Salmonellosis: Zoonotic Pathogen

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Abstract

Salmonella is most common zoonotic foodborne pathogen which becomes a reason of approximately 94 million deaths and one lakh fifty thousand sickness per year. Salmonella was named on the name of scientist Daniel E salmon. Salmonella was classified in *S. enterica*, *S. bongori* that have several different serovars. Some of the species of salmonella are non-pathogenic while some are pathogenic and cause outbreaks. Main sources of transmission are uncooked meat and eggs, uncompromising system, week handling and unpasteurized dairy products. Preventive controls are important in handling poultry or poultry products. The aim of this chapter is to focus on classification, transmission and sources, epidemiology, pathogenesis, biochemical identification, prevention and treatment of salmonella.

Keywords: Salmonella, *S. enterica*, Culture, O, H, K antigen, Catalase test

Cite this Article as: Kousar S, Zaman MA, Asif H, Batool F, Bukhari SA, Rehman AU, Ehtisham-ul-Haque S, Hussain T, Khan AU and Abass KS, 2025. Salmonellosis: Zoonotic pathogen. In: Ismael SS, Nisa QU, Nisa ZU and Aziz S (eds), Diseases Across Life: From Humans to Land and Sea. Unique Scientific Publishers, Faisalabad, Pakistan, pp: 308-314. https://doi.org/10.47278/book.HH/2025.340

A Publication of Unique Scientific Publishers

Chapter No: 25-044

Received: 14-Feb-2025
Revised: 12-Apr-2025
Accepted: 10-May-2025

Introduction

Salmonella was first discovered by Soholerin in 1839 It was first isolated by Eberth from a person who died with typhoid fever from infected mouse salmonella typhimurium was first isolated by Loeffler in 1892 (Rahman et al., 2018) In Enterobacteriaceae salmonella is major foodborne pathogens which cause gastroenteritis in human and animals. Major transmission through contaminated water and food. Salmonella is gram negative, motile, non-spore forming, non-capsulated nonacid fast, facultative pathogen but it can easily stain with common dyes like methylene blue and carbol-fuchsin. For movement they used peritrichous flagella some salmonella also has fimbriae their optimum temperature for growth is 37°C and optimum PH is 4 to 9 They can also endure in freezing water for long period of time and also live in fecal material, moist soil and stream sediment (Amin et al., 2018; Rahman et al., 2018). Salmonella infection is known as salmonellosis which can cause many symptoms like diarrhea, vomiting, abdominal pain headache, chills, fever, nausea, and blood in stool but in very severe cases. Salmonella can spread through contaminated food, contaminated water, undercooked meat and poultry eggs, unpasteurized dairy products, person to person contact, animal contact and improper hygiene. Many animals act as a carrier for salmonella, however, chicken is major source for the transmission of salmonella through undercooked food. Worldwide Salmonella has 2,500 serovar while inflammatory diarrhea caused by salmonella enteritidis. Important significance of infection in immunocompromised patients due to Entertidis. Birds also act as carrier of salmonella mostly *S. entrica* and *S. typhimurium*. *S. bangori* and *Salmonella typhimurium* are the 2 main species of genus. Salmonella enterica confines subsequent 6 species. *Entrica* (subspecies), *houtenae* (subspecies iv)1), *salamae* (subspecies ii), *ubspecies. Arizonae* (subspecies iiia), *subspecies. Diarizonae* (subspecies iiib), indica (subspecies vi). S. Enterica as a greater serovar and shows the most important in human pathology. Most common serovar of salmonella enteritidis, *typhimurium*, *newport*, and *javiana*, serovars *typhi* and *paratyphi*. Typhiod fever and paratyphiod fever caused from typhiodial salmonella (Hendriksen et al., 2011). *S. typhioia* is restricted to humans. Salmonella distributes through cross contamination from infected handler or raw meat. Salmonella is comparatively more convenient to isolate in microbiological laboratory. Some strains form hydrogen sulfide which is easily isolate by growing in respective media that contain ferrous sulphate for example Triple sugar iron. The serotypes are divided on the base of O antigen carbohydrate component of lipopolysaccride

Classification and Taxonomy

Classification is based on Kauffman-White scheme based on its O, H, K antigen the classification is as followed in Table 1, 2.

