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Salmonella in food surveillance: PCR, immunoassays, and other rapid detection and quantification methods

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ABSTRACT

Foodborne diseases caused by *Salmonella* have always been a significant health burden to many countries. Recent epidemiological data have indicated that *Salmonella* was the most common bacterial etiologic agent in food poisoning outbreaks both in the United States and in Asian regions like Hong Kong. In the past, labor-intensive traditional standard culture methods with long turnaround time have always been employed by many laboratories of public health services for the detection of *Salmonella* in Food Surveillance Programmes. To cope with the enormous volume of sample received for *Salmonella* detection, recent advances in nucleic acid- and immunoassay-based methods, and the subsequent commercialization and automation of the technologies have provided more rapid, specific, and productive alternatives for routine applications in testing laboratories. Fluorogenic or real-time PCR methods are able to generate results in a day, whereas immunoassay-based methods can produce negative results in 1–3 days. Some of these rapid methods have already been validated and accepted by international authorities as standard methods and have become increasingly popular among testing laboratories.

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1. Introduction: Foodborne disease outbreaks and food surveillance for *Salmonella*

Food poisoning is usually caused by the consumption of contaminated food or water containing various bacteria, viruses, parasites or toxins of biochemical or chemical nature. As salmonellae are ubiquitous in the environment, they are common causative agents of food poisoning. Foodborne illnesses caused by *Salmonella* are common in the United States and in many European countries, and have always been a significant health burden worldwide (Allos, Moore, Griffin, & Tauxe, 2004; Bäumler, Hargis, & Tsolis, 2000; Brands et al., 2005; Busani et al., 2005; Davies & Wales, 2010; Magnino et al., 2009; Patel et al., 2010). The most recent analysis of epidemiologic data on foodborne disease outbreaks in the United States indicated that *Salmonella* was the most common bacterial etiologic agent, accounting for 112 (52%) outbreaks attributed to bacteria (CDC, 2009). Among the more than 2500 *Salmonella* serotypes, *Salmonella* Typhimurium and *Salmonella* Enteritidis accounted for 46% and 24% outbreaks caused by *Salmonella* and bacteria, respectively (CDC, 2009). In the United States, these two serotypes were also the two most frequently reported serotypes (33% of isolates) from human sources (CDC, 2005; Vugia et al., 2004).

In Hong Kong, both food poisoning outbreaks and typhoid fever are statutorily notifiable under the Prevention and Control of Disease Ordinance. In 2009, a total of 1540 persons were affected in 407 food poisoning outbreaks (Lam, Tam, & Wong, 2010). However, only in about 18% of the outbreaks the causative agents had been confirmed. The top three causative agents were identified as *Salmonella* spp. (42%), *Vibrio parahaemolyticus* (40%) and noroviruses (3%). During 2000–2004, 22% of reported food poisoning outbreaks in Hong Kong were caused by *Salmonella* (Lam, 2005). As in the United States, among all the *Salmonella* isolates, *S*. Enteritidis and *S*. Typhimurium had been the most prevalent serotypes in Hong Kong, followed by *S*. Derby during 1999–2004. On the other hand, fourteen local cases of a typhoid fever outbreak due to the consumption of contaminated food were also reported to occur between late 2005 and early 2006 in Yuen Long district (Ma, 2006). In Hong Kong, a laboratory-based *Salmonella* Surveillance Programme, the reports of foodborne disease outbreaks due to *Salmonella*, and the Food Surveillance Programme provide the epidemiological data on salmonellosis.

