Chapter 1

INTRODUCTION

Bacterial infection is a leading cause of mortality worldwide (O’Connell et al., 2005) and among the bacterial infections, food borne pathogens are the principal cause of illness and death in less developed countries, killing approximately 1.8 million people annually (Faruque, 2012).

The Members of the Food and Agriculture Organization of the United Nations (FAO) and of the World Health Organization (WHO) have expressed concern regarding the level of safety of food both at the national and the international levels.

Salmonella is prominent among the bacterial pathogens. In recent years problems related to Salmonella have increased significantly, both in terms of incidence and severity of the cases of human salmonellosis. Non typhoidal Salmonella ranks second (11% of the total diseases) among the causative agents of domestically acquired food borne illness. It ranks first in contributing to hospitalization (35%) and cause of death (28%) resulting from domestically acquired food borne illnesses. (http://www.cdc.gov/foodborneburden/2011-foodborne-estimates.html).

Each year, approximately 40,000 Salmonella infections are culture-confirmed, serotyped and reported to the United States Centers for Disease Control and Prevention (CDC). Of the total salmonellosis cases, an estimated 96% are caused by contaminated food (Mead et al., 1999). Illnesses caused by the majority
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of Salmonella serotypes range from mild to severe gastroenteritis, and in some patients, bacteremia, septicemia and a variety of associated long-term conditions. Extensive varieties of food have been implicated in food borne illness attributable to Salmonella enterica. Food of animal origin, especially poultry, poultry products and raw eggs, are often implicated in sporadic cases and outbreaks of human salmonellosis (Bryan and Doyle, 1995; Humphrey, 2000). Poultry is widely acknowledged as a reservoir of Salmonella infections in human due to the ability of Salmonella to proliferate in the gastrointestinal tract of chicken (Poppe, 2000) and subsequently survive on commercially processed broiler carcases.

The beginning of the 1980s, saw the emergence of Salmonella strains resistant to a range of antimicrobials, including first-choice agents for the treatment in human, which became a serious public health problem. Multi-drug resistance to "critically important antimicrobials" are further compounding the problems (Ribot et al., 2002; Faldynova et al., 2003). Antimicrobial resistance or the ability of microorganisms to withstand treatment with drugs, they were once susceptible to, is a significant and multifaceted public health problem. In addition, the scarcity of new antimicrobial agents and the dearth of new agents in the drug development pipeline limit treatment options, particularly for patients with infections caused by multidrug-resistant (MDR) organisms. The societal and financial cost of treating antimicrobial-resistant infections place a significant human and economic burden on society, as individuals infected with drug-resistant organisms are more likely to remain in the hospital for a longer period of time and to have a poor prognosis (Lee et al., 1994).

Antimicrobial agents are currently used for three main purposes: (1) to treat infections in human, animals and plants; (2) prophylactically in human, animals, and plants; and (3) subtherapeutically in food animal as growth promoters and for feed conversion (Angulo et al., 2000). When antibiotic use became the norm in both
human and animal medicine, selection pressure increased the bacterial advantage of maintaining and developing new resistance genes that could be shared among bacterial populations (Matthew et al., 2007). The first suggestion that antibiotic use in livestock led to antibiotic-resistant bacteria was in 1951. Starr and Reynolds (1951) reported streptomycin resistance in generic intestinal bacteria from turkeys that were fed with antibiotic.

MDR strains of *Salmonella* are encountered more frequently now and the rates of multidrug-resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug-resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug-resistant genes even when antimicrobial drugs are no longer used, a condition wherein other resistant strains would typically lose their resistance.

