



Isolation and Molecular Identification of *Salmonella* in Organic and Inorganic Eggs in District Abbottabad

Ayub Jadoon¹, Habiba Ejaz², Syed Wajid Ali Shah³, Aroosa⁴

¹⁻⁴Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad, KP, Pakistan.

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Correspondence to: Habiba Ejaz, Department of Microbiology, Abbottabad University of Science and Technology, Abbottabad, KP, Pakistan.

Email: habibaejaz247@gmail.com

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ABSTRACT

Salmonella is a gram-negative bacterium responsible for salmonellosis. A disease transmitted by the consumption of contaminated poultry products such as eggs and raw chicken meat. Pakistan is presently producing around 18 billion table eggs per annum but unfortunately the country's per capita consumption is just 65-70 eggs annually against the world average usage of around 160 eggs. The present study is aimed at the identification of bacterial contaminated eggs especially with *salmonella* infection. The *salmonella* serovars present on the surface of marketed eggs in district Abbottabad are currently unknown. The present study also aimed at the antibiotic resistance on *salmonella* isolates. A total of 300 eggs samples were collected at random from variety of chicken farms (Havelian, Salhad, Sultanpur, Mandian) and processed for isolation of salmonella. The identification was done in the lab by microscopic examination' biochemical tests (vp, Indole, simmon citrate, methyl red, sugar iron) and molecular method by PCR. All isolates were further confirmed on the basis of cultural and biochemical characteristics and by targeting 16s rRNA gene of *salmonella*. All *salmonella* isoates were also examined for their antimicrobial drug susceptibility pattern against commonly used antibiotics. Out of to be 300 samples, the prevalence of salmonella in in organic and inorganic eggs was found 65% and 35% respectively, with an overall prevalence of 13%. Polymerase chain reaction (PCR) targeting 16s rRNA gene of *salmonella* showed positive result with 40 isolates. All 40 salmonella isolates were found to be highly sensitive to ampicillin while 70%, 55% and 40% were sensitive to tetracycline, amoxicillin and penicillin respectively. The isolates were 68% and 50% isolates were resistant to chloramphenicol and gentamycin, respectively. Out of 40, 14 isolates had multiple antibiotic resistance index equal to or more than 0.2. *Salmonella* in in organic and inorganic eggs samples is prevailing in the areas of sampling due to poor hygienic conditions and secondly antimicrobial resistance including several drug resistant phenotypes. Therefore, the present study emphasizes the need for continued surveillance of zoonotic foodborne pathogens including antimicrobial-resistant variants throughout the food production including eggs.

INTRODUCTION

Eggs and egg products are nutrient-dense foods that are essential to the human diet. However, egg consumption has been related with unfavorable health effects. Mishandled eggs and egg products can be a cause of food-borne illnesses, such as salmonellosis. Salmonellosis is one of the most prevalent and widespread food-borne illnesses (Woodward & Kirwan, 1996). *Salmonella* spp infection is one of the most common reasons why people get sick from eating something they shouldn't have. *Salmonella* is spread to people when they eat foods that have it, like raw eggs and egg products. *Salmonella enterica* has about 2500 different strains, but *Salmonella enteritidis* and *Salmonella typhimurium* are the ones that cause most diseases in people around the world. *S.*

enteritidis, on the other hand, does not come from *S.* strains (D. P. Smith & Musgrove, 2008). *Typhimurium*, which is often found in people who get sick from eating eggs, has filled this need. Salmonellosis has become three times more common in the last ten years, which is a major public health concern (Almaro, 2014). *Salmonella* is a Gram-negative facultative anaerobe that is non-spore producing, motile, and belongs to the Enterobacteriaceae family (Humphrey, Whitehead, Gawler, Henley, & Rowe, 1991). *Salmonella* is a sugar fermenting, oxidase-negative bacteria that may convert nitrates to nitrites. *S. enteritidis* and *S. typhimurium* are most frequent sources of accidents salmonellosis, and their distribution is controlled by them (Bekele & Ashenafi, 2010). *S. enteritidis* is a prominent serotype that has been

