Microbiology Research International
Vol. 10(3), pp. 32-39, December 2022
DOI: 10.30918/MRI.103.22.024
ISSN: 2354-2128
Review

A short review on *Salmonella* detection methods

Chala Guyassa* and Chala Dima

Animal Health Institute, Ethiopia.

Accepted 21 November, 2022

ABSTRACT

Salmonellosis is one of the leading causes of food-borne illnesses worldwide, in addition have negative economic impacts due to the cost of surveillance, investigation, treatment and prevention of illness and antimicrobial resistance. Antibiotic resistance in microorganisms can be inherited or acquired through antibiotic exposure. The majority of antibiotic resistance is caused by mutation or genetic material transfer between microorganisms. A variety of biochemical and physiological mechanisms contribute to the development of resistance. Recent advances in technology have made the detection of food-borne salmonella pathogens more rapid and convenient, while achieving improved sensitivity and specificity in comparison to conventional methods. Therefore, there is an ongoing need to develop more advanced detection methods that can identify *Salmonella* accurately and rapidly in foods before they reach consumers. There is also a need of continuous surveillance data for *Salmonella* among countries worldwide to ensure the effectiveness of control programs. Since the effects of *Salmonella* on public health and the economy continue to occur, improving safety of food products by early detection of food-borne pathogens would be considered an important component for limiting exposure to Salmonella contamination. The purpose of this review is to discuss an overview of *Salmonella* detection methods.

Keywords: *Salmonella*, detection, methods.

*Corresponding author. E-mail: guyasachala34@gmail.com.

INTRODUCTION

Foodborne diseases are a continuing challenge to human health (Eng et al., 2015). Over the past two decades, the epidemiology of foodborne diseases has changed rapidly as a consequence of changes in the social environment and the ability of pathogens to adapt to new niches. Food-borne pathogens are the causes of illness and death in developed and developing countries, which results in the loss of the labor force which could in turn have an impact on economic growth. *Salmonella* infection remains a major public health concern worldwide, contributing to the economic burden in both industrialized and underdeveloped countries through the costs associated with surveillance, prevention and treatment of the disease. Gastroenteritis is the most common manifestation of *Salmonella* infection worldwide, followed by bacteremia and enteric fever (Eng et al., 2015; O'Doherty et al., 2015).

Salmonella also causes diseases in food animals which can in turn be potential sources of zoonotic emerging and re-merging human infection (Nielsen et al., 2012). Common clinical manifestations of *Salmonella* infection in cattle include diarrhea, pneumonia, abortion, and death. Infection in dairy cattle is also associated with decreased milk production (O'Doherty et al., 2015). Although cattle can be infected by several *Salmonella enteric* serotypes, the majority of infections are attributed to serovar *Dublin,* which tends to cause more systemic infections, and serovar *Typhimurium*, more often causing enteritis in young calves (Costa et al., 2012). *Salmonella enteric* is also a significant public health interest with zoonotic potential. The majority of infections in humans are associated with products derived from food animals and contaminated products, which have been linked to domestic and feral animal populations (Haack et al., 2016).

The natural habitat of *Salmonella* is the intestinal tract

of humans and other animals. Both water and foods of animal origin have been identified as vehicles for the transmission of the organism. Approximately 95% of cases of human Salmonellosis are associated with the consumption of contaminated animal products such as meat, poultry, eggs, milk, seafood, and fresh products (Haack et al., 2016).

Salmonella species are the leading causes of acute gastroenteritis in several countries and remain an important public health problem worldwide, particularly in developing countries. It is the most common foodborne disease in developing countries, although incidence rates vary according to the country. The fecal wastes from infected animals and humans are important sources of bacterial contamination of the environment and the food chain (Pui et al., 2011).

