

## CHARACTERIZATION OF SALMONELLA SPS FROM CHICKEN SAMPLES

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**Abstract:** *Salmonella* infections are one of the most prevailing diseases in poultry causing food borne infections in humans. A study was carried out from few samples of retail chicken meat with an aim to characterize the *Salmonella* species both phenotypically and genotypically. The isolation of *Salmonella* sps from liver and intestines of poultry chicken is higher when compared to spleen, gizzard and other tissue samples. Phenotypic characterization was carried out by morphological appearance, motility and biochemical characterization tests like Methyl red, Citrate, TSI with or without hydrogen sulphide production were positive and negative for Indole, Voges proskauer, Urease suggesting firmly for confirmation of *Salmonella* infections. Genotypical confirmation was carried out by PCR amplification, the suspected isolates produce a product size of 550bp proving the presence of paratyphoid *Salmonellae*.

**Keywords:** Chicken, *Salmonella*, PCR, Antibiogram.

### Introduction

*Salmonella*, a Gram negative bacilli, belonging to Enterobacteriaceae family including 2541 serovars worldwide and in India nearly 235 serovars have been reported [1,11] so far. Chicken meat is mostly infected with different serovars of paratyphoid *Salmonella* which mainly include S. Typhimurium, S. Enteritidis and S. Heidelberg [4]. These *Salmonella* sps are responsible for economic losses in poultry and also pose public health significance [3].

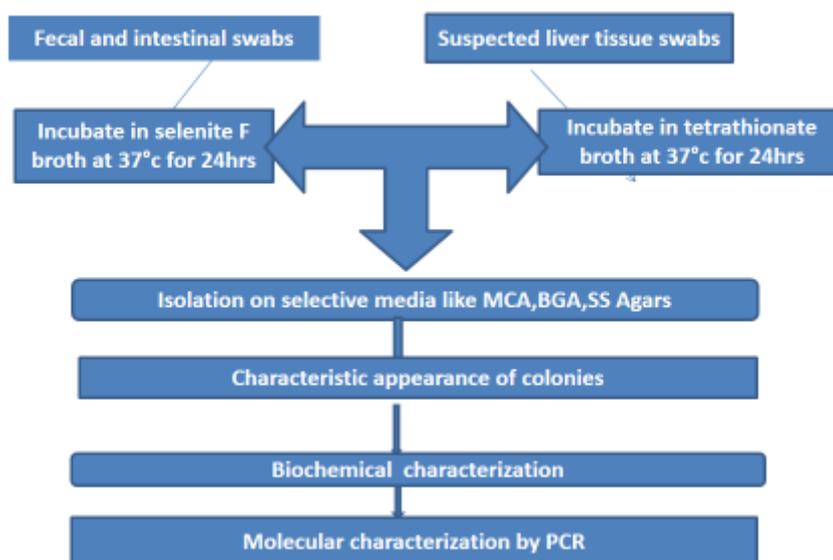
Conventional culture methods for the isolation of *Salmonella* sps is cumbersome and time taking and also includes biochemical characterization. Due to public health significance of *Salmonella*, it requires a rapid diagnosis and faster method for confirmation [10] PCR plays a promising role in arriving a conclusion of the *Salmonella* sps and aids in diagnosis of the infection [12] Hence the present study aimed at characterization of isolated *Salmonella* sps from retail chicken samples both phenotypically and genotypically.

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## Materials and Methods:

Samples: Samples of chicken meat mainly liver and intestines were collected from a retail chicken shop of Gannavaram, Krishna district. The samples were collected in sterile pre enrichment broth specific for *Salmonella* like Tetrathionate broth and selenite F broth.

## Materials and Methods



### Isolation of *Salmonella* sps on selective media:

The isolation of *Salmonella* species from pre enrichment broths was carried out by incubating at 37°C for 24 hrs. Cloudy growth was appreciated after a period of 24 hrs of incubation. Cultural isolation on MaConkey agar, Brilliant green agar and Salmonella shigella agar plate was done by streaking with the incubated samples from pre enrichment medium [7]

### Biochemical characterization:

The colonies from Salmonella Shigella agar were subjected to different tests like Indole, Methyl red, Voges proskauer, Citrate, Urease and TSI.