identified from eggshells and egg yolks in food contamination involving eggs and egg products around the world (S. Foley, Lynne, & Nayak, 2008). If *Salmonella* is swallowed, it adheres to oropharyngeal and intestinal epithelial cells via type 1 fimbriae or pili (Labbé & García, 2013). *Salmonella* pathogenesis is aided by the type III secretion system encoded by *Salmonella* pathogenicity islands 1 (SPI-1) and other SPIs (Humphrey et al., 1991), that play a major role in *Salmonella* human infection. In most regions of the world, *S. Enteritidis* is a prevalent route of transmission illness caused by contaminated eggs (A. D. Wales & R. H. Davies, 2011). Cleaning and sanitizing routines, flock size, flock age, stress, nutrition, vaccination, egg production method, storage, and handling of eggs are all factors that influence Bacterial contamination of external and internal eggs during chicken production (H. Whitley & Ross, 2015). Egg poisoning can happen in one of two ways: vertically or horizontally. Vertical transmission can occur (F. T. Jones, Rives, & Carey, 1995) when *Salmonella* colonizes the reproductive organs (ovary and oviduct) during shell production, while transmission happens when *Salmonella* penetrates the eggshell layer and toxins the egg contents after the egg is placed (Tessema, Bedu, Ejo, & Hiko, 2017). The capacity of *S. Enteritidis* to colonize the sexual organs of egg production and infect the developing egg is very well recognized (F. Jones & Richardson, 2004). According to investigations (Lublin & Sela, 2008), egg shell infection was substantially higher in hens exposed with *S. Enteritidis* than that in hens treated with *S. Typhimurium*. *Salmonellosis* could be contracted by consuming contaminated eating undercooked eggs, and it has been recognized as a global public health problem around the world (Cohen & Tauxe, 1986). Food-borne disease-causing microorganisms are a serious and growing public health concern (Humphrey et al., 1991). Egg quality can be harmed by microorganisms in eggs and egg products, which can lead to pathogen transmission and spoiling (Hald et al., 2012). Consumers may become infected or intoxicated as a result of this (Dunlop et al., 2012). People consume eggs all across the world today, and they remain a basic meal in the human diet (Humphrey, Baskerville, Mawer, Rowe, & Hopper, 1989). They're considered a healthy and inexpensive source of protein, and they're eaten all around the country (Evangelista, Truant, & Bourbeau, 2001). Eggs get infected with numerous kinds of microorganisms when exposed to natural conditions such as soil, faeces, and dirty nesting materials, despite the fact that freshly placed eggs are normally free of microorganisms (Ellen, Bottcher, Von Wachenfelt, & Takai, 2000). *Salmonella Enterica* subsp (Juven et al., 1984). *Enterica* is a common cause of foodborne human gastroenteritis, an illness characterized by intestinal inflammation and diarrhea (Humphrey et al., 1991). The usage of infected food products such as meat, eggs, and egg-related products are the most common sources of *Salmonella* infection. *Salmonella* spp. are thought to be responsible for 155,000 deaths and 93.9 million cases of gastroenteritis each year around the world (Parker, 2021). *Salmonella Enteritidis* is a superior serotype in commercial poultry businesses that is regularly isolated from eggs and is frequently connected with egg-related food illness in humans (Doorduyn, Van

Den Brandhof, Van Duynhoven, Wannet, & Van Pelt, 2006). Foodborne outbreaks linked to raw eggs or egg-related products are a serious public health concern, highlighting the importance of laying hen egg contamination (Humphrey et al., 1991). In light of the disease's importance (Humphrey et al., 1991), the current study aims to determine the molecular basis of *Salmonella* in organic and inorganic eggs in Abbottabad. Several intervention strategies have been developed to reduce the risk of *Salmonella* at farm level (Cohen & Tauxe, 1986). These intervention strategies include biosecurity, vaccination, cleaning, disinfection, prebiotics, probiotics, feed and water acidification with organic acids, and egg (Little et al., 2006). Although the application of these 13 strategies has reduced the *Salmonella* contamination level, complete elimination of this pathogen is yet to be achieved (S. L. Foley, Johnson, Ricke, Nayak, & Danzeisen, 2013). In the poultry industry, several commercial organic acid products are commonly used in feed and water as preventive measures against *Salmonella* contamination (Grijspeerdt, Kreft, & Messens, 2005). Due to increased antimicrobial resistance and a ban on antibiotic growth promoters in many countries, organic acids are a suitable alternative for pathogen control, under a wide variety of food processing conditions (Gast, Guraya, Guard, & Holt, 2011). Organic acids are relatively stable and naturally present in living organisms, especially in the gut environment (F. Jones & Richardson, 2004). Organic acids are either metabolized by food animals or may be excreted unabsorbed (Chousalkar & Willson, 2022), and thus do not present an issue of residues in food. In addition to antimicrobial action, organic acids have ability to reduce biofilm development of foodborne pathogens on different surfaces (Guard-Petter, 2001).

Poultry products are widely consumed in Pakistan to meet the country's important dietary protein requirements, which are mainly met by the poultry industry. Nevertheless, there is indeed a risk for pathogenic bacteria in chicken, that can be extremely expensive. Foodborne outbreaks linked to raw eggs or egg-related products are a serious public health concern, highlighting the importance of laying hen egg contamination. In light of the disease's importance, the current study aims to determine the molecular basis of *Salmonella* in organic and inorganic eggs in Abbottabad.

Furthermore, no published data on egg quality in the Abbottabad area exists. The suggested research into this matter would center on farm-raised eggs, as those are the ones most directly affected by the problem.

METHADODOLOGY

Study Design and Sampling

A potential investigation of the presence of *Salmonella* in organic and non-organic eggs produced at a number of poultry farms in Abbottabad is scheduled to take place between February and July of 2022. A total of 300 egg samples will be collected at random from a variety of chicken farms and brought to Abbottabad. The eggs are going to be packed in sterile plastic bags before being delivered to the laboratory that is located at the Veterinary Research and Disease Investigation Center Mandian in Abbottabad.

