Review Article
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Rapid Molecular Detection of Fowl Typhoid and Avian Paratyphoid in Poultry: A Review

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Abstract

Fowl typhoid (FT) and Avian paratyphoid (AP) are diseases caused by non-motile bacterium *Salmonella enterica* subsp. *enterica* biovar Gallinarum and motile non-typhoidal serovars (NTS) of *Salmonella* other thanGallinarum and Pullorum. The NTS serovars are non-host specific, associated with subclinical infection in poultry and foodborne diseases in humans. Eradication of FT in commercial poultry in some parts of the world was achieved through improved surveillance and culling. However, FT is an endemic disease of poultry in India with occasional outbreaks. Avian paratyphoid (AP) is an important bacterial disease of chickens worldwide. It is one of several types of diseases caused by infection with *Salmonella* spp. Chicks from hatcheries are most at risk. Infection may occur in birds of all ages. It can also cause huge morbidity and mortality loss. Accurate precision diagnosis of the pathogen is a prerequisite for formulating effective control measures for these infections. The conventional methods of detection of causative pathogens are laborious, less sensitive and time-consuming. Control measures by treatment and vaccination can reduce morbidity and mortality from salmonellosis in birds but do not eradicate infection. Improvement in detection methodology and adoption of rapid DNA-based detection techniques of the major *Salmonella* serovars is of utmost necessity. Rapid Salmonella detection methods of important *Salmonella* serovars were reviewed.

Keywords: *Salmonella*, Fowl typhoid, Paratyphoid**,**Non-typhoidal serovar

Introduction:

Salmonellosis in poultry can present three disease conditions-fowl typhoid, pullorum disease and avian paratyphoid. Fowl typhoid (FT) caused by Salmonella *enteric* subsp. *enteric* biovar Gallinarum is of major economic significance in many countries of Asia, Africa, Central and South America (Barrow and Freitas Neto, 2011). Pullorum disease by Salmonella *enterica* biovar Pullorum was last detected in India during 2007-08 (Kumar et al., 2012). In contrast,Salmonella*enterica* biovarGallinarum remains a major pathogen in many developing poultry industries including Asia and South America (Shivaprasad, 2000).Non-typhoidal serovars (NTS) of *Salmonella* other thanGallinarum and Pullorum are usually established in subclinical infection. However, eradication of serovar Gallinarum from domestic fowl in the United States and England during the mid-20th century opened up the ecological niche for serovar Enteritidis. Since serovar Enteritidis is usually asymptomatic in chickens, contaminated eggs have entered the human food supply and cause outbreaks of Enteritidis-associated salmonellosis(Matthews et al., 2015).*S.* Gallinarum (43.7%), being the most frequent, followed by *S.* Enteritidis (30.6%) and *S.* Typhimurium (21.9%) were the most prevalent serovars in poultry samples from January 2011 to October 2016 received at National Salmonella and Escherichia Centre (NSEC), Central Research Institute, Kasauli, India (Kumar et al., 2019).The most common serotypes associated with human illness are *Salmonella* Typhimurium and *S.* Enteritidis in the United States and European countries (Lee et al., 2015). This paper is to review the progress in rapid methods for efficient and reliable Salmonella detection methods usingemerging technologies-conventional culture methods, immunology-based assays, nucleic acid-based assays, and biosensors.

Fowl typhoid and avian paratyphoid in India:

Seropositivity (14.69%) was observed in commercial breeder flocks by rapid serum agglutination test with crystal violet stained antigen in seven states of India (Baksi et al., 2017). However, serological cross-reaction with Salmonella serovar Pullorum and Enteritidis (9, 12:gm) limits its application in clinical diagnosis (OIE, 2018). Rajagopal and Mini (2013)reported an outbreak of FT inthree different poultry farms in Kerala, India.A similar outbreak was reported in West Bengal in backyard poultry (Dey et al., 2016). The prevalence of *Salmonella* inchicken broilers in the Tarai region of Uttarakhand in India was documented in correspondence byKumar et al.(2014).This work involved the isolation of *Salmonella*from a total of 343 faecal samples of poultry and pigs, and from 100 tissue samples of broilers collected between January 2011 and July 2012. Thetotal prevalence of *Salmonella* in poultry was 12.28%(8.4% of cloacal samples and 22.0% of tissuesamples). The detected poultry serovars, in decreasingorder of

frequency, were *S.* Typhimurium, *S.*Enteritidis, and *S.* Gallinarum. In a study by Kumariet al.(2013), 23 *Salmonella* isolateswere reported of which 19 samples were identified as *S.* Gallinarum (9, 12) and 4 samples as *Salmonella* Enteritidis(9, 12: gm) from 134 dead poultry birds collected from23 different farms of Haryana.Kumar et al.(2012) identified *Salmonella* Gallinarum (53), *Salmonella* Pullorum (16), *Salmonella* Enteritidis (13) and *Salmonella* Typhimurium (06) in Hisar and adjoiningdistricts, viz. Jind, Bhiwani, Sirsa, Fatehabad and Rohtak regions of Haryana state of India in the year 2007–08 in from dead broiler birds.

