Salmonella spp. infection – a continuous threat worldwide

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Abstract

Acute diarrheal disease remains a major public health issue. *Salmonella* spp. infection is one of the leading causes of acute diarrheal disease despite the preventive measures implemented. The clinical picture of salmonellosis varies from a common gastroenteritis to enteric fevers which are life-threatening diseases requiring a prompt and correct antibiotic treatment. In this review we present recent salmonellosis outbreaks and point out that *Salmonella* infections continue to be an important health issue. Numerous outbreaks of *Salmonella* spp. have been reported worldwide in recent years, indicating that prevention and control programs need to be improved as well as the infectious diseases surveillance, all over the world.

Keywords ***Salmonella*, outbreak, prevention and control**

Introduction

Acute diarrheal diseases are associated with significant mortality and morbidity. According to the World Health Organization (WHO), more than 2 billion people worldwide suffer from diarrheal disease annually. Regarding the source of infection, in a third of the cases, food is involved.[1] Salmonellosis is one of the most common foodborne infections. Infections caused by *Salmonella* should be divided into minor and major disease. Minor salmonellosis caused by nontyphoid *Salmonella* strains is characterized by self-limiting diarrhea, rarely leading to bacteremia or meningitis.[2] Major salmonellosis is represented by typhoid fever. The clinical picture of typhoid fever includes fever, headache, malaise and sometimes cough.[3]

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Salmonella was first discovered by an American bacteriologist, D. E. Salmon, in 1884. The organism was isolated from porcine intestine.[4] Up to 80% of salmonellosis cases are not recognized as part of a known outbreak and are considered sporadic cases. Moreover some are not diagnosed at all.[5]

Salmonella nomenclature is complex and many classifications have been used. The first classification was based on biochemical characteristics.[6] The classification proposed by Kauffman-White according to O and H antigens was based on the one serotype-one species principle. This classification leads to the subdivision of the *Salmonella* genus into more than 2500 species.[7] Nowadays the *Salmonella* taxonomy includes two species, *enterica* and *bongori*. Based on the analysis of the genome and biochemical characteristics, *S. enterica* contains 6 subspecies, *enterica*, *salamae*, *arizonae*, *diarizonae*, *houtenae* and *indica*. The subspecies are subdivided into serogroups (O antigen) and serovars (H antigen) based on antigenic characteristics. Thus, more than 2500 serovars are known.[8]

Recent research has focused on serotyping by molecular methods, superior to traditional serotyping through a better standardization and reproducibility. In addition, the ability to discriminate is higher. Molecular techniques can be classified into three main categories, methods that detect serovars based on molecular subtype, methods that detect serovars based on specific

genomic markers and direct methods that identify specific genes encoding certain antigens.[9] While molecular methods have advantages related to traditional methods which depend on antigen expression, equipment, reagents are still less accessible for developing countries.[10]

Salmonellosis - routes of transmission

The main reservoir of *Salmonella* is the intestine of humans and animals, but the organism has also been identified in reptiles and insects. There is a wide range of sources of *Salmonella* infection, eggs, meat, dairy products, vegetables and water are the most important.[8] In developed countries the most common source of infection is food. The identification of food source causing disease is sometimes difficult, but it is the most important measure to prevent the spread of the infection.[11] A recent study published in PLoS One draws attention to the growing number of cases of *Salmonella* infection in Australia. They observed a sustained increase of the cases between 2000-2013, involving both *Salmonella* Typhimurium and *Salmonella* non-Typhimurium. They estimate that in Australia in over 70% of cases the source was food.[12]

Water could represent a source of contamination.[13] Pires et al. analyzed several outbreaks and observed that eggs and meat (chicken, pork) remain the most important sources, but vegetables and fruits should not be neglected.[11] Chicken and other birds can carry the microorganism and exposure to these birds has been associated with the acquisition of the infection.[9] Cases of infection have also been reported after contact with pets. Often, the animal is asymptomatic. Person to person transmission is possible.[5]

Some serotypes are host specific while others infect any warm-blooded animal. Thus, for example, *S. typhi* infects only humans.[8] Around 50 serovars are involved in the occurrence of diseases in humans and animals.[13]

Recent outbreaks of *Salmonella* spp.