Isolation of Sample

This had been carried out used standard method by (Guard-Petter, 2001; Ochei & Kolhatkar, 2000). To prevent the spread of bacteria throughout the contents of the eggs, the outside of each egg had been sterilized with an ethanol solution that was 70 percent strong. An opening had been formed in the eggs, and the contents had been thoroughly mixed with a sterile spatula. A needle and syringe that had been sterilized had been used to inject 0.1 milliliter of the egg white and yolk mixture into a plate containing salmonella-shigella agar. The plates were gone have been incubated for a whole day at 37 degrees Celsius. After the incubation period was up, the plate had been examined too looked for the typical salmonella colonies

Identification of Isolates

In ordered to correctly identified each single isolate, we would use morphological characteristics, gram staining, and other types of biochemical testing, including triple sugar iron (tsi) simmon citrate ' indole ' voges proskauer ' methyl red (Burkhalter, Müller, Lüthy, & Candrian, 1995; Soomro et al.).

Glucose Iron (TSI)

The top of an isolated colony was punctured with a straight inoculating needle. Inoculate TSI by first smearing it across the top of the agar slant, then smearing it from the center of the medium to the bottom of the tube. Keep the top loose and incubate the tube for 18 to 24 hours at 35° to 37°C in the open air. Media that has a red or yellow slant indicates successful outcomes (Brigmon, Zam, & Wilson, 1995).

The Simmons Citrate Test

A specialized medium for figuring out if an organism can survive on citrate alone. Citrate is used as a carbon source by some microorganisms. Using citrate as their sole carbon source, bacteria were tested using the citrate method. The metabolism of citrate in bacteria is carried out by enzymes called citrates or citrate desmolase. Based on how much citrate they consumed, these gram-negative bacteria were separated using this agar (Carrique-Mas & Davies, 2008). To make Simmons citrate agar, 4.5 g of agar were mixed with 125 ml of deionized water. The mixture was heated to 121°C for mixing before being autoclaved. The liquid was placed in tubes and plates when it had cooled. The colonies were transferred to Simmons citrate agar using a sterile inoculating loop. Negative results were obtained when tubes were incubated for 24 hours at 37°C with a loose top. No growth was seen.

Indole Test

Another biochemical test for determining if Gram-negative bacteria can convert tryptophan to indole is the indole test. The "tryptophanase" system, which was made up of an intracellular enzyme chain, carried out this test. The Kovac's reagent was used in this experiment. When it came to detecting anaerobic indole production in non-fermenters and subsequently tracking the outcomes, this reagent was more accurate. To assess different bacterial species' capacity to break down the amino acid tryptophan and create indole, the indole test was used (Carrique-Mas & Davies, 2008). Examined bacteria were injected with tryptone through a clean loop, then they were cultivated at 37 degrees Celsius overnight. A few drops of Kovac's

reagent must be added. When detecting anaerobic indole synthesis in a non-fermenter and analyzing the end product, this reagent was noticeably more sensitive.

Examine Methyl-Red

The methyl red test is predicated on the idea that by breaking down the bacterium's glucose and creating organic acid, the bacteria will lower the pH of the medium (below 4.4). This examination is used to distinguish between distinct bacterial species and genera. Methyl red test results: salmonella positive, klebsiella pneumoniae negative (Berrang, Cox, Bailey, & Blankenship, 1991). From the bacterial colony, microorganisms were extracted and put in a glucose-phosphate broth for analysis. Tubes were incubated in an oven set to 37 degrees Celsius for 1 to 7 days. The methyl red reagent was added the next day, drop by drop, and carefully mixed. In the medium, a red color denotes a successful outcome while a yellow one is unfavorable. Methyl red reagent; it produced a yellow color at pH 6.0 and below and a red tint at pH 4.4 and lower.

VP-Test

This test's objective is to recognize different bacterial species. A chemical called acetyl methyl carbinol is produced when some bacteria metabolize glucose (acetoin). Neuronal byproducts are frequently utilized in identification. Acetyl methyl carbinol will oxidize to diacetyl in the presence of potassium hydroxide (KOH) (Day et al., 2009). When mixed with alpha naphthol, this chemical produces a scarlet color (creatine, arginine or creatinine). The plant was used to make Glucose-Phosphate Broth after the target bacteria were removed from the colony of the colony. Tubes were incubated in the oven for 1 to 7 days at 37 ° C. After incubation, 3 ml of 5% alpha naphthol and 1 ml of 40% KOH were added, and they were then mixed. Within 2 to 5 minutes, a copper tint develops, denoting a failed test.

Antibiotic Susceptibility

The Kirby-Bauer disc diffusion method had been utilized to conduct the antimicrobial susceptibility tests that would take place on Mueller-Hinton agar (MHA) (Blais & Martinez-Perez, 2008). After allowing them to air dried at room temperature for three to five minutes, the diseased plates would then have antibiotic discs placed on top of them. On salmonella isolates, antimicrobials including ampicillin (James et al.), chloramphenicol (C), gentamycin (CN), amoxicillin, and tetracycline had been investigated for their effects (T). The cleared zones that were created as a result of antimicrobial inhibition of bacterial growth was measured in millimeters used a straight-line ruler following an overnight incubation at 37 degrees Celsius. Antibiotic resistance had been evaluated on a scale from susceptible to intermediate too resistant

Molecular Identification of *Salmonella* Isolates

At the microbiology laboratory of veterinary researched and disease investigation center Abbottabad the molecular identification of salmonella had been done by used PCR from the extracted DNA isolates (Van Immerseel et al.)

