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Review

Current and emerging technologies for rapid detection and characterization of *Salmonella* in poultry and poultry products

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ABSTRACT

Salmonella is the leading cause of foodborne illnesses in the United States, and one of the main contributors to salmonellosis is the consumption of contaminated poultry and poultry products. Since deleterious effects of *Salmonella* on public health and the economy continue to occur, there is an ongoing need to develop more advanced detection methods that can identify *Salmonella* accurately and rapidly in foods before they reach consumers. Rapid detection and identification methods for *Salmonella* are considered to be an important component of strategies designed to prevent poultry and poultry product-associated illnesses. In the past three decades, there have been increasing efforts towards developing and improving rapid pathogen detection and characterization methodologies for application to poultry and poultry products. In this review, we discuss molecular methods for detection, identification and genetic characterization of *Salmonella* associated with poultry and poultry products. In addition, the advantages and disadvantages of the established and emerging rapid detection and characterization methods are addressed for *Salmonella* in poultry and poultry products. The methods with potential application to the industry are highlighted in this review.

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1. Introduction

Foodborne illnesses continue to be a serious concern as a public health issue for the food industry. The Centers for Disease Control and Prevention (CDC) have estimated that 48 million cases of foodborne illnesses occur in the United States (US) annually and approximately 128,000 cases require hospitalization and 3,000 cases result in death (Scallan et al., 2011). The CDC reported that viruses are major causative agents for foodborne illnesses (59%), followed by bacteria (39%), and parasites (2%); however, bacterial agents are associated with the more severe cases, being responsible for most of the hospitalizations (63.9%) and deaths (63.7%). In particular, *Salmonella* species were considered as the leading cause for these more severe cases resulting in 35% of the hospitalizations and 28% of the deaths (Scallan et al., 2011).

Most human salmonellosis cases are associated with consumption of contaminated egg, poultry, pork, beef and milk products (Geimba et al., 2004; Zaki et al., 2009). The CDC regularly reports *Salmonella* outbreaks that are associated with poultry and poultry products (Patrick et al., 2004; Altekruse et al., 2006; CDC, 2007; CDC, 2009a; CDC, 2010) and these food products are generally recognized as a primary source of salmonellosis (De Boer and Hahne, 1990; Braden, 2006; Linam and Gerber, 2007). Poultry and eggs are considered one of the most important reservoirs from which *Salmonella* is passed through the food chain and ultimately transmitted to humans (Oliviera et al., 2002; Ricke, 2003a; Maciorowski et al., 2004; CDC, 2009b; Finstad et al., 2012; Howard et al., 2012). With increasing consumption of poultry and poultry products, the number of salmonellosis associated with poultry continues to be a public health issue in the US. Since

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Salmonella is a major causative agent for poultry-associated foodborne illnesses, improving safety of poultry products by early detection of foodborne pathogens would be considered an important component for limiting exposure to *Salmonella* contamination. This monitoring of poultry and other related products for *Salmonella* contamination could be made significantly more effective by employing rapid and sensitive detection systems. Transmission of *Salmonella* to humans typically occurs when ingesting foods that are directly contaminated by animal feces or cross-contaminated by other sources (Gantois et al., 2009; Modaressi and Thong, 2010). *Salmonella* contamination of poultry in pre-harvest environments can usually be traced to production issues that include contaminated poultry feed or pathogen introduction to the facilities via a wide range of carriers including house pets, wild animals as well as insects (Jones et al., 1991; Singer et al., 1992; Butcher and Miles, 1995; Murray, 2000; Heyndrickx et al., 2002; Maciorowski et al., 2004; Okoli et al., 2006; Park et al., 2008).

Many of these environmental sources have been reviewed extensively elsewhere but poultry feed has been discussed in more detail than most other sources (Murray, 2000; Maciorowski et al., 2004; Park et al., 2008; Dunkley et al., 2009; Jarquin et al., 2009; Davies and Wales, 2010; Jones, 2011; Ricke et al., 2013a). There are several reasons for the extensive focus on poultry feeds as a source of *Salmonella*. First of all, since one *Salmonella* organism per gram of feed can colonize in young chicks, low or undetectable numbers of *Salmonella* represent a high risk for infection in these birds that is further enhanced by the increased feed mixing and incorporation of individual feed ingredients from a multitude of sources (Milner and Shaffer, 1952; Schleifer et al., 1992). This becomes of particular concern if breeder flock hatchlings are exposed since they represent the starting point for all commercial flocks (Jarquin et al., 2009). In addition, *Salmonella* can linger in feed for extended time periods with reports of bacterial cells remaining viable for several weeks up to 16 months in dry feed stored at 25 °C (Williams and Benson, 1978; Juven et al., 1984; Ha et al., 1998a, b; Petkar et al., 2011). This is further confounded when feeds are treated with antimicrobials such as organic acids where *Salmonella* either can become acid tolerant or their recovery and/or subsequent enumeration accuracy using conventional plating methods is influenced by carryover of antimicrobial compounds into the media (Kwon and Ricke, 1998; Ricke, 2003b; Carrique-Mas et al., 2007; Davies and Wales, 2010). Contaminated feed is also regarded as a source of infectious transmission of *Salmonella* among flocks (Veldman et al., 1995; Huehn et al., 2009). This is further accentuated by the larger numbers of birds housed in confinement resulting in an increase in more birds being infected simultaneously via aerosols and other routes (Nakamura et al., 1997; Murray, 2000; Maciorowski et al., 2006; Park et al., 2008).

