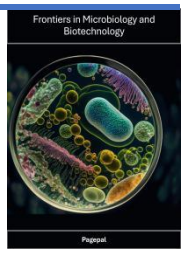
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Poultry-Associated *Salmonella*: Crossroads of pathogenicity and antimicrobial resistance: A comprehensive review

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Abstract

Salmonella is a gram-negative, rod-shaped bacteria that causes a range of barriers to public health, agriculture, and food security. This review pays attention to the topics including taxonomy, characteristics of Salmonella spp., their pathogenicity, and potential ramifications on public health, with a special emphasis on poultry association and food-borne contamination. The way virulence factors such as Salmonella Pathogenicity Islands (SPIs) help the bacterium to avoid immune responses and form infections are considered. Also, in this review, the author addresses the growing problem of AMR in Salmonella, mainly attributed to the misuse of antibiotics in poultry, resulting in MDR and ESBL-producing bacteria. Novel detection technologies including genomic hotspot scanning and whole-genome sequencing, as well as other approaches that can be regarded as innovative are discussed, including phage therapy and infection control and biosecurity measures. This approach, with a focus on the One Health concept, identifies future directions for sample control and prevention of human and animal health risks associated with Salmonella infection

Introduction

Salmonella is a gram-negative, rod-shaped bacterium capable of surviving in the wide range of conditions, growing through binary fission (Naushad, Ogunremi, & Huang, 2023). The classification of *Salmonella* under the genus and its two species *S. enterica* and *S. bongori* are described, accompanied by numerous serovars (Sang, Ren, & Yao, 2024). Over 2,600 serovars of these have been identified and characterized and due to the similarities in their phenotypic traits, their identification is very complicated (H. Wang *et al*., 2023). *Salmonella* is a highly pathogenic organism, whose pathogenicity is associated with its effector molecules like SPIs, which help the bacteria to invade the host cells, avoid immune response, and establish an infection (M. A. S. Khan *et al*., 2024).

Poultry as well as eggs and other foodstuffs are key sources of human infections extreme infections traced to contaminated food products often originate from poor handling of food or inadequate cooking (Hafez & Hauck,

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2023). As poultry is a crucial player in the economy, but remains a stronghold for *Salmonella*. It can be transmitted from one generation of birds to another, through eggs, and from bird to bird through contaminated environments and feeds hence its spread within the poultry population and to humans (Leroy, Charmpi, & De Vuyst, 2023). Proper management practices such as enhanced hygiene measures, better feed preparation, better monitoring and control measures, etc are implemented with the goal of combating constant reportage on the disease and its effects (Ovuru, Izah, Ogidi, Imarhiagbe, & Ogwu, 2024).

The primary causes of AMR in *Salmonella* are overuse and misuse in the poultry industry involving use of the antibiotics as growth promoters and for disease prevention within subtherapeutic concentrations (Mithuna *et al*., 2024). Such practices put selection pressure on the environment thus increasing the emergence of multi-drug resistant (MDR) strains. There are also new studies that have found that horizontal transfer of extended-spectrum beta-lactamase (ESBL)-producing *Salmonella* strains which are resistant to the beta-lactam antibiotics including the third-generation cephalosporins (Irfan, Uzair, Khan, & Saeed, 2024; Kang *et al*., 2024). The authors also found resistance to tetracycline, ciprofloxacin, and nalidixic acid in South Asia due to weak implementation of rules and regulations about the use of antibiotics in livestock and poor monitoring of this problem (Samad). Vigorous implementation of rules regarding antibiotic usage in agriculture, better biosecurity, and regular and effective surveillance programs have been suggested (Poudel *et al*., 2024). National and global networking and sharing of information through the joint World Health Organization (WHO) and the Food and Agriculture Organization (FAO) are also enabling the fight against AMR (Organization, Programme, & Health, 2023).

Present review focuses and discusses the current state of knowledge on *Salmonella*, with an emphasis on its biology, epidemiology, and control. the review is intended to present a guide to managing and preventing this pathogen to researchers, public health officials, and policymakers.

Salmonella

The rod-shaped, gram-negative bacteria *Salmonella* is made up of cytoplasm, cell membrane, ribosomes, plasmids, and a nucleoid region. Its length is 2-5 µm, and its diameter is around 0.7-1.5 µm. Its flagella, which aid in movement, are present. *Salmonella* is a chemoorganotroph, a facultative anaerobe that gets its energy via oxidizing reduced organic molecules. *Salmonella* reproduces via binary fission, which starts after the epithelium is colonized and involves DNA molecule replication and attachment to the cell membrane. Once the bacterium doubles in size, the cell membrane starts to compress inward and a cell wall develops between the two DNA molecules to split the original cell into two identical daughter cells (Chong *et al*., 2021). The bacteria either stays in the gut or travels to the blood or lymphatic systems once it has multiplied. *Salmonella* may survive for many weeks in the moist free environments without a live host and for many months in moist environment. It is often unaffected by very cold temperatures. Since the bacteria can only be killed at temperatures over 75°C, fresh and undercooked food as well as improperly cleaned fruits and vegetables are major sources of the infection's spread (Naushad *et al*., 2023).

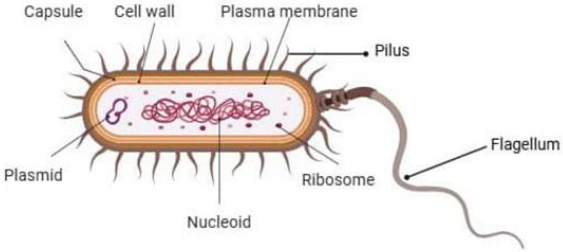


Figure1. Structure of bacterial cell

Classification

Salmonella belongs to the order Enterobacteriales, family Enterobacteriaceae, phylum Proteobacteria, class Gamma-Proteobacteria, order Enterobacteriales, and genus *Salmonella* under the Kingdom Monera, also known as Eubacteria. Two widely used taxonomical approaches have been adopted to award *Salmonella* names due to the genus' taxonomy's confusing and disputed nature. The Judicial Commission of the International Committee on the Systematics of Prokaryotes issued Opinion 80 to rectify the discrepancies in *Salmonella*'s taxonomy categorization. This Opinion aims to address these discrepancies. However, Opinion 80 was limited to issues about organism nomenclature, and it was meant to give a transparent