Bacterial DNA Extraction and Gel Electrophoresis

A pellet was suspended into 100 ml of TE buffer (Tris HCL

EDTA) or double distilled water, heated through a thermal cycler at 98 °C for 4 minutes, centrifuged at 4400 rpm for 7 minutes, and the supernatant was utilized for PCR amplification after identification and sub-culturing in LB broth. For 30 minutes at 110 volts, the sample was tested on a 1% agarose gel to validate the PCR amplified products by band formation and band size comparison with the common DNA marker band. The samples that tested positive or had a visible DNA band were forwarded for sequencing.

RESULTS AND DISCUSSION

Collection of Egg Samples and Initial Isolation of Bacteria

A potential investigation of the presence of *Salmonella* in organic and non-organic eggs produced at a number of poultry farms in Abbottabad is scheduled to take place between February and July of 2022. A total of 300 egg samples were collected from different areas of Abbottabad district according to **table 4.1** below. The eggs were packed in sterile plastic bags before being delivered to the laboratory that is located at the Veterinary Research and Disease Investigation Center Mandian in Abbottabad.

Table 4.1

different areas of Abbottabad district selected for samples collection

Areas of Abbottabad district				
NO of samples	Sultanpur	Hevellian	Salhad	Mandian
	75	75	75	75

This had been carried out used standard method by (Otomo et al., 2007). To prevent the spread of bacteria throughout the contents of the eggs, the outside of each egg had been sterilized with an ethanol solution that was 70 percent strong. An opening had been formed in the eggs, and the contents had been thoroughly mixed with a sterile spatula. A needle and syringe that had been sterilized had been used to inject 0.1 milliliter of the egg white and yolk mixture into a plate containing salmonella-shigella agar. The plates were gone have been incubated for a whole day at 37 degrees Celsius. After the incubation period was up, the plate had been examined too looked for the typical salmonella colonies (Fig 4.1 \$ 4.2).

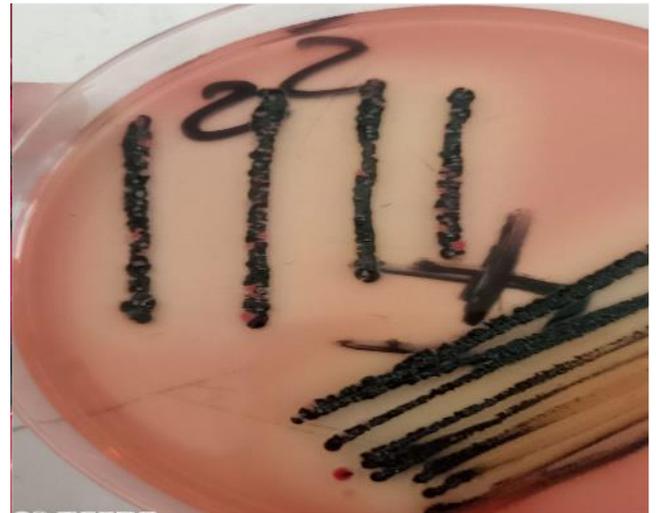
Figure 4.1

Purified colonies of salmonella on EMB media



Figure 4.2

Purified colonies of *salmonella* on media

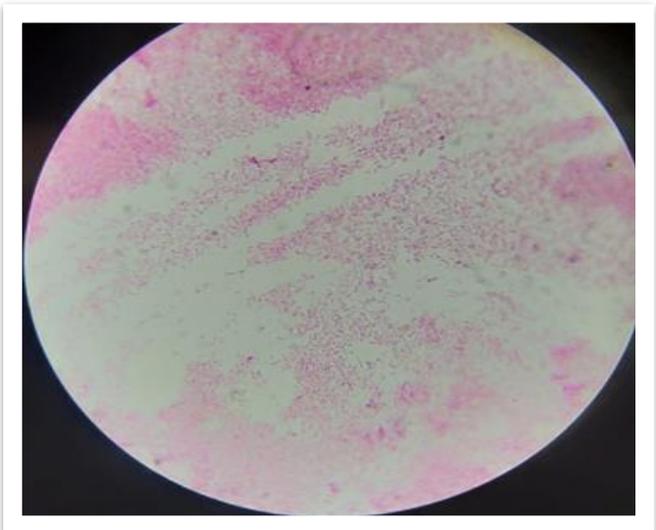


Microscopic Analysis

Salmonella colonies that were specially purified and separated from organic and inorganic egg samples were identified using the Gram staining technique. Slides were stained with gramme after smear creation, and they were then examined under a microscope. *Salmonella* I was described as having short, gram-negative pink rods arranged in an unusual way. (Fig. 4.3)

Figure 4.3

Microscopic picture of salmonella



Biochemical Tests

In order to establish the *Salmonella* strain's biochemical characteristics, rapid biochemical identification procedures were utilized. In total, there were six different biochemical analyses carried out as part of these identification methods. The battery of tests known as the IMVIC included the Simon's citrate test, the sugar iron (tsi) test, the VP test (Voges Proskauer test), the indole test, and the methyl red test (Soomro et al.). In the assessments, pH change and overarching principles of substrate consumption were both taken into consideration. When a bacterial culture is inoculated into a test medium and allowed to grow in that medium, the test medium itself

goes through changes due to the metabolic activity of the bacteria. These changes can be seen as a change in color and interpreted visually, or they can be measured by adding the appropriate test reagent after the incubation process has been completed.

