# PREVALENCE OF *SALMONELLA* IN VARIOUS FOOD SOURCES OF A LOCAL MARKET IN PALLURUTHY, KOCHI.

A DISSERTATION SUBMITTED TO ST TERESAS'S COLLEGE (AUTONOMOUS), ERNAKULAM IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF

**DEGREE OF MASTER OF SCIENCE IN ZOOLOGY**

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## CERTIFICATE

This is to certify that the dissertation title entitled **"Prevalence of *Salmonella* in various food sources of a local market in Palluruthy, kochi"**. Is an authentic record of original project work carried out by **Sainaba Anees** (Reg no: SM23ZOO007), during the academic year 2023-2025, under my guidance in partial fulfillment of the requirement of the Degree of Master of science in zoology from St. Teresa's College (Autonomous), Ernakulam.

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# CERTIFICATE

This is to certify that the research work presented in this thesis entitled **"Prevalence of salmonella in various food sources of a local market in Palluruthy, Kochi."** is based on the original work done by Ms. **Sainaba Anees** under my supervision at Enfys Lifesciences Pvt. Ltd, Kochi, in partial fulfilment of the requirements for the award of the degree of Master of science in zoology from St. Teresa's college (Autonomous) Ernakulam and that no part of this work has previously formed the basis for the award of any degree, diploma, associateship, fellowship or any similar title or recognition.

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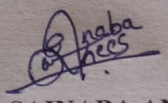
I hereby declare that the dissertation entitled **"Prevalence of *Salmonella* from a local market in Palluruthy, Kochi."** submitted to St. Teresa's College (Autonomous). Ernakulam in partial fulfillment of the requirements for the award of the Degree of Master of Science in Zoology is a record of original research work done by me under the supervision and guidance of Dr. Jean Mary joy, Assistant Professor. Department of Zoology. St. Teresa's College (Autonomous). Ernakulam and to the best of my knowledge and belief, this project contains no material previously published or written by another person, except where due reference is made.



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## ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| °C | Celsius |
| Spp | Species |
| Etc | And other small things |
| Eg | Example |
| BSA | Bismuth sulphite agar |
| XLD | Xylose lysine deoxycholate |
| SS | Salmonella shigella |
| TSI | Triple sugar iron |
| Et Al | And other |
| Hrs | Hours |
| +Ve | positive |
| -ve | Negative |
| Rs | Red slant |
| Ys | Yellow slant |
| Yb | Yellow butt |
| pH | Potential of Hydrogen |
| C | Chicken |
| F | Fish |

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# ABSTRACT

Salmonella spp. is one of the very common foodborne pathogens which has a substantial number of causative agents of gastrointestinal infections. In May 2024, raw chicken and eggs, fish, frozen burger patties, chicken breasts were collected from a local market in Palluruthy. These were first inoculated into the lactose broth and Rappaport-Vassiliadis (RV) medium and then spread-plated on BSA agar. All the three samples, fish, chicken and burger patty, after 18 hours incubation developed black colonies with a metallic sheen characteristic of Salmonella colony morphology. Same three samples on Xylose Lysine Deoxycholate (XLD) agar showed colonies having black centre and slightly reddish translucent zone. Confirmatory biochemical tests done on the specimens included the indole test, triple sugar iron (TSI) test, oxidase test, catalase test, and urease test, and all these tests were positive except for the test carried out on the fish, which therefore confirmed the presence of Salmonella in the sample of the fish.



Keywords: Salmonella, Foodborne pathogen, screening, food source, Palluruthy

# INTRODUCTION

Foodborne infections are the source of numerous illnesses that have a big impact on both the economy and human health. The traits of the most prevalent pathogenic bacteria, include *Salm*onella species, *Shigella* species, *Staphylococcus aureus, Cronobacter Sakazakii, Escherichia Coli, Listeria monocytogenes, Campylobacter jejuni, Clostridium botulinum, Clostridium perfringens, Cronobacter cereus.* Foodborne illness happens when a toxigenic pathogen establishes itself in a food product and produces a toxin that is subsequently consumed by the human host, or when a pathogen is consumed with food and establishes itself (and typically multiplies) in the human host. Consequently, foodborne illness is typically divided into two categories: (a) foodborne infection and (b) foodborne intoxication. Compared to foodborne intoxications, foodborne infections typically have a much longer incubation period before symptoms appear. Among these, salmonella is one of the major foodborne pathogens , which is a causative agent of salmonellosis , primarily a food poison syndrome. Salmonellosis is a bacterial infection that affects the intestinal tract and causes acute gastroenteritis. It is caused by *Salmonella* bacteria, which live in the intestines of many animals, including birds,poultry,pigs and cattles. Humans can contract salmonellosis through contaminated food, contact with infected animals,or person- person to transmission.
Contaminated foods include raw or undercooked meat,poultry,eggs and unpasteurized milk.

Palluruthy is a densely populated locality with a population density of 6785 people per $km^2$ where people rely on the local markets for food needs. Large outbreaks of salmonella can result from a minor salmonella exposure in these areas. Food products like raw poultry,eggs, seafood and processed meat items are often implicated in salmonella outbreaks, especially

when proper handling and storage practices are not followed. As *Salmonella* infection can lead to severe health complications,including enteric fever and septicemia, effective detection and control measures in food sources are critical Routine surveillance in local markets, especially for widely consumed products such as fish, chicken,eggs and processed meat, provides valuable data for understanding the prevalence and spread of this pathogen. In
Palluruthy such a monitoring can be beneficial.The food items in local markets are often distributed through diverse, sometimes informal supply chains, creating the potential for contamination.

Microbiological control in the food industry plays critical role in preventing *salmonella* outbreaks.Test and media are used for identification of salmonella take advantage of unique aspects of *salmonella* physiology or biochemistry relative to other genera within the family of enterobacteria. The majority of the bacteria of the genus salmonella are facultative anaerobes. gram negative rods, oxidase- negative, catalase - positive. Most strains are motile and ferment glucose with the production of both acid and gas. The media used currently for the differentiation and identification of *salmonella* are still based on the detection of carbohydrate fermentation indicated by pH indicator, the detection of proteloytic activity, hydrogen sulphide production and selectivity. Here we used bismuth sulphite agar (BSA) for primary screening and xylose lysine deoxycholate (XLD) and Salmonella shigella agar (SS).

The main difficulty in identifying salmonella was that species resemble *Salm*onella in both morphology and cultural properties showed positive results in *salmonella* specific media like salmonella shigella (SS)agar, bismuth sulphite agar (BSA) agar and xylose lysine deoxycholate (XLD), which resulted in falsely positive *Salmonella* identification results. Biochemical tests that are specific for *Salmonella* ,such as the urease indole triple sugar iron, oxidase and catalase tests,are utilized to overcome this difficulty. Isolates that test positive in these tests are then further validated by sequencing.

Understanding the pathogenic potential of *Salmonella* greatly depends on being able to identify the specific species through identification. Because the severity and degree of disease caused by different *Salm*onella species can vary. And also correct identification is vital in establishing treatment options because some strains may have antibiotic resistance to

medications which might interfere with treatment operations. The primary objective of study is screening of *Salmonella* from various food sources.

## AIM OF THE STUDY

The screening of *Salmonella* from various food sources such as chicken, fish eggs, frozen chicken products (chicken breast, burger patties).

## OBJECTIVES

- Screening of *Salmonella* from various food sources.
- Isolation and enrichment of *Salmonella* in lactose broth and RV medium.
- Primary screening in bismuth sulphite agar
- Secondary screening in xylose lysine deoxycholate and Salmonella shigella agar
- Biochemical characterization
- Molecular confirmation

## RELEVANCE OF THE STUDY

The study underlines the importance of careful food safety monitoring, as salmonella remains a common and possibly deadly contamination in variety of local food sources.

