

## Salmonellosis: Food-borne Plague

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### INTRODUCTION

Food-borne plague or food-borne illness also termed as food poisoning is a result of the intake of food or drink contaminated with microbiological agents like bacteria, viruses, parasites, and toxins. These pathogens act as a source of vulnerable illness causing a substantial loss of time and the treatment cost is also very high. According to the World Health Organization, a huge amount of people dies every year due to diarrheal infections which occur because of contaminated food intake (Borrás S et al. 2007). *Salmonella* as a foodborne plague has prime importance in causing illness in human food (Pires et al. 2011; Shah et al. 2011). Not only live animals, but also products of animal origin i.e., meats, eggs, milk, cheese, ready-to-eat products, and vegetables are the foremost carriers of *Salmonella* (Cellucci et al. 2010). In the case of animals, *Salmonella* causes asymptomatic infections while in humans salmonellosis results in gastroenteritis and systemic diseases (Su and Chiu 2007).

### Etiology

*Salmonella* is a Gram-negative, predominantly motile, non-spore-forming, and facultative anaerobic bacilli. Many species belonging to the genus *Salmonella*, the family Enterobacteriaceae. Genus *Salmonella* is broadly divided into major species named enterica and bongori which are further divided into subspecies. *Salmonella (S.) enterica* has six subspecies (McQuiston et al. 2008). This well-known genus of *Salmonella* is also subdivided due to the presence of

special surface molecules. The surface molecules with etiological importance are O-antigen represented as O-Ag available in lipopolysaccharide and the second one is H-antigen (H-Ag) found in the flagellar complex, flagellin protein. If we focus only on the serotypes of *Salmonella*, there are twenty-five hundred different serotypes of this genus. All these serotypes of *Salmonella* can produce infections in humans, resulting in either morbidity or mortality in a large population (Grimont and Weill 2007). Table 1 shows different serotypes of *Salmonella* with respect to their host.

### Transmission

*Salmonella* is a disaster if it is present in a population of humans or animals as it can be transmitted through both horizontal and vertical routes. In the case of vertical transmission, the *Salmonella* serotypes move from the parent to the infant (Sanabria et al. 2017). On the other hand, horizontal transmission is done by aerogens and is also transmitted through feco-oral route. Fomites, polluted drinking water, contaminated food, and cages that are not properly cleaned are the pronounced source of transmission (Yanestria et al. 2019).

As a major cause of foodborne infection, *Salmonella* is transmitted in humans through an extensive range of food products including beef, poultry, and eggs as shown in Fig. 1 (Bell et al. 2016; Ximenes et al. 2019). The pathogen transmits not only through food products, but also live birds in humans as most people are unaware of this fact about the transmission of *Salmonella* (Behraves et al. 2014). It resides in poultry birds and is then transferred to other animals and humans resulting in a high rate of mortality (Suardana et al. 2014).

*Salmonella* is not only transferred through food products such as meat and eggs but it is also transmitted through contaminated water and contact with households. *Salmonella* causes severe abdominal pain, high temperature, vomiting, and diarrhea in the host (Ximenes et al. 2019).

### Pathogenesis

The distal ileum and proximal colon become colonized with *Salmonella* after consuming tainted food or drink (Hocking 2003; Lönnermark et al. 2015). Salmonellosis has an infective dosage of  $10^5$  to  $10^6$  cells which is sufficient to cause infection in the small intestinal mucosa (Xu et al. 2010). The target cells are enterocytes and it reaches them through the process of chemotaxis using its flagella. After reaching the target cell, it attaches to the human enterocytes using type I fimbriae. There are two types of fimbriae named

**Table 1:** Different serotypes of *Salmonella* and their specific hosts (Baumler et al. 1998).

No.	Subgroups of <i>S. enterica</i>	Specific host	Infection
1.	<i>S. typhi</i>	Humans	Typhoid fever
2.	<i>S. paratyphi</i>	Humans	Bacteremia & fever
3.	<i>S. typhimurium</i>	Humans, Bovines, Equine, Ovines, Chicken, Mice	Fever, diarrhea.
4.	<i>S. enteritidis</i>	Human, Chicken, Mice	Septicemia, fever, gastroenteritis
5.	<i>S. Dublin</i>	Bovines, Swin, Ovines	Septicemia, fever, gastroenteritis, abortion
6.	<i>S. derby</i>	Birds, Swine	Diarrhea, Bacteraemia
7.	<i>S. gallinerum</i>	Chicken	Septicemia, gastroenteritis
8.	<i>S. abortusovis</i>	Ovines	Septicemia, abortion
9.	<i>S. abortusequi</i>	Equine	Abortion
10.	<i>S. choleraesuis</i>	Swine	Fever, Bacteraemia

