Chapter

Non-Typhoidal Salmonellosis: A Major Concern for Poultry Industry

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Abstract

Salmonella is the most important gastrointestinal pathogen distributed ubiquitously. The major serovars involved in Non-typhoidal salmonellosis are *S.* Typhimurium and *S.* Enteritidis. In the viewpoint of ban in the export and import of the *Salmonella* contaminated poultry food and poultry products, the need for rapid detection and mitigation of *Salmonella* has increased mani-folds. The major problem associated with its control is the growing incidence of antimicrobial resistance, which has been reported worldwide in the recent years. From causing self limiting gastroenteritis they have found to be responsible for several fatal diseases like endocarditis, meningitis, lung infestations, appendicitis, pneumonia, and cerebral abscess in human beings. Targeting several proteins such as adhesive proteins, lipoproteins, outer membrane proteins (Omps) etc. as vaccine candidates may pave a way in its control. So, continuous monitoring using one health approach and development of effective treatment and control strategies are critical.

Keywords: non typhoidal *Salmonella*, gastroenteritis, multiple drug resistance, poultry, vaccine

1. Introduction

Non-typhoidal Salmonellosis is caused by bacteria belonging to Enterobacteriaceae family. In poultry, *Salmonella* is known to be present in the gastrointestinal tract without showing any symptoms [1]. This leads to an undetected condition at farm level and after consumption of such poultry products like meat and eggs, humans gets infected at fork end. Domestic animals act as a reservoir for the food-borne spread of host-generalist serovars, which accounts for worldwide incidence of non-typhoidal *Salmonella* (NTS) infections. The range of symptoms varies from self limiting gastroenteritis to various dreadful diseases like endocarditis, meningitis etc. Generally condition becomes severe in children, geriatric and immunocompromised individuals [2]. NTS accounts for 93 million enteric infections and 155,000 deaths globally on annual basis [3]. The two factors contributing to majority of NTS infections are its broad host range and multiple drug resistance (MDR), which has been reported universally in recent years [4]. In developing countries, the situation is grimmer due to poor hygienic conditions. Near about 100 cells of virulent *Salmonella* are sufficient to cause infection in humans, which will further depend upon the health condition of an individual [5], hence it is critical

to adopt multiple intervention strategies. Vaccination is considered as an effective tool to control the disease [6], but the available vaccines has their own restrictions such as short term immunity etc. which limits their applicability. So, there is a must requirement to develop a suitable vaccine against NTS. Several proteins such as lipoproteins, outer membrane proteins (Omps) and polysaccharides have been targeted to evaluate their potential as suitable vaccine candidates. This chapter aims to present a brief overview on some such valuable information on NTS.

2. Non typhoidal *Salmonella* strains and its transmission

Till now, more than 2500 serotypes of *Salmonella* have been identified [7]. Non typhoidal salmonellosis is caused by all serotypes of *Salmonella* except for Typhi, Paratyphi A, Paratyphi B and Paratyphi C. Poultry can get infected either with host-specific *Salmonella* serovars, like *S.* Pullorum and *S*. Gallinarum, which cause a typhoid-like systemic disease or wide ranged NTS. Wide range NTS represents *Salmonella enterica* subspecies *enterica* serovar Enteritidis and Typhimurium together with serovars such as *S.* Newport, *S*. Heidelberg and *S*. Javiana etc. Broad host ranged *S.* serotypes get colonize [8] in host and carry infection asymptomatically. *Salmonella enterica* serotype Enteritidis and Typhimurium are the two most important NTS serotypes transmitted from animals to humans in most parts of the world [9].