## REVIEW OF THE LITERATURE

Martin Busse (1995) investigated the function of selective enrichment media in salmonella isolation. He discovered that the isolation of *Salmonella* depends heavily on selective enrichment media. For almost a decade, Rapport vassiliadis medium has been utilised as a selective enrichment medium for *Salmonella. It* was a novel and feasible medium for selective enrichment of *Salmonella* and motility enrichment, a very promising technique that is also essential for *Salmonella* identification.

The uses and mechanism of eleven enrichment and eight plating media, as well as the incubation temperatures and duration and serotype specificity involving enrichment and plating media,were published in the study by Diane J.et al., (1976). Additionally, the type of the sample, the amount and proportion of *Salmonella, and* the enrichment emulsifying agent used during incubation. Additionally, they looked at additional recovery- related elements such Media brand, storage media; all of these provide suggestions for further research.

Classification by serotyping is the crucial first step in characterizing *Salmonella* isolation and is importance for surveillance, source tracking and outbreaks detection, according to the study by Catherine yoshida et al., (2016). All rapid and high throughput molecular *Salmonella* serotyping techniques are utilized to enhance the detection and lessen the impact of salmonellosis. They contrast the salmonella geno serotyping array (SGSA)created in their labs with three commercially available kits: check & trace (check - points), xMAP(xMAP)

*Salmonella* serotyping assay) and Salm SeroGen (Salm sero- Genotyping AS-1 kit). They made use of 321 isolates, which are representative of frequently reported serovars from both non-human and human sources worldwide. Of the isolates evaluvated ,73.8% to 94.7% were successfully identified using four methods. The clinically significant *Salmonella* serovars Enteritids and Typhimurium were accurately identified by the approaches in 85 % to 98% of cases respectively. 75% to 100% of the non- typhoidal broad host range *Salmonella* serovars such as Heidelberg, Hadar, Infantis, Kentucky and others were accurately identified by the procedures. It was predicted that serotyping would be eventually replaced by whole genome sequencing in public health labs.

According to a study on 1995 by Monika Tietjen et al., which highlights the strict importance of strict food safety regulations. Also highlight the use of recent advanced diagnostic technology such as the commercial available methods and kits that make use of new technologies to make it easier to identify the Salmonella in food. These inculde automated instrumentation DNA/RNA probes, antibody dependent assays, new media formulations, miniaturized biochemical assays and polymerase chain reaction are opposed to the conventional techniques for identifying and isolating Salmonella in food that depends on the pre-enrichment, selective enrichment, serological confirmation and biochemical assays are used to identify and isolate salmonella.

In a study by katsuyuki Gabai et al., (1997) six commercial kits were compared to the Japanese standard method for *Salmonella* isolation in foods and the US Food and drug administration (USFDA) method. Many of these approaches worked effectively when they just screened *Salmonella* serovars. However, only USFDA approch and immuno magnetic sepration combined with Xylose lysine brilliant green agar method (MS-XLBG) were able to detect *Salmonella* serovars in food that had been artificially contaminated. The MS-XLBG approach identified *Salmonella* from six samples, and USFDA method identifies all the seven wild - type *Salmonella* serovars.

Fahim shsltout et al., (2019) study to determine the amount of salmonella present in certain meat and poultry products. Thirty samples of fresh chicken cut - up (skinless and boneless breast and thigh) 15 of each were selected from various markets in the Menoufia Governate, Egypt and total of ninety random samples of various chicken meat items were used.30 samples

of cooked chicken products (Shawarma and fahita Sandwiches) and 30 samples of frozen breaded half cooked chicken products (Chicken nuggets and chicken panne). While *Salmonella anatum* was found in 7% of nugget samples, *Salmonella typhimurium* was found in 13.3%,20%,6.7% of the breast, thigh, panne samples and 13.3 % of breast samples had *Salmonella enteritidis.* 6.7% of the tested samples contained isolated strains of *Salmonella heidelberg, muenster and kentucky.* They Came to the conclusion that everyone who had access to the food, including consumers and food handlers, need to made aware of the value of hygiene.

A study by J O ogah et al., (2015) demonstrates that food workers are crucial in the spread of foodborne illness. In lagos. Southwest nigeria, they sought to ascertain the frequency of salmonellosis among food handlers and its effect on consumer health. They gathered data using conventional procedures ,235 blood samples were drawn from food handlers at different places on Victoria Island and Bargia and placed in EDTA vials. To assess 235 respondents (food handlers) for understanding their knowledge on food safety, questionnaires were given out. According to IgG and IgM anti- *Salmonella* immunoglobulin, 31.5% of the population under the study had either a recent or past *Salmonella* infection. Whereas 68.5% had neither a recent or past infection. Ninety-three of the respondents were men and 142 were women in the 11-60 age range who were actively employed. Of the 93 males who were screened for *Salmonella enterica* serovar typhi and paratyphi infections, 26 were infected ,67 was not and 48 of the 142 females were infected. They came to the conclusion that food handlers on lagos southwest nigeria were potential risk factors rather than the cause of foodborne illness.

The prevalence of *Salmonella* from raw vegetables in china was examined by Xiaojuan yang et al., (2020). They looked into molecular traits and frequency of *Salmonella* isolates from the raw vegetables in China. They gathered 406 samples of raw vegetables from most chinese provincial capitals. *Salmonella* contamination levels were less than 1 MPN/g and the overall prevalance was 3.4%. The largest contamination rates were seen in lettuce (6.0%) and coriander (7.8%). Fifteen multilocus sequence typing patterns and fourteen distinct servoras were found among the 31 *Salmonella* isolates that were isolated from 14 positive samples. Every serovar that has been found infected people before, and some of them have also been connected to outbreaks of diseases linked to raw vegetables. According to this study, a systemic surveillance of the prevalence of *Salmonella* in Chinese raw vegetables is necessary.

Mondal and colleagues (2008) The investigation was conducted to define duck *Salmonella* using a biochemical test and to isolate and identify the *Salmonella* organisms from both diarrheal and seemingly healthy ducks. Duck from three distinct areas - char Nilokhuya, BAU poultry farm and Boyra were used to gather a total of 65 cloacal samples. Nine of the the 65 samples were tested positive. The antibiotic sensitivity pattern revealed duck isolates were extremely resistant to chloramphenicol but extremely sensitive to ciprofloxacin, kanamycin etc.

M J Islam et al., (2016) conducted a study to isolate the associated *Salmonella spp* from cloacal swabs of broiler and their antibiogram studies. They collected a total of 80 cloacal swabs comprising 50 samples of apparently healthy broiler and 30 samples of diarrheic broiler were collected from different poultry farms at Sylhet, Bangladesh during January to June 2013. These samples were then subjected for isolation and identification of Salmonella spp. They conducted a series of conventional bacteriological studies like study of morphology, staining properties, and biochemical characteristics. Their results shows that 48% (n= 24/50) swab samples of healthy broiler and 66.7% (n= 20/30) diarrheic broiler were found to be associated with Salmonella spp. Among that the 44 positive Salmonella isolates 47.73% (n= 21) were Salmonella pullorum, 36.36% (n= 16) isolates were *Salmonella gallinarum* and the rest 15.91% (n= 7) isolates were Salmonella typhimurium. They conducted sensitivity test against 10 commonly used antibiotics, of which Penicillin-G, Erythromycin, Ampicillin, and Bacitracin were found to be resistant, and Ceftriaxone, Gentamicin, and Chloramphenicol showed considerably better sensitivity as compared to others. They concluded that *Salmonella spp* are present in broilers, and bacteria can be clinically controlled by using Ceftriaxone, Gentamicin, and Chloramphenicol.

Nesa et al., (2011) conducted a study on isolation and identification of *Salmonella* serovars from human stool and characterization of the isolated serovars using biochemical, serological, molecular and antimicrobial sensitivity techniques. A total of 25 samples were collected of which 16% were positive to Salmonella serovars. Results of their antimicrobial susceptibility testing showed that the isolated Salmonella serovars were highly sensitive to ciprofloxacin and moderately sensitive to chloramphenicol, kanamycin, cotrimoxazol and nalidixic acid. The

positive isolates were resistant to erythromycin. This study indicates that ciprofloxacin can be used as a first line therapy for the treatment of Salmonella gastroenteritis.

