CHAPTER I
INTRODUCTION

India has vast livestock resources and it plays an important role in Indian economy. In addition it provides livelihood to two-third of rural Indian population. It also secures employment to about 8.8% of the population in India. Livestock sector contributes 4.11% to total GDP and 25.6% of total Agriculture GDP of India (Dash, 2017). But, infectious diseases are significant threat to livestock.

Bacterial infections cause considerable economic losses through decreased production, costs in diagnosis and treatment. Globally, use of antibiotics raised from 2 tonnes/year in 1974 to 45 tonnes/year in 2014. In India, due to antibiotic overuse, abuse or misuse (misdiagnosis) 55% of patients arriving from emergency treatments had antimicrobials in their urine and the figures in animals are even worse and alarming (Van Boeckel et al., 2015).

Though effective antibiotics are routinely used in the treatment, the emergence of antibiotic resistance remains constant constraint and the pace of development of new antibiotics has also drastically come down in recent years. So, there is a pressing need for the development of some alternatives to antibacterial therapy to combat multi drug resistant (MDR) bacterial infections (Kwiatek et al., 2012).

In the dairy industry, mastitis is a widespread problem responsible for heavy losses in milk quality and quantity. Mastitis is defined as inflammation of parenchyma of mammary glands and is characterized by physical, chemical and usually bacteriological changes in milk and pathological changes in glandular tissues. Clinical outcomes of this infection in cattle can range from being acute and life-threatening to chronic and sub-clinical. Mastitis can cause irreversible damage to the udder tissue and less occasional fatalities. It destroys mammary cells, which is replaced by scar or connective tissue, resulting in a permanent loss of production ability.

Mastitis can be caused by many different microorganisms, but one of the most important organisms is Staphylococci (Rebhun, 1995). After diagnosis of mastitis, the standard treatment regimen includes segregation of the diseased cow and treating it with antibiotics. However, this approach has obstacles, such as its high cost and the
elimination of harmless or beneficial organisms due to the lack of specificity of antibiotics.

Staphylococci strains are more risky to dairy animals, as they possess properties to produce enzymes, toxins and intrinsic virulence factors which cause invasion and tissue damage to mammary gland. It can also survive in the keratin of the teat canal of healthy cow and have capacity to resist phagocytosis due to protein-A. On the other hand, there are low level of complements and opsonizing antibodies specific to Staphylococci in milk (Green and Bradley, 2004).

Ability of Staphylococci to resist antibiotic therapy is due to production of beta-lactamases, a group of enzymes that inactivate penicillin and closely related antibiotics. Production of β-lactamases and low-affinity penicillin-binding protein 2a (PBP2a) are responsible for Methicillin Resistance. Strains of Staphylococcus spp. resistant to beta-lactam antibiotics are known as Methicillin Resistant Staphylococci (MRS). Methicillin resistant staphylococci strains have been observed to be multidrug resistant, such as tetracyclines, aminoglycosides, macrolides, lincosamides etc. The patterns of MRS transmission between animal and human have emerged globally, as a major zoonotic threat.

Staphylococcus aureus is a pathogen of pyogenic inflammatory diseases, food poisoning, and toxic-shock syndrome; it is also a major causative agent for opportunistic and/or nosocomial infections, often with a high mortality rate (Noble, 1998). According to one report, most of the S. aureus isolates in Japan today are multidrug resistant, typically known as methicillin-resistant S. aureus (MRSA) (Hashimoto, 1994). Moreover, certain MRSA strains also have already acquired resistance to vancomycin [vancomycin-resistant S. aureus (VRSA)], a unique antibiotic previously considered to be effective against MRSA (Hiramatsu et al., 1997). MDR bacterial infections in the hospital setting are a serious concern, particularly for immune-compromised or intensive care unit (ICU) patients.