NTS transmission usually occurs through consumption of contaminated food i.e. chicken, eggs, pork, beef, dairy products, and water contaminated with animal feces. However, contact with animals such as reptiles and animal environment are equally important sources [10]. Majority of reptiles are known to carry *Salmonella* as part of their natural intestinal flora. In poultry, mode of transmission can be vertical or horizontal. Vertical transmission occurs when parent poultry is suffering from systemic infection or transovarian infection which results in infection of infants. *S.* Enteritidis serovar have a particular preference to this mode of transmission. Polluted feed and drinking water, dirty cages, fomites etc. includes horizontal mode of transmission. Colonization of *Salmonella* in poultry without showing any sign and symptoms is common, hence, its transmission in layers (vertical) and broilers (horizontal) can occur at primary production level [11]. Transmission through eggs and meat from such healthy poultry with colonized NTS is common [12]. The degree of *Salmonella* colonization depends on parameters specific to *Salmonella* and effects of environmental stimuli on gene expression. Factors such as age, environmental and physiological stress, diet, and survival of *Salmonella* through gastric barrier, use of antimicrobials in the farm, chicken health, and genetic background of the chicks could possibly influence the colonization [13]. In poultry farms transmission can also occur through workers, vehicles, clothing, footwear, garbage, insects, rodents, wild birds, pets, equipment, and many other factors. In humans, factors contributing to susceptibility of NTS infections include pernicious anemia, any previous gastric surgery, excessive use of medications responsible for gastric barrier reduction etc. [14]. Other associated susceptibilities include homozygosity for sickle cell anemia [15], HIV [16], malaria [17], malnourished infants, and young adults [18].

3. Global disease epidemiology

In many countries, over the past years, the incidence of NTS has increased markedly. In western countries, the predominant serotypes are *S.* Typhimurium

and *S.* Enteritidis. In United States, each year NTS causes approximately 1.35 million illnesses, 26,500 hospitalizations, and 420 deaths [19]. As per the fact sheets of World Health Organization, NTS is 1 of 4 key global causes of diarrhea. The burden is so substantial that every year 33 millions of lives are lost. In Europe, NTS is the second most investigated zoonosis responsible for causing gastrointestinal infections in humans. As per the European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDPC) reports, the number of confirmed cases of salmonellosis in Europe still remains high, with a total of 91,857 cases reported in 2018 [20]. The estimation of the total number of NTS infections is a difficult task in developing and under-developed countries, a possible reason to this may be non-reporting of the diseased cases to hospitals. The epidemiological pattern has been variable over the past decade in African countries. Sub-Saharan African region is principally affected region in Africa. According to the hospital based studies of Africa, NTS is the second most frequently occurring pathogen in children and is leading cause of bacteremia in adults [21]. A population-based surveillance data reported the incidence to be between 0 and 54 cases per 100,000 person-years of observation in 13 surveillance sites [22]. The disease incidence ranged from 1.4/100,000 population/year in South Africa (all ages) to 2,520/100,000 population/year in Ghana (<5 years of age) [18]. The community acquired NTS bacteremia prevalence varied from 8% in Nigeria to 45% in Central African Republic [18]. From Kenya, documented incidences were found to be 4134/100,000 person-years [23]. According to the Statistical Committee of the Republic of Armenia, a total of 4,392 cases of salmonellosis were reported during the period 2010–2019, comprising at least 50% of patients below 6 years of age [24]. There is a scarcity in data related to NTS infections from several regions of Asia, limited reports are available from India [25], and Taiwan [26]. A hospital-based multicenter study from Indonesia, Thailand, and Vietnam, investigated NTS positivity rates of 27.5% and 11.7% in children and adults respectively from bacteremia cases [27]. From 2009 to 2013, the prevalence rate of NTS was found to be limited 20/12,940 in bacteremia patients with 25% case fatality report in Bangladesh [28]. In Malaysia, reported prevalence was 16.2%, among which most of the affected cases were from children below 1 year of age [29]. A variety of NTS serovars are known to be present in South-East Asia [30], even some less common serovars are also known to be prevalent such as occurrence of *S. enterica* Weltevreden from the farms of Vietnam [31].