Out of 300 total 45 samples where isolates were positive for Tsi and MR although negative for VP, indole and simmon citrate. As a result, all were identified as belonged to the species *salmonella*. From Mandian 13 samples were positive for salmonella out of 75, sultanpur 9 out of 75, havelian 16 out of 75 and salhad 7 out of 75 was indicated the presence of *salmonella* specie (Fig. 4.4 & 4.5).

Table 4.2

No of samples positive for *salmonella*

Area of sampling	no of samples	Positive for salmonella
Havelian	75	16
Salhad	75	7
Sultanpur	75	9
Mandian	75	13
Total no of samples	300	40

Figure 4. 4

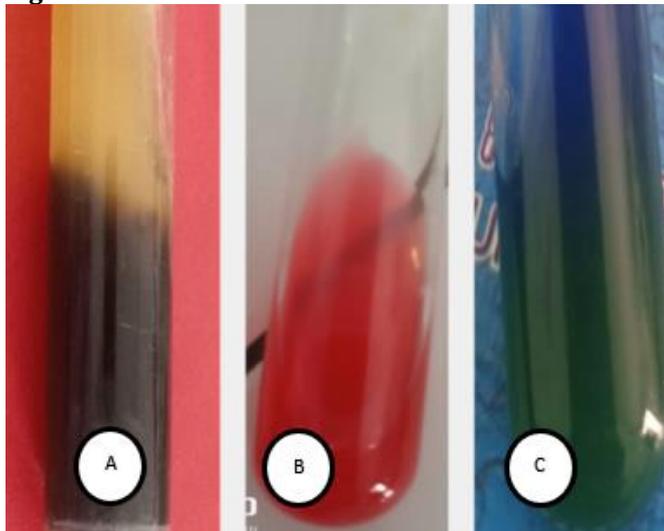
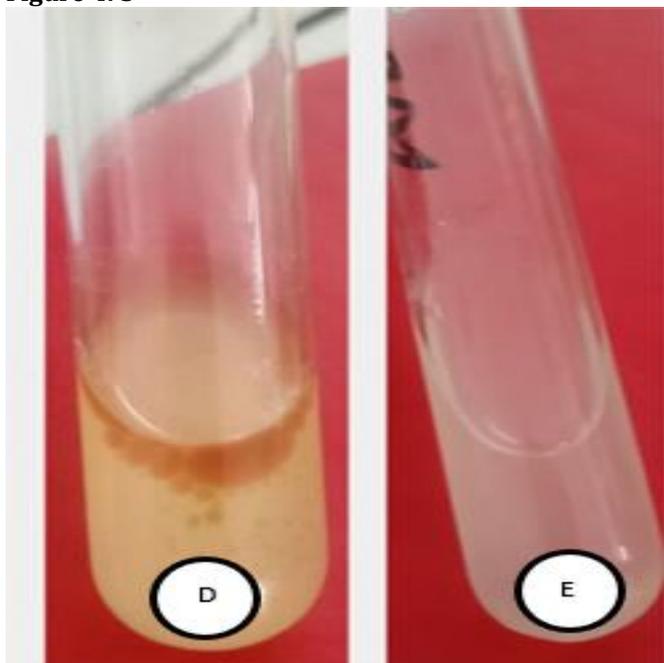


Figure 4. 5



Sampling from organic and inorganic eggs has been demonstrated to be a reliable indicator of *Salmonella* contamination in poultry flocks, and there is a strong correlation between the level of environmental contamination and the frequency of caecal infection, the degree of internal egg contamination, and the resulting human disease (Martelli & Davies, 2012).

Salmonella are the major cause of food-borne illnesses and a serious public health concern. The majority of human disease outbreaks are associated with consumption of contaminated food particularly egg and egg related products (Otomo et al., 2007).

Salmonella contaminations in Abbottabad district have never before been discovered. This analysis was carried out for the first time in order to identify and assess *Salmonella*'s status in the eggs. All four areas were included, and an average contamination percentage of 65.8% was found. When compared to wealthy nations, this is a sizable percentage. It might be seen as a worrying development for the welfare of the chickens as well as for general public health. Additionally, no regulation has been proposed to keep an eye on the feed mills and the chain of production for poultry. In contrast, Finland has been introducing legislation for decades to regulate *Salmonella* in livestock and feed materials.

Testing For Antibiotic Sensitivity

Different bacteria's susceptibility to antibiotics and their antibiotic resistance were examined. Antibiotic-resistant bacteria are currently such a major global public health issue that they have led to an increase in morbidity and mortality as well as longer hospital stays and more expensive medical care.

Only *salmonella*, which exhibited resistance to chloramphenicol in both organic and inorganic egg sample isolates, was determined to have the highest level of resistance during the current study's measurement of the bacterial zone of inhibitions. *Salmonella* isolates from organic eggs and those from inorganic eggs that are susceptible to amoxicillin showed the greatest intermediate resistance. *Salmonella*, however, demonstrated the greatest sensitivity to Ciprofloxacin in both organic and inorganic samples. According to comparative studies from Rwanda, Nigeria, and Ethiopia, the prevalence of *salmonella* was 13.3% vs. 31.6%, 8.1% vs. 19.1%, and 33% vs. 0%. *Salmonella* strains resistant to ciprofloxacin comprise 45.4% of the total. A study conducted in Bangladesh indicated that the resistance frequency was 40.9%. However, a higher resistance rate of 87.5% was reported in a study done in Ethiopia (Chang, 2000).