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explanation and comprehension of *Salmonella*'s division into two well-known species. Since over 2000 *Salmonella* species are identical to one another, it might be challenging to identify each one correctly (Naushad *et al*., 2023) The Centres for Disease Control and Prevention (CDC) have identified two strains of *Salmonella*, *Salmonella enterica,* and *Salmonella bongori,* to expedite this procedure. Six subspecies of *Salmonella enterica* exist: *S. Enterica* ssp. enterica (I), *S. Enterica* ssp. salutations (II), *S. Enterica* ssp. arizonae (IIIa), *S. Enterica* ssp. *S. diarizonae* (IIIb). Enterica ssp. *houtenae* (IV), as well *as S. Enterica* ssp. *dica*. The White-Kauffmann-Le Minor classification, which has been updated by the Pasteur Institute in Paris, France, and the Collaborating Centre for Reference and Research on *Salmonella* of the World Health Organisation, divides the species and subspecies into many serotypes. With changes in their somatic O antigens (lipopolysaccharide), H antigens (flagellar proteins), and Vi antigens (capsular polysaccharides), the more than 2600 serovars that make up the *Salmonella* genus may be identified. *Salmonella* is the classification used for the majority of serovars. *Enterica ssp. enterica (I),* with serogroups A, B, C1, C2, D, and E being the most common (Silva, Campos, Guimarães, & Oliveira, 2023). It is best to write names in Roman form with a capital initial letter to avoid confusion and to differentiate between species-level and serovar designations. It is standard practice in scientific literature to include the name of the genus first, then the name of the species, the word "serovar" (abbreviated as "ser."), and at the end, the specific name of the serovar. To write the scientific nomenclature *Salmonella enterica* subsp. is one example. *Enterica serovar Typhimurium (or ser).* This long-written convention may be streamlined by putting the genus name first, followed by the name of serovar with an initial capital letter. This will reduce the laborious character of naming. *Salmonella* enterica subsp, for example. *Salmonella Typhimurium* is another name for *enterica serovar Typhimurium* (Teklemariam *et al*., 2023). *Salmonella* can live in a wide range of environments and may be divided into three groups according to how well it can adapt to its surroundings. *Salmonella* Typhi, *Salmonella* Paratyphi A, B, C, and *Salmonella* Sendai are among the Group 1 *Salmonella* serovars that have developed to infect humans and higher primates. *Salmonella* Dublin in cattle, *Salmonella Gallinarum* in poultry, *Salmonella Abortusequi* in horses, *Salmonella Abortusovis* in sheep, and *Salmonella choleraesuis* in pigs are only a few examples of the animal hosts that Group 2 *Salmonella* are highly specialized towards. A wide range of hosts, including humans, animals, and the environment, are susceptible to *Salmonella* Group 3. This comprises the two most common serotypes of *Salmonella* that are often spread to people worldwide: *Salmonella Typhimurium* and *Salmonella Enteritidis* (Yada, 2023).

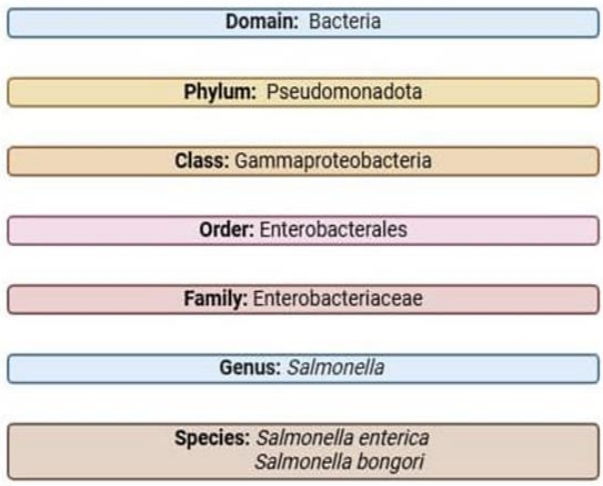


Figure 2. Classification of *Salmonella*

Virulence factors and pathogenicity mechanisms

Salmonella's pathogenicity is a complex process that is defined by many elements working together. It includes a variety of structures, including effector proteins, flagella, and fimbriae. *Salmonella* may also enter the body, proliferate, and avoid detection by the host's immune system. *Salmonella* Pathogenicity Islands (SPIs) are specific areas of the genome that contain the bulk of genes linked to the pathogen's propensity to cause illness. *Salmonella* Pathogenicity Islands, or SPIs, are large chromosomal regions that are home to a large number of virulence-causing genes. There are now 17 SPIs that have been found. Both SPI-1 and SPI-2, which have been the subject of many studies, include the genetic code for the Type III Secretion System (T3SS), which opens a hole in the host cell's membrane. This hole may allow bacterial effector proteins to penetrate the inside of these cells. The actin cytoskeleton is altered by these proteins, allowing the pathogen to enter and proliferate within the host. Initially, SPI-1 is categorized as an invasion island. The essential

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genes needed for nonphagocytic cell penetration have been identified for each species and subspecies of *Salmonella* (A. M. Dos Santos, Ferrari, Panzenhagen, Rodrigues, & Conte-Junior, 2021). The gene cluster is responsible for iron uptake, the production of T3SS, and the stimulation of innate immune pathways. The ability of the bacteria to survive in phagocytic cells and proliferate within the SCV (*Salmonella* Containing Vacuole) in eukaryotic cells is linked to SPI-2. The encoding of fimbriae, protein synthesis, adhesin and toxin production, and macrophage viability are the main functions of the other SPIs (Lou, Zhang, Piao, & Wang, 2019). as their natural settings, bacteria usually live as grouped colonies called biofilms. These biofilms help the bacteria in the environment to remain stable, proliferate, and remain protected. Furthermore, bacteria might strengthen their resistance to sanitizers and antimicrobial agents thanks to biofilms. Exopolysaccharides (EPS), a combination of polysaccharides and proteins, make up the biofilms. Changes in these components might interfere with the viruses' ability to spread disease. The extracellular matrix of biofilms is essential to *Salmonella*'s capacity to survive under harsh environmental conditions. The fimbriae of *Salmonella* spp. are essential. cellulose provides chemical, mechanical, and biological defense against the environment while adhering to the target cell (PT, 2020).