4. Clinical manifestations

NTS infections can cause several clinical symptoms depending on the type of serovar and host factors in humans. NTS symptoms are generally non-specific and hence their identification is a challenging task particularly in areas where laboratory diagnosis facilities are not accessible. Most commonly, *Salmonella* causes self limiting gastroenteritis in human beings. After an incubation period of 6–72 h (mean 24 h), there is sudden onset of nausea, vomiting, abdominal pain and tenderness, followed by mild to severe watery diarrhea and sometimes diarrhea may contain blood and mucus. The stool examination reveals a moderate number of polymorphonuclear leukocytes and blood. Fever is seen in about 70% of patients. Usually, symptoms subside within 2–7 days in healthy children. In certain high-risk groups, like in neonates, young infants, and immunodeficient individuals symptoms may persist for several weeks. As a complication of gastroenteritis, transient bacteremia may occur in some patients (reported incidences in approximately 5% of the patients) [32]. Certain serotypes i.e. *S*. Choleraesuis and *S*. Dublin show a

higher predisposition for bacteremia in humans [33]. After gaining entry to the bloodstream, *Salmonella* get metastasize to different organs and cause focal suppurative infection. In sickle cell anemic patients a common finding as a result of NTS is osteomyelitis [34]. Less frequent occurrence of meningitis has been observed specially in infants [35]. Despite of antibiotic therapy, patients may develop rapid neurological deterioration. Other feared lethal complications include development of endarteritis [36], endocarditis [37], meningitis [38], lung infestations [39], appendicitis [40], pneumonia [41], bone and joint defects [15] and cerebral abscess [42].

5. Multiple drug resistance

In current scenario, there has been an extensive increase in documentation of antimicrobial resistance in NTS. Multiple drug resistance (MDR) is the antimicrobial resistance shown by the microorganism to at least three different groups of antimicrobials. Some *Salmonella* strains are characterized by carrying several antimicrobial resistance. The possibility of having MDR in bacteria is due to the presence of several different resistance genes or a single resistance gene that shows resistance to more than one antibiotic. Some important factors that could cause MDR in microorganisms include selective pressures, proliferation of multiple resistant clones, and inability to detect emerging phenotypes. The overuse or misuse of antimicrobials for the treatment of human disease, in agriculture, and in-home disinfectants comes under selective pressure [43]. The development of the antimicrobial resistance in bacteria is as a result of the genetic modifications of a microorganism for its own survival either spontaneously or acquired. In spontaneous mutation, a genetic modification occurs naturally which helps to survive from the lethal effects of antimicrobials. The reason behind the occurrence of spontaneous mutations is unknown, but the exposure to the antimicrobials may provide selective pressure for antimicrobial resistance [44]. Acquired resistance eventuate from gene transfer from other bacteria [45]. High resistance rate have been reported from *S.* Typhimurium DT104, resistant to five antimicrobial agents i.e. ampicillin, chloramphenicol, streptomycin, sulphonamide, and tetracycline [46]. Resistance to some extended spectrum antibiotics like cephalosporins and fluoroquinolones have been increasingly reported [47]. The emergence of *S*. Choleraesuis resistance to multiple antibiotics including ciprofloxacin has posed serious public health concerns [48].

Bacteria develop MDR by three different mechanisms. In first mechanism of resistance, the bacteria are known to produce certain specific proteins such as hydrolytic enzymes, which destroy the antimicrobials present in their surroundings. An example to this is penicillin resistance, where *Salmonella* produces β-lactamases enzymes which cleave the β-lactam ring of active penicillin and convert it into its inactive form [49]. The second mechanism of resistance is the presence of an active efflux pump system in the cell which actively pumps out the antimicrobials before they become effective [45]. *Salmonella* have energy-dependent efflux pumps for tetracycline and chloramphenicol which inhibit protein synthesis in bacteria by binding to tRNA to the A-site of the 30S subunit of the ribosome [50]. The third mechanism of resistance is called as the receptor modification in which the bacteria tends to chemically modify or mutate the target of the antimicrobial agent. For example vancomycin is the antibiotic which binds with D-Ala-D-Ala on the cell wall and inhibits the peptidoglycan synthesis of the cell wall of bacteria. But vancomycin-resistant enterococcus mutates its terminal peptide to D-Ala-D-Lac that has a lower affinity for vancomycin [45]. The mechanism of action adopted by *Salmonella* for different antimicrobial classes has been enlisted in **Table 1**. Plasmid mediated