In recent years, *Salmonellosis* has increased considerably both in incidence and severity. Efforts to prevent and control this disease are important because of many reported human cases and thousands of deaths every year. *Salmonella* Enteritidis and *Salmonella* Typhimurium are the most important serovars that are transmitted from animals to humans and vice versa (Andrews et al., 2013). Salmonella infection has spread over the world, through globalization, live animals and international trade in animal feed. The control of *Salmonella* is thus a critical challenge confronted by veterinary services and producers who want to produce safe food of animal origin. Studies show that the source of disease in more than 80% of all cases occurring are individual basis rather than as an outbreak (Forshell and Wierup, 2006).

Effective antimicrobial stewardship is contingent, in part, on ongoing surveillance of antimicrobial resistance trends in food products, animals and humans. This information can better inform public policy as well as clinical practice regarding appropriate antimicrobial use. Trends in antimicrobial resistance of Salmonella isolates from food animals are of public health concern given the potential for the spread of resistant microorganisms to humans (Valenzuela et al., 2017).

It is possible to isolate and identify *Salmonella* either from tissues collected aseptically at necropsy or from feces, milk, blood, rectal swabs, or environmental samples. When infection of the reproductive organs or concepts occurs, it is necessary to culture fetal stomach contents, placenta and vaginal swabs, and, in the case of poultry, egg contents. However, *Salmonellosis* is particularly difficult to determine in clinically normal carrier animals. It requires an important prerequisite for the detection of the source of infection and the route of transmission (De Oliveira et al., 2012). Various biochemical and serological tests can be applied to the pure culture to provide definitive confirmation of an isolated strain. Therefore, the objective of this review is to give a general overview of the detection methods of *Salmonella*.

LITERATURE REVIEW

Classification and nomenclature of *Salmonella*

Salmonella makes up a large genus of Gram-negative bacilli within the family *Enterobacteriaceae* and it constitutes a genus of more than 2500 serotypes that are highly adapted for growth in both humans and animals and that cause a wide spectrum of diseases (Hans et al., 2006). The growth of *S. typhi* and *S. paratyphi* is restricted to human hosts, in whom these organisms cause enteric (typhoid) fever. The remainder of *Salmonella* serotypes, referred to as non-typhoidal *Salmonella* can colonize the gastrointestinal tracts of a broad range of animals, including mammals, reptiles, birds, and insects (Fuaci and Jameson, 2005).

Salmonella pathogen was first discovered and isolated from the intestines of pigs infected with classical swine fever, by Theobald Smith in 1855. The bacterial strain was named after Dr. Daniel Elmer Salmon, an American pathologist who worked with Smith. The nomenclature of *Salmonella* is controversial and still evolving. Currently, the Centers for Disease Control and Prevention (CDC) use the nomenclatural system of *Salmonella* recommended by the World Health Organization (WHO) Collaborating Centre (Popoff et al., 2003).

According to this system, the genus *Salmonella* is classified into two species, *Salmonella enterica* (type species) and *Salmonella bongori*, based on differences in their 16S rRNA sequence analysis. *S. enterica*, can be further classified into six subspecies based on their genomic relatedness and biochemical properties (Eng et al., 2015). The subspecies are denoted with roman numerals: I, *S. enterica* subsp. *enterica*; II, *S. enterica* subsp. *salamae*; IIIa, *S. enterica* subsp. *arizonae*; IIIb, *S. enterica*subsp. *diarizonae*; IV, *S. enterica* subsp. *houtenae*; and VI, *S. enterica* subsp. *indica*. Among all the subspecies of Salmonella, *S. enterica* subsp. *Enterica* (I) is found predominantly in mammals and contributes approximately 99% of *Salmonella* infections in humans and warm-blooded animals. In contrast, the other five *Salmonella* subspecies and *S. bongori* are found mainly in the environment and also in cold-blooded animals, and hence are rare in humans (Brenner et al., 2000).

Historically *Salmonella* had been named based on the original places of isolation such as Salmonella London and Salmonella Indiana. This nomenclature system was replaced by the classification based on the susceptibility of isolates to different selected bacteriophages which is also known as phage typing (Bhunia, 2008). Phage typing is generally employed when the origin and characteristic of an outbreak must be determined by differentiating the isolates of the same serotype. It is very

reproducible when international standard sets of typing phages are used. More than 200 definitive phage types (DT) have been reported so far. For example, *S. Typhimurium* DT104 designates a particular phage type for Typhimurium isolates (Andrews and Baumler, 2005).