PiruthivirajKumar et al. (2015) confirmed seven isolates as *Salmonella* Gallinarumand three isolates as *Salmonella*Pullorumfrom samples of poultry originating in several Indian states Andhra Pradesh, Telangana, Tamil Nadu,Karnataka, Maharashtra, Haryana,Uttar Pradesh.In a study byArora et al. (2015), 253 *Salmonella*isolates were recovered from disease outbreaks in broiler chickens from January 2011 to December 2013 indifferent parts of Haryanaand these isolateswere grouped into 3 groups namely *Salmonella* Gallinarum (183), *Salmonella* Enteritidis (41) and *Salmonella* Typhimurium (29).Samanta et al.(2014) identified 22 *Salmonella* isolates (6.1%) from cloacal swabs of 6 birds (15%, n = 40), from 4 feed samples (10%, n = 40), 8 drinking water samples (20%, n = 40), and 4 eggs (10%, n = 40) in birds reared in backyard method. The isolates belonged to serovars *Salmonella* Enteritidis (6) and *Salmonella* Typhimurium (2).Besides fowl, the disease FT was also reported in ducks in Thrissur, Kerala (Chacko et al., 2017).

Salmonella detection methods:

a) Conventional Culture method: Traditional detection methods include non-selective and selective enrichment,biochemical characterization, and serological identification (Table 1). Suspected samples of faeces, heart blood, liver,bile etc. are to be collected aseptically, processed withpre-enrichment 2% buffered peptone water or selective enrichment with Rappaport–Vassiliadis (RV) broth, selenite broth or tetrathionate broth at 42°C for 48 hours.Commonly used selective solid media are MacConkey's lactose agar (MLA), Xylose Lysine Deoxycholate agar (XLD),salmonella shigella agar (SSA), and brilliant green agar (BGA).Cultural characteristics on solid media wereused for the initial identification of *Salmonella*.Presumptive *Salmonella* colonies are tested in triple sugar iron agar (TSI) forglucose fermentation and lysine iron agar (LIA) forlysine decarboxylase reactions followed by a urease test for screening *Salmonella* spp.These colonies are further subjected to biochemicaland serologicalconfirmation (Rajagopal and Mini, 2013; Dey et al., 2016).

Chromogenic (BBL CHROM/HiChrome^{TM} agar Salmonella) and fluorogenic mediahave improved conventional culture methods with faster detection and identification.Kits for rapid biochemical characterization of *Salmonella* are commercially available, including API 20E (bioMerieux, France), Hi Salmonella identification kit (HiMedia,India).

Table 1: Conventional detection methods of *Salmonella* serovars used by different researchers

Source	Selective enrichment	Isolation media	Serotype	Positivity (%)	Reference
Faecal samples	RV Broth	Xylose-Lysine-Tergitol-4 (XLT4) agar	*Salmonella* Arizona (35: z24: z23: -)	6/585 (1.02%)	Kar et al., 2020
Spleen, liver, heart, blood	None	MLA, followed by BGA	SG (1,9,12:--)	Farm outbreak investigation	Dey et al., 2016; Pal et al., 2017
Spleen,liver,gall bladder, heart blood	RV broth	MLA, BGA	ND	Farm outbreak investigation	Rajagopal and Mini, 2013
Cloacal swab	Selenite broth, RV Broth	BGA	*S*. Enteritidis (9,12:g, m,-) *S*. Typhimurium (4,12:i:1,2)	6/360 (6.1%)	Samanta et al., 2014
Heart blood,organs	RV Broth	MLA, BGA, SSA, XLD agar	SG (1,9,12:- -), *S.* Enteritidis (9,12:g, m,-)	23/134 (17.16%)	Kumari et al., 2013
Bile,Heart blood,liver	None	MLA, BGA	SG (9,12:-:-), SP (9,12:-:-),		Kumar et al., 2012

spleen,ova			*S*.Enteritidis (9,12:g,m:-), *S*.Typhimurium (4,12:1:1,2)		
Liver,intestine,spleen,egg	Selenite cystine broth, Tetrathionatebrilliant green broth	MLA, BGA, Hektoen enteric agar	*S*.Heidelberg (1,4,5,12:r:1,2) *S*.Typhimurium (4,12:i:1,2), *S*.Ayinde (1.4,12,27:dz6), *S*.Essen (4.12:gm:-), *S.* Kastrup (6,7:e,n,z15:1,6)	7/260 (2.7%)	Menghistu et al., 2011
Liver, lungs, spleen, heart, intestines and Bursa	Tetrathionate broth (TB)	XLD, (BGA	SG (9,12:-:-), untyped	42/182 (23.08%)	Kashani et al., 2021