Promedmail.org was used to select several worldwide outbreaks of *Salmonella* reported in recent years. European outbreaks are discussed in a separate section.[14]

Salmonella causing diarrheal disease

In 2015, an outbreak linked to frozen raw breaded chicken products occurred in Canada, affecting 4 provinces, Ontario, Quebec, Nova Scotia, and Newfoundland and Labrador; 44 cases were reported.

In 2016, an outbreak took place in South Australia. 230 *Salmonella* cases were reported and the source of infection was raw mung bean sprouts. In addition, in the same year, an outbreak in New South Wales (Australia) was linked to the consumption of rockmelon; 97 cases were reported. In 2016, an outbreak was reported in the USA involving multiple states and over 27 people infected with *Salmonella* Virchow. The cases were linked to the consumption of raw meal organic shake. In the same year, an outbreak of *Salmonella*, the Poona serotype was reported in the USA related to cucumbers imported from Mexico, which involved 40 states and a large number of infected people (907). Most outbreaks reported by promedmail.org in 2019 took place in the USA.

During 2017, several outbreaks were recorded in Australia, in restaurants and in some cases the source remained unknown. In Japan, a total of 87 kindergartners reported cases of fever and vomiting; *Salmonella* was the most common isolated pathogen. In the last years, in Sub-Saharan Africa emerged an invasive and virulent strain of *Salmonella* Typhimurium known as sequence type (ST)313. In 2017, it was also reported in Brazil (actually, the strain was isolated several years ago in Sao Paolo). In 2017, several outbreaks have also been reported in the USA. The US CDC reported 24 cases of *Salmonella* infection in 16 states.

In 2018, more than half of the posts reported outbreaks of *Salmonella* in the USA. The sources of infection were raw turkey products, ground beef, eggs, fast food, pre-cut melon, frozen coconut, etc. In 2018, Israel reported an outbreak of *Salmonella* Concord (40 cases) linked to tahini products. Also, in Australia, 49 persons developed acute diarrheal disease caused by

Salmonella after consuming chicken-sandwich products.

In November 2019 in Chile, a salmonellosis outbreak was reported, affecting 80 people. The source of infection was sushi, improperly prepared. In the USA, in August 2019, an outbreak related to the contact with backyard poultry was investigated. More than 1,000 people in 49 states become infected. A similar outbreak involving several states was reported in May, the source of infection being fresh vegetables.

Until 24 June 2020, 473 more ill people were reported in USA (*Salmonella* Braenderup, Muenchen, Thompson, and Typhimurium). The reports registered 938 cases from 48 states as of 28 July 2020. Table 1 summarizes the data presented above.

"Real-time" data is difficult to collect, interpret and report. Yet, the end of 2020 and beginning of 2021 saw multiple other outbreaks emerge, with *Salmonella* Newport, *Salmonella* Thompson, *Salmonella* Enteritidis, *Salmonella* Potsdam and *Salmonella* Miami being identified. They account to nearly 200 people sick. The last two outbreaks are still ongoing.

Salmonella causing enteric fever

In 2015, in several states of the USA, cases of illness have been reported after eating raw fish, being isolated *Salmonella* Paratyphi.

An outbreak caused by *Salmonella* enterica serotype Typhi, resistant to chloramphenicol, ampicillin, trimethoprim-sulfamethoxazole, fluoroquinolones, and 3rd-generation cephalosporins was reported in Pakistan between November 2016 and September 2017. The outbreak involved 5,372 extensive-resistant (XDR) Typhi cases and 5 travel-related cases in the USA. In 2017, there were 250 cases of multidrug-resistant (MDR) typhoid fever in Pakistan among children. Resistant *Salmonella* Typhi strains have been also identified in Kenya.