Kauffmann-White scheme classifies *Salmonella* according to three major antigenic determinants composed of flagellar H antigens, somatic Oantigens and virulence (VI) capsular K antigens. This was adopted by the International Association of Microbiologists in 1934. Agglutination by antibodies specific for the various O antigens is employed to group Salmonellae into the 6 serogroups: A, B, C1, C2, D and E. For instance, *S. Paratyphi* A, B, C and *S. Typhi* express O antigens of serogroups A, B, C1 and D, respectively (Costa et al., 2012). More than 99% of Salmonella strains that cause human infections belong to *Salmonella enterica subspecies enterica*. Although not common, cross-reactivity between O antigens of *Salmonella* and other genera of *Enterobacteriaceae* occurs. Therefore, further classification of serotypes is based on the antigenicity of the flagellar H antigens which are highly specific for *Salmonella* (Scherer and Miller, 2001).

General characteristics of *Salmonella*

Like other members of the family *Enterobacteriaceae*, they produce acid on glucose fermentation; reduce nitrates to nitrite, and do not produce cytochrome oxidase (Getenet, 2008). They are Gram-negative, oxidase-negative, non-spore-forming, facultatively anaerobic, rod shape and motile by peritrichous flagella (Rivoal et al., 2009). In addition, all Salmonellae except *S. gallinarum-pullorum* are motile by means of peritrichous flagella, and all but *S. typhi* produce gas (H_2S) on sugar fermentation. Salmonella are non-capsulated except *S. Typhi, S. Paratyphi* C and some strains of *S. Dublin* (Getenet, 2008).

It was lipids, polysaccharides, proteins and lipoproteins that compose the cell wall structure of *Salmonella*. The lipopolysaccharide portion of the cell wall and lipid A is endotoxin. Endotoxin is responsible for the biological effects. The common center monosaccharides and polysaccharides of endotoxin are also called somatic O antigens. *Salmonella* has about 60 O antigens that are nominated by numbers. Furthermore, there are some, unlike flagella (H) antigens that are recognized by numbers and letters. Based on these somatic antigens, *Salmonella* may be divided into groups that are using specific antisera (Haack et al., 2016). *Salmonella* grows readily on MacConkey agar or Eosin-Methylene blue. Bismuth Sulfate agar or desoxycholate agar should be used for the identification of *Salmonella* and its ferments glucose and mannose but not lactose or sucrose (Hoorfar, 2011).

They do not require sodium chloride for growth but can grow in the presence of 0.4 to 4%. Most *Salmonella* serotypes are able to grow at a temperature and pH range of 5 to 47°C and 4 to 9 respectively. They are sensitive to heat and are often killed at a temperature of 70°C or above. They require high water activity between 0.99 and 0.94 yet can survive at water activity less than 0.2 such as in dried foods. Complete inhibition of growth occurs at temperatures less than 7°C, pH less than 3.8, or water activity less than 0.94 (Pui et al., 2011).

Detection methods of *Salmonella*

Culture methods

The traditional *Salmonella* culture method involves pre-enrichment, selective enrichment, isolation of pure culture, and biochemical screening which requires 5 to 7 days to complete. The USDA and FDA recommended method involves a 6 to 24 hours pre-enrichment step in a nonselective broth such as lactose broth, tryptic soy broth, nutrient broth, skim milk, or buffered peptone water with a recommended incubation temperature of 37°C (Pui et al., 2011). The selective enrichment step requires additional 24 hours' incubation in Rappaport- Vassiliadis (RV) broth, selenite cystine (SC) broth, or Muller Kauffmann tetrathionate broth. The inoculation temperature of 41.5 ± 1°C for RV broth and 37 ± 1°C for SC and MKTT broth is used. Bacterial cells are isolated from selective agar plates such as Hektoen enteric agar (HEA), xylose lysine deoxy-cholate (XLD), and/or brilliant green agar (BGA). Biochemical testing is done using triple sugar iron agar and lysine iron agar, which requires an additional 4 to 24 hours (ISO, 2002).