The Food Surveillance Programme in Hong Kong is implemented by the Food and Environmental Hygiene Department (FEHD, 2010). Ready-to-eat foods available in local retail outlets are under surveillance for the presence of *Salmonella* organisms and other foodborne pathogens. Laboratory detection, isolation and identification of *Salmonella* from food samples are provided by the Public Health Laboratory Services Branch (PHLSB) of the Department of Health. PHLSB, therefore, plays a key role in the contribution of *Salmonella* epidemiological data. According to the Microbiological Guidelines for Ready-to-eat Food, the presence of *Salmonella* in 25 g of a sample examined

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is regarded as potentially hazardous to consumers and is unacceptable for consumption (FEHD, 2007). In 2004, PHLSB isolated *Salmonella* from eight (32%) of 25 raw meat or meat products samples, and one (7%) of 14 raw fish samples examined in the Food Surveillance Programme. Out of the more than 6000 cooked food samples analyzed, seven (0.1%) were *Salmonella*-positive (Lam, 2005). In 2010, about 5300 ready-to-eat food samples were scheduled for *Salmonella* detection by PHLSB in the Food Surveillance Programme. Traditionally, labor-intensive and time-consuming conventional standard culture methods, which usually require at least 3 working days to generate the results, have always been employed by many laboratories to detect *Salmonella* in foods (Cheung, Kwok, & Kam, 2007). In food surveillance, regarding the enormous volume of sample received for *Salmonella* detection, it is obvious that, when compared to the culture method, rapid methods with shorter turnaround time (1–2 days) for *Salmonella* detection would significantly reduce the resources required in routine laboratory operations, and enhance the overall efficiency and productivity of public health laboratory services.

2. Detection of *Salmonella* in food samples by conventional and real-time PCR methods

The long turnaround time of existing conventional standard culture methods for the detection of pathogens from foods often leads to low productivity and analytical flexibility of testing laboratories. These standard culture methods, for example, the ISO 6579:2002 and the HPA F13 of Health Protection Agency, require three working days to produce a negative result and five working days for a confirmed positive result (HPA, 2008; ISO, 2002). During 1990s, advance in nucleic acid-based technologies had led to development of the more rapid and specific PCR methods for the identification or characterization of *Salmonella* pure isolates from different samples (Abouzeed, Hariharan, Poppe, & Kibenge, 2000; Soumet et al., 1999). PCR methods for *Salmonella* detection employ primer pairs specific or complementary only to DNA sequences of *Salmonella* spp. genes. During PCR amplification, the *Salmonella* DNA sequence flanked by a pair of the species-specific primers is amplified with DNA polymerase. After 30 to 40 cycles of PCR amplification, the resulting PCR products, which are of the same size, could be easily detected by agarose gel electrophoresis. DNA sequences of other species, if present in the sample, would not be amplified as the primers could not anneal to the sequences of non-*Salmonella* DNA under the selected PCR conditions. Some early conventional gel-based PCR methods had been developed based on the sequence of different *Salmonella* genes, such as, the major fimbrial subunit encoding gene (*fimA*), a *Salmonella* invasion gene (*invA*), and a virulence gene (*spvC*) (Cohen, Mechanda, & Lin, 1996; Swamy, Barnhart, Lee, & Dreesen, 1996). Recently, *Salmonella*-specific primers, Itsf and Itsr, for the internal transcribed spacer region of the 16S–23S rRNA gene had also been designed for use in a multiplex PCR that also included the detection of several other common pathogens such as *Escherichia coli* O157:H7, *Staphylococcus aureus*, and *Listeria monocytogenes* in food samples (Park, Lee, & Kim, 2006). In this study, four sets of different primers specific to the four pathogens, respectively, were used for simultaneous detection of the bacteria in one PCR tube. Amplification of the DNAs resulted in products of four different sizes, which could be readily separated and visualized by agarose gel electrophoresis. Multiplex PCR, therefore, allows a more rapid and cost-effective detection of more than one pathogen that may be present in a single food sample. At around the same time, different enrichment methods were evaluated and used in conjunction with PCR to enhance the detection of pathogens in environmental and food samples (Brasher, DePaola, Jones, & Bej, 1998; Carli, Unal, Caner, & Eyigor, 2001; Li & Mustapha, 2002; Liu et al., 2002).

