2.1 *STAPHYLOCOCCUS AUREUS*

Staphylococci were first observed in human pyogenic lesions by Van Reklinghanses in 1871. Louis Pasteur in 1880 obtained liquid cultures of the cocci from the pus and produced abscesses by inoculating them into rabbits thus, demonstrated their pathogenicity. Sir Alexander Ogston, in 1880 established conclusively the causative role of the Staphylococcus in abscesses and other pyogenic lesions. He also named them a ‘Staphylococcus’ (*Stphyle*, meaning a bunch of grapes; *Kokkos* meaning a berry) from the typical occurrence of the cocci in the grape like clusters in pus and in cultures.

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes. Bacteriological culture of the nose and skin of normal humans invariably yields staphylococci. In 1884, Rosenbach described the two pigmented colony types of staphylococci and proposed the appropriate nomenclature: *Staphylococcus aureus* (yellow) and *Staphylococcus albus* (white). The latter species is now named *Staphylococcus epidermidis*. Although, more than 20 species of *Staphylococcus* are described in Bergey's
Manual (2001), only \textit{Staphylococcus aureus} and \textit{Staphylococcus epidermidis} are significant in their interactions with humans. \textit{S. aureus} colonizes mainly the nasal passages, but it may be found regularly in most other anatomical locales. \textit{S. epidermidis} is an inhabitant of the skin.

2.1.1 Pathogenicity

The innate ability of pathogen known to cause the diseases generally known as pathogenicity of that pathogen. Pathogenicity cannot be explained in terms of a single factor. Many products have similar biological effects and it is the interaction of the total army of the \textit{S. aureus} that makes it a potential pathogen or virulent (Anderson, 1976).

\textit{S. aureus} expresses many potential virulence factors: (1) surface proteins that promote colonization of host tissues; (2) invasins that promote bacterial spread in tissues (leukocidin, kinases, hyaluronidase); (3) surface factors that inhibit phagocytic engulfment (capsule, Protein A); (4) biochemical properties that enhance their survival in phagocytes (carotenoids, catalase production); (5) immunological disguises (Protein A, coagulase, clotting factor); (6) membrane-damaging toxins that lyse eukaryotic cell membranes (hemolysins, leukotoxin, leukocidin; (7) exotoxins that damage host tissues or otherwise provoke symptoms of disease (SEA-G, TSST, ET) and (8) inherent and acquired resistance to antimicrobial agents.
For the majority of diseases caused by *S. aureus*, pathogenesis is multifactorial, so it is difficult to determine precisely the role of any given factor. However, there are correlations between strains isolated from particular diseases and expression of particular virulence determinants, which suggests their role in a particular disease. The application of molecular biology has led to advances in unrevealing the pathogenesis of staphylococcal diseases. Genes encoding potential virulence factors have been cloned and sequenced, and many protein toxins have been purified (Todar, 2002).

![Virulence determinants of *S. aureus* – (Todar, 2002)](image)

### 2.1.2 Pathogenesis

Pathogenesis is the process of establishing infection with specific clinical features. *Staphylococcus aureus* causes a variety of suppurative (pus-forming) infections and toxicoses in humans. It causes superficial skin lesions
such as boils, styes and furunculosis; more serious infections such as pneumonia, mastitis, phlebitis, meningitis, and urinary tract infections; and deep-seated infections, such as osteomyelitis and endocarditis. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and infections associated with indwelling medical devices. *S. aureus* causes food poisoning by releasing enterotoxins into food, and toxic shock syndrome by release of superantigens into the blood stream.