Importance of *Salmonella* in foodborne diseases

Foodborne infections are a major public health concern on a global scale. Each year, about 1.2 million cases of foodborne diseases occur in the United States; *S. Typhimurium*, *S. Enteritidis*, and *S. Newport*. *Salmonella* was reported to be the cause of 11,581 (30.7%) human outbreaks in 2018, an increase from the 20.6% occurrences in 2017 according to the European Food Safety Authority (Garces-Vega, Ryser, & Marks, 2019). Other materials may potentially be contaminated with *Salmonella*, in addition to meat, eggs, and milk. The frequency of instances of salmonellosis associated with meals low in water activity is becoming more widely acknowledged. Many of these foods are readily digestible, don't need preparation, and keep well at room temperature for an extended period of time. The fact that businesses utilize the reduction of water activity (aw) as a control tool expressly to prevent the development of dangerous microorganisms in food is another important factor. Initially, foods such as flour, almonds, chocolates, dried fruits, and powdered milk were considered safe to eat since they prevented the development of bacteria. They either experience processes including crystallization, dehydration, desiccation, and lipid oxidation, which are unfavorable for microbes, or they have a low water activity (aw) value. However, there are a few viruses that may survive under these conditions for months or even years. This is a serious reason for concern for many different companies (He *et al*., 2023).

Poultry as an economical source

In Pakistan, the poultry industry plays a critical role in mitigating the imbalance between the supply and demand of protein. Commercial chicken farming started in Pakistan in the early 1960s and continued to grow significantly over the next several years. The promotional activities of the government and the community of poultry farming tenacity may have contributed to the early growth of the industry (Sajid, Asghar, Tariq, Wilk, & Płatek, 2023). The government offered particular incentives to encourage this business since it understood that the chicken production chain was an essential part of the food processing industry. As a result, the chicken industry received temporary exemptions from import tariffs, sales and income taxes, and both. As a result, the sector had annual growth rates of 20–30% in the early 1970s and 10–15% in the 1980s. A robust home market, which led to an annual growth rate of chicken meat consumption of over 4%, was the main driver of this development. Pakistan's poultry business is quite active and well-managed; it makes up 26.8% of the country's total meat output, 5.76% of the agricultural sector, and 1.26% of the GDP. For about 1.5 million people, the poultry business has grown significantly over the last few years and is now an important source of employment (M. W. Khan & Abbas, 2023). Pakistan's human population is expanding at a rate of 2.03% each year. There is a strong 89.0% correlation between the number of people on the planet and the growth of poultry in Pakistan. This estimate (Hussain, Rabbani, Aslam, & Ahmad, 2015) shows the sector's potential growth in Pakistan over the next years. Pakistan is now the world's eleventh-largest poultry producer, according to statistics and numbers from the Pakistan Poultry Association. 19.170 billion eggs and 2.028 million tons of meat are contributed by the commercial poultry industry. Still, Pakistani people only have access to 5 kg of chicken meat and 51 eggs annually per person. This is far less than what is available per capita in industrialized countries, where there are 300 eggs and 41 kg of meat annually (Survey, 2017). The public is misinformed

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about professionally produced grill meat and eggs from commercial layers, despite the fact that the chicken business is a major source of animal protein. Given that this contributes to the development of antibiotic resistance in human diseases, there is rising concern over the practice of giving large doses of antibiotics to hens. Hormones are also given to chickens in order to accelerate their growth and help them grow to greater sizes. It is crucial to remember that improvements in environmental circumstances and genetic selection have contributed to the development, livability, and health of chickens (McCulloch, 2017; Ronquillo & Hernandez, 2017).

Poultry as a reservoir of *Salmonella*

A wide variety of domestic animals, including sheep, cattle, poultry, and pigs, may get *Salmonella* serovars. Globally, *Salmonella* is the most common cause of foodborne illness, and the main sources of contamination are eggs and chicken meat. The symptoms that the infected animals may show might vary widely, from mild gastroenteritis to, in the worst cases, deadly consequences. Numerous serotypes, such as *S. typhimurium* as well as *S. enteritidis*, which are potentially contagious to humans, are mostly seen in poultry. The incidence of *Salmonella* in poultry varies throughout countries. In some developed countries, one percent of chickens test positive for *Salmonella*. However, a number of factors might affect the prevalence of *Salmonella*, such as countries with complex epidemiological issues reporting a 10% positive rate in the flock. Chicken infections with *Salmonella* may occur at various developmental stages and via a variety of routes, including as vertical (parent to child) and horizontal (individual to person) transmission pathways. Different forms of *Salmonella* serovars are detected in poultry sources, and their spread is location- and time-dependent. There are several ways that *Salmonella* might enter flocks of chickens. The majority of cases of *Salmonella* infection in humans, poultry, and feed come from insects and other animals. *Salmonella* infections may originate from contaminated feed and spread to animals and insects, or they can infect flocks of chickens directly. In their 2022 paper, Shariat, Larsen, Schaeffer, and Richardson investigated the possibility that turkeys' pelleted feed served as a *Salmonella* reservoir (Shariat, Larsen, Schaeffer, & Richardson, 2022). Additionally, a link between tainted animal feed and human *Salmonella* epidemics was found. *Salmonella* was effectively recognized by researchers in samples of poultry and feed. They discovered that the *Salmonella* serotypes detected in feed and poultry were same by using pulsed-field gel electrophoresis and serological analysis (Meteab & Abed, 2018).

Salmonellosis

The primary source of is present in both human and animal digestive tracts, namely in swine and poultry. The organisms are released in faeces once they become intestinal forms. From there, they may find their way to water, dirt, and kitchen surfaces, among other places, via the actions of insects and other creatures. *Salmonella* infections mostly affect humans who consume eggs, poultry, and raw meat products. S is the most often found serovar that causes food-borne illnesses. Typhimurium in addition to *S. Enteritidis*. The most common way for people to get *Salmonella* disease is via eating or drinking contaminated food or drinks (J. Wang *et al*., 2023). Every food item has the capacity to make people sick. Even in the absence of contaminated food or drink, direct human-to-human or animal-to-human contact may transmit *Salmonella*. This kind of gearbox is uncommon, nevertheless. It is difficult to pinpoint the precise incidence of *Salmonella* disease. Since only large outbreaks are investigated and documented, the reported cases only represent a small portion of the actual number. Consequently, sporadic cases are not well recorded since only those with persistent diarrhea seek medical attention for microbiological testing. Infectious diseases caused by food or water are common, uncomfortable, and sometimes fatal conditions that impact millions of people globally (Shaji, Selvaraj, & Shanmugasundaram, 2023). The Centers for Disease Control and Prevention (CDC) estimate that 76 million Americans get food-borne illnesses each year, leading to over 325,000 hospital admissions and more than 5000 deaths. Foodborne infections result in substantial financial costs. It is estimated by medical professionals that the yearly cost of food-borne diseases in the US is between $5 and $6 billion, taking into account both the direct medical expenses and the lost productivity. The yearly economic impact of *Salmonella* infections alone, including direct and indirect healthcare costs, is one billion dollars. Salmonellosis, both typhoid and non-typhoid, is still a major public health problem and is without a doubt the most economically significant foodborne infection. The prevalence of typhoid salmonellosis is still present (Raut, Maharjan, & Fouladkhah, 2023).