long polar fimbriae (Lpf) and thin aggregative fimbriae (Tafi). In the case of *S. typhi*, the attachment with the host cells is done by using type IV pili (Wagner and Hensel 2011). After the attachment process, it gets entry into the host cell through the process of phagocytosis by using its pathogenic chemicals i.e., type III secretion systems (T3SSs). This collection of pathogenic chemicals of *Salmonella* is called *Salmonella* pathogenicity islands (SPIs) (Wagner and Hensel 2011). After this, the cells of *Salmonella* are collected by macrophages, dendritic cells, and polymorphonuclear cells as they are present in the interstitial space of lamina propria after the process of exocytosis. Then the *Salmonella* cell is captured into the host efferent lymph in the mesenteric lymph nodes (Fig. 2) to stop their entry into the bloodstream (Velge et al. 2012). When *Salmonella* gets attached to the epithelial cells of lamina propria, it produces inflammatory reactions and releases pro-inflammatory cytokines. Acute inflammatory reactions brought on by pro-inflammatory cytokines resulted in diarrhea, ulceration, and the death of mucosal cells. Mesenteric lymph nodes, liver, and spleen play vital roles in the ongoing infection of Salmonellosis (Baron et al. 1996).

It has been examined that almost all subspecies of *Salmonella* produce enterotoxin and cytotoxin. The composition of these toxins resembled that of the cholera toxins. These toxins are heat labile or heat stable and are related to diarrhea (Song et al. 2013). The production of enterotoxins results in the buildup of fluid in the ligated murine ileal loop. The damage caused by cytotoxins includes inflammatory diarrhea, damage to the intestinal mucosa, and hinder protein synthesis (Hocking 2003). The toxins produced by *S. typhi* cause typhoid fever and chronic infections (Chong et al. 2017). Another investigation shows that the virulence of both the mutant *Salmonella* with toxin phenotype and wild-type *Salmonella* with toxin phenotype is almost similar (Nakano et al. 2012).

The pathogenesis of *Salmonella* infections involves the O antigen lipopolysaccharide (LPS). All components of LPS play a crucial role in *Salmonella*'s pathogenesis, although O side chains' length, structure, composition and surface roughness can have an impact on the pathogen's virulence. The pathogenicity is reduced when a whole length of chain is not produced. The resistance of *Salmonella* strains to the lytic action of the complement cascade depends on the length of the chain. The strains with smooth surfaces face a little bit of resistance while on the other hand strains with a rough

surface experience more resistance. This is due to the steric barrier of *Salmonella* to the complement cascade's ability to bind (Hocking 2003).

The pathogenicity of *Salmonella* is enhanced by the virulence plasmids and virulence genes. The virulence plasmids of *S. typhimurium*, *S. Dublin*, and *S. enteritidis* cause systemic infection to the liver, spleen, and mesenteric lymph nodes. The virulence plasmids can be isolated from human or animal organs and blood (Hocking 2003).

The virulence factors of *Salmonella* are enlisted as flagella and flagellin, superoxide dismutase, and iron acquisition systems. The invasiveness of flagella increases as the motility increases and the inflammatory response has been shown by the flagellin. Superoxide dismutase of *Salmonella* affects host cells by inactivating the bactericidal reactive oxygen species. Another feature of the virulence of *Salmonella* is that it creates iron acquisition systems for the transfer of iron, magnesium, zinc, and potassium with low concentrations in the environment. Enterobactin and salmochelin are two siderophores that *Salmonella* generates. To access the host's scarce iron, these siderophores are essential. *Salmonella* also acquires a little amount of magnesium through the CorA, MgtA, and MgtB systems. For the uptake of zinc and potassium, ZnuABC and Trk systems are employed. Each of these ions is essential for *Salmonella*'s survival and pathogenicity (Ibarra et al. 2009).

## Treatment

In addition to lowering the death rate linked to bacterial infections, antibiotics are essential in the treatment of infectious disorders and have significantly enhanced quality of life. Antibiotics are selective against invasive germs: which guarantees maximum eradication of the target microorganisms while causing the least possible harm to patients (Nami et al. 2015).

