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**Research Article**

# Rapid recovery of Salmonella from chicken meat and poultry fecal samples by selective pre-enrichment

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**ABSTRACT**

Salmonella is Gram negative; rod shape and non-lactose fermenter bacteria belong to Enterobacteriaceae family. Two most important Serotypes of *Salmonella enterica* transmitted from animal to human. Salmonella cause pullorum disease in chicken and cause huge economic loss in poultry industries. Chicken and chicken meat products are major sources of Salmonella outbreaks. Salmonella detection based on culture-based protocol. Conventional method is time consuming take 4-5 days for isolation of Salmonella. In this study Salmonella recovered from chicken meat and fecal samples by culturing samples in pre-enrichment media that contain selective chemicals that enhance the growth of Salmonella only and retard the growth of another micro biota. Chicken meat samples were collected from different chicken meat shops and poultry fecal sample were collected from poultry farm. Samples were treated with selective pre enrichment broth. Biochemical test was performed for conformation for isolation of Salmonella. Selective pre-enrichment method is efficient for rapid recovery of Salmonella. Disc diffusion method was used to check antibiotic susceptibility of isolates. The salmonella isolates that were recover from poultry feces were more resistant to antibiotics than those isolates from chicken meat.

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## Introduction

Salmonella is Gram negative rod shape non lactose fermenter intracellular facultative pathogen, 2 to 3 micrometers in size have ability to grow at different temperature (8 to 45) and pH (4 to 9.5) belong to Enterobacteriaceae family (Wang et al. 2013). Salmonellosis characterized by abdominal pain, diarrhea, fever and vomiting (WHO, 2018). Salmonella is transmitted by eating of contaminated food and water. Salmonella is major cause of foodborne disease worldwide. Salmonella spp. cause intestinal infections in human and animals (Hoffmann et al. 2015). According to WHO, each year 100,000 people dead due to Salmonella associated food poisoning worldwide each year (Hoelzer et al. 2011). Every year 78 million people are infected and 59 thousands dead by *Salmonella enterica* worldwide (Havelaar et al. 2015)

Salmonella also effect the economy and damage the poultry industry and huge expanses on Salmonellosis medication because $3.7 billion lose each year (Bierschenk et al. 2017). Major source that involve in spread of Salmonella in poultry are soil, contaminated water, animal feed and feces of infected chicken. Salmonella is transmitted into animal by fecal oral route by ingesting contaminated food and water (Abda et al. 2021). Chicken and chicken products are main source of Salmonella outbreak (Rasamsetti et al. 2021). Salmonellosis in human beings is occurring due to ingesting contaminated chicken meat and chicken meat products (Rasamsetti et al. 2021). Salmonella contamination occurs during slaughtering and processing process from contaminated equipment's (Chotinun et al. 2014). GIT associated microorganisms spread by poultry slaughtering and

processing especially during defeathering (Boubendir et al. 2021).

Contaminated chicken meat is major cause of Salmonellosis, report show that 48% Salmonella bacteria is isolated from fresh chicken meat sold in Thailand (Chotinun et al. 2014). Salmonella detection is based on conventional method. It includes several steps and time consuming, conventional method takes 4 to 5 days (Rasamsetti et al. 2021). Some study claimed that 6 hours are sufficient for pre enrichment. Buffered peptone water contains malachite green and Novobiocin increase the recovery of Salmonella were reported Salmonella Bile salt, brilliant green and malachite green add into selective broth reduces the growth of other bacteria (Flockhart et al. 2017).

Due to poultry associated salmonellosis in human beings rapid detection of Salmonella is very needed for testing of poultry feed, live chicken, chicken meat and poultry products to prevent Salmonella infection in humans and poultry farms (Crump et al. 2002; De Medici et al. 2003; Mumma et al. 2004; koyuncu et al. 2010). Malachite green retard the growth of coliform bacteria (Rappaport et al. 1956). Malachite green is resistant to high osmotic pressure, low pH level help Salmonella to proliferate (Dwivedi et al. 2014). Malachite green is effective for isolation of Salmonella (Elgea et al. 2005).

Against Gram positive bacteria bile salt has very high antimicrobial effect (wells et al. 2000). Bile salt kill microorganisms by oxidative DNA damage (Bakker et al. 1992., Bernstein et al. 1999). Gram negative bacterial cell wall contain oligopoly saccharides that is resistant to action of bile salt (Cremers et al. 2014).