Antimicrobial class	Antimicrobial mechanism of action by *Salmonella*	Reference
Aminoglycosides	Decreased drug uptake, drug modification, and modification of the ribosomal target of the drug	[51]
Beta-lactams	Secretion of β-lactamase enzymes	[50]
Phenicols	Enzymatic inactivation of the antibiotic by chloramphenicol O-acetyl-transferase and removal of the antibiotic by an efflux pump	[52]
Quinolones	Inducing mutations in the quinolone resistance determining region, increased efflux pumps expression and decreased outer membrane permeability.	[53]
Tetracycline	Produce energy dependent efflux pumps to remove tetracycline out from the bacterial cell	[54]
Sulfonamides and trimethoprim	Expression of *sul* genes i.e. *sul1* or *sul2* for sulfamethoxazole resistance and dihydrofolate reductase (*dfr*) genes for trimethoprim	[55]

Table 1.
Antimicrobial mechanisms of Salmonella *for different antimicrobial class.*

resistance determinants (genes) to antimicrobials have been known to be responsible for the worldwide dissemination of several *Salmonella* serotypes i.e. Enteritidis, Heidelberg, Typhimurium, Infantis, Virchow, Kentucky. The most common genes found in poultry and its meat products are β-lactamases, *CTX-M* (*CTX-M-1, -2, -9* and *- 15*), *TEM-52*, *AmpC-type CMY-2*. The transmission of these genes is associated with diverse plasmid families such as Incl l ($bla_{\text{CTX-M-1}}$, $bla_{\text{TEM-52,}}$ $bla_{\text{CMY-2}}$), Incl A/C ($bla_{\text{CMY-2}}$), Incl H12 ($bla_{\text{CTX-M-2,}}$ $bla_{\text{CTX-M-9}}$). Plasmid mediated quinolone resistance is governed by *QnrB2*, *QnrB19, QnrS1* genes. The genes mediating R-type ACSSuT in NTS are commonly clustered together in *Salmonella* genomic island 1 (SGI-1), a chromosomal genetic element.

6. Antimicrobial resistance in poultry food chain

The practice of using antimicrobials in food animals is rigorous, it may be either for growth promotion, prophylactic, therapeutic or metaphylactic reasons and this results in MDR. In poultry sector, the use of antimicrobials as growth promoters, such as bambermycin, bacitracin, chlortetracycline, penicillin, tylosin, fluoroquinolones and cephalosporins is concerning [56]. Consumption of low doses of antibiotics in poultry feed for rapid poultry growth is a general practice. Use of antibiotics not only kill majority of the gut microbiota, but, some resilient bacteria survive and become resistant. Over time, these resistant bacteria transfer antibiotic resistant genes to other susceptible microbial population. The situation is crucial in developing countries where laws to control the sale and use of antibiotics are not strict. For therapy, antimicrobials like erythromycin, fluoroquinolones, gentamycin, neomycin, penicillin, spectinomycin, tetracyclines and tylosin are commonly used in poultry [56]. The minimum time period from administering the last dose of medication to the production of meat or other animal-derived products for consumption purpose is referred to as withdrawal period. The withdrawal period for antimicrobials should be followed strictly, in order to prevent the detrimental effects of drug residues in food. There are numerous programmes to reduce the flow of foodborne pathogens from animals to humans, for instance programs for meat and poultry inspection, Hazard Analysis Critical Control Point (HACCP) system and standard operating procedures for sanitation.