Epidemiology of Salmonellosis

The epidemiology of salmonella hinges on the species of salmonella. Globally enteric fever resulting from *S. typhi* or *S. paratyphi* has catastrophic effects primarily in low-income countries. Non-typhoidal salmonella is the root of infections in both industrialized and non-industrialized countries (Radostitiset al., 2007; Westrell et al., 2007). Approximately NTS cases were nearly 94 million out of which one lakh fifty-five thousand deaths were recorded yearly (Majowicz et al., 2010). According to WHO studies 80% of reported cases were of *S. enteriditis*

and *typhi* (Galanis et al., 2006; Vieira et al., 2009). NTS have a zoonotic background eggs and poultry are the main sources, another carrier are rodents and reptile's feline and canine (Braden et al., 2006) (Hohmann et al., 2001). A case analysis was regulated in US 6% cases were in Herpetofauna (Smith et al., 2024). *S. enteritidis* and *S. typhiwere* present in 26and 25% of the samples sequentially in Africa and 38, 8 and 21% of *S. enteritidis* isolates were present in Asia, Europe, America sequentially (Scallan et al., 2011). Figure 1 shows isolates of *S. enteritidis* in different countries.

Table.1: Classification of salmonella on the basis of kauffman-white scheme

S. enterica subspecies				
Type	Subspecies	Serovars	Suspected individual	References
Sub specie I	enterica	1435	Human and warm-blooded animal	(Gordon et al., 2008; Wattiau et al., 2011)
Sub specie II	Salamae	485	Cold blooded animal	(Carter et al., 2004)
Sub specie III	Arizonae Diarizonae	94	Reptiles and avian specie	(Al abidy et al., 2005)
Sub specie IV	Houtenae	96	Cold blooded animals	(Al abidy et al., 2005)
Sub specie V	Indica	11	Poultry	(popoff et al., 1997)
S. bongori				
Sub specie	Serovars	Suspected individual		References
S. cholerasuis	1	swine		(Lake et al., 2002)
S. enteriditis	2000	Animal and human		(Rahman et al., 2017)
S. typhi	1	Human		(Carter et al., 2004)

Table 2: On the basis of host preference:

Host	Sub specie	References:
Man	*S. typhi, S. paratyphi*	(Tortora et al., 2004)
Animal host	*S. choleraesuis* and *servors of S. enteriditis* e.g. *S. pollurum, S. gallinarum and S. dublin*	(Rahman et al., 2017)
No host	2000 serovars of *S. enteriditids*	(Tortora et al., 2004)