Human staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defenses. The portal may be a hair follicle, but usually it is a break in the skin which may be a minute needle-stick or a surgical wound. Foreign bodies, including sutures, are readily colonized by staphylococci, which may makes infections difficult to control. Another portal of entry is the respiratory tract. Staphylococcal pneumonia is a frequent complication of influenza. The localized host response to staphylococcal infection is inflammation, characterized by an elevated temperature at the site, swelling, the accumulation of pus, and necrosis of tissue. Around the inflamed area, a fibrin clot may form, walling off the bacteria and leukocytes as a characteristic pus-filled boil or abscess. More serious infections of the skin may occur, such as furuncles or impetigo. Localized infection of the bone is called osteomyelitis. Serious consequences of staphylococcal infections occur when the bacteria invade the blood stream. A resulting septicemia may be rapidly fatal; a bacteremia may result in seeding
other internal abscesses, other skin lesions, or infections in the lung, kidney, heart, skeletal muscle or meninges.

**Sites of infection and diseases caused by S. aureus** – (Todar, 2002)

### 2.2 DRUG RESISTANCE

Since, their discovery during 20th century, antimicrobial against (antibiotics and related medicinal drugs) have substantially reduced the threat passed by infectious diseases. The use of these ‘wonder drugs’, combined with improvements in sanitation, housing, and nutrition, and the advent of widespread immunization programmes, has led to a dramatic drop in deaths
from diseases that were previously widespread, untreatable, and frequently fatal. Over the years, antimicrobials have saved the lives and caused the suffering of millions of people. By helping to bring many serious infectious diseases under control, these drugs have also contributed to the major gains in life expectancy experienced during the recent past. These gains are now seriously jeopardized by another recent development. The emergence and spread of microbes that are resistant to cheap and effective first-choice, or ‘fist-line’ drugs. The bacterial infections which contribute most to human disease are also those in which emerging and microbial resistance in most evident: diarrhoeal diseases, respiratory tract infections, meningitis, sexually transmitted infections, and hospital–acquired infections. Some important examples include penicillin–resistant *Streptococcus pneumonia*, vancomycin–resistant enterococci and methicillin – resistant *Staphylococcus aureus* (WHO, 2002).

2.2.1 Drugs

An antibiotic is a substance produced by various species of microorganisms that suppresses the growth of other microorganisms and may eventually destroy them. However, common usage often extends the term antibiotic to include synthetic antibacterial agents, such as sulfonamides and quinolones, which are not products of microbes. Chemicals were in use of for treatment of infectious diseases since time immemorial. Sulfanilamide introduced in clinical use in 1936, proved to be effective against systemic
infections caused by Streptococcus, Staphylococcus, Pneumococcus. The clinical potential of microbial products as therapeutic agents against infections was recognized by Pasteur and Joubert in 1877. They demonstrated that one of the ‘common’ bacteria of the air when introduced to urine previously inoculated with anthrax bacilli inhibited the growth of anthrax bacilli. However, it was not till 1928 that these observations could be translated into clinical advantage. Sir Alexander Fleming observed that contamination with a fungus, Pencillium notatum, prevented the growth of surrounding bacterial colonies, on culture plates. He cultivated the fungus in a broth and showed that the filtrate inhibited the growth of number of gram-positive organisms. This substance was named ‘penicillin’.

Sulfonamides were the first effective antimicrobials. There usage was peak during the Second World War and they were found capable of coping with vast number of infections associated with warfare. However, this picture has changed with the bacteria becoming resistant to the sulfonamides. Sulfonamides act by interfering with bacterial synthesis of folic acid from para amino benzoic acid (PABA). Folic acid is ultimately needed for production of nucleic acids. Resistance to sulfonamides develops as a result of adaptive modifications of the enzyme systems in the bacteria, which enable the bacteria to produce folic acid by other means. Sulfonamides are bacteriostatic but have wide spectrum of activity against gram positive and gram negative bacteria.
β -Lactam antibiotics include the penicillins, cephalosporins and carbapenems. These antibiotics have a common structure and mechanism of action. The inhibition of synthesis of peptidoglycan cell wall is the key factor for β -lactams antibacterial action. Peptidoglycan is a heteropolymeric component of the cell wall, having a high cross-linked lattice work structure, which provides rigidity and mechanical stability.

