Food for Public Health Series

Salmonella in the Food Chain: Public Health Risks and Detection Methods

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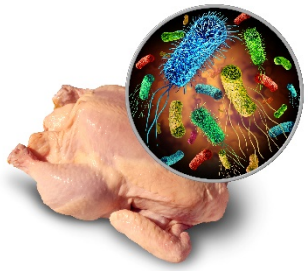
Problem of *Salmonella*

With over a million cases reported each year, *Salmonella* is considered as the major bacterial pathogen responsible for foodborne disease episodes in the United States. It is believed to be second leading cause of foodborne illnesses and the leading cause of foodborne hospitalizations and deaths (1) with more than 1.35 million human infections, 26,500 hospitalizations, and 420 fatalities annually in the United States according to the report from Centers for Disease Control and Prevention (2). The economic burden of *Salmonella* alone in the US economy including medical expenses, reduced productivity and premature deaths is estimated to be around $4 to 11 billion each year with vulnerable groups like people with weakened immune system, children, pregnant women, and aged senior citizens bearing a disproportionate amount of this burden (3-5). *Salmonella* strains can contaminate a wide variety of food products including fresh fruits, vegetables, meat and poultry (6). According to a recent CDC report, more than 3/4th (79.7%) of the reported *Salmonella* illnesses were linked to food categories like chicken (19.7%), fruits (14.6%), seeded vegetables (12%), pork (11.9%), other produce (9.4%), beef (6.9%) and turkey (5.2%) whereas less than 1/4th (20.3%) are linked to food categories like eggs (4.5%), fish (3.7%), vegetable row crops (3.4%), sprouts (3.2%), dairy (2.4%), other sea food (1.7%), grains/beans (0.8%), other meat/poultry (0.6%) and oils or sugars (<0.1%) (7). Typically, an American faces a 1 in 40 likelihood of acquiring a *Salmonella*-related illness from the consumption of poultry during their lifetime, and a 1 in 100 likelihood from the consumption of beef or pork (8).



Overview of *Salmonella*

Salmonella is a Gram-negative, rod-shaped bacterium measuring 0.2–1.5 × 2–5 μm, characterized by its non-fastidious and non-spore-forming nature and is categorized within the class Gammaproteobacteria and the family Enterobacteriaceae. This bacterium exhibits chemo-organotrophic characteristics and is facultatively anaerobic (they are primarily aerobic but can endure anaerobic conditions) and generally motile. (9,10). Initially, *Salmonella* serovars were classified as separate species and designated based on the diseases they induced, the animals in which they were identified, or after the individual who discovered them or the site of their initial identification (11,12). However, according to the most recent classification, the *Salmonella* genus consists of only two species: *Salmonella enterica* and *Salmonella bongori*, where only the former is typically considered as a pathogen capable of causing illness in humans (13). The species-*Salmonella enterica* is subdivided into six distinct subspecies: enterica, salamae, arizonae, diarizonae, houtenae and indica which is differentiated based on biochemical characteristics and denoted by Roman numerals I to VI (14-16). *Salmonella enterica* I is subdivided into two categories: typhoidal

Salmonella comprising *S. typhi* and *S. Paratyphi*, and non-typhoidal *Salmonella* comprising *S.* Typhimurium and *S.* Enteritidis. The former exclusively impacts humans, whereas the latter can infect both humans and animals (17). Currently, its taxonomic classification adheres to the Kaufmann-White scheme. This system identifies various *Salmonella* strains by analyzing their surface and flagellar antigen features. Nowadays *Salmonella* types are usually recognized by the name of their serovars (18) and as of now, more than 2600 different serovars of *Salmonella enterica* and *Salmonella bongori* have been recorded and characterized (19,20). Based on serotype differences, the strain may be classified as human-specific, animal-specific, or non-specific, contingent upon the host required for its survival. These bacteria possess the ability to adapt and flourish in a variety of animal hosts, including humans (21). All *Salmonella* serovars, with the exception of *Salmonella* Pullorum and *Salmonella* Gallinarum, possess peritrichous flagella that facilitate multidirectional motility (22).

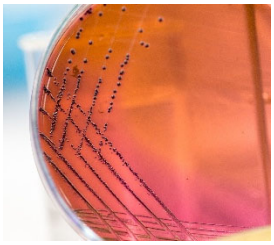
Salmonella is a ubiquitous and persistent bacterium that can survive several weeks in a dry environment and potentially for several months in water. However, it exhibits sensitivity to heat and can be inactivated at temperatures of 70 °C or higher. Most serovars of *Salmonella* can survive at temperatures between 5 to 47 °C whereas certain serovars can endure temperatures from 2 to 4 °C up to 54 °C (23). However, the optimal growth temperature is 37 °C (24). *Salmonella* is capable of surviving at pH levels between 4.05 and 9.50, but it thrives best in an optimal pH range of 6.5 to 7.5. It requires a water activity level between 0.94 to 0.99 for multiplication, yet it can endure in products with lower water activity for prolonged durations. Generally, *Salmonella* does not proliferate at pH below 3.8, water activity lower than 0.94, or temperatures above 70°C (9,23).