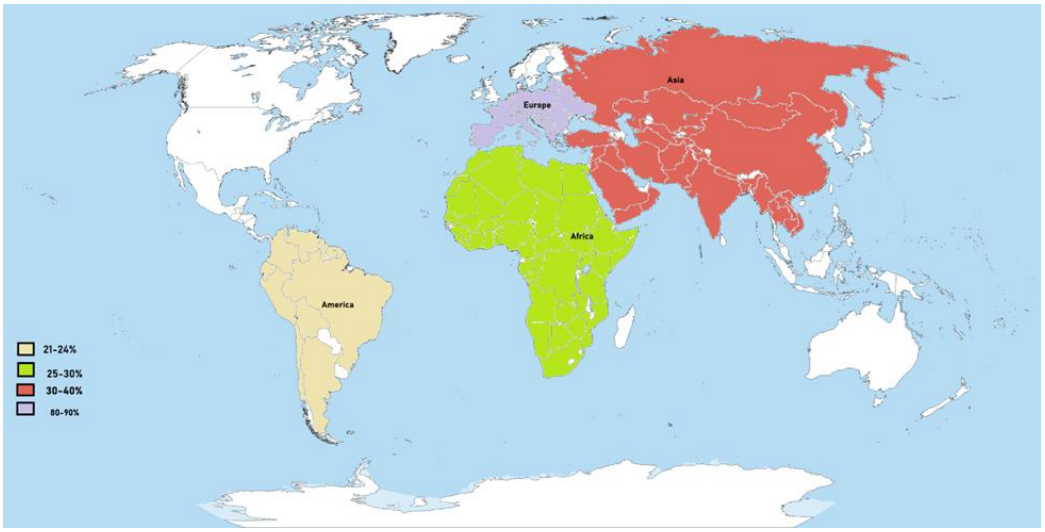

Fig. 1: Isolates of *S. enteritidis* in different countries

According to the study conducted in hospitals in sub-Sahara Africa the main root for systemic infections were S. enteritidis more usually than *S. typhi and paratyphi.* Invasive NTS was more prevelant and death rate was high in toddlers and adults with immuno-depressed system due to HIV. The cases of NTS were as well reported in developed countries and that was becoming a point of interest. The approximate case rate in Europe of NTS disease per year was 690 cases out of 100,000 people. In Israel 52-102 cases were supported out of 100,000 people annually. In 2010, an outbreak was reported in 16 countries caused by enteritidis infected eggs with 1939 cases that lasted for a year. Approximately 380 million eggs were transported in US alarmed US food and drug authority to take actions. From 2005-2010 more cases than usual was reported. In 2014, 300 number of cases were reported by *Salmonella infantis* in live poultry in USA. It was stated that people have contact with poultry that becomes a cause of the salmonellosis outbreak. In 2014, 425 cases were reported in raw yellowfin tuna by serovar salmonella bareilly in USA. It was reported in frozen raw yellowfin tuna also known as nakochi scrape (CDC, 2012)

Salmonella source and Transmission

The main source of salmonella is contaminated food and water. Its source of transmission is also animals and birds, especially pigs, turkey chicken. It is also contagious and can spread through person to person especially in sexual contact there is a large range of salmonella infection especially in eggs, dairy products and unpasteurized milk under cooked vegetables. A Study published in PLos marked a crucial increase in salmonella infection in Australia between 2000 to 2013 it's also found that *salmonella Typhimurium and Salmonella non-Typhimurium* can

cause rapidly increase during this period It also estimated that more than 70% cases are linked to food sources (Popa et al.,2021).

The Centers for Disease Control and Prevention (CDC) estimates that salmonellosis causes more than 1.2 million illnesses each year in the United States of America, with more than 23,000 hospitalizations and 450 deaths (CDC, 1986)

Sources

Fecal oral route: It is the most common way of spreading salmonella in which food and water is contaminated with feces. Other possible sources of infection include contaminated lettuce and other leafy vegetables, sprouts, spices, and seeds, as well as contaminated meat and eggs. (Pal et al., 2007; Zaheer et al., 2018)

Salmonella is also spread through fomites (food processing equipment and kitchen tools) mechanical vector (insect or flies) (Palet al., 2007)

Most animals carry salmonella without showing symptoms containing in their intestine and gallbladder and periodically spreading in their feces (Jensen et al., 2006)

Raw or Undercooked eggs

However, eggs that have shells on them appear to be perfect, barrier but affected hen can pass salmonella to egg contaminating albumen or yolks (Feasey et al., 2012; Bjelland et al., 2020).

Weakened Immune System

Salmonella can also affect people which has weakened immune system also spread through human to human especially in family gathering also increased risk while traveling

Unpasteurized dairy Products

Raw milk and dairy products form from raw milk can be infected.

Poor Handling

Poor waste disposal and cleanliness of workers in food handling and preparation activities would also cause infection.

Transmission routes of Salmonella

Humans

Salmonella has two types of non-typhoidal salmonella which is zoonotic and typhoidal, also known as enteric salmonella (Gast et al., 2003). The main mode of spread for non-typhoidal salmonellosis is infected water and food. Some healthcare workers or farmers encounter infected animals while taking care of them, which can be a main cause of non-typhoidal salmonella (McCall et al., 2001). But if we talk about typhoidal salmonella also known as gastroenteritis salmonellosis is caused by the intake of water infected with feces of human or by direct contact with humans (Khan et al., 2010).

Avian livestock

In avians there are two routes vertically (through mother birds to their eggs) and horizontal transmission (through direct contact, intake of infected water, food and eggs) (Jordain et al., 1996). Eggs remained a big cause of outbreaks of salmonellosis as they pass through the cloacae of infected bird feces are present at that surface which attach to the outer layer of shell (Herenda et al., 1996). So, the major route is the fecal-oral route. Animals and pets especially, reptiles' lizards, snake and rodents' cats, dogs and birds like turkey, chicken have salmonella in their feathers fur or skin and in feces also further if people touch contaminated pet or food or even surface where animal shelter touch their mouth without washing hands properly (Quinn et al., 2002)