In 2017, in India after continuous rains, the number of cases of typhoid fever increased. People consumed polluted water and therefore hundreds of cases were reported. An outbreak of typhoid fever has been also reported in Zambia due to a water crisis in the area. New Zealand, in 2017, reported an outbreak of paratyphoid fever, the incriminated product being mussels.

In 2018, numerous cases of typhoid fever were reported in Syria, in the Al-Hol camp, for Iraqi refugees and displaced Syrians. The source of infection was drinking water. In 2018, multiple cases of typhoid fever were reported in India, in the Woraiyur area also due to the consumption of contaminated water. According to the CDC, about 350 cases of typhoid fever are reported each year in the United States. A 2019 Promed post reported that a police detective was diagnosed with typhoid fever in the USA (California) and 6 colleagues had similar symptoms.

After typhoon Lingling hit the North Korea in 2019, an outbreak of paratyphoid fever was reported. The cases were registered in South Hamgyong and South Pyongan provinces. A summary is presented in Table 2.

Salmonella Paratyphi B was reported at the end of 2020, causing illness in 18 people. The etiological agent was not identified, and the outbreak is classified as closed.

A deeper view of *Salmonella* outbreaks in Europe

Salmonella causing diarrheal disease

In Europe, starting with 2007, the number of cases of salmonellosis has decreased, being implemented several preventive measures regarding the control of food products and hygiene. However, the European Centre for Disease Prevention and Control (ECDC) reports that "Compared with 2015, a 23.6% increase in the number of outbreaks due to S. Enteritidis was reported at EU-level in 2016, with 13 Member States".[15] The most important *Salmonella* serovars involved in outbreaks are Enteritidis followed by Typhimurium.[16]

It should be noted that in recent years, the increase in the consumption of fresh produce has led to an increased number of outbreaks associated with the ingestion of contaminated fresh produce. The first outbreak related to fresh produce involving an increasing number of states was registered in the USA in 1990, the responsible food being tomatoes. Subsequently,

Table 1. Summary of worldwide *Salmonella* causing diarrheal disease

Year	*Salmonella*	Source	Country	Region(s)	No. of cases
2015	Unidentified	Frozen raw breaded chicken products	Canada	4 provinces	44
2016	Unidentified	Raw mung bean sprouts	Australia	South Australia	230
2016	Unidentified	Rockmelon	Australia	New South Wales	97
2016	*Salmonella* Virchow	Raw meal organic shake	USA		27
2016	*Salmonella* Poona	Cucumbers (possible import from Mexico)	USA	40 states	907
2017	Unidentified	Kindergarten outbreak	Japan		87
2017	Unidentified	Unknown source	Australia		
2017	Unidentified	Multiple outbreaks	USA	16 states	24
2017	*Salmonella* Typhimurium	Unidentified	Sub-Saharan Africa Brazil		
2018	*Salmonella concord*	Tahini products	Israel		40
2018	Unidentified	Chicken sandwich	Australia		49
2019	Unidentified	Sushi	Chile		80
2019	Unidentified	Backyard poultry	USA	49 states	>1,000
2019	Unidentified	Fresh vegetables	USA	Several states	>1,000

Table 2. Summary of worldwide *Salmonella* causing enteric fever

Year	Salmonella	Source	Country	Region(s)	No. of cases
2015	*Salmonella* Paratyphi	Raw fish	USA		
2016	*Salmonella* Typhi XDR	Unidentified	Pakistan, USA		5,377
2017	*Salmonella* Typhi MDR	Unidentified	Pakistan		250
2017	*Salmonella* Typhi	Water	India		
2017	*Salmonella* Typhi	Water	Zambia		
2017	*Salmonella* Paratyphi	Mussels	New Zealand		
2018	*Salmonella* Typhi	Water	Syria	Al-Hol	
2018	*Salmonella* Typhi	Water	India	Woraiyur	
2019	*Salmonella* Typhi	Unidentified	USA	California	7
2019	*Salmonella* Paratyphi	Unidentified	North Korea	South Hamgyong, South Pyongan	
2020	*Salmonella* Paratyphi B	Unidentified	USA		18

several such outbreaks were identified in Europe.[17]