Pathogenesis

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Salmonella interacts with non-phagocytic cells, namely the intestinal mucosa's epithelial cells, after eluding the stomach's immune system and making it to the intestines. The organisms have virulence factors that help spread an infection, such as toxins, fimbriae, virulence plasmids, and flagella. Numerous processes contribute to the pathogenesis of illness, such as endocytosis by bacteria, neutrophil recruitment and migration, cytokine release from epithelial cells, fluid and electrolyte discharge, and systemic infection (Bibek Lamichhane *et al*., 2024). Before *Salmonella* to reach the small intestine of a host, it must first pass through the proventriculus, an acidic area of the digestive tract. Therefore, the ability of bacteria to withstand and survive in acidic conditions is crucial in the context of infection. The bacteria infiltrate and multiply inside the chicks' mononuclear phagocytic systems' cells. The gastrointestinal tract is where these bacteria multiply most often, and as a result of bacterial discharge via feces, the environment becomes heavily contaminated (Farhat *et al*., 2023).

Clinical Signs and Symptoms

Salmonella often infects a wide variety of animals, including both domesticated as well as wild species. This bacteria often affects the gastrointestinal tract of the host, frequently without obvious symptoms. *Salmonella* infections may manifest as either clinical (symptomatic) or sub-clinical (asymptomatic). Depending on the kind of *Salmonella* present, different symptoms might manifest in poultry, and it is possible for hens to harbor the bacteria without displaying any signs of illness (Galán-Relaño *et al*., 2023). For instance, adult birds may have diarrhea, decreased egg production, poor hatchability, and a higher rate of S-related death. Pullorum serovar enterica. The illness results in anorexia, diarrhea, dehydration, and mortality in young poults. On the other hand, septicemia, severe diarrhea, weakness, and sometimes even death are signs of poultry typhoid. However, regardless of the precise form of infection, all *Salmonella* infections in poultry often manifest with catastrophic symptoms such as severe diarrhea, fever, weight loss, dehydration, and even death (Raut *et al*., 2023).

Risk Factors

Animal Risk Factors

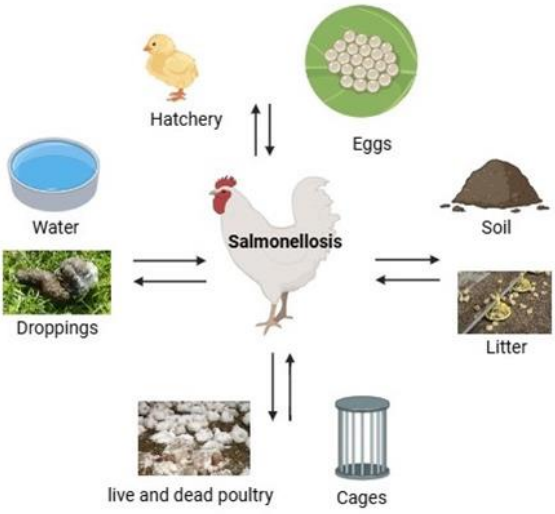


Figure 3. Transmission of Salmonellosis among different sources

Since they are the natural hosts, chickens have developed a high degree of immunity to various illnesses. In addition, chickens may act as carriers of subclinical illnesses, transferring the infections to other chickens via contamination and to their embryos through the egg. The ability of the infection to persist increases with age, with mortality in newly hatched chicks being greatest. As a result, there was a noticeable and statistically significant difference between the seroprevalence and the age of the birds, which showed a positive connection. In particular, the existence of S. Adult hens were shown to have a greater prevalence of pullorum antibodies than newborn chicks, which are known to have a higher death rate just after hatching. Pullorum disease commonly presents clinically in chicks less than three weeks old, which makes it difficult to identify antibodies in these birds unless they live and develop into carriers (Galán-Relaño *et al*., 2023).

Environmental and Management Risk Factors

Filthy surroundings combined with hunger, overcrowding, and other stressful events may aggravate mortality rates and result in a reduction in performance, especially in young birds. The presence of damp organic materials such as chicken dung facilitates the survival and growth of infections by providing essential nutrients and physical shielding. The adoption of a methodical immunization program, efficient ventilation, suitable hen

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building spacing, and the lack of breed mixing in commercial poultry farms may all be responsible for the low frequency in the intensive environment (Ehuwa, Jaiswal, & Jaiswal, 2021).

Pathogen Risk Factors

Salmonella pullorum contributes to the long-lasting infection in these hosts by being viable in a favorable environment for many months or even years. *Salmonella* contains lipopolysaccharide (LPS), an important part of the outer membrane and an important toxin that interacts with the host immune system to cause inflammation, septic shock, fever, and eventually death. In order to lessen the host's innate immune reactions, an organism may alter the O-antigen's structural composition. It is believed that this action increases the microorganism's ability to survive and remain in the host. *Salmonella*'s harmful or pathogenic effects are partially attributed to lipid A. Cytokines may be induced to be produced by lipopolysaccharide (Pees, Brockmann, Steiner, & Marschang, 2023).

Transmission

Both laterally and vertically, *Salmonella*e may spread from infected breeding flocks to their progeny. When eggs are produced that have *Salmonella*e in them, either on the surface or in the contents, it may spread the paratyphoid disease to the progeny of infected breeder flocks. Because feces are present during the laying eggs, paratyphoid *Salmonella*e are often present in the eggshells. If the shell structure is disrupted during hatching, *Salmonella*e may penetrate and pass through the shell and shell membranes, thereafter they may either directly infect the developing embryo or expose the chick to infectious *Salmonella* organisms (Tariq *et al*., 2022). Direct contact with birds, eating contaminated dung or litter, drinking polluted water, coming into touch with workers, contaminated personal and agricultural equipment, and a number of other factors may all result in horizontal transmission. Infected chicken house conditions are often linked to paratyphoid *Salmonella*e infections. It is thought that some species of *Salmonella* are a normal component of the microbes that live in an animal's gallbladder or digestive system. Consequently, these animals could possibly have a direct or indirect role in the virus's dissemination to humans. Poultry and poultry products are the major cause of *Salmonella* outbreaks in human beings. chicken contamination may occur during the processing of corpses if diseased portions such as the liver and intestines are not handled properly (E. J. E. Dos Santos *et al*., 2020).