Current testing of samples for the presence of *salmonellae* can be divided into three steps: detection of the pathogen by plate culture, identification of the isolate and its specific serovar designation, and finally, subtyping of the isolate for association with salmonellosis (Belete et al., 2014). These methods rely on conventional methods that apply serial enrichments with increasing selectivity culminating in the isolation of Salmonella on selective differential agar plates. It always takes up to 5 days to obtain a presumptive positive result. The traditional biochemical testing of nutrient utilization medium is needed for confirmation, and another few days to complete (Andrews et al., 2013).

Although innovative technologies have been applied to subtype salmonella's isolation, at least 24 h is needed for confirmation of *Salmonella* in multiple analytes. DNA fingerprinting techniques are based on DNA size differences on an agarose gel. The digested genomic DNA of the target bacteria is separated on an agarose gel and then hybridized with complementary sequences for identifying the banding pattern. A database of

fingerprint species, serovar, and strain identifications is used for comparison (Guard et al., 2012). The fingerprinting methods include pulsed-field gel electrophoresis (PFGE), ribotyping, and intergenic sequence (IGS) ribotyping. The use of PFGE has greatly increased the ability to track and trace back illness clusters and outbreaks. However, PFGE still requires a pure isolate and a minimum of 3 days to complete (Rivoal et al., 2009).

Culture independent methods

Recent technological advances have made the detection of *Salmonella* pathogens more rapid and convenient while achieving improved sensitivity and specificity in comparison to conventional methods. These methods employing newer technologies are generally referred to as "rapid methods," which include nucleic acid-based or antibody-based assays that are modified or improved compared to conventional methods (Yukawa et al., 2015). These rapid detection methods can be of high value to the food industry by providing several key advantages such as speed, specificity, sensitivity, cost-efficiency, and labor efficiency (Sun et al., 2015).

Immune-based rapid detection methods

Detection of antibodies to Salmonella by Enzyme Immunoassay (EIA)

The detection of antibodies to *Salmonella* by EIA offers a sensitive and cost-effective method for mass screening of animal flocks/herds for indications of a past/present *Salmonella* infection. The limitation of the method is that the immune response of the individual animal is not elicited before 1 to 2 weeks after infection takes place. A number of commercial kits are available for testing poultry, cattle and pigs. An obvious advantage of this method is that it can be automated and no incubation is required to increase the number of bacterial cells (Zamora and Hartung, 2002).

The EIA is a well-established technique for assaying antigens. Antibodies labeled with an enzyme are bound to *Salmonella* antigens, and the level of antigen present is determined by enzymatic conversion of a substrate, usually resulting in a color change that can be read visually or by a spectrophotometer. The EIAs rely on the standard cultural procedures for pre-enrichment and selective enrichment to provide enough *Salmonella* cells for detection. EIA technology that enables detection at an earlier stage of resuscitation and/or culture can provide even more rapid results. Serological tests, such as ELISA, serum agglutination and complement fixation can be used for the retrospective diagnosis of salmonellosis or the detection of carriers (Hans et al., 2006).

Enzyme-linked immunosorbent assay (ELISA)

Serological confirmation and serotyping- Agglutination tests, ELISA, anti-globulin and compliment fixation tests have been used to detect antibody responses to *Salmonella* infections (Quinn et al., 2005). The detection of the presence of *Salmonella* O-, Vi- and H- antigens are tested by slide agglutination with the appropriate sera, from pure colonies and after auto-agglutinable strains have been eliminated. This method relies on the antibody/antigen reaction between a test culture and commercially prepared antiserum. Enzyme-linked immunosorbent assay based approaches are the most prevalent antibody-based assay for pathogen detection in foods (Mandal and Parvin, 2011).