A survey of salmonellosis in the European Union over a period of 15 years (2000-2014) showed that catering services are a very important source, being the second after the cases registered in the household environment. Eggs and egg products were the main source of infection, followed by meat and vegetables. Proper food preparation plays an essential role.[18] Meller et al. conducted a study on Danish meatballs that are distributed through catering service and showed that a temperature of about 70°C at the core of the meatballs, using oven heating significantly reduced the risk of salmonellosis.[19] In 2013, in Germany there was an outbreak with *Salmonella enterica* subspecies *enterica* serovar Infantis, which lasted for a long time, from April to October; 267 people were infected and one death was recorded. The source of the outbreak was represented by raw pork products. The most affected people were the elderly and people receiving medication that decreases gastric acidity.[20] *Salmonella* can adhere to various surfaces such as recipients used by catering services, forming biofilm. Studies have revealed that the degree of adhesion differs depending on the material, teflon, glass, or polyurethane.[18] In 2015, in Bosnia & Herzegovina, 200 children were reported to be infected with *Salmonella* Enteritidis. The source seems to be macaroni with cheese and eggs. In 2016, an outbreak with

Salmonella enterica serovar Enteritidis was reported in Greece, affecting 56 persons. Most persons had severe symptoms that required hospitalization. In addition, symptoms began less than 12 hours after the ingestion of the contaminated food, cheesy penne pasta. It was assumed that the cooking temperature was not adequate and high-fat cheesy penne pasta due to the increased level of fat formed a structure as a capsule around the microorganism. These conditions prevented its destruction by gastric acid. The short incubation period was attributed to a very high infectious dose or a co-infection with *Staphylococcus aureus*. It should be noted that most hospitals in Greece do not have the capacity to screen stools for various pathogens with a short incubation period, therefore, co-infection with *S. aureus* could not be ruled out.[21]

Also, in 2016, there were reported outbreaks involving rare *Salmonella* serotypes. In Europe, a multi-country outbreak of a new *Salmonella* serotype took place. Its antigenic formula was 11:z41:enz15. There were 40 cases reported in Greece, Germany, the Czech Republic and Luxembourg. The outbreak was related to sesame-based products.[22] Also, Germany reported an outbreak of *Salmonella* Stourbridge, a rare serotype identified in Europe; 14 cases were recorded, considering that between 2011-2016 the average was 3 cases per year.[23]

In Europe, in 2017, 92,649 cases of salmonellosis were reported, of which 156 deaths. Thus, 19.6 cases per 100,000 population were registered. The highest rate was reported in the 0-4 age group, 94.1 cases per 100,000 population. Between 2013-2017, in many European countries the number of cases was constant. The countries which reported an increase in the number of cases were Greece, Estonia, Poland, Portugal, Slovakia, Spain and the United Kingdom.[24]

In 2017, in an ECDC report, experts announced "a multi-country outbreak of *Salmonella* Enteritidis linked to eggs from Poland is ongoing in the EU/EEA". Between 1 February 2017 and 28 November 2017, 196 confirmed cases of *Salmonella* Enteritidis infection were reported by 8 EU/EEA countries. 72 probable cases were also discussed. Moreover, prior to February 2017, 340 historical confirmed cases and 374 historical probable cases were reported by 16 countries, the largest number being registered in the United Kingdom.[15] After identifying the source of infection, the number of cases decreased, but then an increase was registered until 2018. These data indicate that although the number of cases decreased due to the application of control methods, the strains continued to contaminate the food.[25]

Another outbreak investigated by ECDC, involving *Salmonella enterica* subspecies *enterica* serovar Agona included 147 cases, 25 were reported between 2014 and 2016, and 122 since January 2017. Most cases have been identified in the United Kingdom (129 cases) followed by Finland with 15 cases. The possible source of infection was ready to eat products containing cucumbers, but the information was not clear. Between 2012-2016, *Salmonella* Agona was reported in 26 EU/EEA countries (378 to 582 cases per year). *S.* Agona is among the 10 most common *Salmonella* serotypes reported in Europe.[26]