The penicillin constitutes the most important group of antibiotics. The basic structure of penicillin consists of a thiazolidine ring connected to a β-lactam ring to which is attached a side chain.

1. Penicillin G and its close congener penicillin V are highly active against sensitive strains of gram positive cocci, but they are readily hydrolysed by penicillinase. Thus they are ineffective against most strains of *S. aureus*.

2. The penicillinase resistant penicillins (methicillin, nefcillin, oxacillin, cloxacillin and dicloxacillin) are effective against penicillinase producing *S. aureus*.

3. Ampicillin, amoxicillin comprise a group of penicillins whose antimicrobial activity is extended to include such Gram-positive microorganisms as *S. aureus, Streptococcus* sp. and *E. coli*.

Combinations of amoxicillin, ampicillin and ticarcillin with the β -lactamase inhibitors calvulinic acid and sulbactam have been tried both experimentally and clinically. Clavulinic acid is a broad spectrum, irreversible,
‘suicidal’ inhibitor of β-lactamase enzyme, which inactivates enzyme and itself gets destroyed in the process. It is effective in the treatment of otitis media, sinusitis, bronchitis, urinary tract infections and skin and soft tissue infection.

Cephalosporins have a common β-lactam ring structure, which closely resemble penicillin nucleus. The most widely accepted and convenient classification of cephalosporins is the generation scheme. This is based on their spectrum of antimicrobial activity, and not on their time of introduction. The cephalosporins have been divided into four generations.

The first generation agents–cephalexin, cephaloridine, cephadroxil and cefclor etc., act mainly on gram positive bacteria; the second generation agents – cefamendole, cefuroxime, cefotaxime acts on some gram negative bacteria; third generation agents – cefotaxime, cefotium and ceftriaxone have a broad spectrum and acts on gram positive and most gram negative bacteria (including multi drug resistant strains of organisms, such as Enterobacter sp. and Pseudomonas sp. etc.); the fourth generation agent – Cefepim is recent introduction.

Aminoglycosides are typified by the presence of aminosugars glycosidically linked to aminocyclitols. All the agents in this group are potent bactericidal agents, used primarily to treat infections caused by aerobic gram negative bacteria. The aminoglycosides primarily inhibit protein synthesis and decrease the fidelity of translation of mRNA at the ribosomes. This is brought about by the binding of aminoglycosides to the 30s ribosomal subunit.
Subsequently the normal cycle of ribosomal function is disrupted at the level of initiation of protein synthesis. This leads to accumulation of abnormal initiation complex, blocking further translation of messages.

The various agents in this group include streptomycin, gentamycin, tobramycin, amikacin, netilmicin, sisomycin, kanamycin and neomycin. The major indication for aminoglycoside is lung abscess, osteomyelitis, middle ear infection and septicemia.

A mechanism of resistance to penicillins is shown by so-called ‘methicillin-resistant’ strains. This is a broad-raging resistance to all penicillins and cephalosporins effected by a reduction in affinity of the penicillin binding proteins of the staphylococcal cell wall for β-lactam antibiotics (Hartman and Tomasz, 1984, Utsui and Yokota, 1985). Methicillin resistance was first noted soon after the introduction of methicillin (Jevons, 1961), but there is good evidence that it existed before the antibiotics was brought into use. Resistant strains were also resistant to several other antibiotics, but for many years they achieved only a local prevalence in the occasional hospital.

Vancomycin is the drug of choice for methicillin-resistant isolates. Patients unable to tolerate vancomycin have been treated with fluoroquinolones, trimethoprim-sulfamethoxazole, clindamycin or minocycline. Each of these drugs has been effective in cases that require bactericidal therapy (Chamber, 1997 and Trucksis et al., 1991). Quinolones with enhanced antistaphylococcal activity have recently become available, but their use may
also be limited by the development of resistance during therapy. A number of potentially active drugs are under investigation, including quinupristin-dalfopristin, a new carbapenem, and a new family of antimicrobial drugs, oxzolidinones (Michel and Gutmann, 1997). The glycopeptide-intermediate strains reported to date have been variably sensitive to chloramphenicol, gentamicin, rifampin, trimethoprim-sulfamethoxazole and tetracycline (Tenover et al., 1988). The initial case involving a glycopeptide-intermediate strain was treated with surgical debridement and ampicillin-sulbactam plus an aminoglycoside (Hiramatsu et al., 1997).