The worldwide spread of pathogenic bacteria that are resistant to a variety of antibiotics and the significant problems involved with their control threaten to reduce modern medicine to a state reminiscent of the pre-antibiotic era. Although novel antibiotics directed against such drug-resistant bacteria can be developed when extensive funds are committed for research, the pathogens will ultimately become
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resistant to the new drugs. To break this vicious cycle, it will be necessary to adopt chemotherapy-independent remedial strategies to combat bacterial infections.

Recently, the medical community is keen in replacements for antibiotics which involve bacteriophages for therapeutic purposes. The field of phage therapy has advanced considerably including a recent surge of interest in Western medicine for bacteriophages as antibacterial agents in alternative to antibiotics (Weinbauer, 2004). The relative ease by which phages can be isolated and produced compared to antimicrobial or antibiotic agents and the increasing resistance of bacterial pathogens to traditional antibiotics both serve as important reasons for the advancement of phage therapy (Gill and Hyman, 2010).

Bacteriophages, also referred to as phages, are a class of viruses that infect bacteria. They are the most abundant living entity on this planet, with estimates of their number between $10^{30}$ to $10^{32}$ viruses (Kutter and Sulakvelidze, 2005). Every location on the planet which contains bacteria possesses bacteriophages. Bacteriophages play a vital role in control of bacterial populations and maintaining colony diversity. To understand the dynamics of a bacterial system, detailed understanding of phages must also be considered. Studies have shown that bacteria and phages have a co-evolutionary relationship, an arms race, as one evolves so does the other to counter the challenges each presents (Lenski, 1984).

Phage population size primarily established from observations made with water samples that are simple to collect and quantify (Suttle, 2007). Although terrestrial environments may contribute a relatively minor part of the total numbers of phage particles in the biosphere, prokaryotic diversity in soil samples is very high (Fierer and Jackson, 2006). Quantification of phage population in soil and terrestrial samples is difficult, but phages are estimated to be present at levels approaching $10^9$ particles per gram of soil (Williamson et al., 2003).

Bacteriophages are a very simplistic entity because they are considered to be at the edge of life and not possessing any biological machinery like other living organisms, they can only replicate when they have infected a host. They take over the cellular operations and use its genetic information to force the cell to produce more phages. After a while the cell begins to swell with the number of phage particles multiplying in the host, and eventually the cell busts open and the phages begin
diffusing for a new bacterial host to infect. In terms of organization, they are the most streamlined. Phages have a protein shell wrapped around their genetic material. Some phages are very simple in shape, but other one complex in structure.

The International Committee on the Taxonomy of Viruses (ICTV) recognizes several groups of phages, on the basis of shared morphological traits, such as the shape, size, and structure of the virions, with some consideration of other molecular properties, such as the structure of genomic nucleic acid sand similarities in the signature genes (Virus Taxonomy). For example, tailed bacteriophages with double-stranded DNA genomes classified under the order Caudovirales and are divided into three main families according to their tail morphology: Siphoviridae (long non-contractile tail), Myoviridae (long contractile tail), and Podoviridae (short tail).

Lytic bacteriophages (phages) are viruses that specifically infect and lyse the bacteria. Phage therapy is a method of harnessing phages as bio-agents for the treatment of bacterial infectious diseases. Phage therapy was originally introduced 80 years ago by Felix d’Herelle, a discoverer of phages (Summers, 1999). Despite all his efforts, this therapeutic initiative was later abandoned in Western countries, in part because of the subsequent highly successful discovery and mass production of many kinds of effective antibiotics in the 1940s. Furthermore, most early research into the therapeutic use of phages was poorly organized or uncontrolled, and our understanding of the basis for phage biology was immature. These factors combined to produce a negative outcome for phage therapy (Ho, 2001). Nevertheless, phages have been used for practical purposes in the former Soviet Union and Eastern Europe to the present day (Weber-Dabrowska et al., 2000). The ongoing active bacterial evolution of multidrug resistance also has recently motivated the western scientific community to re-evaluate the therapeutic potential of phages for diverse bacterial infections that are virtually incurable by conventional chemotherapy.