Because of the importance of *Salmonella* in foodborne disease, numerous typing methodologies have been developed and have been used to trace salmonellosis outbreaks to the contaminated source and to delineate the epidemiology of *Salmonella* infections. Some of the typing techniques include serotyping and phage typing. These techniques are useful for defining relationships between strains. This immunological approach has been used to detect *Salmonella* in poultry production (poultry feed, feces, litter, carcass rinsing, and water samples) and has provided a better sensitivity and shorter time frame than that of culture-based methods (Maciorowski et al., 2006).

Improvements by combination with other advanced technologies have been made to the basic ELISA method for *Salmonella* detection. For example, the incorporation of monoclonal antibodies can improve the sensitivity of the assay, and it can quantify *Salmonella* among poultry probiotic bacteria such as *Villanelle* (Dill et al., 1999). The detection limit for *S. Typhimurium* was determined to be 5.5×10^4 cells/ml in pure culture. Dill combined monoclonal and polyclonal antibodies and a commercial filtering system to detect *S. Typhimurium* cells in a chicken rinsate, with a detection limit of fewer than 100 *S. Typhimurium* cells (Hoorfar, 2011; Dill et al., 1999). As the advantages of ELISA methods for *Salmonella* detection in foods and animal feeds, they are now widely used for the detection of *Salmonella* in animal-producing foods (Hoorfar, 2011).

Serotyping: Serotyping is based on the O and H antigens using the slide agglutination test. Most serotypes exhibit diphasic flagellar antigen expression by alternately expressing two genes, *fli*C (phase 1) and *flj*B (phase 2) which encode flagellins of different antigenicity. *Salmonella* serotyping methods recognize 63 district phase 1 flagellar antigenic factors and 37 phases 2 flagella antigenic factors although the latter is not always

present (Murgia et al., 2016). Bacterial growth for serotyping should be taken from a triple sugar iron (TSI) agar slant or nutrient agar as culture from selective media is often unsuitable for typing (Toro et al., 2016).

A loop full of the culture of *Salmonella* to be serotyped should be suspended in a drop of saline on a microscope slide and examined for auto-agglutination (Vibbert et al., 2015). This can occur with rough strains and will invalidate the serotyping. Smooth-rough dissociation occurs after subculture and most frequently from media containing carbohydrates. Smooth *Salmonella* to be serotyped is emulsified in a drop of 0.85% saline on a clean microscope slide. A drop of antiserum is added to and mixed well with the *Salmonella* suspension. The slide is rocked gently for about 30 seconds and the antigen-antibody mixture is examined for agglutination. *Salmonella* is first tested against antisera to the O (somatic) antigens and then the H (flagella) antigens (Toro et al., 2016).

Phage typing: Phage typing is based on the specificity of a given phage for its host bacterium, and this relationship allows one to use known phages to identify their specific hosts. Therefore, phage typing of *Salmonella* isolates is based on the sensitivity of particular isolates to a series of bacteriophages at appropriate dilutions. This can be useful to determine whether isolates, which come from different places at different times, are similar or different in their reactions to specific sets of phages used for typing (Quinn et al., 2003).

Nucleic acid-based assays

Polymerase chain reaction (PCR)

Developments for the further improvement of PCR methodology to enhance *Salmonella* detection sensitivity and specificity are generally focused on identifying the specific gene(s) that represent optimal targets for differentiation among *Salmonella* serovars and strains (Arun, 2008). The application of WGS and related technologies has been particularly useful for identifying new PCR typing targets. Developing a PCR primer based on the *flhB* gene which encodes for the membrane protein FlhB, a part of the flagellar secretion system. Their rationale was based on the premise that since *S. Pullorum* and *Gallinarum* are the only non-motile serovars, their *flhB* gene may have unique characteristics. To determine this, they examined the *S. TyphimuriumflhB* nucleotide sequence as a potential PCR target for identifying *S. Pullorum/Gallinarum* and differentiating these serovars from non-S. Pullorum/Gallinarum isolates. They based the construction of the PCR primer *flhB* sequence on an NCBI non-redundant database search using the basic local alignment search tool algorithm (Ricke, 2017).