**Indole test:** The incubated culture is inoculated into peptone water and incubated for 24 hrs at 37°C. Upon addition of Kovacs reagent a positive reaction results in formation of pink colour ring in the presence of indole formation by the tryptophanase enzyme released by *Salmonella* bacterium.

**Methyl red test:** The incubated culture is inoculated into MR-VP medium and incubated for 24 hrs at 37°C. Release of acid upon fermentation of glucose decreases the pH below 4.0 and addition of pH indicator turns the medium red in color.

**Voges proskauer test:** The test result depends on the digestion of glucose to acetylmethylcarbinol in the presence of *Salmonella*. If glucose is being broken down, it will react with alpha-naphthol and potassium hydroxide to form a red color.

**Citrate test:** Citrate utilization test is used to determine the ability of bacteria to utilize sodium citrate as the only carbon source. The carbon dioxide released will subsequently react with water and the sodium ion in the medium to produce sodium carbonate, raising the pH towards alkalinity. Bromothymol blue indicator present in citrate agar turns the medium from green to blue.

**TSI:** Depending on the sugars fermented by *Salmonella* the TSI slant and butt produces different colors in the presence of phenol red indicator with or without H<sub>2</sub>S production.

**Urease test:** Ability of *Salmonella* bacterium to hydrolyse urea for production of ammonia and CO<sub>2</sub> resulting in a pH shift from light orange (pH 6.8) to magenta pink (pH 8.1) in the presence of phenol red.

**Direct microscopic examination:** Grams staining was performed to examine the smears prepared from the selective plates.

**Genotypic characterization: PCR** was used for the amplification of the genomic DNA, boiling method was employed for the extraction of DNA as per the method of Sambrook.

**Primers:** The primers used were as follows [6]

Forward primer – 16SF1(5'TGTTGTGGTTAATAACCGC3')

Reverse primer – 16SIII (5'-CACAAATCCATCTCTGGA3')

The components of PCR reaction mix is carried as following Master mix - 10 µl, Forward primer - 1 µl, Reverse primer - 1 µl, Bacterial DNA - 5 µl, Milli Q water - 3 µl. A total of 20 µl reaction mix was carried out for PCR amplification in Gene Amp PCR system 9700.

The PCR cyclical conditions were followed as per [2]. which include an initial denaturation of 94°C for 4 min, 35 cycles of 94°C for 1 min (denaturation), 58°C for 1 min (annealing) and 72°C for 30s (Polymerization) followed by 72°C for 10 min (final extension). The amplification products were checked for the presence of bands on 1.2% agarose gel.

**Antibiotic resistance pattern:**

The antibiotic disc diffusion method was done to test sensitivity of *Salmonella* isolates as per the Kirby and Buear method. The Muller-Hinton agar plates were incubated at 37°C for 48 hrs and the zone of inhibition of bacterial growth by the antibiotic discs was noted in comparison with the standard charts.

## **Results**

### **Direct microscopic examination:**

The cultural examination of the colonies formed on selective media were subjected for Gram's staining which revealed gram negative slender pink coloured rods. The culture from pre enrichment broth was subjected to motility test by hanging drop method and well defined actively motile slender rods of *Salmonella* are observed.

### **Phenotypic characterization**

On *Salmonella* Shigella agar – Transparent or translucent black color colonies

On Brilliant Green agar – Red color colonies were observed

On MaConkey agar - Pale color colonies were observed.

### **Biochemical characterization**

The morphologically confirmed *Salmonella* were biochemically characterized. The results of the biochemical tests were presented in table no. 1.

**Indole test:** Absence of Pink colour ring at the interface indicates absence of *Salmonella*.

**Methyl red test:** Large amounts of acid production by *Salmonella* turns the medium red in color upon addition of methyl red indicator.

**Voges proskauer test:** *Salmonella* does not ferment glucose thereby acetoin is not produced thus VP test is negative for salmonella

**Citrate test:** *Salmonella* utilizes citrate and produces sodium carbonate, raising the pH towards alkalinity. Bromothymol blue indicator present in citrate agar turns the medium from green to blue giving a positive reaction.