Combining traditional enrichment methods with the conventional PCR methods could shorten the turnaround time for *Salmonella* detection to one or two days. However, conventional PCR methods require laboratory space, time and labor for post-PCR gel electrophoresis and analysis. To reduce the demand for laboratory resources and the risk of contamination by PCR amplicons, automated fluorogenic PCR assays that eliminate the need for gel electrophoresis were developed in the late 1990s and early 2000s for *Salmonella* detection in foods (Bailey & Cosby, 2003). One example was the commercial automated BAX PCR system (Dupont Qualicon, USA) that combined all the essential PCR reagents, such as, DNA polymerase, nucleotides, primers, internal positive control, and fluorescent dye (SybrGreen), into a single small lyophilized pellet in each PCR tube, to which the sample cultures could be added. At the end of the PCR amplification, the same instrument could perform both PCR product detection and a melting curve analysis. Amplification of the internal positive control could be used for determining whether a negative result was a failed reaction owing to PCR inhibition. The melting curve profiles showing *Salmonella*-specific temperature peaks could also eliminate the possibility of getting false positive results. Including the overnight pre-enrichment step, the turnaround time of *Salmonella* detection could be reduced to about 24 h. The system significantly simplified and streamlined the process of *Salmonella* detection in food samples by reducing the time and labor required for reagent transfers and gel electrophoresis, and eliminated the potential for technical error and cross-contamination. The use of the BAX system for the detection of *Salmonella* in food was validated and approved by authorities, e.g., the Association of Official Analytical Chemist International (AOAC), and Health Canada, as standard methods (Silbernagel, Jechorek, Carver, Barbour, & Mrozinski, 2003). Reports on the comparison of performance between the BAX system and other analytical methods, for example, the immunoassay-based and the standard reference culture methods, for *Salmonella* detection are also available. The BAX system was found to have very low false-positive and false-negative rate and generated results comparable with those of the standard culture methods, ISO 6579, HPA F13, and MFHPB-20 (Cheung et al., 2007; D'Aoust & Purvis, 1998; D'Aoust et al., 2007; Tomazelli et al., 2008). Furthermore, for raw samples with higher background flora inoculated with *Salmonella* Typhi, positive results could be obtained with the BAX system, whereas false negatives were generated by the immunoassay-based Tecra Unique™ Salmonella test and the standard culture method (Cheung et al., 2007).

To identify the risk and factors that influence food safety and to estimate the burden of disease that a pathogen can cause, use of analytical methods that can produce quantitative data are necessary (Malorny, Löfström, Wagner, Krämer, & Hoorfar, 2008). With the advance of automated fluorogenic PCR assays, real-time monitoring of PCR amplicons had become feasible and led to the possibility of quantification of bacterial DNA concentration initially present in the test sample. Soon after its launching, the BAX system was upgraded to a real-time PCR platform — the BAX system Q7, which allowed a more specific probe-based detection and pathogen quantification (Koyuncu, Andersson, & Häggblom, 2010). Other real-time PCR platforms or kits commonly employed for the detection and quantification of salmonellae from foods or environmental samples include the TaqMan *Salmonella enterica* detection kit (Applied Biosystems, USA), the molecular beacon-based iQ-Check *Salmonella* II kit (Bio-Rad, USA), and the LightCycler *Salmonella* Detection Kit (Roche Diagnostics GmbH, Germany) (Cheung, Chan, Wong, Cheung, & Kam, 2004; Eyigor, Temelli, & Carli, 2010; Koyuncu et al., 2010; Perelle et al., 2004).

In TaqMan PCR assays, the amplification mixture contained a TaqMan probe, which was an oligonucleotide with a reporter dye at the 5′ end and a quencher dye at the 3′ end. Fluorescence of the reporter dye was suppressed by the quencher when the probe was intact and hybridized to the target sequence of the amplicons during amplification. In subsequent PCR cycles, the DNA polymerase cleaved the TaqMan probe and released the reporter dye, resulting in an increase of the

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