Vertical Transmission

The term "vertical transmission" describes the genetic transfer of an illness or characteristic from a parent to their progeny. It is unclear how important vertical transmission is for chicken typhoid. As *S. gallinarum* Although has been detected in eggs, it has been difficult to recreate in an experimental environment the vertical transmission to offspring. Additionally, very sensitive poultry usually manages to eradicate *S. gallinarum* if they get it through and don't show signs of being carriers. Prolonged carriage has been seen in hens that were resistant to clinical indications, as they have been able to carry the organism for at least 14 weeks. *S. Gallinarum* may cause salmonellosis in eggs and can infect reproductive organs, which can result in the virus being passed from parent to child (Liu, Zhang, Ding, Bin, & Zhu, 2023).

Horizontal Transmission

Chickens may get *Salmonella* from their local environment in a variety of ways, which can then infect additional chickens. In addition to carrying supplies like water and food, these vehicles may also hold wild animals. *Salmonella* may go from one species of animal to humans via contaminated excrement. The bacterium is stored in the feces of infected chickens, who then excrete it for lengthy periods of time. Therefore, the waste products of infected birds act as a major source of infections for healthy birds. Birds may also spread among themselves by mating, eating eggs, cannibalism of unwell birds, and skin wounds. Contamination will also occur. Transmission of illnesses between bird populations may occur via contaminated transport vehicles and the entry of diseased birds or flocks. Moreover, it is often believed that the main natural environment reservoirs for *Salmonella* include insects, rodents, mammals, and wild bird species. Bird droppings may contain *Salmonella* germs, which can taint egg shells. Bacteria might penetrate the egg if the shell fractures (Galán-Relaño *et al*., 2023).

Diagnosis

Clinical Diagnosis

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Clinical signs, flock background, mortality rates, and post-mortem investigation are the main factors that define the preliminary diagnosis. When pullorum disease strikes, the most common clinical signs seen in affected birds are huddles near a heat source, anorexia, depression, and white feces stuck to their vents. When the chicks are 2-3 weeks old, the mortality rate hits 100%. The lesions are characterized by unabsorbed yolk, white nodes dispersed throughout the lungs, and localized necrosis of the liver and spleen (Farhat *et al*., 2023).

Serological Test

Serology is a helpful technique for detecting animal populations that are infected and assessing the level of infection within a particular population. The slide agglutination test (SAT), fast whole blood agglutination test, rapid serum agglutination test, tube agglutination, and ELISA are among the serological tests. A complex corpuscular antigen (like bacteria) and specific antibodies that form an antigen-antibody network are necessary for the agglutination test to be performed. As a result, the antigen begins to clump together visibly. These aggregates sink to the bottom of the microtiter cup due to gravity, which makes the previously hazy liquid above transparent (Diaz *et al*., 2022).

Differential Diagnosis

The differentiation between signs of disease and lesions resulting from *S. pullorum* (pullorum illness). *S. gallinarum*, or chicken typhoid, is not conceivable because of these similarities. The antigenic and biochemical characteristics of these bacteria also show a great deal of similarity. However, fully grown birds are the majority of those affected by chicken typhoid. When white nodules are seen in internal organs, they might be misinterpreted for hepatic lesions caused by respiratory tract lesions or Marek's disease. It's critical to differentiate them from joint diseases with synovitis and bursitis brought on by various bacteria or viruses, aspergillosis, and Yersinia pseudotuberculosis (Kabeta, Tolosa, Duchateau, Van Immerseel, & Antonissen, 2024).

Methodologies for rapid detection of *Salmonella*

Often referred to as "rapid methods," the use of these cutting-edge technologies for detection involves assays based on nucleic acids or antibodies that have been modified or improved over conventional protocols. These rapid detection technologies may provide a host of significant advantages to the food industry, including labor efficiency, cost-effectiveness, sensitivity, speed, accuracy, and labor efficiency. With advancements in detection technology, it is now possible to diagnose certain foodborne diseases faster and with more detail thanks to analysis. Furthermore, this has improved the precision of a rapid method for identifying particular pathogens that coexist with benign species in food or other complex biological environments, even to the point of detecting minute genetic differences between strains (Aladhadh, 2023). In the end, the sensitivity has been raised to detect even tinier concentrations of active bacteria in food or other complex substances. Even at extremely low concentrations, these microorganisms have the ability to infect highly susceptible people. Quick detection systems have also seen tremendous development, which makes them ideal for automation and high-throughput outcomes. In addition to minimizing errors made by humans, this lowers expenses by enabling more tests to be run at once. While classic culture-based methods sometimes take 4-5 days, cutting-edge molecular and immunological techniques may detect the target pathogen in food samples in only a few hours on average. The next sections outline current and emerging rapid methods for detecting *Salmonella* in chicken (Wang *et al*., 2021).

Culture methods

Although *Salmonella* is often diagnosed using traditional culture-based methods, these techniques are frequently labor-intensive, time-consuming, and impractical for large-scale or on-site testing. However, these methods have been used since the diagnosis of enteric fever and are generally regarded as the most accurate and dependable. These techniques for detecting *Salmonella* have been standardized by the International Organization of Standards, and regulatory agencies all around the globe utilize them extensively. Similar instructions have also been provided by the FDA via its Bacteriological Analytical Manual (BAM) (M. S. Awang *et al*., 2021). The first step in most classic culture procedures for the food samples is pre-enrichment in a non-selective liquid medium, such as buffered peptone water. The pre-enrichment process is then put into the two selective-enrichment media that successfully stop the development of undesirable microorganisms: Muller-Kauffmann Tetrathionate-Novobiocin (MKTTn) broth and Rappaport Vasiliadis Soy Broth (RVS). The material is then inoculated

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onto two or more selective differential agar media, e.g., BrillianceTM *Salmonella* Agar, Xylose Lysine Deoxycholate (XLD), Xylose Lysine Tergitol-4 (XLT-4), Brilliant Green Sulfa (BGS), Bismuth Sulfite (BS), and other comparable alternatives. *Salmonella* bacteria may proliferate and distinguish themselves from other background microbial flora thanks to this technique. Verifying the existence of suspected positive *Salmonella* colonies is the last step in the standard cultural methods process (Lee, Runyon, Herrman, Phillips, & Hsieh, 2015).