The largest advance toward faster detection of salmonellae has been in the realm of molecular biology, where polymerase chain reaction and quantitative PCR (qPCR) are predominantly being applied as the methods of choice for the detection. Different protocols targeting different specific genes or gene regions specific to salmonellae have been published. Numerous studies have been conducted to detect and characterize Salmonella in poultry, poultry products, and feeds using PCR assays to target selected antibiotic resistance or virulence genes along with genus-, species-, and serotype-specific genes (Murgia et al., 2016).

Quantitative PCR, also referred to as qPCR, possesses the ability to label and cumulatively quantify the generated PCR products at each cycle during the amplification process, resulting in improved detection sensitivity of up to 100% depending on the food matrix. Because the enhanced sensitivity associated with qPCR leads to increased susceptibility to the inhibitors that are likely to exist in food samples, optimizing DNA extraction methods has been examined to limit PCR inhibitor contaminants (Ricke, 2017). Several extraction approaches have been developed, and commercial DNA extraction kits are readily available for the recovery of DNA suitable for qPCR and pathogen capture approaches such as immune magnetic separation (De Oliveira et al., 2012).

Real-time quantitative polymerase chain reaction using PCR (Q-PCR), reverse transcriptase PCR (RT-PCR), and nucleic acid sequence-based amplification (NASBA) have been used for the detection of *Salmonella* from various food matrices. *Salmonella* enterica was detected at 1 CFU ml^{-1} after a culture enrichment of 8 to 12 hours in the TaqMan-based Q-PCR using *invA* gene as target. NASBA method has been used for the detection of viable *Salmonella* cells and it has been demonstrated to be more sensitive than RT-PCR, and it requires fewer amplification cycles than the conventional PCR methods (Arun, 2008).

As we all know, the quality and quantity of target DNA, PCR template, are important factors during the design of a PCR assay. Although a well-designed PCR primer and a good PCR template can bring high specificity of the target detection, it is still not sufficient to overcome the side effects of PCR inhibitors in samples, such as denatured proteins, organic chemicals, and sucrose (Ricke, 2017). Moreover, the presence of DNA and cells other than those from the targeted organism can affect the efficiency of the PCR methods. To overcome this, an enrichment step is commonly performed to enhance assay sensitivity by ensuring the detection of viable pathogens before the PCR reaction. Ferretti et al. (2001) reported that PCR with a 6 hours non-selective enrichment could detect various *Salmonella* serotypes in salami stuff as low as 1 CFU in 100 ml of food

homogenate.

Myint et al. (2006) reported a PCR method for *Salmonella* detection in contaminated poultry tissue samples, and false negative results were obtained without enrichment. However, a positive rate of 90% was observed after enrichment. Generally, culture enrichment is recommended in order to distinguish live cells from dead cells before PCR (Myint et al., 2006). Maciorowski et al. (2006) investigated different enrichment times to detect indigenous *Salmonella* in poultry dietary samples using PCR. It was found that it could not be detectable for *Salmonella* with 7 h enrichment, and the sensitivity for detection was 25 and 50% with 13 h enrichment and 24 h enrichment, respectively (Maciorowski et al., 2006). Improvements have also been made to the basic PCR technology as well. In particular, two primary PCR-based methods have emerged over the past several years, such as multiplex PCR and real-time quantitative PCR (Kawasaki et al., 2005).

Metagenomics

Metagenomics is a recently developed methodology. It can be used to directly analyze the microorganisms within a sample by sequencing all the genomes in the sample and comparing the genomic data to those of known microorganisms (Toro et al., 2016). In addition to identifying the bacteria present in the sample, this method can also be used to analyze the genetic relationship among the organisms assessed, identify putative virulence factors and explore new or rare pathogens. Metagenomics functions as pooling all technologies for detection, identification, and subtyping of Salmonella into a single assay thus it reveals microbial community directly from food samples or after pre-enrichment stage within 24 h (Jarvis et al., 2015).