**TSI:** *Salmonella* produces pink color alkalineslant and yellow color acidic butt with H<sub>2</sub>S production.

**Urease test:** *Salmonella* does nohydrolyse urea thus magenta pink was not observed.

**Table No. 1 Biochemical characterization of Isolated *Salmonella* species**

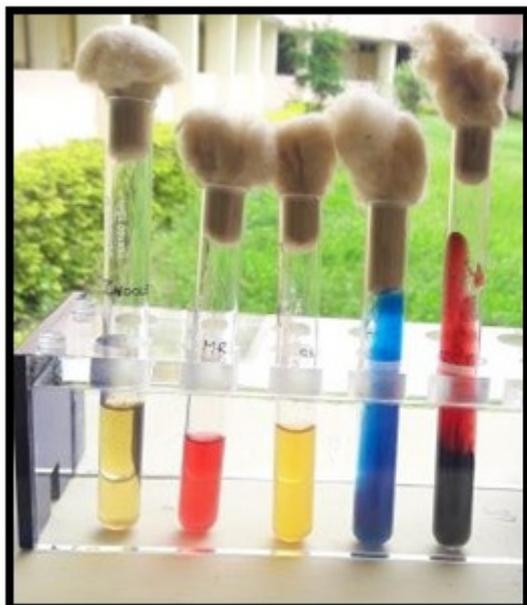
| Biochemical test                                  | Result of both Liver and Intestines samples                  |
|---|--|
| Indole test                                       | Negative   |
| Methyl red test:                                  | Positive   |
| Voges proskauer test:                             | Negative   |
| Citrate test:                                     | Positive   |
| Urease test:                                      | Negative   |
| Growth in Pre enrichment broth                    | Growth with cloudy turbidity                                 |
| Growth at 37°C on Macconkey                       | Round, smooth, translucent colonies with dew drop appearance |
| Growth at 37°C on Brillinat Green Agar            | Red color colonies were observed                             |
| Growth at 37°C on <i>Salmonella</i> Shigella agar | Pale color colonies were observed.                           |
| Motility  | Motile slender rods  |



**Black translucent colonies on *Salmonella Shigella* Agar**



**Red colour colonies on *Brilliant Green* Agar**

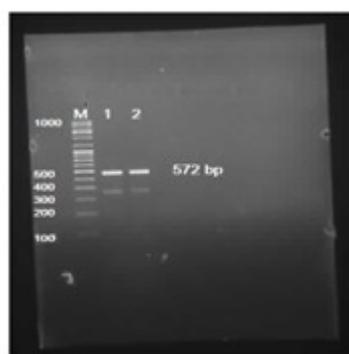


**Indole, methyl red, Voges-proskauer, Citrate and TSI Tests**



**Urease Test  
Salmonella (right) Negative  
A positive urease**

**Genotypic characterization:** The detection of *Salmonella* sps for various serotypes by PCR had been specific by using 16SF1 and 16SIII and positive bands were obtained at 572bp confirming the presence of Paratyphoid *Salmonella* sps. Serotyping was not done to confirm the specific serotype present in the samples.



Agarose gel stained with Ethidium bromide, with PCR products of *Salmonella* isolates (M:100bp DNA ladder; 1: *Salmonella* isolate from intestine sample; 2: *Salmonella* isolate from liver sample.

**Antibiogram:** A total of six antibiotic discs were used namely C (chloramphenicol), CIS (Ceftriaxone/sulbactam), S (Streptomycin), TE (Tetracycline), Na (Nalidixic acid), AMP (Ampicillin)

Out of six antibiotic discs tested, the susceptibility of the *Salmonella* sps was observed towards Chloramphenicol, Ceftriaxone/sulbactam, Streptomycin, Tetracycline and were resistant to Nalidixic acid and Ampicillin.

**Discussion:** *Salmonella* infections are one of the most important bacterial pathogens causing high morbidity and have public health significance too. Morphological, cultural and biochemical characterization of the isolated samples are suggestive of similar characters of salmonella infections. Isolation of *Salmonella* bacterium from liver and intestines samples is usually more common when compared to other samples [5,8]. The most reliable and confirmative diagnostic test targeting the specific gene has become a powerful tool in arriving a confirmative conclusion of the pathogens. In this study the isolates produced a product size of 572bp indicating the presence of Paratyphoid Salmonellae.

In conclusion, this study showed the presence of *Salmonella* bacterium from the samples of retail chicken shops mainly liver and intestines. Conventional isolation methods for detection of *Salmonella* are time taking thus application of PCR plays a promising role in confirmation of the pathogen at an early period as it is highly specific and sensitive.

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