Typically, antibiotics are not needed to treat NTS infections. Antibiotic therapy is used against the complications like meningitis and septicemia and the frequently used antibiotics against these are ciprofloxacin, ceftriaxone, and ampicillin (Medalla et al. 2016). The antibiotics used against the life-threatening side effects of *Salmonella* specie are cefixime, chloramphenicol, amoxicillin, trimethoprim/sulfamethoxazole (TMP-SMX), azithromycin,

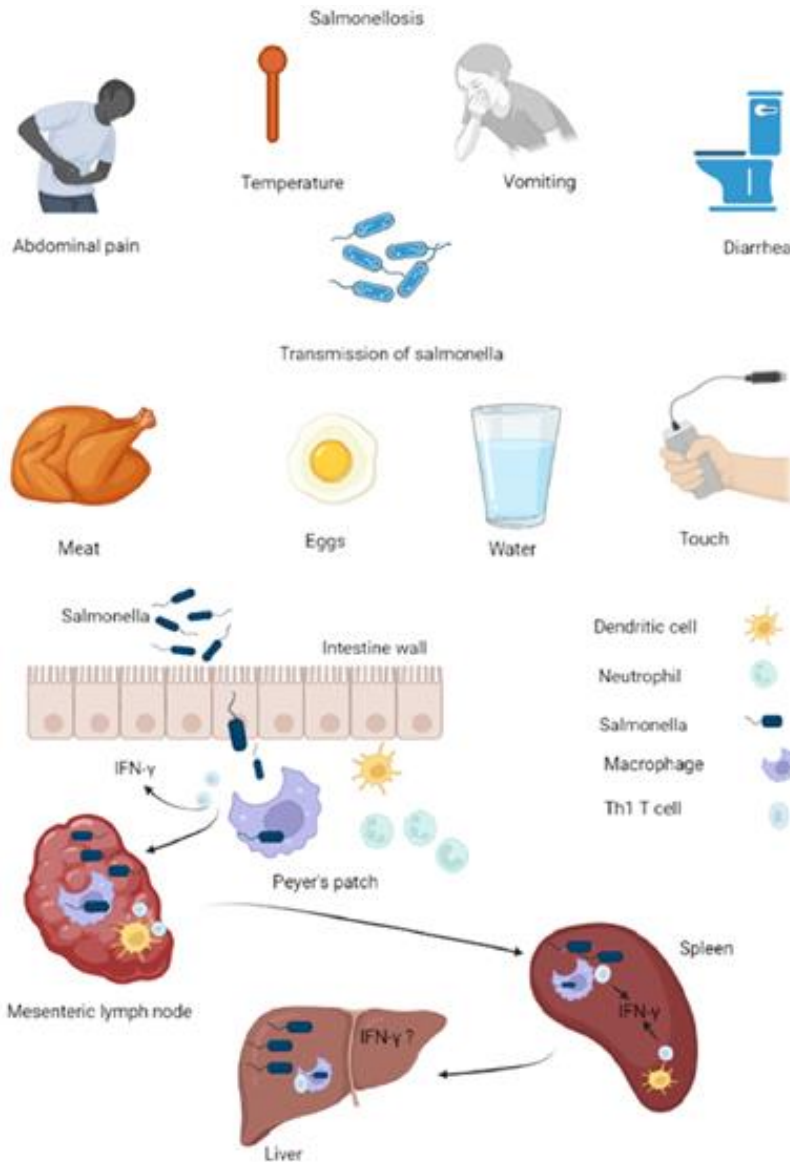


Fig. 1: Different ways for transmission of Salmonellosis.

Fig. 2: Role of different organs in *Salmonella* infection.

aztreonam and cefotaxime or ceftriaxone. These antibiotics are specially used against the *S. typhi* and *S. paratyphi* to save the life of animals. Steroidal therapy is also preferred in conditions like coma, stupor, obtundation, shock, and delirium. The most common steroids used in these complications are dexamethasone and corticosteroids (Kumar and Kumar 2017).

### Innovations

The use of antibiotics to treat salmonellosis produces excellent results but to some extent, the excessive use of antibiotics in patients can produce antibiotic resistance which is a global issue these days. So, the researchers develop and create different techniques and protocols to treat salmonellosis i.e., the use of yeast as a therapeutic agent against *Salmonella*. For the confirmatory diagnosis, different

biomarkers are now available in the market for instance oligonucleotides.