According to the 2018 ECDC report, *Salmonella* was the most common cause of foodborne outbreaks in Europe. Over 60% of *Salmonella* outbreaks were recorded in Slovakia, Spain and Poland. "Findings from our latest Eurobarometer show that less than one third of European citizens rank food poisoning from bacteria among their top five concerns when it comes to food safety. The number of reported outbreaks suggests that there's room for raising awareness among consumers as many foodborne illnesses are preventable by improving hygiene measures when handling and preparing food" said EFSA's chief scientist Marta Hugas".[27]

Recently, between August 2018 and February 2019, there was an outbreak with *Salmonella enterica* serotype Poona, with 32 confirmed cases, 30 cases in France, one case in Belgium and one case in Luxembourg. The investigation showed that they consumed an infant formula product based on rice proteins, commercialized by the same brand. According to the reports in the European Surveillance System, *Salmonella* Poona is the 36th most common *Salmonella* serotype

associated with human infection. Between 2013-2017, 23 EU/EEA countries reported an annual case number ranging from 378 to 582. A third of the cases were reported in France and 37% were reported in children aged 0-4 years.[28]

A recent outbreak in Romania took place in Iaşi in 2018. There were 134 cases of gastroenterocolitis with *Salmonella* group D. The incubation period varied between 8 and 24 hours. The source of infection was fast food that contained mayonnaise from a public foodservice (Table 3).[28]

Salmonella causing enteric fever

Typhoid fever is rare in Europe, most of the reported cases are related to travel. In 2016, more than a third of typhoid fever cases were reported in the United Kingdom, travel related to India. In France the infection was acquired while traveling to Africa and Asia. Worldwide, 11-21 million cases of typhoid and paratyphoid fever are reported annually.[30]

In Georgia a hospital-based surveillance that included 6 hospitals between 2008-2011, enrolling 537 patients revealed among patients with febrile illness that 3 of them had *Salmonella* Typhi infection. Antibodies were detected in 0.6% of those investigated.[31] The analysis of 192 strains of *Salmonella* Typhi in Switzerland isolated between 2002 and 2013 showed that 11.5% had multidrug resistance. An important problem is the resistance to fluoroquinolones.[32]

In 2014, a case of typhoid fever has been reported in Spain, in a traveler returning to Spain from Guatemala. *Salmonella enterica* serovar Typhi producing an extended-spectrum β-lactamase (ESBL) has been identified.[33] In the Czech Republic between 1997 and 2017, 53 cases of typhoid fever were reported. Of these, only 7 were autochthonous, the rest were imported. For example, in 2017 it was reported the case of a 25-year-old girl who participated in the Rainbow Gathering in Italy, where she slept in a tent and drank unboiled water.[34]

Methods to prevent *Salmonella* outbreaks

Restaurants represent a common place for the onset of an outbreak. The correct washing of employees' hands remains a very important action in preventing these outbreaks.[35] A recent study showed a 100% reduction in *Salmonella* pathogens when the hands were washed properly. The use of contaminated equipment, the storage of food in inadequate conditions or cooking it at inappropriate temperatures are significant risk factors.[36,37]

The restaurant industry as well as poultry and other meat farmers need to maintain high standards of food security. A recent analysis indicates that not only the prevalence of Salmonellae but also factors related to virulence of the strain is important. The author cites temperature abuse, the level of undercooking, contamination of ready-to-eat food, but also high-