2.2.2 Development of drug resistance

Obviously, if a bacterial pathogen is able to develop or acquire resistance to an antibiotic, then that substance becomes useless in the treatment of infectious diseases caused by that pathogen (unless the resistance can somehow be overcome with secondary measures). So, as pathogen develop resistance, we must find new (different) antibiotics to fill the place of the old one in treatment regimes. Hence, natural penicillins have become useless against Staphylococci and must be replaced by other antibiotics. Tetracycline, having been so widely used and misused for decades, has become worthless for many of the infections that once designated it as a ‘wonder drug’ (Todar, 2002).

Antibiotic resistance results from gene action. Bacteria acquire genes conferring resistance in any of three ways. In spontaneous DNA mutation, bacterial DNA (genetic material) may mutate (change) spontaneously. In a
form of microbial sex called transformation, one bacterium may take up DNA from another bacterium. Penicillin resistant gonorrhea results from transformation. Most frightening however, is resistance acquired from a small circle of DNA called plasmid that can flit from one type of bacterium to another. A single plasmid can provide a slew of different resistances. Though bacterial antibiotic resistance is a natural phenomenon, social factors also contribute to the problem. These factors include increased infection transmission, coupled with inappropriate antibiotic use (Lewis, 1995).

Antimicrobial resistance is not new, but the number of resistant organisms, the geographic locations affected by drug resistance, and the breath of resistance in single organisms are unprecedented and mounting (Levy, 2002). Diseases and disease agents that were once thought to be controlled by antibiotics are returning in new leagues resistant to these therapies. It was focused on the underlying principles and ecological factors that affect drug resistance in bacteria. It should be stressed, however, that antimicrobial resistance is also evident in other microorganisms too (Ash, 1994).

Management of Methicillin Resistant *Staphylococcus aureus* (MRSA) infections include strict infection control policies, including contact isolation. In contrast to Vancomycin Resistant *Staphylococcus aureus* (VRSA), there is evidence that oral/tropical antibiotics can eliminate the carrier state (Segal et al., 1996). Methicillin–resistant Staphylococcal infections generally are treated with intravenous vancomycin. Alternatives may include fluoroquinolones plus
rifampin, trimethoprim–sulfamethoxazole, or monocytic. Although, vancomycin resistance among Staphylococci has been described in vitro for several years, the recent isolation a VRSA from hospitalized patient is an ominous sign (Columbus, 1998).

When microbes began resisting penicillin, medical researchers fought back with chemical cousins, such as methicillin and oxacillin. By 1953, the antibiotic armamentarium included chloramphenicol, neomycin, terramycin, tetracycline, and cephalasporins. But today, researchers fear that we may be nearing an end to the seemingly endless flow of antimicrobial drugs. At the center of current concern is the antibiotic vancomycin, which for many infections is literally the drug of ‘last resort’. Some hospital acquired Staph infections are resistant to all antibiotics except vancomycin. Now vancomycin resistance has turned up in another common hospital bug, enterococcus. And since bacteria swap resistance genes like teenagers swap T-shirts, it is only a matter of time, many microbiologists believe until vancomycin resistant staph infections appear “Staph aureus may pick up vancomycin resistance from entrococci, which are found in the normal human gut”, and the speed with which vancomycin resistance has spread through enterococci has prompted researchers to use the word “crisis” when discussing the possibility of vancomycin resistant Staphycocci (Lewis, 1995).

