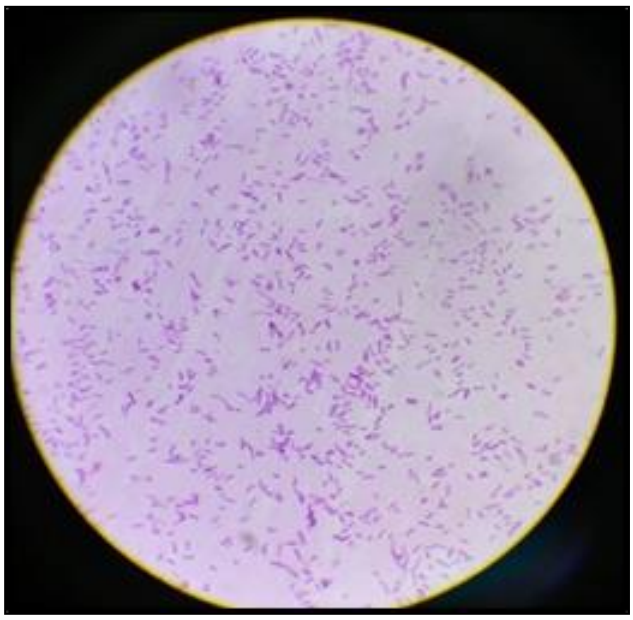

**Fig 2:** *Salmonella* colony showing Gram negative rods in Gram's staining

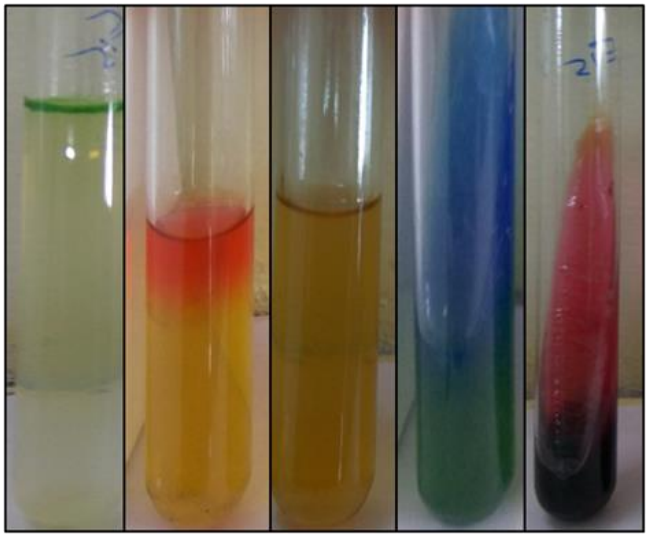

**Fig 3:** IMViC (-/+/-/+) and TSI utilization (alkaline slant and acidic butt with $H_2S$ production) pattern of *Salmonella* isolates

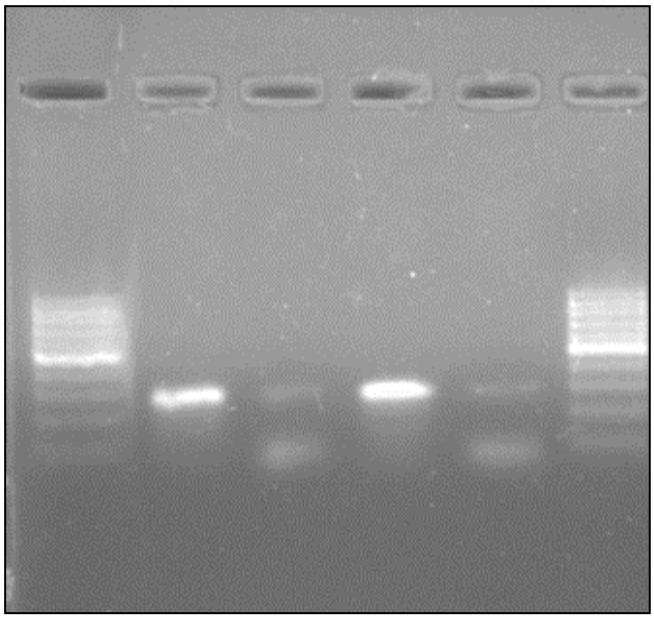

**Fig 4:** PCR amplification of *invA genes of Salmonella* isolates. Lane M represents 100 bp molecular weight marker, lane 2 and 4 represents negative control, lane 1 and 3 represent *Salmonella* isolate

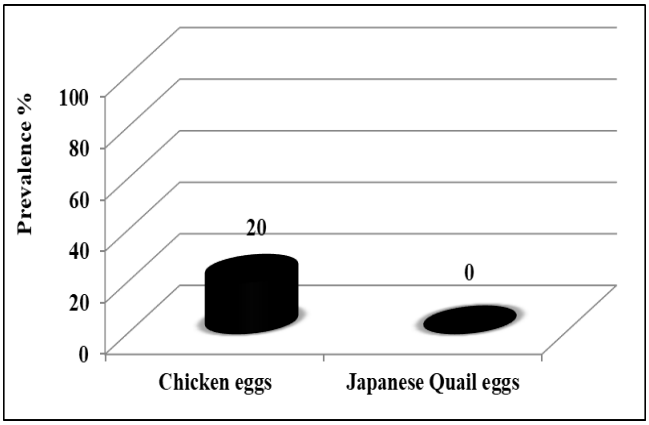


**Fig 5:** Prevalence of *Salmonella* in the chicken and Japanese quail eggs sold in Thanjavur district

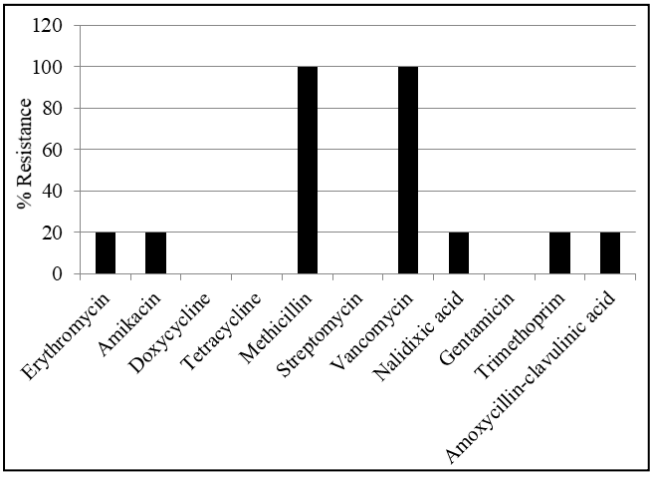


**Fig 6:** Percentage of antibiotic resistance among the *Salmonella* isolated from the study

## Conclusion

The results of this study indicated the *Salmonella* serotypes prevailing in the poultry eggs sold in retail markets. Hence, effective salmonellosis control strategies should be followed to break the egg borne transmission. In addition, proper hygienic food safety practices while handling and storing of foods needs to be employed for controlling salmonellosis. The presence of antibiotic resistance in *Salmonella* suggests that there is appreciable risk of spread of antibiotic resistance to other bacteria.

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