Table 3. Summary of European *Salmonella* outbreaks causing diarrheal disease

Year	*Salmonella*	Source	Country	Region(s)	No. of cases
2015	*Salmonella* Enteridis	Macaroni with cheese and eggs	Bosnia & Herzegovina		200
2016	*Salmonella* Enteridis	Cheese pasta	Greece		56
2016	New *Salmonella* serotype 11:z41:enz15	Sesame-based products	Europe	Greece, Germany, Czech Republic, Luxembourg	40
2011-2016	*Salmonella* Stourbridge	Unidentified	Germany		13
2017			Europe		92,649
2017	*Salmonella* Enteridis	Eggs from Poland	Europe	8 countries	196
2014-2017	*Salmonella* Agona	Possibly cucumbers	UK, Finland		269
2018-2019	*Salmonella* Poona	Infant formula	France, Belgium, Luxembourg		32
2018	*Salmonella* group D	Mayonnaise from fast-food vendor	Romania	Iaşi	134

risk consumption behaviors and high-risk customers as linked to infections. Fresh and raw food are also a major concern.[38]

Eggs are an important source for *Salmonella* infections. Various egg decontamination methods are available such as gamma irradiation, freeze drying, hot air, microwave heating, etc., but these methods have many effects on the properties of the eggs. Pasteurization remains the most commonly used method. Currently microbiologically decontaminated eggs are recommended for groups of vulnerable individuals, for example in hospitals. Keerthirathne et al. suggest the use of a method for decontaminating eggs without altering their structure.[39]

Lastly, environmental (water sources) contamination may be implicated. *Salmonella* is one of many pathogens that can be transmitted through drinking water. The well-known but often overlooked recommendation to prevent traveler's diarrhea "Boil it, peel it, cook it, wash it (with proper water) or forget it!" still rings true today.[40,41]

A great many lessons may be drawn from published case reports and bulletins about infectious outbreaks, are fundamentally due to hygiene procedures not being followed.

Our aim is not to review safety practices (household, environmental or industrial) to prevent *Salmonella* infections. Rigorous hygiene practices must be implemented in the food processing and preparation industry, especially around environmental controls in ready-to-eat food production, and ready-to-cook products.

Recent research has focused on the study of methods to reduce the degree of contamination of food with pathogens. The use of phages in bioactive packaging materials could be useful. Promising results have already been obtained regarding the use of anti-*Listeria monocytogenes* and anti-*E. coli* phages, the phages being free or immobilized in packaging materials to prevent contamination of fresh seeds and vegetables.[42] The review by Lievres et al. highlights the usefulness of phages in the prevention of contamination of dairy products with *Salmonella*. Adding anti-*Salmonella* phages to *Salmonella*-contaminated milk or goat cheese has led to a lower rate of contamination. Phages may be added in the matrix of the dairy products as food additives.[43]

A recent research on a mouse model has revealed that probiotics can be useful for the prevention of *Salmonella* infection. *Salmonella* spp. exhibit mechanisms of evasion of the immune system and it seems that probiotic bacteria inhibit these mechanisms. It has been shown that after the administration of probiotics the lymphocyte activation occurs and splenocyte apoptosis decreases.[44]

Data on *Salmonella* infections are not aggregated from worldwide resources. The lack of communication and preparedness is evidenced even in the response to the SARS-CoV-2 pandemic. This has focused the world's attention on one disease, but it begs the question: When the pandemic will eventually subside, will we again go back to ignoring infectious disease surveillance and communication?

Conclusions

Salmonella remains a major cause of acute diarrheal disease. The most important source remains the food. Enteric fever, and severe infections were reported worldwide. "Infections do not respect customs". Even though most reports come from developing countries, cases are identified everywhere in the world. In this context, the implementation of more rigorous preventive and control measures is necessary.

There is a continuous need to support and develop the public health units (human resources, equipment, reagents) and for a better collaboration between public health authorities inside and outside countries. New surveillance methods should be implemented, as PulseNet network that use DNA fingerprints of bacteria from patients to find clusters of disease and prove outbreaks, all over the world. By identifying ongoing foodborne outbreaks, health officials can stop an outbreak, and industry and regulatory agencies can make changes to improve food and water safety.[45] The described salmonellosis situation demonstrates that infectious diseases still represent an important concern, surveillance having a major importance and impact.

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