Salmonella in the current investigation had the greatest sensitivity to Ampicillin in both organic and inorganic isolates. Comparative data revealed that *salmonella* had a 52.27% resistance to ampicillin (Durand, Barnard, Swanepoel, & Engelbrecht, 1987).

In current study *salmonella* showed maximum resistance against Gentamicin from organic isolates and intermediate from inorganic isolates. Comparative findings *salmonella* resistance to Gentamycin were found to be 90.9% (Burki et al., 2015). Gentamicin resistance was found in 22.7% of *salmonella* strain (Eng et al., 2015).

In current study *salmonella* showed maximum resistance against Penicillin from in inorganic isolates and sensitive from organic isolates. Comparative findings *salmonella* resistance to Penicillin were found to be 50% Penicillin resistance was found in 70% of *salmonella* strains(Sun, Selmer, & Andersson, 2014).

In current study *salmonella* showed maximum resistance against Tetracycline from organic isolates and in inorganic isolates. Comparative findings *salmonella* resistance to Tetracycline were found to be 90.9%(Sun et al., 2014) . Tetracycline resistance was found in 22.7% of *salmonella* strains.

In current study overall found that salmonella showed sensivity against Ampicillin and Ciprofloxacin. Highest resistant against Tetracycline and Chloramphenicol. in case of Penicillin, Gentamycin, and Amoxicillin salmonella showed intermediate resistant, sensitivity and resistant (Fig 4.6 & 4.7)

Figure 4. 5
Antibiotic sensitivity test in percentage.

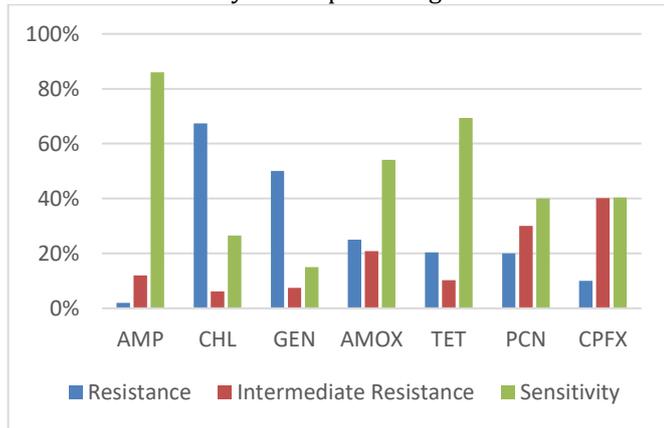


Figure 4. 6
Zones of inhibition of *salmonella* against selected antibiotics.



The *Salmonella* isolates used for this investigation revealed a modest but broad spectrum of antibiotic resistance, according to comparative results. Of the 145 *Salmonella* isolates examined, 133 (or 91.72%) were sensitive to every antibiotic that was used. Overall, there was resistance to tetracycline (4.13%), cephalothin (2.06%), trimethoprim (0.68%), amoxicillin and ampicillin (5.51%), and cephalothin (4.13%). No isolate shown resistance to cefotaxime, ceftiofur, ciprofloxacin, chloramphenicol, gentamycin, neomycin, or streptomycin.

Molecular Characterizations

Colony PCR was utilized for both the extraction of DNA and the amplification of 16SrRNA. Just these bacterial isolates with antibiotic sensitivity tests were subjected to DNA extraction, PCR amplification, and DNA sequencing prior to having their antibiotic sensitivity determined. The DNA that was extracted was examined using UV transilluminator bands while it was running on an Agarose gel. The diagram shows the DNA bands of several samples in comparison to ladder DNA. (Fig 4.8)

Figure 4.8
bands of *salmonella* on agarose gel.



DISCUSSION

The presence of *Salmonella* in poultry products, particularly eggs, represents a significant public health concern worldwide, and investigations into its prevalence in specific geographical locations such as District Abbottabad provide valuable data for understanding regional contamination patterns and informing local food safety policies. The isolation and molecular identification of *Salmonella* from organic and inorganic eggs necessitates a comprehensive discussion that encompasses the public health implications of such contamination, the methodological approaches employed for detection, the comparative analysis of findings with regional and global studies, the factors influencing contamination in different production systems, and the broader recommendations for surveillance and control measures that emerge from this research. *Salmonella* is widely recognized as a major zoonotic pathogen, and its presence in eggs and egg products serves as a continuous threat to human populations, with contaminated eggs being internationally significant sources for the transmission of *Salmonella* infection to humans for several decades (Sajid et al., 2015; Gast, 2023). The public health burden of salmonellosis is substantial, with estimates suggesting that between 200 million and 1 billion cases of *Salmonella* infections occur worldwide annually, resulting in approximately 93 million cases of gastroenteritis and 155,000 deaths, with about 85% of these cases being foodborne related (Lamichhane et al., 2024). The economic impact is equally concerning, with the annual cost of salmonellosis reaching approximately \$4.1 billion in the United States, about €3 billion in the European Union, and AUS\$811 million in Australia (USDA, 2021; Li et al., 2025; Simpson et al., 2018). These figures underscore the importance of conducting localized studies such as the one undertaken in District Abbottabad, as they contribute to the global

understanding of *Salmonella* epidemiology and support the development of targeted intervention strategies.