Ozbey G et al., (2006) conducted a study to identify *Salmonella spp* from the samples such as carcass, intestine,liver,gallbladder from Elazig province. The identity of Salmonella spp. was further proved by culture and by polymerase chain reaction (PCR). A total of 1250 samples were tested. They collected all samples were collected from different commercially reared chicken flocks. Chicken carcasses, intestines, livers, gall bladders and spleen were found positive with proportions of 12%, 7.2%, 4%, 2% and 1.6% respectively, by both culture and PCR. The Salmonella detection rate was the highest (12%) in chicken carcasses and the lowest (1.6%) in spleen. They concluded that *Salmonella Spp* were widespread among the chicken population in Elazig.

Jamshidi et al, (2010) Conducted a study to isolate and identify the *Salmonella enteritidis* and *Salmonella typhimurium* from the eggs of retail stores in Mashhad. In this study they collected a total of 250 eggs were collected randomly from 50 retail stores in Mashhad city over a period of 3 months in the summer of 2008.They collected five samples from each store were collected, and transferred to the laboratory. In this study they used conventional culture method - including pre-enrichment, enrichment, selective plating and differential plating was performed to isolate salmonella spp. They used multiplex polymerase chain reaction assay and they use three pairs of primers such as S141 and S139 for the invA gene, specific for the genus of Salmonella; Fli15 and Tym for the flic gene, specific for Typhimurium serovar; and Prot6e-5 and Prot6e-6 for Prot6E gene, specific for Enteritidis serovar to confirm these isolates are salmonella spp and for determining serovars as
Typhimurium and Enteritidis serovars. They concluded that Four out of 250 samples (1.6%) from eggshells were determined as contaminated with Salmonella spp. And isolated colonies were salmonella and their serovar was determined as Typhimurium.

A study by Rosie B E shows that successful isolation of *Salm*onella depends on a large number of factors such as food preparation procedures, the number of organisms present, sample handling after collection, etc. In case of raw meat samples, the competitive flora may be the most crucial factor and it varies from sample to sample and from one kind of meat to another.

In a study by Mridha D et al., they collected a total of 352 samples, including 128 cloacal swabs, 32 whole carcasses, 64 feed, 64 water, and 64 attendants' hand rinses. They used a variety of cultural and biochemical methods for the estimation of prevalence, isolation, and identification of *Salmonella spp*. They also evaluated the difference of *Salmonella* positivity status between two groups of farms, one of which was comprised of good practices adapted in broiler rearing at the project intervened farms, and the other was non-project intervened traditional farms. The findings were verified by polymerase chain reaction.For further characterization, a serogrouping by slide agglutination test and an antimicrobial susceptibility test utilizing disk diffusion techniques were carried out by them the overall prevalence of Salmonella spp. is 31.25%, according to their results. They came to the conclusion that there was a considerable difference in the prevalence of *Salmonella spp* between project-intervened and non-project-intervened farms. This study suggests that, from the perspective of public health and food safety, project-intervened farms are generally safer than non-intervened farms.

According to a 1995 study by Monika Tietjen et al., only a very small percentage of Salmonella cells can be infectious, underscoring the critical significance of stringent food safety laws. Additionally, emphasize the use of modern, sophisticated diagnostic tools, such as commercially accessible techniques and kits that use these tools to make it simpler to detect Salmonella in food. In contrast to the traditional methods for identifying and isolating Salmonella in food, which rely on pre-enrichment, selective enrichment, serological confirmation, and biochemical assays, these methods include automated instrumentation DNA/RNA probes, antibody-dependent assays, new media formulations, miniaturized biochemical assays, and polymerase chain reaction

# MATERIALS AND METHODOLOGY

Sample collection

For this study,food samples, including eggs, chicken, fish, frozen chicken products such as burger patties and chicken breast are collected from local market in Palluruthy, Kochi, Kerala.

Bacterial Isolation and Enrichment

Samples are subjected to a two-step enrichment process based on standard protocol for salmonella isolation.

Pre-enrichment: samples were first inoculated in lactose broth (Table 1) and incubated at 37°C for 24 hours (Aktar.et al.,2016).

Table 1. Composition of lactose broth

| Ingredients | gm/liter |
|---|---|
| Peptone | 5.00 |
| HM Peptone B # | 3 00 |
| Lactose | 5.00 |

| | |
|---|---|
| Final pH (at 25°C) | 6.9±0.2 |

Selective enrichment: Following pre- enrichment, samples were inoculated into Rappaport - vassiliadis (RV) medium (Table 2) and incubated at 42° for 24 hours (Rahman et al.,2019).

Table 2. Composition of RV medium

| Ingredients | gm/liter |
|---|---|
| Soya peptone | 4.500 |
| Sodium chloride | 7.200 |
| Potassium dihydrogen Phosphate | 1.440 |
| Magnesium chloride | 36.000 |
| Malachite green | 0.036 |
| Final pH (at 25°C) | 5.2±0.2 |

Primary screening

The enriched samples were first spread plated on bismuth sulphite agar (BSA), a selective medium for salmonella. Colonies were incubated at 37°C for 24 hours, allowing for characteristic black colonies with metallic sheen to form if salmonella was present.

Table 3. Composition of bismuth sulphite agar

| Ingredients | gm/liter |
|---|---|
| Beef extract | 6.0 |
| Peptic digest of animal tissue | 10.0 |

| | |
|---|---|
| Ferric citrate | 0.4 |

| | |
|---|---|
| Brilliant green | 0.01 |
| Bismuth ammonium citrate | 3.0 |
| Sodium sulfate | 10.0 |
| Di sodium hydrogen phosphate | 5.0 |
| D+ Glucose anhydrous | 5.0 |
| Agar | 24.0 |
| pH | 7.6±0.2 |

Secondary screening

To confirm the presence of salmonella, colonies from BSA were plated on xylose lysine deoxycholate (XLD) and salmonella shigella ss agar. Colonies showing a black center on XLD or opaque on ss were selected for further biochemical testing (Rahman et al., 2019).

Table 4. Composition of xylose lysine deoxycholate

| Ingredients | gm/ liter |
|---|---|
| Yeast extract | 3.00 |
| L- lysine | 5.00 |
| Lactose | 7.50 |
| Sucrose | 7.50 |
| Xylose | 3.50 |
| Sodium chloride | 5.00 |

| | |
|---|---|
| Sodium deoxycholate | 2.50 |
| Sodium thiosulphate | 6.80 |
| Ferric ammonium citrate | 0.80 |
| Phenol red | 0.08 |
| Agar | 15.00 |
| pH | 7.4±0.2 |

Table 5. Composition of salmonella shigella agar

| Ingredients | gm/liter |
|---|---|
| Peptone | 5.000 |
| HM peptone B# | 5.000 |
| Lactose | 10.000 |
| Bile salt mixture | 8.500 |
| Sodium citrate | 1.000 |
| Sodium thiosulphate | 0.33 |
| Ferric citrate | 1.000 |
| Brilliant green | 0.33 |
| Neutral red | 0.025 |
| Agar | 15.000 |
| pH | 7.0±0.2 |

Biochemical confirmation

Based on the secondary screening done in SS and XLD colonies which were selected as salmonella Spp.These isolates were purified and subcultured on nutrient agar slant. These slants were considered as working stock and stored at 4°C for further experimentts.

The biochemical confirmation is crucial in salmonella identification because many salmonella like species which share common morphological and cultural properties can be wrongly identified as salmonella and gives false reslut.

For confirmatory testing,five biochemical tests specific for salmonella spp.were performed: The urease test, indole test, triple sugar ion test, oxidase test and catalae test. The samples were inoculated and incubated at 37°C for 24 hours.