The drug-resistant bacteria can be present anywhere, in various environmental samples, farms, and retail meat products. *S.* Enteritidis (88%) isolated from hatching eggs, litter, feed, drinkers, bird rinse, and ceca, were reported to be resistant to drugs, ampicillin, nalidixic acid, and tetracycline [57]. There is a frequent isolation of *Salmonella* serovars such as *S.* Enteritidis, *S.* Infantis, *S.* Typhimurium, and *S.* Heidelberg from broiler carcasses. The antibiotic-resistant *Salmonella* isolates has been found from poultry chiller water and carcasses. The isolated *Salmonella* were resistant to antibiotics including tetracycline, ampicillin, amoxicillin-clavulanic acid, ceftiofur, streptomycin, and sulfisoxazole. Broiler farms with *Salmonella* isolates resistant to multiple antibiotics, i.e. streptomycin (30.9%), gentamicin (12.6%), sulfadimethoxine (20.9%), tetracycline (13.9%), and trimethoprim-sulfamethoxazole combination (8.6%) were recovered. Among these isolates, 67% of *S.* Heidelberg and 54% of *S.* Kentucky isolates showed resistance to five or more antibiotics [11]. These serovars have a high resistance towards ceftriaxone and ceftiofur antibiotics. It has been observed that conversion of conventional farms to organic farms can reduce the prevalence of antibiotic resistant *Salmonella* from 44% to 6% [58]. A comparison between the *Salmonella* isolates obtained from poultry samples of Maryland retail shop from conventional and organic farms revealed that conventional carcass samples were resistant for five to seven antimicrobials, whereas 79% of the isolates from organic carcass samples were susceptible to all 17 tested antimicrobials. Assessment of the status of poultry retail shops is necessary, as in many countries, people prefer to procure the freshly slaughtered chicken. In such retail shops, the likelihood of cross-contamination of poultry carcasses is high and MDR-*Salmonella* has been isolated from retail meat shops as well. The Food and Drug Administration (FDA) have reported the National Antimicrobial Resistance Monitoring System (NARMS), regarding retail meat interim report for *Salmonella*, which includes the antibiotic resistance profile of *Salmonella* sp. in retail poultry meat [59]. The retail meats show high resistance to the common antibiotics such as tetracycline, streptomycin, sulfamethoxazole, and ampicillin.

7. Diagnosis

Salmonella diagnosis requires isolation of bacterium from the clinical samples and its culture in suitable culture media. The most common selective media used for *Salmonella* are SS agar, bismuth sulfite agar, Hektoen Enteric (HE) medium, Brilliant Green agar and Xylose-Lysine-Deoxycholate (XLD) agar. To further confirm diagnosis, biochemical, and serological tests are employed. The biochemical tests include sugar fermentation test, decarboxylation and dehydrogenation reactions, and hydrogen sulphide production. Serological examinations are usually carried out in outbreaks. Suffering from any other ailments makes diagnosis more cumbersome such as in cases of HIV-infected adults [60]. Hence, development of a rapid and sensitive diagnostic test is the need of the hour. A multiplex PCR has been found to be useful to identify NTS i.e. *S.* Typhimurium and variants, *S.* Enteritidis, *S.* Dublin and *S.* Stanleyville with 100% sensitivity and specificity [61]. Presence of low number of bacilli in clinical specimen is a limitation to this. So, to detect low infective loads of NTS, a microwave-accelerated metal-enhanced fluorescence (MAMEF) technique has been developed [62], which is well efficient enough to detect as little as 1 CFU/ml in less than 30 seconds. But, this still needs wider field applicability. A well defined ELISA with a definitive cut-off has not yet being commercialized for detection of NTS. But several researchers have suggested the use of lipopolysaccharide antigens from *S.* Enteritidis (serogroup D) and *S.* Typhimurium (serogroup B) for NTS detection [63].