* ND = not done

b) Serotyping: Serotyping of the biochemically confirmed isolates is performed from facilities at the National *Escherichia* and *Salmonella* Centre, Kasauli, Himachal Pradesh, India (Pal et al., 2019) or National *Salmonella* Centre at Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India (Kar et al., 2020) by Kauffmann –White scheme by slide agglutination with O- and H-antigen specific sera.

c) Serological tests: It employ specific mono- or polyclonal antibodies to bind with somatic, lipopolysaccharide (LPS) or flagella antigens for detection of *Salmonella* spp. in a variety of sample matrices. Many assays including enzyme-linked immunosorbent assay (ELISA), latex agglutination tests, immunodiffusion, and immunochromatography are available commercially. Several commercial validatory bodies (like the Association of Official Analytical Chemists, AOAC, USA or similar organizations) certified tests were designed for rapid detection of Salmonella in kit format with ELISA systems (IDEXX SE Ab X2 test for S. Enteritidis), latex agglutination (Oxoid Salmonella test kit), immunodiffusion for motile Salmonellae (Biocontrol/Merck 1-2 Test® for *Salmonella*).

Bautista et al. (2002) detected 19 of 22 strains of *Salmonella* spp. in an immune-chromatography-strip based diagnostic kit for *Salmonella* but failed to detect *S. worthington, S.* Choleraesuis var. *kunzendorf,* and *S. johannesburg*. Immunochromatography-based tests may be based on dipstick or lateral flow assay format. Animmunochromatographic assay was developed for the simultaneous detection of *S.* Typhimurium and Enteritidis in a single chip by Moongkarndi et al. (2011). After a 6–24-hr enrichment step, contamination of *S*. Typhimurium and *S*. Enteritidis at 1cfu/ml or greater can be detected. The commercially available *Salmonella* lateral flow test is simple, rapid and reliable.

d) Nucleic acid-based assays: The conventional methods of biochemical identification are laborious and time-consuming. Therefore, rapid DNA-based detection techniques of the *Salmonella* serovars Gallinarum and Pullorum were used (Pal et al., 2019).

i) Conventional PCR: For confirmation of *Salmonella* serovars, several studies have developed PCR assays to test their ability to detect these *Salmonella* serotypes (Table 2). Allele-specific PCR assay based on *rfbS* (Shah et al., 2005), polymorphic areas of *glg*C and *spe*C genes (Kang et al*.,* 2011; 2012), fimbrial operon gene *bcfD* (Zhuang et al.,2014), flagellar biosynthesis gene *flhB* (Xiong et al.,2016), fimbrial operon gene *sefA* (Gong et al.,2016), flagellar biosynthesis gene *flhB* (Xiong et al., 2017), SPUL 2693 (Xu et al.,2018), *stn*, I137_08605 and *rat*A genes in multiplex PCR (Xiong et al*.,* 2018) were used by various workers.To increase the accuracy and to decrease the time of analysis, some multiplex PCR methods (Batista et al., 2016) were developed allowing the simultaneous identification of multiple pathogens in one sample within a single reaction (Oliveira et al., 2002;Cortez et al., 2006).

ii) Real-time PCR: Real-time PCR or quantitative PCR facilitates direct detection of PCR products in less than half the time of conventional PCR, with no requirement of post-processing steps.Cheng et al. (2008) developeda real-time PCR method with custom-designed primers and a TaqMan probe to detect the presence of a 262-bp fragment of the *Salmonella*-specific *invA* gene.Thequantitative PCR developed by Silva et al. (2011) includes the detection of *Salmonella* spp. and *S.*

Enteritidis, but it was not able to detect *S*. Typhimurium. Rubio et al. (2017) developed the multiplex qPCR for detecting *S*. Gallinarum and *S*. Pullorum. Heymans (2018) developed and evaluated multiplex qPCR targeting the *invA*, the STM4200, and the SEN1392 genes for the simultaneous detection of *Salmonella* spp., *S. enterica* serovar Typhimurium and *S. enterica* serovar Enteritidis in various (food) matrices. The *invA* gene was expected to be detected in all *Salmonella* strains, whereas the STM4200 and SEN1392 genes were expected to detect *S*. Typhimurium and *S*. Enteritidis strains, respectively. However, one limitation of this qPCR was serovars S. Derby (n = 2), *S.* Goldcoast (n = 1) and *S*. Rissen (n = 5) were also amplified by the STM4200 primer set. The latter two serovars are occasionally identified in poultry meat.Commercial kits based on conventional PCR and real-time PCR are successfully used for routine Salmonella screening in poultry feed, eggs, raw meat etc.