1. Indole test

Indole test demonstrates the ability of certain bacteria to decompose amino acid tryptophan to indole which accumulates the medium.

Test: using a loop a small amount of bacterial culture was inoculated in tryptone broth and incubated at 37°C for 24 hours.

2. Urease test

The test is used to determine the ability of an organism to split the urea through the production of enzyme urease.

Test: using a loop a small amount of bacterial culture was inoculated in urea agar slant and incubated for 37°C for 24 hours.

*3. Triple sugar iron test (TSI)*

Triple sugar iron test is a microbiological test that demonstrates microorganisms' ability to ferment sugar and to produce hydrogen sulphide. Which is used to differentiate among the different groups of enterobacteria. It is often used in selective identification of enteric bacteria.

Test: using a loop a small amount of bacterial culture is inoculated in TSI agar slants and incubated at 37°Cfor 24 hours.

*4.oxidase Test*

This test detects the cytochrome oxidase system that will catalyze the transport of electrons in the bacteria.

Test: Using a loop a small amount of culture was placed on a disc and observe for colour change

5. catalase test

The test detects the presence of catalase, an enzyme that causes the realse of oxygen from hydrogen peroxide by breaking down into water and oxygen gas.
Test: A small amount of catalase enzyme was placed on the top of bacterial culture and observe for bubble production.
TSI, urease, indole inoculated samples are incubated for 24 hours at 37°C.

*DNA Isolation*

Biochemical characterization is followed by sequencing to ensure that isolate was a salmonella spp.

DNA was extracted 6a loopful of well grown bacteria following standard phenol - chloroform method (Sambrook and Russel,2001. Briefly the culture was incubated at 37°C in a lysis buffer (Nacl 400mM, sucrose 750mM, EDTA 20mM and Tris Hcl 50mM), contains 1 mg ml-1 lysozyme. subsequently SDS (1%) and Proteinase k (100µg ml-1) were added to the solution and the incubation was continued. The crude DNA sample extracted with chloroform : isoamyl alcohol ( 700 µl of 24:1 mixture ) twice and the aqueous phase containing DNA was retrieved by centrifugation at 10000 rpm for 10 min.Further,0.6 volume of isopropanol was added and the DNA was allowed to precipitate at -20°C for 60 min.The DNA was pelleted and then washed times with 70% ethanol and dried at room temperature for 20-30 min.The DNA was dissolved in TE buffer/ Milli Q Water (~30 µl) and quality of DNA was confirmed by Agarose gel electrophoresis.

*PCR*

The 16s rRNA gene from the bacterial DNA was ampilified in 25 µl reaction volume containing 1 µl DNA (10-50ng) 1 µl of each forward and reverse primers (10 picomoles µl-1) and 10 µl emerald Amp GT PCR master mix ( Takara).The cycling conditions used are as follows: initial denaturation at 95°C for 2 min, followed by cycle denaturation at 95°C for 40s annealing at 55°C for 40 s extension at 72°C for 1.5 min. For a total of 30 cycles and a final extension for 10 min at 72°C.The success of PCR reaction was confirmed by running a 5µl PCR product on 1% of agarose gel (impregnated with ethidium bromide) at 120V; for ~45 min in 1X TAE

buffer. The image of the gel was recorded with the UV gel documentation system for further reference.

*Primers used*

| SI.NO | PRIMER NAME | PRIMER SEQUENCE | REFERENCE |
|---|---|---|---|
| 1 | 27F | AGAGTTTGATC(AC)TGGCTCAG | Lane,1991 |
| 2 | 1492R | GTTACCTTGTTACGACTT | Lane,1991 |

*Preparation for sanger sequencing*

*PCR Product clean up*

The 1500bp PCR product was gel purified and then was used as template for sequencing PCR

*Sanger sequencing PCR*

Sequencing PCR was done with ABI PRISM Big due terminator ready reaction mix( Life technologies USA).

*Purification*

The cycle extension products were purified following ethanol/EDTA/Sodium acetate precipitation.

*Machine*

Applied biosystems AB1 3730xl DNA

*Bioinformatics analysis*

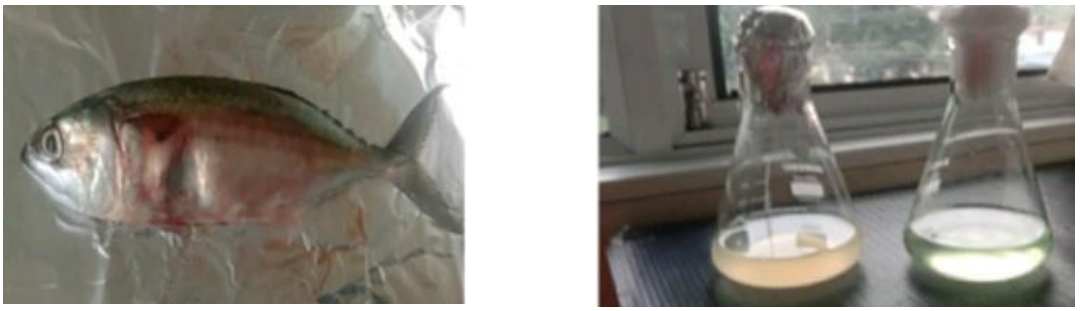
Import AB1 FILES
|
Quality
|
Export trimmed sequence as fasta
|
NCBI Blast analysis

The sequences were quality checked, trimmed and assembled using the software sequencer V4.10.1 (Gene codes corporation, Anno Arbor, MI USA). Out of the 12 samples sequenced (2D) nine samples were good enough for assembly. Assembled sequence were searched in NCBI using BLAST tool and identity of the samples were confirmed based on percentage similarity and query coverage of the nearest neighbours

## RESULTS

Sample collection and enrichment

All the samples (Egg, chicken, Fish. Chicken breast, chicken burger patties and water samples were successfully enriched in both lactose broth and RV medium. Figure 1 showing representative images of samples enriched in both broth, demonstrating adequate bacterial growth for further screening.



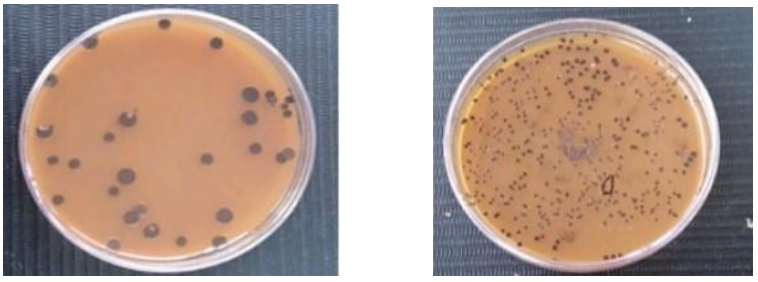
**(a)** **(b)**

Figure 1: (a) fish sample collected (b) sample enriched in lactose broth and RV medium.

Primary screening in bismuth sulphite agar

After plating in bismuth sulphite agar **(**BSA) and incubating, colonies with black pigmentation and metallic sheen, characteristic of salmonella, were observed on the plates of chicken, fish, burger patties. Figure 2 demonstrates the characteristic morphology of these colonies, confirming successful initial isolation. The use of selective and differential plating media is

very important part of standard cultural methods for the isolation of salmonella from food and environmental samples ( Warburton et al.,1994).Bismuth sulphite agar are used in the standard methods of different agencies in Canada, the United States and Europe (Flowers et al., 1992).BIS uses bismuth sulphite and brilliant green as inhibitory against competing microorganism and ferrous sulphate as the indicator of H2S production (Difco,1984).



**(a)** **(b)**

Figure 2: (a) chicken spread plate in BSA (b) Egg spread plate in BSA.