Enzyme-linked immunosorbent assay (ELISA)

For the purpose of identifying pathogens in food, the most used antibody-based technique is the enzyme-linked immunosorbent test (ELISA). An immunological approach has been used to identify *Salmonella* in various chicken production samples, such as water, feed, feces, litter, and corpse rinse. When compared to culture-based approaches, this technology has shown increased sensitivity and quicker results. The ELISA method, which is the foundation for *Salmonella* detection, has been improved with the introduction of more advanced technologies. For example, the test's sensitivity was increased by measuring and detecting *Salmonella* in poultry probiotic bacteria like Veillonella, which breaks down lactate into propionate and acetate, using monoclonal antibodies (Gao *et al*., 2023). The bare minimum number of *S. Typhimurium* cells per milliliter that can be detected in a pure culture using a monoclonal antibody is 5.5 x 104. In the rinsed chicken, fewer than 100 Using a mix of monoclonal and polyclonal antibodies together with commercial filtering equipment, *S. Typhimurium* cells were discovered. *Salmonella* in food-producing animals may now be found using ELISA methods, which were first created to identify the bacteria in food and animal feed. The research highlights the advantages of the ELISA platform over the conventional culture-based method. However, depending on sample times and processing techniques, commercial ELISA sensitivity may vary greatly, sometimes leading to false negative results (Yin *et al*., 2023).

Polymerase Chain Reaction (PCR)

The ability of *Salmonella*-specific primers to bind to the target sequence specifically via complementary DNA base pairing is what this test is based on. The two strands of the DNA segment between the two annealed primers are replicated simultaneously when Taq polymerase, a thermostable DNA polymerase, recognizes the template primer complex as a substrate. Depending on the Taq polymerase's thermostability, the denaturation, annealing, and elongation steps happen in a cyclical fashion until the target sequence is amplified to a detectable level. The DNA, primers, polymerase, deoxynucleotide triphosphates (dNTPs), and a buffer are mixed in a reaction tube before the thermocycler's first cycle is started. Three steps are repeated, each time amplifying a particular region of the *Salmonella* genome: (a) Heating the double-stranded DNA to make it into single strands. (a) The freezing procedure used to affix specific, complementary, brief DNA sequences known as oligonucleotide primers to the single-stranded molecule. (c) The technique of lengthening primers with enzymes to produce an exact duplicate of the original double-stranded DNA sequence. This process is repeated from (a) to (c) in cycles of thirty to forty. The last step involves identifying the amplified target DNA using agarose gel electrophoresis. It is often necessary to culture the bacteria on an enrichment medium, separate and purify the DNA, and then do PCR. A state-of-the-art technique for identifying pathogens in food samples, including *Salmonella*, is PCR-based testing (Mohd Syafiq Awang *et al*., 2021; Naushad *et al*., 2023).

Whole-genome sequencing methods (WGS)

The study of foodborne *Salmonella* infections and microbiology have been transformed by whole-genome sequencing (WGS) methods. WGS provides more information and accuracy by enabling whole genome sequencing and analysis. In addition, this method could provide a thorough and effective means to determine the genotype, resistance, and virulence genes of bacteria in a single laboratory procedure. Some countries, like the United States and England, have included whole genome sequencing (WGS) in their food safety standards (Stevens *et al*., 2022).

Antimicrobial Resistance

Antimicrobial resistance is a global problem that is characterized by a dearth of evaluations of *Salmonella*'s resistance to antibiotics and a lack of adequate laws. Furthermore, antimicrobials are freely available without a prescription, which results in patients not following their treatment plans, which may be detrimental to the course of therapy. Extended spectrum beta-lactamase (ESBL) genes have been developed and persist because of the widespread and unreasonable use of antimicrobial drugs in chicken

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husbandry. This poses a serious risk to the public's health as well as the poultry industry. Research on salmonellosis conducted in Ethiopia has shown that *Salmonella* is becoming more resistant to common antimicrobials used in the veterinary and public health domains. The nation's history of eating raw meat in conjunction with the pervasive, unfiltered use of antibiotics highlights the high incidence of salmonellosis in the nation. Treatment of clinical cases may be significantly jeopardized if resistance to two or more antimicrobials is present since a considerable proportion of *Salmonella* isolates have shown resistance to commonly recommended antibiotics (Malijan *et al*., 2022). Numerous bacteria have developed resistance to antibiotics as a result of the widespread and careless use of these medications. Antibiotic-resistant bacteria are encouraged to survive by selection pressure created by the usage of antimicrobials in each particular situation. Antibiotic-resistant bacteria have become a major problem in the food chain due to the widespread use of antibiotics on domestic animals to promote growth and prevent sickness. Antimicrobial resistance has become a major problem in many illnesses, especially with the emergence of drug-resistant strains or variants. Because they may pick up and distribute plasmids, transposons, phages, and other genetic elements, several environmental infections have shown increased resistance to antibiotics (Fatima *et al*., 2023).

AMR of *Salmonella* in South Asia

Antimicrobial resistance, or AMR, is a serious problem in South Asia, and its increasing incidence is cause for alarm. Antimicrobial resistance (AMR) in animal feed, along with its consequent widespread and unregulated usage, is acknowledged as a major factor in AMR in the context of human health. The poultry industry is well acknowledged in the realm of animal health for its notable escalation in the application of antibiotic agents and the consequent emergence of antimicrobial resistance (AMR). Antibiotic-resistant bacteria proliferate in South Asian fields because farmers there utilize antibiotics to compensate for poor sanitation and hygiene standards (Hoque *et al*., 2020). The risk of bacterial resistance in humans may be increased by the presence of drug-resistant microbes in the chicken business, poor antimicrobial agent management, poor sanitation and hygiene standards, and a lack of effective infection prevention and control methods. Given that people live near to chicken farms in South Asia, this is very alarming. A worrying level of antimicrobial resistance (AMR) has been shown by a number of point prevalence studies carried out in nations including Bangladesh, Pakistan, India, and Nepal (Talukder *et al*., 2023). These point prevalence studies in South Asian chicken farms are limited to looking at resistant bacteria in chicken carcasses, dressed poultry meat, and chicken flesh. Because of this, the literature on the existence of resistant microorganisms in live poultry birds and the environment of poultry farms is scarce (E. Khan, Hafeez, & Ikram, 2018). As a result, it is still unknown how common drug-resistant bacteria are on chicken farms and in the surrounding area, as well as how specifically these microbes contribute to the growth in antibiotic resistance in both people and chickens. Furthermore, a comprehensive analysis of the level of antimicrobial resistance (AMR) in South Asian chicken farms is conspicuously lacking. Inadequate government monitoring and surveillance mechanisms may be the reason for the paucity of comprehensive data on antimicrobial resistance (AMR) in South Asian chicken farms (Britto, John, Verghese, & Pollard, 2019).

