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Isolation and identification of *Salmonella* spp. from layer poultry farms of North western districts of Tamil Nadu

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Abstract
Salmonellosis is a bacterial disease of poultry affecting primarily chickens and turkeys. Other poultry such as pheasants, quail, ducks, guinea-fowl and peafowl are also susceptible. Antimicrobials in feed formulations are regularly used at sub-therapeutic levels in commercial poultry business to prevent the occurrence of gastro intestinal, respiratory and reproductive diseases caused by bacteria or / and viruses. In this context, a pilot study was undertaken in three districts of Tamil Nadu to understand the technical knowledge of antimicrobial usage by the farmers and the antimicrobial resistance exhibited by Salmonella isolated from apparently healthy layer flocks. A total of 900 cloacal swab samples were collected from 10 layer farms from each district of North western agro climatic zone and screened for the presence of *Salmonella* spp. isolation is by non-selective pre-enrichment followed by selective enrichment before plating on selective and differential agars. Twelve Salmonella isolates were isolated and Kirby-Bauer disk diffusion susceptibility test was performed against various antibiotics.

Keywords: Salmonella, poultry, media

Introduction
Salmonellosis in layer poultry is an important disease of concern because of its ability to get vertically transmitted from parent flock to commercial chicks. It is also considered as an important food borne pathogen that can be transmitted by poultry products such as meat and egg. Salmonella comprises 2541 serovars [1] and more than 53 serovars are distributed in poultry [2]. They are gram negative, non-lactose fermenting and non sporing bacteria. All serovars of Salmonella are motile except *Salmonella pullorum* and *Salmonella gallinarum*.
Surveillance of poultry for the presence of *Salmonella* will ensure access to safe food by the customer because meat and eggs are considered to be major sources of transmitting Salmonella to humans. Almost 40% of the infection is attributed to consumption of poultry egg and meat. Although Salmonella contains more than 2300 serotypes but about 10% of the serotypes were of poultry origin.
Hence, this study was conducted to perform surveillance of layer poultry flocks located in North Western districts of Tamil Nadu for the presence of *Salmonella* spp. (ii) Isolation, identification of isolated *Salmonella*.

Materials and Methods
Sample collection
Cloacal samples were taken from thirty poultry layer flocks from each district of North western agro climatic zone namely Namakkal, Dharmapuri and Krishnagiri districts. Ten samples from each farm were taken using sterile cotton swabs by unbiased random sampling method.
Cultural methods for detection of Salmonellae involve a nonselective pre-enrichment, followed by selective enrichment and plating onto selective and differential agars. Suspected colonies are confirmed biochemically and by PCR.

Culture method
Briefly, the swab were added into 5 ml of buffered peptone water (BPW) (HiMedia, India) and incubated at 37 °C for 18 h. One ml of pre-enriched broth was transferred into tubes containing 10 ml selenite cystine broth (HiMedia, India) and 10 ml tetrathionate brilliant green

broth (HiMedia, India) and incubated at 37 °C for 24 h. After 24 h of incubation, a loop full of culture from each of the broth were streaked onto MacConkey and Hektoen enteric agar (HEA) [4]. After inoculation, the plates were incubated at 37 °C for 24 h and observed for typical Salmonella colony growth. Typical transparent and colourless colonies and greenish blue colonies with black centre appear on MacConkey and Hektoen enteric agar respectively. The suspected colonies were confirmed by conventional biochemical methods. The confirmed colonies were submitted to National Salmonella and Escherichia Centre, Kasauli for serotyping.

Results

Prevalence of Salmonella in layer poultry

A total of 900 cloacal swabs collected from healthy poultry flock which do not show any signs of Salmonellosis were processed during the study period. Out of these, 12 samples (0.01%) were found positive for Salmonella by conventional method of isolation and identification as recommended by ISO 6579:2002 standards [3].

Cultural and Morphological characterization

All the Salmonella isolates appeared colourless and transparent because of their non-lactose fermenting character. In Gram's staining, the bacteria appeared small rod shaped, Gram negative, either single or paired. On triple sugar iron slant tubes, most of the Salmonella isolates showed glucose fermentation, gas production from glucose and H2S formation. (Fig. 1 & 2)

Biochemical characterization

All suspected colonies of Salmonella were subjected to the following biochemical tests viz., indol formation, methyl red reaction, voges-proskauer reaction, citrate utilization, nitrate reduction, urea hydrolysis, triple sugar iron agar and pnenyl alanine deamination (table.1).

Serotyping result

All the twelve Salmonella isolates that were isolated were identified as *Salmonella enteritidis*.

