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Avian salmonellosis is an economically important bacterial disease ailment inflicting serious difficulty to the growth of poultry industry (Rajagopal & Mini, 2013). It is caused by *Salmonella*, a member of the family Enterobacteriaceae, a Gram-negative facultative intracellular pathogen that is able to cause different disease syndromes in a broad range of hosts. It constitutes two species *Salmonella bongori* (*S. bongori*) and *Salmonella enterica* (*S. enterica*) (Reeves, et al., 1989). Although there are greater than 2,600 *Salmonella* serovars (Rainier, et al.

, 2013), relatively few serovars cause infection in most animals (Saeki, et al., 2013; Zhu et al., 2015). Hence, *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.*

Enteritidis), serovar Gallinarum biovars Gallinarum and pullorum and *S. Typhimurium* are generally isolated from poultry. Among these serovars *Salmonella enterica* serovar Gallinarum (*S. Gallinarum*) consists of two biovars, Gallinarum and Pullorum that cause fowl typhoid and pullorum disease in adult and young, respectively (Hossain, et al.

, 2006). Salmonellosis is one of the major challenges of poultry farming that hinder its growth and development. Therefore, it is necessary to screen virulence genes of *Salmonella* at the molecular level for development of rapid and fast disease diagnosing techniques. Poultry industry is uplifting as a profitable sector in Nepal. Since 1974, the remarkable improvement of commercial poultry in Nepal has had results in tremendous development of this sector over the recent period of time.

Since, 1974 the remarkable improvement of commercial poultry in Nepal had been started (FAO, 2012). In our country, 50 - 55 % of poultry birds are commercially managed. And, poultry industry contributes about 3.5% in GDP.

Additionally, provides an employment opportunity creating an income along with improving the nutritional level of the country. This also provides fulltime employment to about nine thousand and partial employment to about ninety nine thousand people (CBS, 2015). Usually bacterial culture methods are used to identify *Salmonella* and require at least 3-11 days. The standard protocol for isolating *Salmonella* species includes non-selective pre-enrichment followed by selective enrichment and plating on selective and differential media. These procedures are time consuming and labour intensive (Menghistu, Rathore, Dhama, & Agarwal, 2011). In recent years, particularly in developed countries, several methods such as Enzyme Linked Immunosorbent Assay (ELISA), latex agglutination, immunodiffusion, Polymerase chain reaction (PCR), and real time PCR (RT PCR) had been introduced.

In comparison to other methods, polymerase chain reaction (PCR) technology and real time PCR (RT PCR) have allowed the specific amplification of particular target portions of DNA, which can be used for the diagnosis of pathogens of veterinary importance. PCR tests have demonstrated their utility as screening tools for *Salmonella* broiler and layer samples to reduce workloads and shorten time for *Salmonella* evaluation (Bautista et al., 2011).

S.

enterica consist of several virulence genes which encode products that help the organisms to express its virulence in the host. Among the virulence genes, 16srna encode for the confirmation of Salmonella at genus level whereas invA for adhesion and invasion of the pathogen in the host system, spv for systemic disease state in the host cells, speff for ornithine decarboxylase of Salmonella gallinarum, sdi and hilA encode for protein belonging to the transcriptional regulators, fim H for fimbriae like protein, avrA to modulate host cellular functions, agf for diagnosis of Salmonella arrayed on hydrophobic grid membrane filters, sivh gene for outer membrane protein and Stn for enterotoxin genes of host pathogenic processes. While spvA, spvB, and SpvC virulence gene codes for plasmid of pathogenic organism. Thus, precise and systematic method should be adopted for screening virulence genes from S.

enterica isolates originated from the infected samples (Murugkar et al., 2003). Similarly, Polymerase chain reaction (PCR) had been discovered as a high-throughput approach with a high degree of sensitivity and specificity for pathogen detection. Therefore, this research was undertaken in the clinical cases of broilers to screen the virulence gene responsible for salmonellosis along with the antibiotic resistance pattern of these samples. Salmonella isolates will be screened based on virulence gene profiling, focusing on virulence determinants associated with SPIs, plasmids, toxins, fimbriae, and flagella that were positive for the infection by Salmonella as an intracellular pathogen.