Table 2: Conventional PCR assays for molecular detection of *Salmonella* serovars

Gene	Primers	Oligonucleotides (5'-3')	Amplification product (bp)	Positivity in Salmonella serovar	Reference
invA	forward S139 reverse S141	GTGAAATTATCGCCACGTTCGG GCAA TCATCGCACCGTCAAAGGAACC	284	All serovars	Rahn et al., 1992
glgC	SG-L SG-R	GATCTGCTGCCAGCT CAA GCGCCCTTTTCAAAACATA	174	SG	Kang et al., 2011
speC	SGP-L SGP-R	CGGTGTACTGCCCGCTAT CTGGGCATTGACGCA AA	252	SP,SG	Kang et al., 2011
9R22C9	9R-L 9R-R	CTTTACGGGCAAACCACAGT TGCTGCTCTTTTTCCATCTCA	119	SG strain 9R	Kangetal., 2012
SPUL 2693	Forward Reverse	CGGGGTACCATGGATAAGCGTC ATGAGA CCGGAATTCTCATTTCTGTCCCT CCTCAATGGCT	2160	SG	Xu et al., 2018
fliC	Fli15 Typ04	CGGTGTTGCCCAGGTTGGTAAT ACTGGTAAAGATGGCT	620	Typhimurium	Oliveira et al., 2002
sefA	A058 A01	GATACTGCTGAACGTAGAAGG GCGTAAATCAGCATCTGCAGTA GC	488	SP/SG,*S.*Enteritidis, *S.*Berta	Oliveira et al., 2002

SG – *Salmonella* Gallinarum, SP – *Salmonella* Pullorum

Table 3: Comparative analysis of *Salmonella* detection methods

Method	Reaction time	Sample	Commercial kits	Remarks
Culture	3 step methods-Pre-enrichment, selective enrichment,plating on selective media,then bio-typing, total of 5-6 days	Faeces,organs	Enrichment broth,selective agar	Confirmatory,low-sensitivity
ELISA	8 hrs	Serum, Enrichment broth culture	IDEXX SE Ab X2 test for SE(gm-flagellin based),*Salmonella* Antigen ELISA Kit,Creative Diagnostics	Screening test, specificity issues
Latex agglutination tests	Pre-enrichment, selective enrichment step	Enrichment broth culture	Oxoid Salmonella test kit	Presumptive Screening test, non-motile strains not

				detected
Immunodiffusion	Pre-enrichment, selective enrichment step, 2 days	Enrichment broth culture	Merck 1-2 Test® for Salmonella	Motility based test
Lateral flow assay	Pre-enrichment, selective enrichment step, 20 mins test time	Boiled enrichment broth culture	Merck Singlepath®	Presumptive,Point-of-care tests
Conventional PCR	Pre-enrichment, selective enrichment step, DNA extraction,2 days	DNA from Broth culture	Hi-PCR® Salmonella Semi-Q PCR Kit,HiMedia	Highly sensitive,qualitative
Realtime PCR	Pre-enrichment step of a few hours, selective enrichment in RV broth(optional), followed by DNA extraction(30 mins) and PCR amplification(70 mins)-total 24-48 hrs.	DNA from Broth culture	IDEXX Real PCR™*Salmonella* spp.; MicroSEQ® *Salmonella* spp. Detection Kit,Applied Biosystem, TaqMan® *Salmonella enterica* Detection Kit, Applied Biosystem; iQ-Check Salmonella, Bio-Rad	Highlysensitive, detect 1−3 colony forming units (cfu) per25 grams of sample

iii) Loop-mediated isothermal amplification (LAMP) assay: Several assays have been successfully established to detect *Salmonella*,however, most of these assays are unable to determine *Salmonella* serovars such as LAMP assay based on *InvA* (Yang et al.,2013), LAMP assay based on *bcfD* (Zhuang et al.,2014).

e) Biosensors:A number of biosensors that use antibodies as a recognition element for *Salmonella* sp. were developed, but more and more devices are now also reported based on nucleic acids-based DNA aptamers by several researchers as reviewed by Paniel and Noguer (2019).

Conclusion:

The traditional methods for Salmonella detection are based on cultural, serological, and biochemical properties using selectivemedia.However, rapid methods for *Salmonella* detection have become increasingly important; many are approved bycountries with advanced rearing systemsand are considered desirable as a future approach.Sample processing techniques in the two steps ofpre-enrichment and enrichment also appeared to affect the sensitivity of *Salmonella* detection in low-concentration, viable but not culturable (VBNC) samples,particularly with the presence of disinfectants. More reliable and efficient new assays with precision are likely to replace the existing conventional methods.

Conflict of interest:

The authors declare that no conflict of interest exists.

Author's contribution:

SD prepared the manuscript and KB, SNJ, and IS critically reviewed the draft and approved the same.

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