The findings of isolation studies conducted in regions geographically and ecologically similar to District Abbottabad provide important context for interpreting local research results. A comprehensive study spanning three years and examining 1,747 random samples from 56 locations in Islamabad and Northern Punjab, which includes the Abbottabad region, reported an overall *Salmonella* prevalence of 9.27% across all sample types, with isolation rates of 8.04% in chicken organs and 11.20% in allied sources including eggs and feed ingredients (Sajid et al., 2015). Specifically, this study found a *Salmonella* prevalence of 9.64% in egg samples, highlighting that eggs represent a significant vehicle for potential *Salmonella* transmission in this region (Sajid et al., 2015). The study further identified that the maximum isolation prevalence was recorded in meat meal at 19.35%, followed by fish meal at 17.54%, hatchery fluff at 14.63%, livers at 13.17%, and poultry litter at 10.89%, indicating that multiple sources within the poultry production continuum contribute to the contamination of final products (Sajid et al., 2015). These findings emphasize the potential public health hazard posed by *Salmonella* contamination in the region and underscore the significance of establishing continuous surveillance systems to understand the ever-changing epidemiological patterns of *Salmonella* serovars, as the endemic prevalence of various serovars can cause outbreaks of human salmonellosis due to the consumption of contaminated meat and eggs, as has already been reported worldwide (Sajid et al., 2015). The prevalence rates observed in Northern Punjab are consistent with global patterns of *Salmonella* contamination in poultry products, although significant regional variation exists depending on production practices, biosecurity measures, and environmental conditions.

The comparison of *Salmonella* contamination between organic and inorganic eggs represents a particularly relevant aspect of research conducted in District Abbottabad, as consumer demand for organic and specialty eggs has increased substantially in recent years. Organic eggs, which are produced by hens raised according to certified organic standards that prohibit the use of synthetic pesticides, fungicides, herbicides, and fertilizers in feed, and require that hens be cage-free or free-range with access to outdoors, present unique food safety considerations compared to conventionally produced eggs (Gast et al., 2023). The term "pasture-raised" has emerged as a newer category in the egg industry, referring to sustainable agriculture practices where animals are raised on pasture, grazing and foraging while receiving supplementary diets, and it is important to distinguish this from "pasteurized" eggs, which undergo thermal processing to inactivate pathogens (Gast et al., 2023). Research examining the influence of different housing systems on *Salmonella* contamination risk has revealed that managing environmental and housing conditions for laying flocks is critical for reducing opportunities for the introduction, transmission, and persistence of *Salmonella*, and different hen housing systems for commercial egg production influence these environmental parameters, with unique risk factors and

management challenges characteristic of each system (Gast, 2023). While some studies suggest that free-range and organic systems may expose hens to environmental sources of *Salmonella* through contact with wild birds, rodents, and contaminated soil, other research indicates that the enhanced welfare conditions in these systems may improve immune function and reduce stress-related susceptibility to infection. The comparison between organic and inorganic eggs in District Abbottabad must consider these complex interactions between production systems and contamination risk, as well as the specific management practices employed by local producers. The presence of *Salmonella* in either production system carries significant public health implications, as contaminated eggs can transmit infection to consumers through both vertical transmission, where *Salmonella* colonizes the reproductive tissues of systemically infected laying hens and contaminates the egg interior during formation, and horizontal transmission, where salmonellae penetrate through eggshells after contamination of the exterior surface from environmental sources such as feces, soil, or contaminated equipment (Gast, 2023; Puntang-on et al., 2022).

The molecular identification of *Salmonella* isolates from District Abbottabad eggs enables not only confirmation of the pathogen's presence but also characterization of antimicrobial resistance patterns, which is of critical importance given the global public health crisis of antibiotic resistance. Studies examining antimicrobial resistance in *Salmonella* isolates from poultry environments have documented concerning levels of resistance to commonly used antibiotics. Research conducted in layer farming operations has revealed that *Salmonella* isolates demonstrate resistance to multiple antibiotics, including oxytetracycline, cefotaxime, and enrofloxacin, with one study reporting that *Salmonella enterica* serovar Typhimurium isolates were resistant to cefotaxime, enrofloxacin, and oxytetracycline simultaneously (Puntang-on et al., 2022). The same study detected antibiotic residues in egg samples, with streptomycin found in 36.11% of eggs, enrofloxacin in 11.81%, and tetracycline in 7.64%, suggesting that antibiotic residues in eggs may be related to the treatment of antimicrobial-resistant *Salmonella* isolates and the use of medicated feed with inappropriate antibiotic withdrawal times (Puntang-on et al., 2022). The detection of antibiotic residues in eggs is particularly concerning, as it indicates potential violations of withdrawal periods and raises questions about the judicious use of antimicrobials in poultry production. The presence of antimicrobial-resistant *Salmonella* in eggs destined for human consumption poses direct health risks, as infections caused by resistant organisms are associated with increased treatment failures, prolonged illness, and higher healthcare costs. The 2025 multistate outbreak of *Salmonella* Enteritidis linked to shell eggs in the United States, which resulted in 79 confirmed cases and 21 hospitalizations, demonstrated that resistant bacteria are increasingly emerging in eggs and poultry, with similar reports from China's Guizhou Province indicating that two-thirds of foodborne *Salmonella* strains exhibit resistance to multiple antibiotics (Malesu, 2025). These global patterns underscore the importance of

characterizing antimicrobial resistance in local *Salmonella* isolates, as the information generated can guide empirical treatment decisions and inform antibiotic stewardship policies in both human and veterinary medicine.