Secondary screening on XLD and SS agar

Colonies isolated from BSA that displayed salmonella like morphology were further screened on SS and XLD agar. Colonies of interest on XLD showed black center with a translucent red halo. While SS agar colonies were smooth and opaque.XLD uses sodium deoxycholate, sodium thiosulphate, ferric ammonium citrate and phenol red as selective agents, indicator of H2S production and carbohydrate utilization respectively (Anon,1984). SS agar screens for salmonella by its ability to reduce sulfur compounds to HS (indicated by black colonies) it's inability to ferment lactose (resulting in colourless colonies), and its resistance to bile salts and brilliant green dye. These characteristics make ss agar an effective medium for detecting salmonella in mixed microbial samples (Maddocks et al.,2002).

(a) (b)

(c) (d)`

Fig 3 : Images showing (a) SS Chicken plate (b) XLD Chicken plate (C) SS egg plate (d) XLD egg plate.

A total of 8 colonies were chosen from the secondary screening for biochemical testing: 1 from chicken and 7 from fish.

Table 6: Number of isolates per sample

| Sample name | Number of isolates |
|---|---|
| Egg | 0 |
| Chicken | 1(C12) |
| Fish | 7(F1,2,4,8,9,10,12) |
| Chicken breast (Frozen product) | 0 |
| Chicken burger patties (Frozen product) | 0 |

Biochemical characterization

The biochemical characterization of isolates, including oxidase, catalase, TSI, urease, indole tests, confirmed the presence of salmonella spp in fish isolate (F10)

Table 7: Biochemical results of for chicken (C), fish (F) and water (W) isolates.

| Isolates | Oxidase test | Catalase test | TSI test | Urease test | Indole test |
|---|---|---|---|---|---|
| Postive control | Negative | Positive | RS YB gas +ve or -ve/H2S +ve | Negative | Negative |
| C12 | Negative | Positive | RS YB H2S+ve , gas-ve | Positive | Negative |

| | | | | | |
|---|---|---|---|---|---|
| F1 | Positive | Negative | RS YB H2S+ve, gas-ve | Positive | Negative |
| F2 | Positive | Negative | RS YB ,H2S+ve, gas-ve | Positive | Negative |
| F4 | Positive | Negative | RS YB, H2S+ve, gas-ve | Positive | Negative |
| F8 | Positive | Negative | RS YB, H2S+ve, gas-ve | Positive | Negative |
| F9 | Positive | Negative | RS YB, H2S+ve, gas-ve | Positive | Negative |
| F10 | Positive | Negative | RS YB, H2S+ve, gas-ve | Negative | Negative |
| F12 | Positive | Positive | YS YB, H2S+ve, gas-ve | Positive | Positive |

So, these biochemical tests confirmed the presence of salmonella spp in fish isolate (F10) and differentiated it from closely related organisms. salmonella was found to be oxidase negative and catalase - positive, which is consistent with its typical biochemical profile. The TSI test shown a alkaline slant and acidic butt with hydrogen sulphide (H2S) production indicated by a black precipitate, a hallmark of salmonella sulfur reduction ability. Additional urease test was negative, distinguishing salmonella from urease positive organisms like proteus. The indole test was also negative, aligning with salmonella inability to produce indole. These results underscore the reliability of these biochemical tests in confirming salmonella spp. and differentiating them from other enteric pathogens.

These findings affirm the presence of salmonella in fish samples are consistent with the pathogenic metabolic profile. Further sequencing of isolates will confirm its species level identification.

*DNA isolation*

Good quality high molecular weight genomic DNA suitable for further downstream process were obtained fig 4.

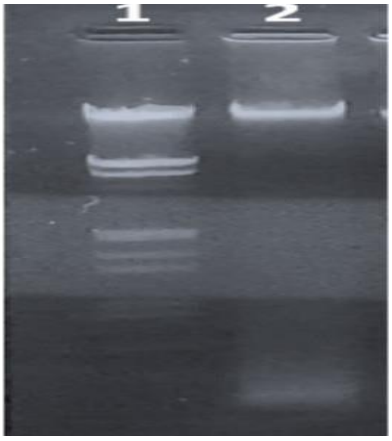



Figure 4: agarose gel image of the genomic DNA (a) Lambda DNA EcoR1 Hind III double digest DNA ladder (1000bp) (b) F10.

*PCR AMPLIFICATION*

Bacterial 16s rRNA gene was successful ampilified from the genomic DNA of the sample F10. The PCR product is approximately 15000bp in size. The concentration of PCR product is good enough for sequencing, without any non-specific amplification products seen in the gel. (Figure 2).

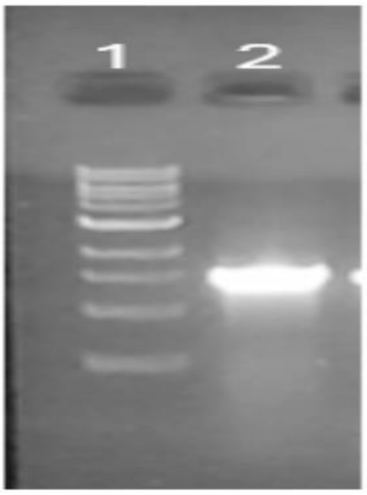


Figure 5 :(a) kb ladder (b).Bacterial 16s rRNA PCR product F10.

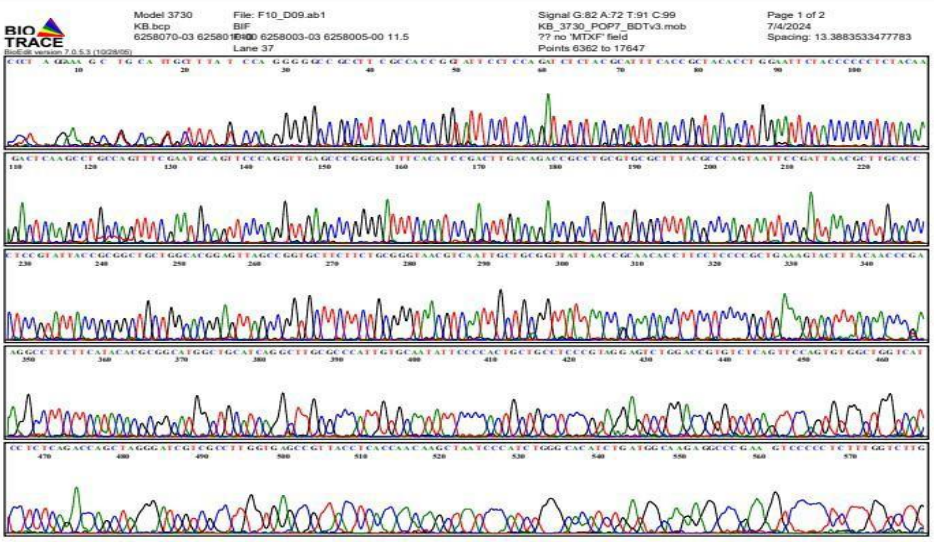
NCBI BLAST result table.