Phage therapy may be an alternative to antibiotics, because it has proved to be medically superior to antibiotic therapy in many ways (Kazmierczak et al., 2014). Phages are very effective for local infections; because, by proliferation through bacteria, they can penetrate into deep infected areas where antibiotics cannot access. In financial terms, the developmental costs of phage therapy are expected to be much less than those involved in the development of novel antibiotics. Besides phage
therapy, staphylococcal phages are also striking candidates for food preservation (Garcia et al., 2009). Most phages selected for phage therapy or food preservation are strictly lytic because of the complications caused by lysogeny.

Another important characteristic of phages selected for therapeutic and biotechnological applications is their narrow host range (Lu and Koeris, 2011). Therefore, it is mandatory to specifically determine the bacterial species responsible for the infection, as well as disposing of a set of phages of known host range, to provide efficient treatment. Traditionally, phage cocktails are used to overcome these issues (Merabishvili et al., 2009). On the other hand this property allows specific treatment against pathogens without affecting the commensal flora.

Growing evidence has suggested 4 different clinical applications for phages, as follows: (1) the administration of living bactericidal (virulent) phages or their natural mutants (Biswas et al., 2002); (2) the topical use of purified phage-encoded bacteriolytic peptidoglycan (cell wall) hydrolases (collectively referred to as endolysin or lysin), such as amidase and transglycosidase (Schuch et al., 2002); (3) the clinical use of phage structural proteins as a metabolic inhibitor to the key enzyme(s) of bacterial peptidoglycan synthesis (Bernhardt et al., 2001); and (4) the use of the phage-display system of the filamentous M13 phage, which expresses coat proteins fused to specific antibodies directed against bacterial antigens. Besides these experimental achievements, clinical trials of phage therapy also may improve its prospects in the west (Sulakvelidze et al., 2000).

The importance of research on phages is evident in recent past with a consortium on phage research begun in 2007 by the Howard Hughes Medical Institute and is currently involving over 85 colleges and universities, which has students discovering unique phages, obtaining images, and purifying phage DNA. A subset of these phage genomes is sequenced and analysed using bioinformatics tools (Louise and Lynn, 2015).

1.1 PRACTICAL UTILITY OF THE RESEARCH PROBLEM

Currently, although antibiotics are successful in the treatment of majority of infections, frontline therapies for field uses are not available readily. Use of antibiotics in treatment and control of bacterial infections is widely adopted strategy
since the discovery of antibiotics in late 1920s. Indiscriminate antibiotic usage including overuse, abuse or misuse (misdiagnosis), substandard antimicrobials/counterfeit drugs and the adaptation evolution governed by natural selection has lead to development of antimicrobial resistance in bacteria. Increasing cost of research in development of new antimicrobial compounds has diminished interest of corporate and public agencies for antimicrobial research funding and has augmented efforts in alternative means for control of bacterial pathogens.

Successful phage therapy reports in human bacterial infections are reported worldwide, but very few reports are available for animal pathogens. There is a need for revitalization of phage therapy in bacterial infections around the world. Once characterized, the phages will be handy and cheapest antibacterial tools. Bacteriophages can also be used in reducing the specific pathogen loads in animal environments in a cost effective manner.

The research work will provide broad picture of multi-drug resistant staphylococci in this region including pathogens of animals. This work will also decipher the information on possible diversity of staphylococci specific phage populations, its morphological & physico-chemical characteristics, host range, reproduction strategy, growth parameters, stability and efficiency against the staphylococci under laboratory conditions which can be a guide for bacteriophage therapy in animal infections involving multi-drug resistant staphylococci and possible use of characterized phages in reducing the pathogen burden from farm and hospital environment. So considering the above facts, the present work is being proposed with the following objectives.

1.2 OBJECTIVES OF THE STUDY

1. To isolate and identify Staphylococci (including MDR) from clinical samples.

2. To isolate and characterize lytic bacteriophage against known Staphylococcus spp. strains.

3. To assess the effect of lytic bacteriophage on clinical isolates.