Xylose lysine deoxycholate agar (XLD) is most common selective and differential agar for isolation of Salmonella (Rasamsetti et al. 2021). Tegretol 4 is present in XLT-4 that retard the Gram positive, Proteus and Pseudomonas species growth (Mallinson et al. 2000). Novobiocin is interfering the synthesis of bacterial DNA. Gram negative bacteria contain LPS in their cell wall that limits the permeability of Novobiocin. So, Novobiocin inhibit the growth of Gram positive bacteria (Bisacchi and Manchester, 2015). LPS present in cell wall of Salmonella is more resistant to Novobiocin than LPS of Proteus, Klebsiella and E. coli species (Elgea et al. 2005)**.** Novobiocin added into BPW and TT broth for increase selection of Salmonella to reduce growth of other micro flora (Jensen et al. 2003). Salmonella show antibiotic resistant that interfere the effective treatment. Antibiotic resistant developed into Salmonella pathogen due to continuous use of antibiotics in poultry due to which Salmonella infection could not treat effectively (Schlundt, Toyofuku, Jansen and Herbst, 2004). Cephalosporin and fluoroquinolones resistant strain of Salmonella were isolated from animal of different countries (Rahimi et al. 2013).

## Materials and Methods

### Sample colletion

For isolation of Salmonella, meat samples were collected from different chicken meat shops of Jhang bazar Faisalabad. After collection of meat samples were transferred into sterile polythene bags. Poultry fecal samples were collected from poultry farm of Faisalabad. After samples collection all samples were transported into Microbiology laboratory for cultivation and isolation in icebox.

### Sample processing

Total 30 meat sample which include chicken liver, gizzard and chest meat pieces were rinsed with buffered peptone water and placed these meat samples into sterile petri plate and collect their drops. Fecal samples were diluted into sterile water make 10-fold serial dilution.

### Isolation and identification

Samples were non-selective pre enriched in buffer peptone water incubate at 37°C for 24 hours. Then transferred 1ml of enriched buffered peptone water into the Tetrathionate broth for selective enrichment incubate at 42°C for 24-h. Take one loopful from the cultured broth and streaked prepared selective agar medium xylose lysine Tegretol agar and incubate at 37°C for 24-h (Andrews et al. 2018). Total 5 Salmonella isolates were recovered from chicken meat and 3 Salmonella sample were recovered from poultry fecal sample by conventional method. Isolates were confirmed by Gram staining and biochemical tests.

### Preparation of selective pre-enrichment media

Selective pre-enrichment media were prepared by mixing 1g bile salt into 1 litter buffered peptone water and autoclaved the mixture than mix 0.018g novobiocin into media. And poured into sterile test tubes.

### Isolation by selective pre-enrichment method

For isolation 1ml of samples were poured into 15ml Buffer peptone water and incubate at 37°C for 4-h. after incubation 1ml cultured buffered peptone water were taken and inoculated into selective pre-enrichment broth incubate at 37°C for 20-h. One loopful from selective pre-enrichment media were streaked on Xylose lysine tergitol-4 agar and incubate at 37°C for 24 hours. Black colored colonies were appeared on cultured plate after incubation.

Total 8 samples of Salmonella were recovered from chicken meat sample and 4 salmonella sample were recovered from poultry fecal sample by selective pre-enrichment method. Microscopic identification was done by Gram staining and observed under oil immersion lens. Pink rods were seen under microscope. Further confirmation of Gram-negative rods was done by biochemical test.

**Table 1:** Results of isolates of salmonella from chicken meat samples by conventional and selective pre-enrichment method

| **Selective Pre-Enrichment Media Ingredients** | **Number of chicken meat samples** | **Number of positive samples** |
|---|---|---|
| Buffered Peptone Water | 30 | 05 |
| Buffered Peptone Water, Bile Salt, Novobiocin | 30 | 08 |

**Table 2:** Results of isolates of salmonella from poultry fecal samples by conventional and selective pre-enrichment method.

| **Selective Pre-Enrichment Media Ingredients** | **Number of poultry fecal samples** | **Number of positive samples** |
|---|---|---|
| Buffered Peptone Water | 10 | 03 |
| Buffered Peptone Water, Bile Salt, Novobiocin | 10 | 04 |

- Combination of Novobiocin and bile salt show effective and 24 hours earlier results.