The high number of poultry-associated *Salmonella* outbreaks in humans highlights the need for rapid, reliable, and cost-effective high-throughput detection methods along the entire production chain from live poultry and feed to poultry products. Adoption of the microbiological testing of poultry products during production and processing could play a significant role in preventing *Salmonella* infection (Crump et al., 2002; De Medici et al., 2003; Mumma et al., 2004; Koyuncu and Haggblom, 2009; Koyuncu et al., 2010). In this review, the current and emerging rapid methodologies and their potential application in detecting and characterizing *Salmonella* in poultry production will be discussed.

2. *Salmonella* serovars commonly associated in poultry and poultry products

The *Salmonella* genus has been divided into two major subspecies including 2579 serotypes: *Salmonella enterica* and *Salmonella bongori* (V). *S. enterica* subdivided into 6 subspecies as *enterica* (I), *salamae* (II), *arizonae* (IIIa), *diarizonae* (IIIb), *houtenae* (IV), and *indica* (VI) (Grimont and Weill, 2007) and several of these serotypes are frequently associated with poultry and poultry products (Grimont and Weill, 2007; Foley et al., 2008, 2011). This is in part due to their marked ability to persist in a wide range of varying environmental conditions. For example, *Salmonella* strains can grow in foods stored at low (2–4 °C) and high (54 °C) temperatures (Balamurugan, 2010). Several *S. enterica* serotypes have the ability to colonize and infect live birds, and are commonly associated with raw poultry and eggs (Ricke, 2003a; Dunkley et al., 2009; Howard et al., 2012; Ricke et al., 2013a). Serotypes such as *S*. Typhimurium, and *S*. Enteritidis can infect a wide range of hosts (De Medici et al., 2003; Seo et al., 2004; Altekruse et al., 2006; Cortez et al., 2006; Malorny et al., 2007a), whereas *S*. Gallinarum and *S*. Pullorum are avian-specific strains (Foley et al., 2011). *S*. Enteritidis is one of the most frequent causes of foodborne illnesses in humans, and it is most commonly implicated with egg and poultry in the US (Olsen et al., 2000). Among *Salmonella* serotypes, *S*. Enteritidis and *S*. Typhimurium represent two of the more prominent *Salmonella* serotypes associated with human infections (Foley et al., 2008).

3. Methodologies for rapid detection of *Salmonella*

Recent advances in technology have made the detection of foodborne pathogens more rapid and convenient, while achieving improved sensitivity and specificity in comparison to conventional methods (Mandal et al., 2011). The detection methods employing these newer technologies are generally referred as “rapid methods” which include antibody- or nucleic acid-based assays that are modified or improved compared to conventional methods (Ibrahim, 1986; Dziezak, 1987; Fung, 1994; Stager and Davis, 1992; Doyle and Beuchat, 2013). These rapid detection methods can be of high value to the food industry by providing several key advantages such as speed, specificity, sensitivity, cost- and labor-efficiency. As detection technology has continued to advance not only has the identification of a particular foodborne pathogen become more rapid but the depth of information generated from the analysis has become more comprehensive. This has also led to improvements in the specificity of a rapid method to detect particular pathogens that are present in a background of non-pathogenic organisms in food matrices or other complex biological environments to the point of defining subtle genetic differences at the strain level. Finally, sensitivity has continued to be enhanced to detect ever fewer numbers of viable pathogens in food or other complex samples that could comprise the lower ranges of infectious doses for the highly susceptible individuals within the human population. In addition, rapid detection systems are now much more amendable to automation and high-throughput outcomes, thus reducing human errors as well as costs by increasing the total number of assays that can be conducted at a particular time point.

Advanced molecular and immunological methods require only a few hours on average to detect the target pathogen from food samples compared to 4–5 days using conventional culture-based methods (Hadjinicolaou et al., 2009). Generally, non-selective or selective enrichment steps are employed to increase the sensitivity when detecting *Salmonella* in poultry and poultry products (Ukeda and Kuwabara, 2009; Mihayara et al., 2010); however, it should be noted that the addition of enrichment steps could increase the total assay time. According to the Food and Drug Administration (FDA), any rapid detection method that indicates the presence of the target foodborne pathogen (positive results) must be confirmed by traditional culture-based methods (FDA, 2001). Some rapid assays have been approved for *Salmonella* detection in poultry by the National Poultry Improvement Plan (NPIP) under USDA (USDA-

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