Microbial community analyses may prove to be useful for the detection of *Salmonella* as well as develop a high-resolution 16S rRNA microbiome profiling approach for the detection of *Salmonella enterica* in food samples using Resphara Insight, an ultra-high resolution taxonomic assignment algorithm and sequence analysis pipeline for species-level 16S rRNA sequence identification.[35] As the authors point out, the development of such approaches takes advantage of the more economical microbiome profiling of microbial communities, which can be tracked in response to shifts in environmental conditions. Simultaneous tracking of *Salmonella* and overall microbial communities may have added importance as there may be interactions between *Salmonella* and animal gastrointestinal populations, such as in the poultry cecal microbiota. The drawbacks of this technology are that Metagenomics is expensive and it requires specific instruments and a long data process time (Jarvis et al., 2015).

Aptamer-based detection assay

Besides antibodies, other biomolecules have been investigated to selectively capture and enrich Salmonella from cultures, among which aptamer is the most prevalent one (Jyoti et a., 2011). Aptamers are single-stranded oligonucleotides, DNA, or RNA that can fold into unique 3D structures based on their primary nucleotide sequence, rendering them capable of binding to specific ligands, like antibodies interacting with an antigen (Ozalp et al., 2015). Aptamers offer some advantages over antibodies in that they are relatively inexpensive to synthesize and they provide more batch-to-batch consistency (Bruno et al., 2014). However, few studies have reported their specific use in detecting *S. Typhimurium* from river water and fecal samples (Singh et al., 2012). Bacteriophages have also been explored as a means to capture Salmonella cells. Phages may offer some advantages over antibodies given their inherent specificity for host cells, their ease of production in bacteria versus animals or eukaryotic cell culture, and their relative stability in harsh conditions such as pH and temperature extremes (Laube et al., 2014).

Relative to culture-independent detection, researchers have focused on methods to concentrate whole cells within the sample before the pre-enrichment step. The enriched whole Salmonella allows for direct detection from food and environmental samples. The enrichment steps mainly rely on filtering liquids, reinstates, or mechanically disintegrated (i.e., blended or stomached) samples. Therefore, this approach has been widely used in large volumes of water, but the testing of food samples was problematic due to the food particles difficulty going through filter membranes (Vibbert et al., 2015). To overcome this problem, endopeptidases have been added to apply in food samples. These degrade the small, soluble proteins and peptides so that they are unable to clog the filter and pass through with permeate. The United States has awarded the method with a grant prize. The Food and Drug Administration also recommends the method for the food safety guard, which signified its potential to greatly enhance the detection of Salmonella directly from foods (Bruno et al., 2014).

CONCLUSION AND RECOMMENDATION

The knowledge about *Salmonella* and its evolution is important to ensure the safety and quality of food. In order to meet the current requirement of rapid detection, it is clear that several approaches have emerged including PCR-based, Aptamer-based, antibody-based, and other approaches encompassing those stemming from the current genomic era. A clear character of the method development direction is moving toward greater automation, cost-saving, and time-saving network

integration. It is important to mention that outputs from one approach would serve to strengthen directly or tangentially other approaches. Intervention strategies are hence important to control *Salmonella* from farm to fork. Control of *Salmonella* in animals, animals' food, and food and human are the important key to controlling antibiotic resistance. The degree of better communication between veterinary organizations and health care providers is important in order to exchange knowledge and relevant information. In order to have control over the spread of salmonellosis and to target antibiotic resistance, international collaboration is needed. Better knowledge about antimicrobial resistance mechanisms is important to design and developing well-organized control strategies for antimicrobial resistance. Therefore, based on the above conclusion the following recommendations were forwarded:

- Developing strategies in order to minimize the expansion of antimicrobial resistance is critically important for protecting both public and animal health.
- Collaboration involving the public, the public health, animal health, and animal agriculture communities on the development and implementation of such control strategies is needed to assure that public health is protected.
- Provision of rapid detection methods can be of high value to animal and human health as well as in the food industry by providing several key advantages such as speed, specificity, sensitivity, cost-efficiency, and labor efficiency.
- Keeping overall (Farm to Flock) hygiene is important to control salmonella transmissions.

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Citation: Guyassa C, Dima C, 2022. A short review on *Salmonella* detection methods. Microbiol Res Int, 10(3): 32-39.