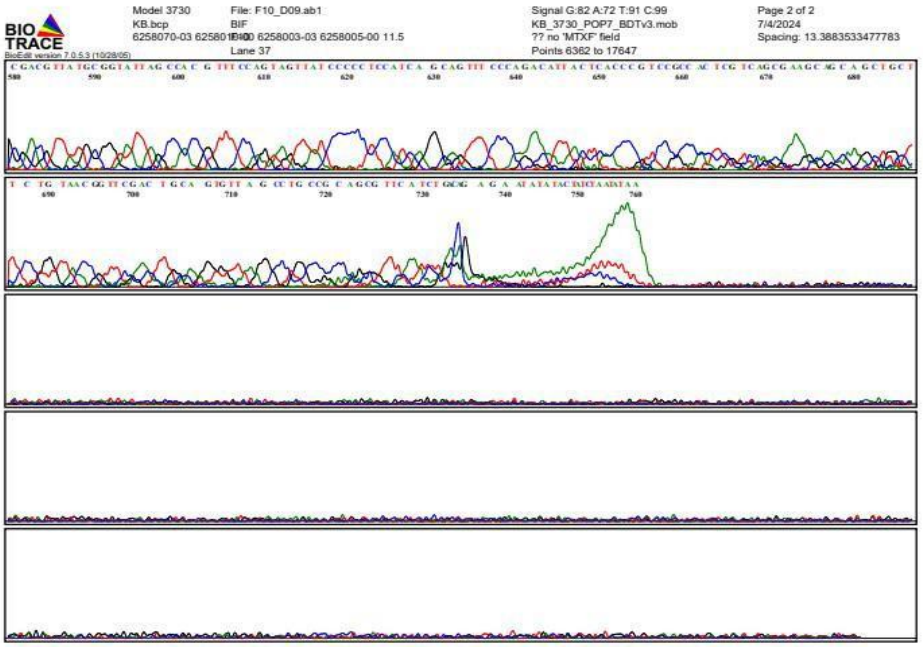
| SI NO | Sample name | Sequence length (bp) | Nearest neighbour | E value | % identity | Query coverage |
|---|---|---|---|---|---|---|
| 1 | F10 | 580 | *Salmonella enterica*<br><br>Subsp.*enterica serovars* | 0.0 | 99.83 | 100 |

Assembled sequence in FASTA format

>F10 AAACGGTGGCTAATACCGCATAACGTCGCAAGACCAAAGAGGGGGACCTT
CGGGCCTCTTGCCATCAGATGTGCCCAGATGGGATTAGCTTGTTGGTGAG
GTATCGGCTCACCAAGGCGACGATCCCTAGCTGGTCTGAGAGGATGACCA
GCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAGTG
GGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTAT
GAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCGGGGAGGAAGGTGTTG
CGGTTAATAACCGCAGCAATTGACGTTACCCGCAGAAGAAGCACCGGCTA
ACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGA
ATTACTGGGCGTAAAGCGCACGCAGGCGGTCTGTCAAGTCGGATGTGAAA
TCCCCGGGCTCAACCTGGGAACTGCATTCGAAACTGGCAGGCTTGAGTCT
TGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCT
GGAGGAATACCGGTGGCGAAGGCGGCCCCC

Figure 6: F10 chromatogram image


Model 3730
KB.bcp
6258070-03 6258015-00 6258003-03 6258005-00 11.5
Lane 37
File: F10_D09.ab1
BIF
Signal G:82 A:72 T:91 C:99
KB_3730_POP7_BDTv3.mob
?? no 'MTXF' field
Points 6362 to 17647
Page 1 of 2
7/4/2024
Spacing: 13.3883533477783


Model 3730
KB.bcp
6258070-03 6258015-00 6258003-03 6258005-00 11.5
Lane 37
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BIF
Signal G:82 A:72 T:91 C:99
KB_3730_POP7_BDTv3.mob
?? no 'MTXF' field
Points 6382 to 17647
Page 2 of 2
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# DISSCUSION

*Isolation, enrichment and screening*

In the present study the samples were pre enriched in lactose broth and incubated at 37°C for 24 hours. And selective enrichment was done in RV medium and samples were incubated at 37°C for 24 hours. The enriched samples were first spread on plates on bismuth sulphite agar and incubated at 37°C for 24 hours ,BIS uses bismuth sulphite and brilliant green as inhibitory agents against competing microorganisms and ferrous sulphate as indicator of H2S production allowing for the characteristic black colonies with metallic sheen to form if *Salmonella* was present and such colonies were formed on plates containing chicken fish burger patties samples. Another study done by Aktar et al.,(2016) reported this same characteristic morphology, round black colonies with metallic sheen at edges which confirms the successful initial isolation.Colonies showing characteristic morphology of *Salmonella* were further screened on XLD and SS agar. XLD uses sodium deoxycholate sodium thiosulphate ferric ammonium citrate and phenol red as selective agents and indicator of H2S production and carbohydrate utilization respectively. Colonies of interest showed black centred with red halo. The same result was shown by the study of Rahman et al.,(2019). While SS agar colonies were smooth and opaque.SS agar screens *Salmonella* for its ability to reduce sulfur compounds H2S which is indicated by black colonies and its inhability to ferment lactose resulting in colourless colonies,and its resistance to bile salts and brilliant green dye. Which are the same characteristics shown by the study of Maddocks et al ,(2002).

*Biochemical confirmation*

In this study 8 colonies were elected from SS and XLD for further biochemical testing. The biochemical confirmation is a crucial step in *Salmonella* identification because many closely related species of *Salmonella* showing similar morphological and cultural properties shows positive results in media specific for *Salmonella* which can lead to falsely positive identification of *Salmonella* in the samples. For confirmatory testing, five biochemical tests specific for *Salmonella* were performed : The urease test, indole test, triple sugar iron test, oxidase test,catalase test. The salmonella is a catalase - positive, oxidase - negative, indole and urease - negative and TSI - positive organism. The catalase test detects the presence of enzyme

catalase, catalase detoxifies hydrogen peroxide by breaking down into water and oxygen gas. The bubbles resulting from the production of oxygen clearly indicate a positive result. In oxidase test artificial electron donors and acceptors are provided when the electron donor is oxidised by cytochrome oxidase it turns dark purple and is considered as positive result. In indole test, tryptophan is hydrolyzed by tryptophan 's to produce three possible end products one of which is indole. Indole production is detected by kovac's reagent which contains 4 (p) dimethyl amino benzaldehyde that reacts with indole to produce a red colour. In urease test hydrolysis of urea produces ammonia and CO2 . The formation of ammonia alkalinizes the medium and the pH shift is detected by the colour change of phenol red from light orange at pH 6.8 to pink at pH 8.1. The TSI test differentiate bacteria based on their fermentation of lactose, glucose, sucrose and the production of hydrogen sulphide. In our study. In our study eight colonies were chosen for a biochemical test out of only one F(10) showed a positive result. Another study by Rahman et al.,(2019).in which all the samples tested shows positive results in biochemical testing. Therefore this ensures that these biochemical tests are highly specific for *Salmonella* species.

In 2010 Jamshidi et al., identified the Salmonella enteritidis and Salmonella typhimurium from the eggs of retail stores in Mashhad.In this study they collected a total of 250 eggs were collected randomly from 50 retail stores in Mashhad city over a period of 3 months .They used multiplex polymerase chain reaction assay and they use three pairs of primers such as S141 and S139 for the invA gene, specific for the genus of Salmonella; Fli15 and Tym for the flic gene, specific for Typhimurium serovar; and Prot6e-5 and Pro T6e-6 for ProtEIN gene, specific for Enteritidis serovar to confirm these isolates are salmonella spp and for determining serovars as Typhimurium and Enteritidis serovars.They results shows that isolated colonies were salmonella and their serovar was determined as Typhimurium.In our study the F(10) isolate which showed the positive result in the biochemical test is further confirmed through molecular sequencing. Another study by Cortez et al ,(2006) in which 6 samples used *Salm*onella isolates were identified by a multiplex-PCR using three sets of primers targeting the invA, pefA, and sefA gene sequences from Salmonella spp., S. Typhimurium and S. Enteritidis, respectively. In our study the F(10) isolate which showed the positive result in the biochemical test is further confirmed through molecular sequencingThe sequencing results and NCBI blast results shows that F(10) isolates *Salmonella enterica.* While comparing with DNA ladder the PCR product size is 1500 bp.