8. Prevention and control measures

The fundamental basis for the control of NTS is food safety at every step from farm to fork. Even antibiotic treatment is not recommended in uncomplicated gastroenteritis cases as this condition is self-limiting. The list for preventive and control measures include good sanitation practices, safer food, and water handling methods, vaccination, public awareness, malaria control, and antiretroviral therapy programmes. To limit the number of infections arising as a result of animal contact it is advisable to wash hands properly after each animal contact, as in many cases the organism is in colonized state in animals without showing any sign and symptoms. Proper food cooking contributes to limit infections. Although irradiation technology has been approved by several health agencies like WHO, CDC, and European commission's Scientific Committee on Food, its use is partially implemented. Curtailment to the indiscriminate antibiotic usage in poultry feed along with better farm managerial practices leads to decreased multidrug resistant bacterial load. One health approach including multiple interventions is mandatory to enhance understanding, prevention, and control of NTS, as human health is completely related to the animal health and their environment. Adoption of different on-farm interventions strategies such as genetic selection of *Salmonella*-resistant birds, regular flock testing, use of natural antimicrobial products such as prebiotics or probiotics and egg washing on farms can reduce infection. The incidences of NTS infections have been observed more in individuals suffering from malaria and HIV because of immune-compromised health status in such individuals. So, adoption of strategies, such as malaria control, and antiretroviral therapy programmes, will not only lower the chances of primary sufferings but will greatly reduce NTS infections also.

Vaccination could be considered as a potential tool to control NTS, but currently no licensed vaccine is available for this in humans. The available typhoidal vaccine does not provide protection against NTS infections. Vaccination in animals may limit transmission of the micro-organism to humans. With this objective researchers are trying different vaccine strategies on livestock for NTS prevention. It includes live attenuated vaccines, killed vaccines, and a combination of both. Oral administration of live attenuated *S.* Gallinarum to chickens prevented not only wildtype infections by *S.* Gallinarum but also infections by *S.* Enteritidis [64]. Delivery of a killed vaccine comprising three different *Salmonella* serogroups i.e. Typhimurium, Mbandaka and Orion to chickens resulted in significant reduction in bacterial load when compared to the unvaccinated groups [65]. Administration of live attenuated *S.* Typhimurium vaccine followed by a killed *Salmonella* serovars Berta and Kentucky into chickens, showed a significant decrease in *Salmonella* sp. in the vaccinated animals when compared to the unvaccinated group [66].

Subunit vaccine development may pave a better way towards control scheme. Such vaccines come with an advantage of raising a protective immune response by using only a part of the infectious micro-organism. Common sub-cellular components of *Salmonella* used for development of vaccines are outer membrane proteins (Omps), porins, toxins and ribosomal fractions. Such vaccines have been tried in different animals and have variable success rates [67–70]. Many of the cell surface carbohydrates of pathogenic bacteria like capsular polysaccharides are important antigenic determinants as in case of Vi-based vaccines against *S.* Typhi in humans. Omps are the surface exposed proteins which play a crucial role in pathogenic processes such as motility, adherence and colonization of the host cells, injection of toxins and cellular proteases, and formation of channels for the antibiotics removal [71]. Administration of Omps of *S.* Enteritidis can elicit high antibody responses and prevent bacterial shedding in chicken challenged with virulent *Salmonella* [72].

These functions make them attractive targets for the development of vaccines. *Salmonella* is an intracellular pathogen and generation of both B-cell and T-cell immune responses are essential. Live attenuated vaccines provide both humoral and cell mediated immune response; but, they may pose a risk in immunocompromised individuals. Whereas, inactivated vaccines induce only humoral immunity. Hence, the development of subunit vaccines after B-cell and T-cell epitope prediction and assessment of peptides with high affinity for class I and II MHC proteins are a better approach, and studies focussing this [73], increase the likelihood of developing a successful vaccine. Successful induction high levels of anti-porin antibodies and enhanced cell mediated immunity against *Salmonella* also have been demonstrated [74].

9. Conclusion

The spread of non typhoidal salmonellosis is ubiquitous and persists in environment for a very long time duration. This poses difficulty in reducing the spread of infection. Infection from the poultry farm to fork level leads to severe complications in humans especially in immunocompromised individuals, children, and elderly. Moreover, the emergence of antimicrobial resistance in NTS is a major challenge in its effective treatment. Furthermore, till now no known vaccine is available which can control all the serotypes of NTS. Hence, in the present circumstances, implementation of one health approach could be a possible answer to prevent NTS infections.

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