Pathogenesis

Pathogenesis of salmonella depends on various factors like age, immune system, less intake of water and operation. The pathogenic factors include:

- O, H antigen
- LPS layer
- Toxins
- Intracellular bacteria

O, H antigens play a role in entering cell O is surface polysaccharide antigen help to escape immune response, H antigen is flagellar antigen which helps the bacteria in the movement. The specific pattern of O and H antigens helps in identification of type of salmonella. LPS layer also plays a role in pathogenicity and because of LPS layer, many antibiotics and detergents do not penetrate cell thus making it easier to cause a disease. There are three types of toxins: enterotoxin, endotoxin and cytotoxin. Enterotoxin causes fluid to accumulate in intestine due to less secretion from epithelial cells. Endotoxin, when induced, causes fever in the infected individual. Cytotoxin stops protein formation and causes iron chelation in intestinal epithelial. Salmonella is intracellular bacteria that can survive host immune response and can reside in macrophages and escape phagocytosis.

Once non-typhoidal salmonella is ingested, it goes to mucus of intestine like caecum, colon and ileum sometimes bacilli pass through the mucus of pyrngal area and attach with the villi in,the intestine which are small finger like appendages that help in the absorbance of water. Then they enter the cell by the process of endocytosis (membrane is folded inward engulfing bacteria and forms a vesicle). Then, after the period of 24 hours, they are present in sub mucosa here are phagocytosed by macrophages (CDC 2012). If the person's immune system is low, they remain in phagocytes and here they multiply. Toxins like enterotoxin and cytotoxin of salmonella contribute to gut damage and cause

inflammation, then prostaglandin which activates CAMP (cyclic adenosine monophosphate) results in increased production of water and bicarbonate, which results in the diarrhea. In the case of *S. typhi* it enters to GIT tract then passes to different body organs by blood and multiply in liver, gallbladder. The inflammation caused by *S. typhi* is very harmful because it causes necrosis of intestine cause ulceration in intestine and results in hemorrhages (Brooks et al., 2001).

Detection and Diagnosis of Salmonella

In animals, Salmonella can be found in feces, blood, fetal tissues, vaginal discharge, the placenta, and many internal organs after necropsy, depending on the disease's form (Adem et al., 2022; Mokeria et al., 2022)

Mainly salmonella is present in a stool sample the salmonella is commonly detected by two methods i.e culturing and Biochemical identification

Culturing Procedure

Feces blood or another sample should be plated on numerous agar i.e. selective or non-selective media for example MacConkey, blood, EMB (eosin methylene blue) salmonella and shigella and brilliant green agars XLD (xylose lysine Deoxycholate)

Isolation of Salmonella

Sample Collection

Feces, blood, egg, water and meat samples can be taken

Enrichment and Pre-enrichment

After sample collection, the next step is pre-enrichment. Buffered peptone water or LB broth is used and then the culture is left for 24 hours incubation. After pre-enrichment the selective media Rappaport Vassiliadis is used for enrichment at 42 centigrade for 24 hours. Next is selective plating culture is grown on XLD (Xylose lysine agar) (Cappuccino et al., 2007)

Salmonella colonies on XLD red colonies with black center and on SS agar colorless colonies with black center (Aseel et al., 2013).

Any growth in enrichment broth is sub-cultured onto various agars, and only doubtful colonies are tested on triple sugar iron agar and lysine-iron agar for biochemical reactions to make a presumptive identification (Al-Abidy et al. 2005).

Microscopic Observation

Under microscope we can see small gram-negative pink color rod shaped bacteria

Biochemical Identification

1. Indole test

Amino acid tryptophan is converted into indole in presence of tryptophanase. If indole is present, it forms a red ring at the top on the addition of kovacs reagent (37% HCL, Para dimethyl amino-benzyldehyde). Salmonella has indole test negative meaning no red ring forms and it also differentiates it from *E. coli* which has indole positive (Hoelzer et al., 2011)

2. MR test

Glucose yields pyruvate some bacteria further ferment glucose to lactic acid, acetic acid also called stable acids which lowers the pH. When methyl red is added at low pH 4 it changes color of media to red and if no acid present media color is yellow. Methyl red test for salmonella is positive (Williams et al., 1981)

3. VP test

Glucose is converted to pyruvic acid and pyruvic acid is then converted to acetone which reacts to 40% KOH which is a reagent converts to diacetyl which then reacts with alpha naphthol gives pink to red color which is positive but in case of salmonella test is negative which means no color.