These findings affirm the presence of salmonella in fish samples, consistent with the pathogen's metabolic profile. Further sequencing of isolates will confirm species-level identification. This study emphasizes the need for vigilant food safety monitoring, as Salmonella remains a prevalent and potentially harmful contaminant in various local food sources

# CONCLUSION

The presence of *Salmonella spp* in various food samples, particularly raw chicken,egg, and processed meat like burger patties chicken breast , highlights a significant food safety concern in the local market of Palluruthy. The detection of characteristic black colonies on selective media coupled with positive biochemical tests confirm the *Salmonella* contamination in fish which is commonly consumed. These findings emphasise the importance of stringent monitoring and handling protocols in food processing and retail sectors to minimize the risk of foodborne infections, ensuring consumer health and safety..It is very important to the identity specific salmonella species which is significantly crucial for understanding its pathogenic potential. Because different salmonella species can cause varying degrees of illness and severity.And also accurate identification is crucial in determining treatment options because some strains may have antibiotic resistance to drugs which can interfere with treatment procedures. Confirming salmonella through biochemical assays

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# PUBLICATION

Organised by St. Teresa's College (Autonomous), Ernakulam, Kerala, India

# Prevalence of *Salmonella* in various food sources of a local market in Palluruthy, Kochi

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**Abstract:**

*Salmonella* spp. is a common foodborne pathogen linked to gastrointestinal infections. In May 2024, samples of raw chicken, eggs, fish, frozen burger patties, and chicken breasts were collected from a local market in Palluruthy. These were first inoculated in lactose broth and Rappaport-Vassiliadis (RV) medium, then spread-plated on BSA agar. After 18 hours, burger patty, chicken, and fish samples developed black colonies with a metallic sheen, characteristic of *Salmonella*. On Xylose Lysine Deoxycholate (XLD) agar, colonies displayed black centres with a slightly reddish translucent zone. Confirmatory biochemical tests—including indole, triple sugar iron (TSI), oxidase, catalase, and urease tests—were conducted. All tests indicated *Salmonella* presence except in the fish sample, which tested negative. These findings confirm *Salmonella* contamination in the analysed food samples.

**Keywords:** *Salmonella, Foodborne pathogen, Screening, Food source, Palluruthy.*

*Salmonella* species are among the most prevalent foodborne pathogens globally, known for causing significant gastrointestinal infections and contributing to public health burdens (Pal *et al*., 2015). Food products like raw poultry, eggs, seafood, and processed meat items are often implicated in *Salmonella* outbreaks, especially when proper handling and storage practices are not followed (Bryan, 1988). As *Salmonella* infections can lead to severe health complications, including enteric fever and septicemia, effective detection and control measures in food sources are critical. Routine surveillance in local markets, especially for widely consumed products such as chicken, fish, and processed meats, provides valuable data for understanding the prevalence and spread of this pathogen (Newell *et al*., 2010).

In Palluruthy, a densely populated locality, such monitoring can be particularly beneficial. The food items in local markets are often distributed through diverse, sometimes informal supply chains, creating the potential for contamination. Our study aimed to isolate and confirm the presence of *Salmonella* in commonly consumed food items from the Palluruthy market, including raw chicken, eggs, fish, and processed burger patties. Using selective media and confirmatory biochemical assays, we sought to determine the prevalence of *Salmonella* spp. and its distribution across different food types.

## MATERIALS AND METHODS

### Sample Collection

For this study, food samples, including eggs, chicken, fish, and frozen chicken products such as burger patties and chicken breasts, were collected from local markets and supermarkets in Palluruthy, Kochi, Kerala.

### Bacterial Isolation and Enrichment

Samples were subjected to a two-step enrichment process based on standard protocols for Salmonella isolation.

Pre-Enrichment: Samples were first inoculated in lactose broth (Table 1) and incubated at 37°C for 24 hours (Aktar *et al.*, 2016).

Table 1: Showing the composition of lactose broth

| Ingredients | gm/ litre |
|---|---|
| Peptone | 5.00 |
| HM Peptone B# | 3.00 |
| Lactose | 5.00 |
| Final pH (at 25°C) | 6.9 ± 0.2 |

Selective Enrichment: Following pre-enrichment, samples were inoculated into Rappaport-Vassiliadis (RV) medium (Table 2) and incubated at 42°C for 24 hours (Rahman *et al.*, 2019).

Table 2: Showing composition of RV medium

| Ingredients | gm/ litre |
|---|---|
| Soya peptone | 4.500 |
| Sodium chloride | 7.200 |
| Potassium dihydrogen phosphate | 1.440 |
| Magnesium chloride | 36.000 |
| Malachite green | 0.036 |
| Final pH (at 25° C) | 5.2 ± 0.2 |

**Primary Screening**

The enriched samples were spread-plated on Bismuth Sulphite Agar (BSA), a selective medium for *Salmonella*. Colonies were incubated at 37°C for 24 hours, allowing for characteristic black colonies with a metallic sheen to form if Salmonella was present. Table 3 details the composition of BSA (Aktar *et al.*, 2016).

Table 3: Composition of Bismuth Sulphite Agar

| Ingredients | gm/ litre |
|---|---|
| Beef extract | 6.0 |
| Peptic digest of animal tissue | 10.0 |
| Ferric citrate | 0.4 |
| Brilliant green | 0.01 |
| Bismuth ammonium citrate | 3.0 |
| Sodium Sulfate | 10.0 |
| Di sodium hydrogen phosphate | 5.0 |
| D+ glucose anhydrous | 5.0 |
| Agar | 24.0 |
| pH | 7.6 ± 0.2 |

### Secondary Screening

To confirm the presence of *Salmonella*, colonies from BSA were plated on Xylose Lysine Deoxycholate (XLD) and *Salmonella shigella* (SS) agar. Table 4 show the compositions of these media. Colonies showing a black center on XLD or opaque on SS were selected for further biochemical testing (Rahman *et al.*, 2019).

Table 4: Showing Composition of Xylose Deoxycholate agar

| Ingredients | gm/ litre |
|---|---|
| Yeast extract | 3.00 |
| L-lysine | 5.00 |
| Lactose | 7.50 |
| Sucrose | 7.50 |
| Xylose | 3.50 |
| Sodium chloride | 5.00 |
| Sodium deoxycholate | 2.50 |
| Sodium thiosulphate | 6.80 |
| Ferric ammonium Citrate | 0.80 |
| Phenol | 0.08 |
| Agar | 15.00 |
| pH | 7.4 ± 0.2 |

### Biochemical Confirmation

For confirmatory testing, five biochemical tests specific for *Salmonella* spp. were performed: the indole test, triple sugar iron (TSI) test, urease test, oxidase test, and catalase test. The samples were inoculated and incubated at 37°C for 24 hours.

## RESULTS AND DISCUSSION

### Sample Collection and Enrichment

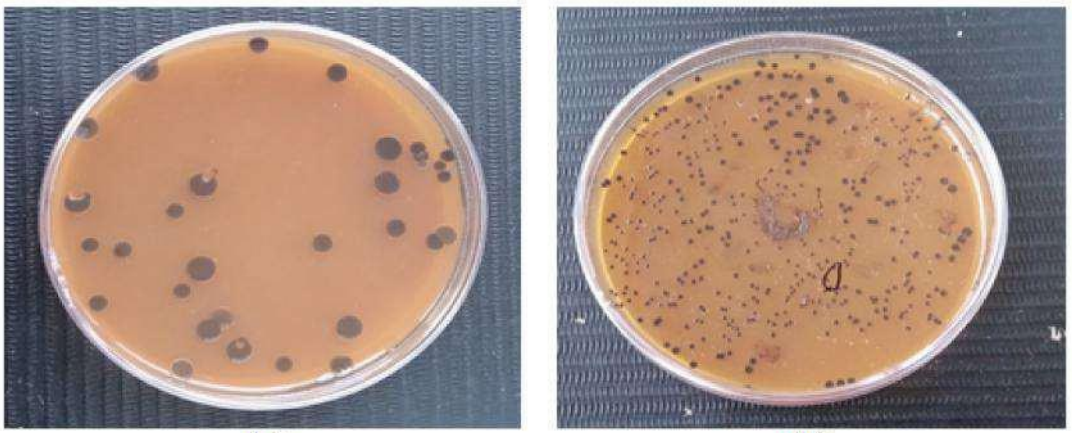
All collected samples (egg, chicken, fish, burger patties, and chicken breasts) were successfully enriched in both lactose broth and RV medium. Figure 1 shows representative images of samples enriched in both broths, demonstrating adequate bacterial growth for further screening.

