2 • Review of Literature
2.1 The first known use of phages to treat bacterial diseases of animals

Felix d’Herelle first time realized the value of phage in veterinary medicine, the co-discoverer of bacteriophages. In the spring of 1919, large outbreaks of lethal fowl typhoid in chickens occurred in the Acrissuraube region of France. D’Herelle analyzed several dead animals from the outbreaks, and he identified S. gallinarum as the etiologic agent of the disease. D’Herelle also isolated bacteriophages from the chickens, and he examined their efficacy in preventing and treating S. gallinarum infections in six experimentally infected chickens (D’Herelle, 1921, 1926). The results of the study were promising: Phage administration prevented the birds from succumbing to the bacterial infection, whereas the two control chickens not treated with phages died after a single dose of the challenge strain. The promising results of the small pilot study prompted D’Herelle to almost immediately initiate larger trials, which he called immunization experiments. One hundred chickens were infected with S. gallinarum and 20 of them were treated (“Immunized”) with S. gallinarum specific phage. The 20 phage-treated birds survived; whereas 60 (75%) of the phage-untreated birds died. Encouraged by these results (and the results of similar studies with rabbits and buffalo), D’Herelle subsequently used other phages in his collection to treat bacterial dysentery of Humans. Also, the results of D’Herelle’s studies examining phage Prophylaxis and treatment of fowl typhoid prompted other investigators to begin examining the utility of bacteriophages in preventing or treating various naturally occurring and experimental bacterial infections in animals. As discussed below, the reported outcomes of those studies varied dramatically, depending on the infectious agents used the animal models of infection, and the lytic potency of bacteriophages.

2.2 Bacteriophages selected as best alternative of antibiotic

2.2.1 Advantages of phage therapy

- Phage therapy was mainly popular in the Western area with the newly discovered antibiotics. The antibiotic resistance problem becomes
headache for number of scientist and clinician and that's why they are again looking at bacteriophages as a therapeutic option in the treatment of bacterial infection. After the success of bacteriophage as a therapeutic agent number of companies through the word wide start to begin investing in the field of virology and start to use phage as a novel approach of treatment. It is easy to see why there is a move in favour of bacteriophages as therapeutic agent, as they appear to offer a number of advantages over the use of antibiotics

- Bacteriophages target only the pathogens of interest and the normal gut microflora are not affected
- Their mechanism of action is completely different from all available antibiotics and so they are effective even against bacteria exhibiting multiple antibiotic resistances. Hence, even if they are not used as first-line therapy, bacteriophages represent a very useful last line of defense
- The pharmacokinetics of bacteriophages therapy is such that the initial dose increases exponentially as the virus multiplies within the susceptible bacterial host and is subsequently released. Often there is no need to carry out repeat dosing. It has been already proved that phage can penetrate poorl blood tissues and can cross the blood–brain barrier (Alisky et. al., 1998, Hanlon., 2007)
- Extensive clinical experience in the former Soviet Union and Eastern Europe has co concluded that with the use of phage as a therapeutic agent reduce the cases of side effect and allergic reaction
- Bacteriophages are at first sight cheap and easy to produce
- In case of phage therapy there is no risk of bacterial resistance because the frequency of phage mutation is significantly higher than that of bacteria
- As every coin has two sides same way some disadvantages also associated with every great achievement

2.2.2 A phage therapy also has some disadvantages such as

- After lysis of host bacterial cell might be release of large amount of bacterial endotoxin
- Some phages may have capacity to produce toxins
Review of Literature

- Lacking the knowledge of pharmacokinetic of phage
- When phage lysate inoculate in patient it might be neutralized by the host immune system may lead to failure of phage therapy
- During the infection of phage if phages convert from lytic phages to lysogenic phages (prophases) leads to bacterial immunity

2.2.3 The disadvantages towards clinical application of phage therapies have been successfully overcome by different approaches

- The preparation of genetically modified phage reduces the problem of side reaction and allergic reaction because of the release of endotoxin
- The single administration of phages are usually enough for the treatment thus the problem of neutralization by the host immune system may be reduce during the clinical application of phage therapies. (Merril et. al., 1996; Smith et. al., 1987; Wang et. al., 2006)
- To get the prevention against phage toxicity it requires thorough study of phage
- The faster mutation rate of phage solved the problem of bacterial resistance toward the phage