Factors Contributing to Pathogenesis and Virulence in *Salmonella*

Salmonella is usually found in the intestinal tract of animals. Although commonly found in animal-based foods like milk, eggs, beef, and poultry, it can also contaminate other types of food, including vegetables and infect humans through the consumption of contaminated food (25). It utilizes various specific virulence genes like invA, spvC, sopE and sseL to execute its pathogenic mechanisms. The genes *invA* facilitates the entry of *Salmonella* into host cells,
spvC aids bacteria in enduring intracellular environment by forming a protective vacuole, *sopE* affects the immune system of host and induces inflammation and *sseL* secretes toxins responsible for harming host cells. The majority of these virulence genes are found on *Salmonella* Pathogenicity Islands (SPIs) which are unique DNA regions that contribute to the pathogenicity of the bacteria. Certain strains of *Salmonella* possess plasmids including virulence plasmids, antibiotic resistance plasmids, conjugative plasmids, IncI1 plasmids and IncF plasmids that confer benefits such as antibiotic resistance or enhanced pathogenicity of the bacterium (26).

Salmonella Detection Methods

Several initiatives have been undertaken to improve the detection of this prevalent and opportunistic pathogen of public health concern (27). Currently, there are several methods being developed for its detection. Traditional culture-based methods are still considered as the gold standard method (28) in numerous countries. They are simple and cost



effective but at the same time they require significant labor and time due to their dependence on the proliferation of microorganisms in various culture media (29). Detection of *Salmonella* takes longer time in samples with minimal quantity and low residue because it generally necessitates bacterial enrichment in a medium (30). This method requires 2-3 days for preliminary identification and more than a week for the confirmation of pathogen's species (31). Additionally, there are constraints related to sensitivity (27), and they are prone to false-negative outcomes because of the existence of viable but non-culturable pathogens (29). Unlike conventional

culture-based techniques, immunological methods such as ELISA (enzyme-linked immunosorbent assay), lateral flow immunoassay, and immunomagnetic bead separation techniques are simpler, quicker and widely used (32). ELISA is preferred for its simplicity, stability and the ability to decrease detection times, but it has some limitations in terms of sensitivity and the management of complex biological samples (28). Similarly, lateral flow immunoassay is also

preferred for its ability to decrease detection times, sensitivity and portability but it has some limitations like variation in colloidal gold products from batch-to-batch, the tendency of the antigen/antibody complex to detach easily from the gold particle surface and the instability of the marker (28). Immunomagnetic bead separation technique is also highly efficient and specific technique (33) but has some limitations like magnetic beads' non-specific adsorption, their easy aggregation and poor rate of recovery, and intricate operational procedures (28). Nucleic acid based detection methods like PCR (Real time, digital), Loop-mediated isothermal amplification (LAMP) are simple, efficient, sensitive and facilitates accurate identification of nucleic acids. However, they also present several challenges, including difficulties in automation, the potential for false-negative results due to PCR inhibitors in samples, the necessity for DNA purification, issues in differentiating between viable and non-viable cells, the critical nature of precise primer design, risks of cross-contamination, and the requirement for trained personnel (28,32). Similarly, Raman Spectroscopy is another highly efficient nondestructive technique but necessitates advanced equipment and expertise. Different types of biosensors are nowadays more popular due to their portability, compactness and ability for multiplex detection, but they struggle with electrode interference and nanomaterial instability (28,34). Similarly, Immunomagnetic chemiluminescent assay that combines immunomagnetic separation with chemiluminescent principles represents another simple and efficient cost-effective technique; however, it requires labeling. (35). Each of the methods possesses distinct advantages and disadvantages. Hence, the choice of methods depends upon several factors like availability of resources and the intended objectives whether to identify a specific strain or to determine how severe the infection is (26).

Conclusions

Salmonella remains a predominant foodborne pathogen and a significant public health issue primarily impacting vulnerable groups such as children below five years of age, pregnant women, elderly, and the people with weakened immune system (36). More than a million cases are reported each year in United States which contributes to the billions of economic burden in addition to the health implications. The ability of *Salmonella* to induce infection is driven by particular virulence genes that augment its pathogenicity and resistance. Hence, detection of *Salmonella* is extremely necessary. Several methods have been developed for the detection and control of *Salmonella* in various food products. However, the selection of those methods depends on the objectives of detection and availability of resources.

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