### 1. Advancements in Diagnostics Techniques

The microbiological culture techniques for the confirmatory detection of *Salmonella* are generally performed but these techniques stick with low sensitivity, intensive labor requirements, time-consuming, not cost-effective, lack trained professionals and the results require weeks to confirm presence and absence of *Salmonella*. The time-efficient methods of diagnosis have prime importance as it is necessary for the treatment of clinical complications and to confirm the origin of that pathogen (Odumeru and León-Velarde 2012; Zaderowska and Chajeka 2012; Almeida et al. 2013; Ahmed et al. 2014).

## 2. Oligonucleotides as a Biomarker for the Detection of Salmonella

The technique used for the detection of *Salmonella* is established on the chemically modified nucleic acids to target *Salmonella*. In this method, we use the universal nuclease activity of *Salmonella* as a biomarker. For the detection strategy, chemically modified oligonucleotides which are flanked at the 5' end and at the 3' end a fluorophore (FAM) and a quencher (TQ2) are used. FAM acts as a reporter molecule, the degradation of the oligonucleotide's probes through nuclease, and the FAM records the activity through fluorescence measurements (Hernandez et al. 2014) (Fig. 3).

## 3. Advancement in Treatment

*Salmonella* species are increasingly resistant to conventional antibiotics as well as complementary therapies (Crump et al. 2015). *Salmonella* and other dangerous bacterial species are becoming increasingly resistant to antibiotics for a variety of reasons, including the overuse of antibiotics due to easy access in some countries (Medalla et al. 2016). Along with poor cleanliness habits, the use of antibiotics to protect crops and promote animal growth in animal husbandry has also contributed to the overuse of antibiotics and resulting in the rise in resistance (Nami et al. 2015; Sabtu et al. 2015; Medalla et al. 2016).

## 4. Use of yeast as a Prophylaxis

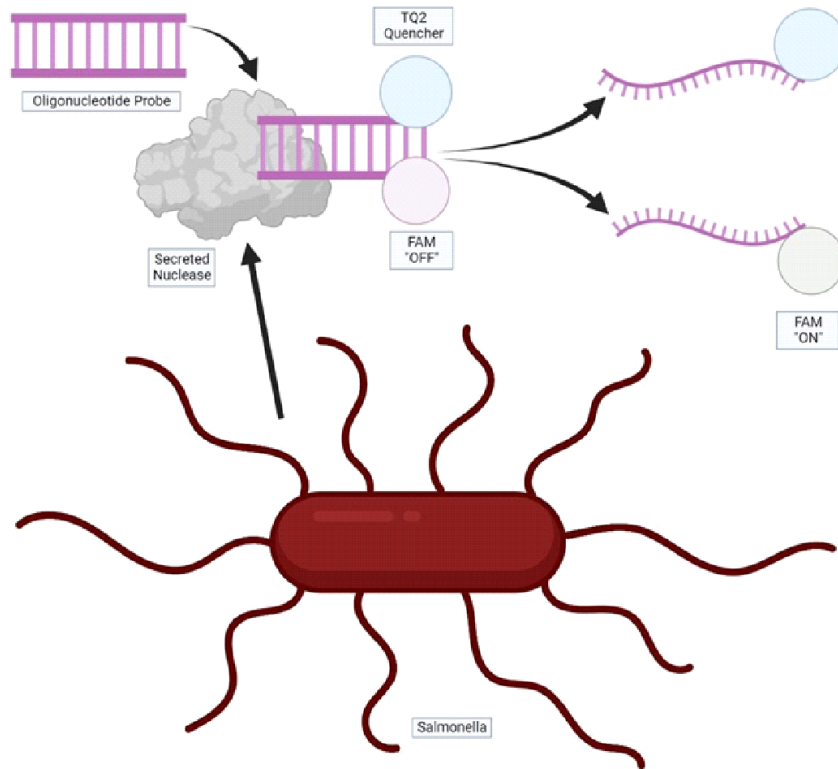
The recent advances in the treatment of *Salmonella* is to use yeast as a preventative agent against typhoid, paratyphoid, and NTS (non-typhoid *Salmonella*). The yeast functioned as a probiotic and its strain *S. boulardii* is most commonly used (Kelesidis and Pothoulakis 2012; Rajkowska and Kunicka-Styczynska 2012; Tomicic et al. 2016). It is also being discussed in many studies which provide knowledge about the effectiveness of different species and strains of yeast (Palma et al. 2015). One of the promising benefits of using yeast as a probiotic is that they are not targeted by the host cells as compared to the bacterial cell (Martins et al. 2009). The yeast functioned as a protective layer for the intestinal mucosa and protected it from the damage caused by pathogens, allergies, and toxins (Guttman et al. 2009). Different studies revealed that *S. boulardii* performs its function by inhibiting the production of pro-inflammatory cytokines (IL-8), and the activation of MAP kinases stops for example Erk 1/2 and JNK/SAPK. The preservation and protection of tight junctions were attributed to the *S. boulardii* anti-inflammatory factor (SAIF). It also generates proteases, which degrade the toxins created by pathogenic bacteria (Pothoulakis et al. 2009).