Over 8 million kg of antibiotics are used annually in animal husbandry, with 70% of that quantity going towards nontherapeutic goals including fostering development and managing illnesses. In comparison, just 1.3 million kg of antibiotics are used annually to treat infections in humans. The widespread use of antibiotics in cattle causes the natural bacteria of the animal and the environment around it to become resistant to the antibiotics, which is then released into the manure. Because MDR pathogens are used unrestrictedly throughout their early growth, effective management of infections is essential (M. A. S. Khan & Rahman, 2022).

Salmonella species, found in eggs often show a high degree of antimicrobial resistance (AMR). The most resistant strains of *Salmonella* to ampicillin and nalidixic acid were found among the several antibiotics used in the chicken production process. A research conducted in India found that compared to duck eggs, commercial layer hen eggs showed significantly higher levels of bacterial resistance to tetracycline (TET), oxytetracycline (OT), and nalidixic acid (NA) (Harsha *et al*., 2011). (Mahmud *et al*., 2013) reported that *Salmonella* discovered on the outside of chicken eggs in Bangladesh showed overall resistance to ampicillin and amoxicillin, with tetracycline, ciprofloxacin, and colistin following suit. *Salmonella* bacteria in Chinese eggs were discovered by Xie *et al*. (2019) to have changing degrees of

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resistance to beta-lactam antibiotics, having amoxicillin, cefazolin, penicillin, and piperacillin. Additionally, they demonstrated resistance to tetracyclines and aminoglycosides, including minocycline, gentamicin, kanamycin, and streptomycin (Xie *et al*., 2019).

Extended-Spectrum-Beta-Lactamases (ESBLs) *Salmonella*

Currently, there is a growing worry about the rising resistance of *Salmonella* species to routinely used antimicrobials. Particularly worrisome are strains that have developed resistance to several drugs, known as multiple drug resistance (MDR), affecting two or more therapeutic agents. While fluoroquinolones like ofloxacin and ciprofloxacin, as well as extended-spectrum cephalosporins like cefotaxime and ceftriaxone, have shown their effectiveness, resistance to these drugs has also developed. The rapid rise and global dissemination of antibiotic-resistant strains, including the identification of the Extended Spectrum Beta-lactamase (ESBL) *Salmonella* species, is increasingly becoming a significant global concern (Suresh *et al*., 2019). ESBLs are enzymes that have the ability to break down penicillin, cephalosporin, and oxyiminino-β-lactam compounds (such as cefuroxime, third-generation cephalosporin, and aztreonam), with the exception of cephamycins and carbapenems. The majority of ESBLs are classified as Ambler class "A" β-lactamases and may be effectively inhibited by β-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam. ESBLs are plasmid-borne and hence readily disseminated throughout the Enterobacteriaceae family. This possibility intensifies the propagation of resistance to β-lactams and other frequently used antibiotics such as quinolones and aminoglycosides (Z. Wang *et al*., 2023).

Management of Antimicrobial Resistance (AMR)

The majority of recommendations for treating salmonellosis use antibiotics. Unfortunately, *Salmonella* spp. are created and spread as a consequence of the non-selective use of antibiotics. that many medications cannot cure. Managing infections caused by multi-drug resistant (MDR) strains will provide considerable hurdles since medicines are becoming more ineffective. In an effort to find an alternative to antibiotics, scientists have turned their attention back to phage therapy (M. A. S. Khan & Rahman, 2022). While the commercial application of phages for *Salmonella* control in chicken is yet in its early stages, the use of lytic phages is considered an environmentally safe, economically viable, and long-term antimicrobial treatment (Atterbury *et al*., 2020). In addition, it has advantages over antibiotics in terms of resistance, genetic flexibility, specificity, and development cost. Studies carried out in lab and field settings indicate that phages may be useful against multidrug-resistant strains of *Salmonella*. However, there are still major barriers to the commercial use of phages, including a lack of information regarding the protection of phage usage, phage stability, and the lack of a regulatory framework (Huang *et al*., 2018).

Surveillance and Preventive Strategies of *Salmonella* Infection

To successfully manage and prevent human salmonellosis, a variety of management approaches are used. Policies pertaining to cleanliness and hygiene, routine testing and diagnosis of food handling staff, ongoing surveillance of animals suspected of harboring pathogens, and treatment of carriers as well as symptomatic people are some of these preventative measures. Testing for *Salmonella* infection is essential at every stage of the animal production chain, including breeding facilities, cars, slaughterhouses, and storage facilities (B. Lamichhane *et al*., 2024). *Salmonella* infections in people and animals may be prevented or controlled using a number of techniques, such as:

Management and Biosecurity Measures

Many tactics, such as those for supervising and carrying out procedures to guarantee the safety and security of biological resources and facilities, may be used to reduce or prevent *Salmonella* infections in people and animals. To effectively monitor and execute biosecurity protocols, agricultural environments must be free of salmonellosis. Accurately identifying the primary source of infection and the many ways it spreads across the farm is essential for putting into place efficient biosecurity measures and halting the spread of disease. The adoption of strategies including the isolation of sick animals, movement restrictions, disinfection, and farm cleanliness upkeep are necessary for an efficient biosecurity program. Both external and internal biosecurity methods fall under this category and may be used to reduce or eliminate the risk of infection entering or leaving the farm. Reducing the entry of diseases that come from sources other than the farm premises requires the use of external biosecurity measures (Galipó *et al*., 2023). These strategies

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include building perimeter walls, controlling vehicle movement to and from the farm, and imposing limitations on the importation of animals. The aim of internal biosecurity protocols is to manage the transmission of *Salmonella* inside the agricultural environment. The strategies include isolating ill animals from healthy ones, changing shoes while leaving the farm, and frequently cleaning the bedding and the vehicles used to transport deceased animals (Trampel, Holder, & Gast, 2014). Veterinarians, stakeholders, salespeople, technicians, and other visitors to farms present a serious risk of infection transmission. Additionally, farmers in particular should be more aware of the need of taking precautions while visiting and leaving the farm since failing to do so might result in the spread of *Salmonella* infections from other farms and the surrounding area. Contamination may result from improper washing or disposal of boots, clothing, and equipment (Renault *et al*., 2018).