**Table 3:** Results of antibiotic sensitivity test of Salmonella isolates

| **Antibiotics** | **Dosage** | **Resistant** | **Susceptible** |
|---|---|---|---|
| Chloramphenicol | 30 | 07 | 5 |
| Erythromycin | 15 | 10 | 2 |
| Ampicillin | 10 | 5 | 7 |
| Cefotaxime | 10 | 3 | 9 |
| Doxycycline | 30 | 08 | 4 |
| Gentamycin | 10 | 10 | 2 |

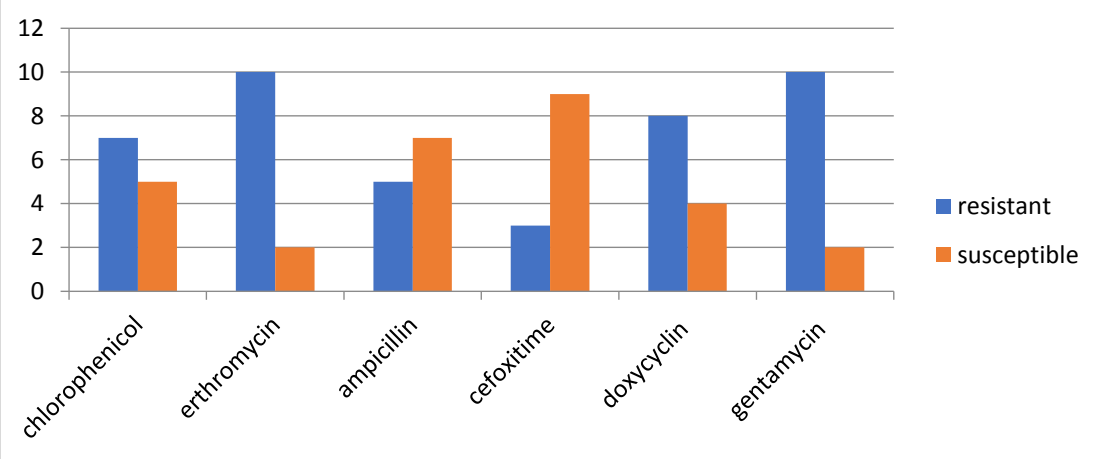


**Figure 1:** Representing Antibiotic Resistance Result

**Antibiotic susceptibility test**

This test is used to check that which antibiotic is most effective against a bacterial infection. This test is helpful for effective treatment of a disease. For determining the antibiotic susceptibility of Salmonella isolates were performed by using disc diffusion method. Total 12 sample were spread on Muller Hinton agar and incubate at 37°C for 24-h. After incubation zone of inhibition were measured.

**Results**

Total 40 samples including 30 chicken meat and 10 poultry fecal samples were collected for isolation of salmonella by conventional method and selective pre-enrichment method. By Conventional method 8 samples were positive for salmonella from 40 samples and salmonella isolation taken 3 days by conventional method then confirmed by biochemical test. By selective pre enrichment method 12 were positive for salmonella from 40 samples with 30% prevalence and salmonella were isolated in 2 days show in (Table 1 and Table 2). All salmonella isolates were treated with antibiotics including erythromycin, ampicillin, doxycycline, cefotaxime, chloramphenicol and gentamycin. out of 12 isolates 3 were resistant to all antibiotics and 5 were multi drug resistant. Sample was susceptible to Cefotaxime, Ampicillin and Chloramphenicol showed in Table 3 and Fig. 1.

**Discussion**

Salmonella species are most significant causing food poisoning in human beings and cause much disease in broiler with high mortality rate worldwide. Most important source of salmonellosis is chicken meat and chicken meat products (Hoffmann et al. 2015). Salmonella isolation is done by conventional method that take 4 to 5 days for isolation. This method is time consuming. Due to which effective treatment and diagnosis could not takes place (Rasamsetti et al. 2021). Among 30 chicken meat samples 5 samples were positive for salmonella that were cultured in non-selective pre enrichment media, 8 samples were isolated from 30 samples by selective pre-enrichment media. The results show that combination of bile salt and Novobiocin increase the selectivity of salmonella in pre enrichment step (Daquigan et al. 2016). This method is helpful for rapid isolation and identification of salmonella. This method take 2 days for isolation of salmonella then biochemical tests were performed (Rasamsetti et al. 2021).

Graph 1 shows that out of 12 isolates 5 were resistant to all drugs, 6 were multidrug resistant, 8 were resistant to more than one drugs. These results show that antibiotic resistance is increasing in salmonella as reported in (Abda et al. 2021). Major reasons of this antibiotic resistance are the misuse of antibiotics in poultry farm by farmers.

**Conclusion**

The result of this study indicates that the selective pre enrichment method is effective, accurate and time saving method for isolation of salmonella. The combination of selective ingredients (bile salt and Novobiocin) in buffered peptone water shows that it is best selective media for selective pre-enrichment of samples for isolation of Salmonella and Salmonella were recovered in 48 hours. Antibiotic sensitivity test indicates that antibiotic resistance is increasing in Salmonella species which is threat signal for human health.

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