The use of antibiotics not only selects for antimicrobial-resistant bacteria, but may also increase the likelihood of disease transmission. In 2006, Bauer-Garland and group researched the transmission of MDR *Salmonella* Typhimurium in broiler chicken under selective-pressure. MDR *S*. Typhimurium strain had significantly increased transmission when chicken were treated with tetracycline, demonstrating that antimicrobial use influences transmission of antimicrobial-resistant pathogens in poultry (Bauer-Garland et al., 2006). *Salmonella* Enteritidis is one of the most common serotypes of *Salmonella* causing human illness that is associated with consumption of egg-containing products and chicken (Voetsch et al., 2009). Since 1996, the number of nalidixic acid (a drug closely related to ciprofloxacin the most commonly prescribed antibiotic for *Salmonella* infections) resistant *S*. Enteritidis, submitted to the National Antimicrobial Resistance Monitoring System (NARMS) is increasing. Of these resistant isolates, 90% also showed decreased susceptibility to ciprofloxacin (2009).
Investigations for new alternative anti-microbials, effective against bacterial pathogens including *Salmonella*, have become increasingly relevant for both human and veterinary applications, among which bacteriophage are a potential candidate (Boyle *et al.*, 2007).

**OBJECTIVES OF THE STUDY**

Bacteriophages (phages) are obligate intracellular parasites that multiply inside bacteria and in doing so make use of the host biosynthetic machinery. In fact, right from the discovery of phages, their potential as a therapeutic agent was introduced by Félix d’Herelle, a French-Canadian microbiologist. But research in this direction was abandoned by the Western scientific community with the discovery of antibiotics. But now, the emergence of antibiotic resistance is one of the motivating factors, pushing the scientists to go back to this long forgotten cure (Matsuzaki *et al.*, 2005). Thus, after several years of abandonment, the use of phages for killing bacteria has drawn recent attention and reappraisal. This has led to a vast phage research, in varied fields, with impressive outcomes (Sulakvelidze *et al.*, 2001). Recent review also hints the potential of phages as alternatives to antibiotics (Thiel, 2004; Greer, 2005; Skurnik and Strauch, 2006).

The first and foremost advantage of phage as an alternative biocontrol is that they are active against bacteria that have become resistant to antibiotics (Alisky *et al.*, 1998; Carlton, 1999; Górski *et al.*, 2007; Skurnik *et al.*, 2007; Kutter *et al.*, 2010) and second is their specificity, thereby preventing secondary bacterial infection which is commonly observed in antibiotic therapy (Kutter and Sulakvelidze, 2005).
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They are relatively harmless supplemented by the fact that we constantly consume them every day (Skurnik et al., 2007; Abed et al., 2010). When well-purified phages are used, not many side effects have been described, for all types of administration (Alisky et al., 1998; Carlton, 1999; Górski et al., 2007; Kutateladze and Adamia, 2010).

Unlike antibiotics, phages are an ‘intelligent’ drug. They multiply at the site of the infection until there are no more bacteria (Inal, 2003). In addition, bacteria that have become resistant to a certain type of phage continue to be destroyed by other types of phages. They are easy to isolate as they are found throughout nature (Skurnik et al., 2007). Thus the search to find new phages when bacteria become resistant to them is effortless as evolution drives the rapid emergence of new phages that can destroy bacteria resistant to existing phages as well as the antibiotic resistant forms (Sulakvelidze et al., 2001). This means that there will be an ‘inexhaustible’ supply of bacteriophages available to exploit. Finally phages can be genetically modified in order to make up for some of their disadvantages as evidenced by the several works (Geier et al., 1973; Cao et al., 2000; Hagens et al., 2004; Fischetti et al., 2006; Yacoby et al., 2007; Lu and Collins, 2009). In addition, individual components of phages (e.g. lysins) can also be used as antimicrobial substances (Borysowski et al., 2006).

The ability of Salmonella phages to effectively reduce the bacterial load in food products have given promising results (Leverentz et al., 2001; Goode et al., 2003; Fiorentin et al., 2004, 2005; Pao et al., 2004; Jianxiong et al., 2009; Ye et al., 2010).

Thus the aim of the present study was to isolate and characterize Salmonella and Salmonella specific lytic bacteriophages from the intestinal content of broiler chicken where they are most likely to be found (Goyal et al., 1987; Silja et al.,...
2010) and to determine the potential application of employing both *in vitro* and *in vivo* assays.

Specific objectives included the following:

1. To isolate *Salmonella* specific lytic phages
2. To characterize the isolated phages
3. To investigate the potential of phages as biocontrol agents