2.3 Alternative of antibiotics

For more than last five decades the human mainly depend on antibiotic to treat infectious disease caused by pathogenic bacteria. But during this treatment the emergence of bacterial resistance to antibiotics in clinical, veterinary, and agricultural usage has made antibiotics less and less effective (Teuber, 2001; Heuer et. al., 2006). In near future it might be possible that all the pathogenic bacteria resistant to most or all available antibiotics. It was red signal given by the World Health Organization that those multiple antibiotic-resistant pathogens would very likely bring the medical field back to the preantibiotic era.
2.3.1 Antimicrobial efficacy of phage therapy

There are so many areas in which phage can be used as a therapeutic agent. Phage can also be used as an alternative to antibiotics for the treatment against food-borne pathogens. Such as *Listeria monocytogenes*, *Campylobacter jejuni*, and *Salmonella* spp. (Greer 2005).

2.3.2 Antimicrobial efficacy of BCWH

The use of Bacterial cell wall hydrolase can also be proven as an alternative to antibiotics for the treatment of various infectious diseases mostly lysozymes (Niyonsaba and Ogawa 2005) and virolysins (Fischetti 2005), have been
extensively used. Lysozymes were very effective towards bacteria, fungi, and viruses (Reddy et. al., 2004; Wang et. al., 2005; Lee-Huang et. al., 2005).

### 2.3.3 Antimicrobial efficacy of AMP

Detail studies on the antimicrobial efficacy of different families of AMP qualifying AMP as an alternative of antibiotic. Prokaryotic AMP, such as bacteriocins, shows the high level of efficacy in eliminating pathogenic bacterial species (Riley and Wertz 2002). Various natural and synthetic AMP have been used in the treatment of infectious disease. (Marr et. al., 2006; Zaiou 2007).

### 2.4 Whole genome sequencing

To finding the nucleotide sequencing of DNA in the genome is the primary aim of DNA sequencing process. With the use of DNA sequencing new gene can be identified and also discovered new metabolic pathway in particular organism. In whole genome sequencing determines the complete DNA sequence of an organism's genome. The chromosomal DNA, mitochondrial DNA and Chloroplast DNA can be used as a sample for the whole genome sequencing. So many varieties of tissue and cell can be used as a source of DNA for the whole genome sequencing. The whole genome sequencing can also be useful method preferred by the Human Genome Project and also used to the identification of novel genes that may influence development of diseases (Mardis et al., 2008). Applied Biosystems' SOLiD technology gives the opportunity of sequencing the DNA fragment which is having fixed length. Before sequencing, the DNA is amplified by emulsion PCR. (Schuster, 2008).

### 2.5 The study of bacteriophages carrying antibiotic resistance genes

In this study Marta Colomerlluch et al., in 2011 concluded that the occurrence of bacteriophages carrying antibiotic resistance genes in fecal sample of cattle, pigs, and poultry. These antibiotic resistance genes can also transmitted through the bacteriophage. Bacteriophage behaves as a vector for the horizontal transmission of antibiotic resistant gene. These genes can be identified and were quantified by PCR.
2.6 Molecular characterization of multi resistant *Escherichia coli* isolates from poultry litter

Elizabeth Ponce-Rivas *et al.*, 2012 shows that in 19 fluoroquinolone resistant *E. coli* strain were isolates from chicken, were the predominant genes in these fluoroquinolone resistant isolates, constituted the most common genes identified and was located on mega plasmids as well on the chromosome. The diversity of *E. coli* was shown in poultry sample and this diversity was studied with the help of pulsed-field gel electrophoresis (PFGE).

2.7 The role of the bacteriophage treatment in reducing Salmonella colonization of poultry

Carlota Bardina, *et al.*, 2012, reported that Salmonella remains the major cause of food infection through worldwide, chickens known to be the main reservoir for these pathogenic bacteria. The use of bacteriophage offers several advantages. In this study, three polyvalent bacteriophages (UAB_Phi20, UAB_Phi78, and UAB_Phi87) obtained from our collection that exhibited a broad host range against various Salmonella spp. A cocktail composed of the three bacteriophages was more effective to reducing the colonization of *S. enteritidis* and *S. typhimurium*. 