## Control

Salmonellosis is a foodborne ailment, so all the factors that contribute to its spread are related to food and food products (Jones 2011). *Salmonella* can hide in a variety of foods, but there are a lot of ways that help to ensure that the bacteria stay away (Fig. 4):

- Don't eat raw or barely cooked eggs or meat. Uncooked food serves as one of the major factors in the transmission of Salmonellosis. Properly cooked food decreases the chances of spreading the infection (Wegener et al. 2003).
- Pasteurization has brought a revolution in the entire food industry and at the same time, it has resulted in a significant decrease in foodborne disease spread (Barrow 1993). Its most important aim is to kill pathogens within perishable food items. So, avoid using unpasteurized food items (Maijala et al. 2005).
- Avoid splashes from raw meat onto other surfaces while washing. These splashes transfer germs onto other food, like lettuce, that will not be cooked to kill germs before eating (DeBusser et al. 2013).
- Wash raw fruits and vegetables well, and peel them if possible (Chambers and Gong 2011). Washing fruits and vegetables tend to decrease the potential transfer of harmful germs such as *Salmonella*. Wash the vegetables thoroughly under fresh running water (Tellez et al. 2012).
- Refrigerate food properly before cooking and after serving it. Refrigeration slows down the growth of many pathogens (Koutsoumanis et al. 2019). *Salmonella* requires a suitable temperature, moisture, and pH for their growth and a refrigerator seizes them to make the food pathogen-free (Revolledo et al. 2006).
- Wash your hands well with soap and warm water before and after handling food (Goldbach and Alban 2006). Washing hands ensure the elimination of all types of pathogens and thus ensures quality health (Sundström et al. 2014).
- Keep kitchen surfaces clean before preparing food on these surfaces. Don't mix cooked food with raw food. Don't use the same utensils to prepare them (Hopp et al. 1999) i.e., don't use the same knife to cut raw chicken and then to slice mushrooms and use different plates or cutting boards to slice them on (Gavin et al. 2018).
- Cook meat to its correct minimum temperature. The minimum temperature which is required to cook the food and eliminate all the pathogens should be attained to ensure its purity. As far as *Salmonella* is concerned, it is massively spread by the consumption of undercooked food. Use a food thermometer to be sure (Vanderwal 1979).
- Wash your hands with soap and water after touching animals, their toys, and bedding (Mead and Barrow 1990). Avoid eating food in an area where animals are present. Minimize contact with animals having diarrhea (Vico and Jaime 2011).





**Fig. 3:** The phenomenon that shows the nuclease of *Salmonella* attacks oligonucleotide and degraded it in response to which FAM records the activity.



**Fig. 4:** Four things should be kept in mind while controlling *Salmonella*: cook meat properly, separate the raw and cooked food, refrigerate the food to slow down the production of bacteria, keep the environment clean and take care of personal hygiene

## Conclusion

The rate of mortality and morbidity due to the human enteric pathogen *Salmonella* is of high magnitude even in the presence of antibiotic drugs. Due to the excessive use of antibiotics, resistance has developed in animals, so we must derive a way to get rid of antibiotics. According to the data of research it is estimated that about 2.8 billion *Salmonella*-associated diarrheas are reported annually. The major problem is the lack of a system through which the by-products of animal origin can be secured from *Salmonella* for the healthy consumption of humans. Due to antibiotic resistance in animals and humans, yeast is used as a complementary therapy for salmonellosis. Precautions should be taken while using meat, eggs, drinking water, and household objects to avoid contact with pathogens. In the control of infections caused by *Salmonella* in both humans and animals, the surveillance of the pathogen is a mandatory step.

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