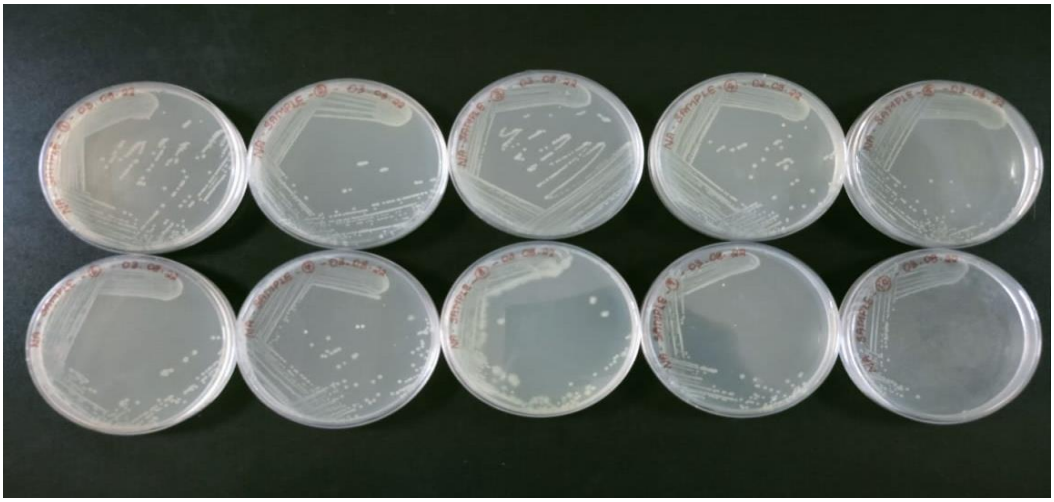

Fig 1: Colonies of isolated *Salmonella* sp. on Nutrient agar were moderately large 2-3 mm in dm, grey white, moist, circular disc with smooth convex surface and entire edge.

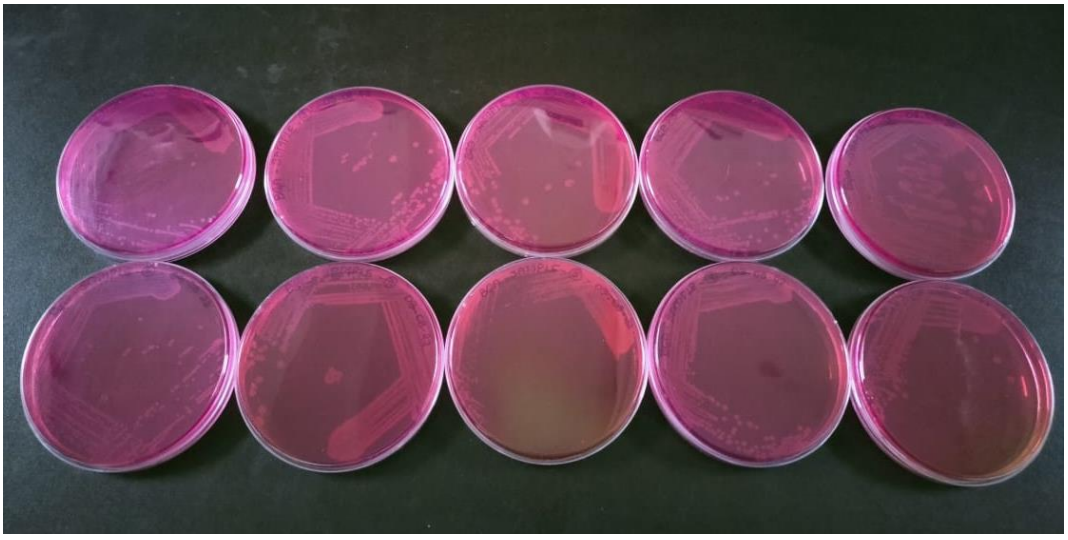

Fig 2: Colonies of isolated *Salmonella* sp. on Brilliant green agar appear as Red- pink-white opaque coloured colonies surrounded by brilliant red zones in the agar.

Table 1: Summary of the biochemical tests results of Salmonella isolates

Sample No	Methyl Red	Voges proskauer	Urease	H_2S	Citrate	Lysine	ONPG	Lactose	Arabinose	Maltose	Sorbitol	Dulcitol
1	Negative	Negative	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Negative
2	Negative	Positive	Positive	Negative	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Negative
3	Negative	Negative	Positive	Positive	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
4	Negative	Negative	Negative	Positive	Negative	Positive	Negative	Negative	Positive	Negative	Positive	Positive
5	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Negative	Positive
6	Positive	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Positive	Positive	Positive	Negative
7	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Positive
8	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Negative	Negative	Negative	Negative	Negative
9	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Negative	Positive	Positive	Negative
10	Negative	Negative	Positive	Positive	Positive	Positive	Negative	Negative	Positive	Negative	Positive	Positive
11	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive
12	Negative	Negative	Negative	Positive	Positive	Positive	Negative	Negative	Positive	Positive	Positive	Positive

Discussion

Poultry is a major source of food borne salmonellosis to human than any other food animals. The layer poultry is regarded as a potential vertically transmitting source for *Salmonella*. The major *Salmonella* serovar is *Salmonella Enteritidis* which is one of the most commonly identified serotype in poultry. In this study, specific biochemical media were used for the detection of *Salmonella* isolates. Majority of isolates were able to produce H_2S gas and none of them fermented lactose. The colony characteristics and the biochemical tests results were in accordance with the earlier available literature.

In conclusion, this study was used for the presence of *Salmonella* in layer poultry and the pattern of susceptibility for the commonly used feed grade antimicrobials give a better and wider understanding on their usage. The antimicrobials that control multiplication of a pathogen is of vital importance in food animals including poultry.

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