4. Citrate test

If glucose is not present bacteria use citrate as a carbon source if they have citrate permease enzyme. Simon citrate agar is used in slant form and culture is stabbed in media; bromothymol blue indicator present in media changes its color to blue if test is positive. Salmonella has a positive citrate test

5. Catalase test

Hydrogen peroxide reacts with culture and if catalase enzyme is present, it forms bubble means positive test salmonella has catalase positive. The bubble formation can be seen immediately after the culture added to hydrogen peroxide.

6. Oxidase Test

In oxidase test if cytochrome c enzyme is present which catalyze electron transport chain purple or blue color forms if positive test within 5-10 seconds or maximum 50-60 seconds, negative test does not show any color. Salmonella shows no color means negative test.

7. Triple Iron sugar

The triple Iron sugar is used for differentia between the members of Enterobacteriaceae family like *salmonella shigella* and *E. coli* etc

We inoculated samples in TSI Tube. This tube has three sugar glucose sucrose and lactose bacteria are allowed to ferment the sugar if we see alkaline slant/ acid butt with H2S (hydrogen sulfide) production and it means salmonella is present in the sample.

Salmonella's presumptive biochemical identification can be confirmed through antigenic analysis of O and H antigens using polyvalent and specific antisera. About 95% of clinical isolates can be identified with available group A-E typing antisera. (Boran et al., 1996; Giannella et al., 1996) After this salmonella should be sent to laboratory for more accurate serological testing and confirmation (Nair et al., 2018)

8. Motility test

The mobility of bacteria can be detected by motility test. The medium used is sulfide indole motility (SIM) media which is a semisolid media. Culture is stabbed in this media. We can see lines away from the stab line after the incubation of 24 hours regarded as positive result. Salmonella is a motile and show movement. While non motile bacteria don't show any movement in the media (Table 3).

Table 3: Different tests and their results

Test	Result	References
Indole	-	(Amil et al., 2014)
MR	+	(Simmons et al., 2022)
VP	-	(Karolenko et al., 2025)
Citrate	+	(Simmons et al., 2022)
Catalase	+	(Sharma et al.,2024)
Oxidase	-	(Karolenko et al., 2025)
TIS	Red/yellow H2S +	(Simmons et al., 2022)
Motility	Red/yellow	(Amil et al., 2014)

Prevention of Salmonella Infection

Salmonella infection can be prevented by proper food handling and cooking, hand washing and avoiding contaminated food and water. Cooking meat and eggs thoroughly will kill Salmonella bacteria as they are heat sensitive (Petri et al., 2008).

There are several ways to keep Salmonella infection away:

- Don't bother to rush into the kitchen if you are sick.
- Ensure hygiene practices while in contact with animals and wash hands properly especially in children under five years age.
- If you are cooking meat or eggs, then avoid contact with other surfaces.
- Refrigerate meat properly and avoid cross contamination.
- People should avoid the use of unpasteurized milk.
- Immunocompromised people are more vulnerable so avoid contact with animals.

Treatment of Salmonellosis

Prognosis of Salmonellosis is always good except when it spread to other parts of body causing sepsis (bacteremia) but it is unusual. Hospitalization is needed in a few cases if a patient gets severe diarrhea (Figueiredo et al., 2015). Plenty of water must be used. Intravenous fluids administration recommended by your doctor when excessive loss of fluids from body occurs due to diarrhea and vomiting. Electrolytes intake must be ensured for speedy recovery (kumar et al., 2017).

If infection is caused by *S. Typhi* and *S. Paratyphi* then it may require antibiotic administration:

- Ciprofloxacin
- Trimethoprim-sulphamethoxazole (TMP/SMX).
- Ceftriaxone.
- Azithromycin

Conclusion

We conclude that salmonella is harmful bacteria for humans as well as animals and it has zoonotic importance. It is most observed in poultry farms where farmers and care takers have direct contact with them. It is mostly observed in the countries with poor hygiene and in underdeveloped countries. Therefore, the farms should be disinfected and cleaned on a daily basis and farmers should take proper precautions. Meat and eggs should be properly cooked and stored at temperatures which avoids multiplication. If faced with diarrhea plenty of water should be taken so the fluid content remains balanced

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