1. To lessen the possibility of employees transmitting infections, certain agricultural safety procedures, including (Tilli, Laconi, Galuppo, Mughini-Gras, & Piccirillo, 2022), may be used.

2. The visitors' movements need to be strictly regulated.

3. It is necessary to supply sanitary outerwear and footwear to both visitors and personnel.

4. It's crucial to often clean out organic waste from footbaths and use disinfectants, especially while working within the farm.

5. Prioritizing the health and welfare of the young, healthy animals before responding to the sicker and elderly ones is crucial while providing care for animals.

6. It is forbidden for laborers to handle food with the same instruments they use on fields.

Vector Control and Eradication

Depending on the World Organization for Animal Health (WOAH), vectors are defined as living organisms that have the capacity to both transmit a virus and assist in dispersing associated ailments across the populace. Certain insects, rodents, and wild birds have been shown to be potential sources of infection as well as factors in the development, dissemination, and maintenance of illness. The feces of rats and wild birds, which may be found anywhere on the farm, including food and water, can transmit diseases to other farm animals. Therefore, it's essential to routinely sanitize the farm and reduce the number of rodents. Maintaining a high standard of cleanliness is essential to keeping mice out of the region. This includes properly disposing of waste and debris and caulking any openings or gaps. In addition, it's critical to store supplies in a transparent area far from the main house building to keep rats out. In livestock farms and holdings, maintaining a high degree of hygiene is essential to managing carrier insects properly. This entails maintaining the environment dry and clear of standing water, making sure that enough air is provided, and eliminating garbage and trash on a timely and regular basis. It is also feasible to regularly apply organophosphates and synthetic chemical pesticides (Van Limbergen *et al*., 2018). permethrin, fenvalerate, dichlorvos organophosphate (DDVP), tetrachlorvinphos (TCVP), methomyl, benomyl, cyromazine, and dimethoate are among the compounds named. It's crucial to remember, however, that these substances pose a serious risk to both humans and animals. As such, it is imperative that certain criteria be followed while using them. Combining bioinsecticides made from natural sources with essential oils that repel or kill insects may prove to be a more cost-effective, environmentally friendly, and efficient choice. Pyrethrin is a botanical chemical derived from chrysanthemum flowers that has a reduced toxicity when applied. Insecticidal properties are recognized for the essential oils of thyme, cinnamon, rosemary, clove, mint, orange, eucalyptus, and tea tree. Due to their lower levels of toxicity, these oils are permitted to be used as parts of naturally occurring insecticides that are sold commercially (Mares, 2017).

Isolation and Quarantine

The primary goal of quarantine is to prevent the transmission of disease to both healthy animals and vulnerable people, including youngsters, the elderly, and immune-compromised patients, both inside the hospital setting and outside of it (Balaraman *et al*., 2021). Enforcing quarantine regulations for ill people or animals and restricting contact with them may dramatically decrease the threat of infection and the transmission of disease between humans and animals. The barns that are not impacted should be separated from the quarantine buildings, and they should be equipped with a working waste disposal system. Regular cleaning is required for the agricultural equipment, feeders, drinkers, and utensils. Following the right protocols for the

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safe handling, transportation, and disposal of sick corpses is also crucial. The length of the quarantine period varies depending on the kind of sickness and level of pathogen exposure (Azizi-Lalabadi, Rahimzadeh-Sani, Feng, Hosseini, & Jafari, 2023). The length of quarantine for people who have been exposed and are in good health should match the length of time the illness takes to incubate. In contrast, the length of the quarantine for sick animals should be based on the time it takes for symptoms to appear as well as the findings of laboratory tests. Implementing these preventive measures, often known as "biosecurity and sanitary management," may improve food safety and the health of both people and animals (Isman, 2020).

Surveillance of *Salmonella*

There is a great deal of variation in *Salmonella* monitoring among developing countries, and it is often not as robust or thorough as it is in wealthier ones. In less developed countries, the national public health agency or ministry of health is itself in charge of *Salmonella* surveillance. However, owing to a compromised infrastructure and resources, laboratory testing and data collection are limited. Additionally, inadequate sanitation, poor infrastructure, and compromised information and education about food safety and good cleanliness practices among the contributing population to the worsening of *Salmonella* transmission in developing countries (Naushad *et al*., 2023). However, in order to successfully limit the spread of these germs, it is essential to participate in international cooperation and aid programs, as well as to make investments in education and capacity development. Following good hygiene practices is essential for managing salmonellosis. This includes boiling food at the desired temperature to eradicate any possible *Salmonella* present and thoroughly washing of hands with soap and water before usage in food. It's critical to preserve food finely and prevent cross-contamination. Using different cutting boards and cutlery for raw and cooked meals might help accomplish this (Demiray & Sayar, 2024). Furthermore, since reared animals might serve as a reservoir for infection, controlling the spread of *Salmonella* in these animals is essential. Prioritizing the identification of the organism's origins and the ways in which it is transmitted to people is crucial for the successful management of *Salmonella*. The One Health method is the most effective way to do this since it entails paying enough attention to food and animal sources, the environments in which the organisms live, and the routes by which they are transferred to people. Certain preventative measures must be taken, including proper feeding and husbandry practices for animals, meticulous cleaning of animal placing and using equipment, vigilant observation of wildlife sources, especially bird species, and strict adherence to food safety regulations (Kirti, Krishna, & Shukla, 2024).

Conclusions

In this review, *Salmonella* figured out to be a serious pathogen of utmost importance to world health and food security. These characteristics, namely, various serovars, multiple strategies of pathogenesis, and the ability to exist in various environments are the evidence in favor of further dissemination and infection. Another challenge in the management of *Salmonella* species is the rise in the AMR which puts both clinical and public health outlets at risk. Newer technologies such as whole-genome sequencing of specimens have improved the understanding of the resistant strains, but the successful application of these tools at large remains a problem. Healthy management, phage therapy, and One Health appear to present the best strategies in order to reduce the impact of *Salmonella*. The next steps should be concerned with the improvement of these strategies and strengthening of the regulatory environments to counteract the increasing problem of AMR threatening worldwide food security and health.

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Conflict of Interest

The authors declare no conflict of interest.

Authors' Contribution

Conceptualization, Mudassar Mohiuddin; Writing—original draft preparation, Hira Hameed, Atiqa Riaz, Zakir Hussain, Muhammad Mujahid Bhutta; Writing—review and editing, Hira Hameed, Atiqa Riaz, Zakir Hussain; supervision, Mudassar Mohiuddin; All authors have read and agreed to the published version of the manuscript.

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