The identification of specific *Salmonella* serovars through molecular methods provides essential epidemiological information for understanding transmission dynamics and implementing targeted control measures. Globally, *Salmonella enterica* serovar Enteritidis is recognized as the predominant pathogen associated with egg contamination, and it has been the main cause of egg-transmitted illness for several decades (Gast, 2023; Malesu, 2025; Xiong et al., 2022). However, other serovars including *S. Heidelberg* and *S. Typhimurium* are also implicated in egg-borne outbreaks, and regional variation in serovar distribution is well documented (Gast, 2023).

Risk factors associated with *Salmonella* contamination in layer farming environments have been extensively studied, and the findings of such research provide valuable guidance for interpreting local results and developing intervention strategies. A comprehensive risk factor analysis conducted in a modern layer farm with a closed-housing system equipped with evaporative cooling, automatic feeding, and egg collection belts revealed that the highest *Salmonella* prevalence rates were found in samples from layer pen floors at 65.5%, followed by the egg sizing machine at 52.5%, and eggshells at 15% (Pumtang-on et al., 2022). Statistical analysis identified that rodent presence at the farm and the frequency of changing disinfectant foot dips were significant factors related to *Salmonella* contamination on pen floors, with odds ratios of 22.5 and 24 respectively, while hand-washing before sorting and cleaning of the egg sizing machine were significant factors for contamination at that site, with an odds ratio of 13 (Pumtang-on et al., 2022). These findings demonstrate that *Salmonella* prevalence in layer farming is intimately related to effective biosecurity and hygiene practices, including rodent control, cleaning of farm equipment, and good worker hygiene. The application of such risk factor analysis to the District Abbottabad context would help identify specific practices and conditions that contribute to *Salmonella* contamination in both organic and inorganic production systems, enabling the development of targeted interventions tailored to local circumstances. The importance of biosecurity is further emphasized by the understanding that contamination of eggs can occur through both vertical transmission from infected breeders and horizontal transmission from environmental sources such as contaminated equipment, dust, feed, or drinking water, making comprehensive biosecurity programs essential for *Salmonella* control (Pumtang-on et al., 2022).

The implications of *Salmonella* isolation and molecular identification research extend beyond academic interest to practical applications in public health and food safety policy. The findings of such studies can inform the development of risk-based surveillance programs, guide the implementation of hazard analysis and critical control point systems in egg production facilities, and support the design of educational campaigns targeting consumers and food handlers about safe egg handling practices. Research

has demonstrated that managing storage temperatures is vital for limiting the growth of *Salmonella* inside eggs, and that different interventions throughout the egg production cycle, applied in combination, represent the most effective overall strategy for controlling *Salmonella* in layers (Gast, 2023). The 2025 *Salmonella* outbreak investigation demonstrated how rapid genomic surveillance using whole-genome sequencing and coordinated public health action can identify contamination sources, prevent further illness, and inform global food safety strategies, with investigators able to match human infections to the outbreak strain through genetic fingerprinting (Malesu, 2025). This highlights the importance of incorporating advanced molecular techniques into routine surveillance programs, enabling rapid source traceback and timely intervention. For District Abbottabad, the establishment of molecular identification capabilities for *Salmonella* would enhance local public health infrastructure and contribute to the global network of foodborne disease surveillance. Furthermore, the integration of findings from local studies into national and international databases supports the One Health approach to addressing zoonotic threats, recognizing that human health, animal health, and environmental health are interconnected and require coordinated interdisciplinary collaboration for effective prevention and control of foodborne pathogens (Malesu, 2025).

CONCLUSION

Salmonella contaminations have never before been found in Pakistan's Abbottabad area. The goal of this experiment was to identify and assess the presence of *Salmonella* in both organic and inorganic eggs. With regard to all egg samples, the average contamination ratio that was found was 65.8%. The difference in handling/hygiene condition of the eggs at the markets may be the cause of the eggs in the sample of the market that was studied. When compared to wealthy nations, this is a sizable percentage. It might be seen as a worrying development for the welfare of the chickens as well as for general public health. Additionally, no regulation has been proposed to keep an eye on the feed mills and the chain of production for poultry. In contrast, Finland has been introducing legislation for decades to limit *Salmonella* in livestock and feed (Gantois et al., 2009). This investigation may be related to minimizing bacterial contamination by maintaining the sanitary state of egg collectors. Long-term storage of unsold eggs increases the danger of egg contamination, which may be brought on by improper shipping and storage practices. However, our findings imply that in order to decrease *salmonella* transmission, effective hygiene measures must be implemented in chicken farms and on market eggs. Reduced human infection rates and improved public health and safety can be achieved by reducing *Salmonella* contamination at the farm level. As one of the controls to lower the risk of *Salmonella* contamination in the poultry sector, 134 commercial organic acid compounds are widely utilized in feed and water. It is now understood that *Salmonella* a sporological success, which allows it to tolerate harsh environmental conditions, is partly attributed to its capacity to produce biofilms, a major global problem with food safety.

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