(a) (b)

Figure 1: (a) Fish sample collected (b) Sample enriched in lactose broth and RV medium

**Primary Screening on Bismuth Sulphite Agar**

After plating on Bismuth Sulphite Agar (BSA) and incubating, colonies with black pigmentation and a metallic sheen, characteristic of *Salmonella*, were observed on plates of chicken, fish, and burger patties. Figure 2 demonstrates the characteristic morphology of these colonies, confirming successful initial isolation. The use of selective and differential plating media is a very important part of standard cultural methods for the isolation of *Salmonella* from foods and environmental samples (Warburton *et al.*, 1994). Bismuth Sulphite Agar are used in standard methods of different agencies in Canada, the United States and in Europe (Flowers *et al.*, 1992). BIS uses bismuth sulfite and brilliant green as inhibitory agents against competing microorganisms and ferrous sulfate as the indicator of $H_2S$ production (Difco, 1984).



(a) (b)

Figure 2: (a) Chicken spread plate in BSA (b) egg spread plate in BSA

**Secondary Screening on XLD and SS Agar**

Colonies isolated from BSA that displayed *Salmonella*-like morphology were further screened on XLD and SS agar. Colonies of interest on XLD showed black centers

with a translucent red halo, while SS agar colonies were smooth and opaque (Figure 3). XLD uses sodium desoxycholate, sodium thiosulfate, ferric ammonium citrate and phenol red as selective agents, indicators of $H_2S$ production and carbohydrate utilization, respectively (Anon, 1984). SS Agar screens for *Salmonella* by its ability to reduce sulfur compounds to H S (indicated by black colonies), its inability to ferment lactose (resulting in colourless colonies), and its resistance to bile salts and Brilliant Green dye. These characteristics make SS Agar an effective medium for detecting *Salmonella* in mixed microbial samples (Maddocks *et al.*, 2002).

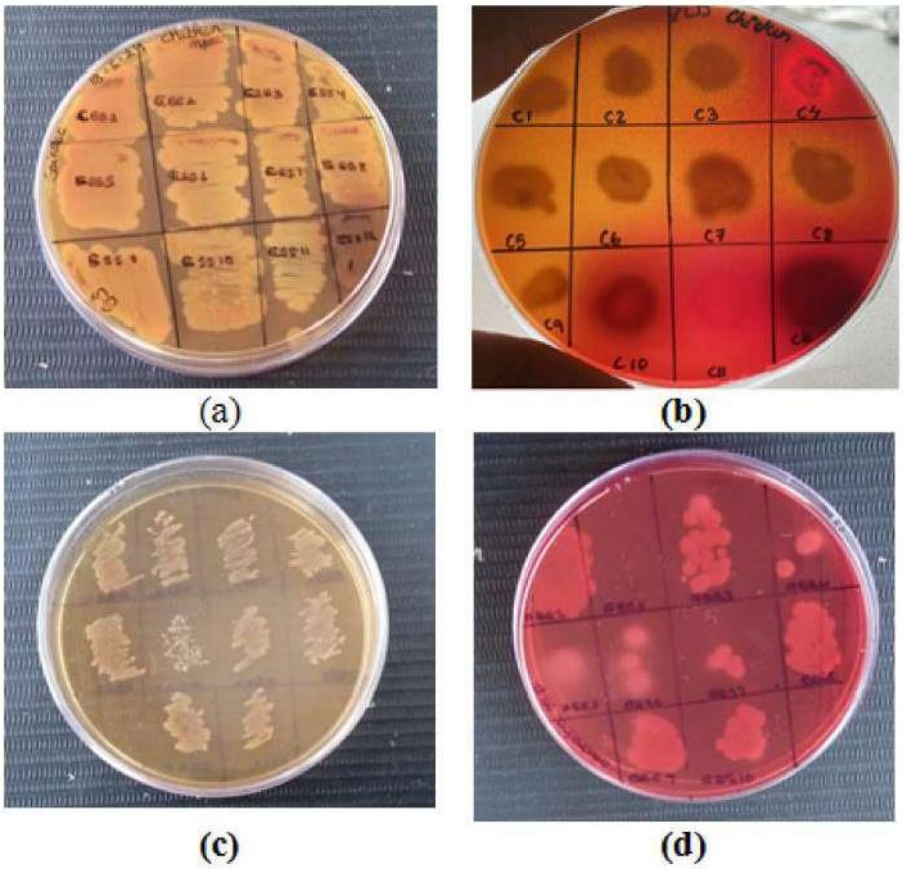



Figure 3: Images showing (a) SS chicken plate (b) XLD chicken plate (c) SS egg plate (d) XLD egg plate

A total of 8 colonies were chosen from the secondary screening for biochemical testing: 1 from chicken and 7 from fish (Table 6).

Table 6: Number of Isolates per Sample

| Sample name | Number of isolates |
|---|---|
| Egg | 0 |
| Chicken | 1 (C12) |
| Fish | 7 (F1, 2, 4, 8, 9, 10, 12) |
| Chicken breast (Frozen product) | 0 |
| Chicken burger patty (Frozen product) | 0 |

**Biochemical Characterization**

The biochemical characterization of the isolates, including Oxidase, Catalase, TSI, Urease, and Indole tests, confirmed the presence of *Salmonella* spp. in the fish isolate

Table 7: Biochemical test results for chicken (C) and fish (F) isolates

| Isolates | Oxidase test | Catalase test | TSI test | Urease test | Indole test |
|---|---|---|---|---|---|
| Positive control | Negative | Positive | RS YB gas +ve or - ve/ h2s +ve | Negative | Negative |
| C12 | Negative | Positive | RS YB, h2s +ve, gas -ve | Positive | Negative |
| F1 | Positive | Negative | RS YB, h2s +ve, gas -ve | Positive | Negative |
| F2 | Positive | Negative | RS YB, h2s +ve, gas -ve | Positive | Negative |
| F4 | Positive | Negative | RS YB, h2s + ve, gas -ve | Positive | Negative |
| F8 | Positive | Negative | RS YB, h2s +ve, gas -ve | Positive | Negative |
| F9 | Positive | Negative | RS YB, h2s +ve, gas -ve | Positive | Negative |
| F10 | Positive | Negative | RS YB, h2s +ve, gas -ve | Negative | Negative |
| F12 | Positive | Positive | YS YB, h2s +ve, gas -ve | Positive | Positive |

(F10) and differentiated it from closely related organisms (Table 7). *Salmonella* was found to be oxidase-negative and catalase-positive, which is consistent with its typical biochemical profile. The TSI test showed an alkaline slant and acid butt with hydrogen sulfide ($H_2S$) production, indicated by a black precipitate, a hallmark of *Salmonella*'s sulfur reduction ability. Additionally, the urease test was negative, distinguishing *Salmonella* from urease-positive organisms like *Proteus*. The indole test was also negative, aligning with *Salmonella*'s inability to produce indole. These results underscore the reliability of these biochemical tests in confirming *Salmonella* spp. and differentiating them from other enteric pathogens. The findings highlight the importance of employing such tests in food safety laboratories for the detection and monitoring of *Salmonella* contamination, particularly in seafood, to mitigate the risk of foodborne illness.

These findings affirm the presence of *Salmonella* in fish samples, consistent with the pathogen's metabolic profile. Further sequencing of isolates will confirm species-level identification. This study emphasizes the need for vigilant food safety monitoring, as *Salmonella* remains a prevalent and potentially harmful contaminant in various local food sources.

## CONCLUSION

The presence of *Salmonella* spp. in various food samples, particularly in fish, highlights a significant food safety concern in the local market of Palluruthy. The detection of characteristic black colonies on selective media, coupled with positive biochemical test results, confirms *Salmonella* contamination. These findings emphasize the importance of stringent monitoring and handling protocols in food processing and retail sectors to minimize the risk of foodborne infections, ensuring consumer health and safety.

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