**2.3 PHAGE THERAPY**
Ernest Hankin, a British bacteriologist, reported in 1896 the presence of marked antibacterial activity (against *Vibrio cholerae*) in the waters of the Ganges and Jumuna rivers in India, and he suggested that an unidentified substance (which passed through five porcelain filters and was heat labile) was responsible for this phenomenon and for limiting the spread of cholera epidemics. Two years later, the Russian bacteriologist Gamaleya observed a similar phenomenon while working with *Bacillus subtilis* and the observations of several other investigations are also thought to have been related to the bacteriophage phenomenon (Helboort, 1992). However, none of these investigators further explored their findings until Frederick Twort a medically trained bacteriologist from England, reintroduced the subject almost 25 years after Hankin's observation by reporting a similar phenomenon and advancing the hypothesis that it may have been due to, among other possibilities, a virus (Twort, 1915). However, for various reasons Twort did not pursue this findings, and it was another two years before the bacteriophages were "officially" discovered by Felix d'Herelle, a French-Canadian microbiologist at the Institute of Pasteur in Paris (Kutter, 2001; Sulakvelidze, 2001). After the discovery of bacteriophages 85 year ago, it was hoped that they would be useful in the treatment of bacterial infections. Phage therapy was initiated in 1921 by Bruynoghe and Maisin in the treatment of Staphylococcus infections. Although the results were promising, little was accomplished in this field during the following years. The idea of potential applications of phage therapy
was abandoned after the introduction of sulphonamides and then antibiotics into medical practice (Weber-Dabrowisk et al., 2000). Prior to the development of antibiotics, research into and the practice or phage therapy was a substantial enterprise in Europe, parts of Asia and North and South America, and continues to be a viable, if not thriving, industry in some countries of Eastern Europe (Radepsky, 1996 and Alisky et al., 1998).

2.3.1 Development

Felix d’Herelle (seated) – Pioneer in phage therapy

d'Herelle became fascinated with the apparent role of phages in the natural control of microbial infections and the possibilities of lacking advantages of their action. The first attempt of bacteriophages as therapeutical agents was conducted at the hospital Des Enfants-Malades in Paris in 1919 (Summer, 1999). The phage preparation was ingested by d'Herelle, Hutinel, and several hospital interns in order to confirm its safety before administering it the next day to a 12 years old boy with severe dysentery. The boy went from
12 bloody stools a day to no symptoms by the next morning; thereafter other children were later treated with equal success. Therefore, the first reported application of phages to treat infectious diseases of humans came in 1921 from Richard Bruynoghe and Joseph Maisin, who used bacteriophages to treat *Staphylococcus* skin infections (Sulakvelidze, 2001 and Kutter, 2001). The bacteriophages were injected into and around surgically opened lesions, and the authors reported regression of the infections within 24 to 48 hr.

'd'Herelle's work with plague in Egypt, was impressed by a group of British medical officers and officials managed to arrange for extensive field trials in India, led by Lt. Colonel J. Morison, director of the Haffkine institute in Bombay. The major target was cholera. An Indian physician, Dr. M. N. Lahiri, carried out extensive characterization of 361 isolates of *Vibrio cholerae*, and applied this newly developed therapy in treating cholera patients in the Campbell Hospital, Calcutta and in Lahore. Of the 33 patients treated with the then standard fluids and salts, 13 died. In contrast, 16 patients were given two ounces each by mouth of d’Herelle’s most active phage preparation and none of them died. Malone and d'Herelle extended this work to several villages in the Punjab. Over a period of six weeks, they collected data on 198 cases of cholera. Only 8% of the 74 patients treated with phage died, while the mortality rate in those not given the phage was 63% (Kutter, 2001).

In 1931, the Council on Pharmacy and Chemistry of the American Medical Association (Eaton and Stanhope, 1934) commissioned a review of
bacteriophage therapy. The commercialization of therapeutic phage preparations to treat bacterial infection in humans was started in France by d’Herelle and in the United States in the 1940s by the pharmaceutical company Eli Lilly. However, because of controversial results and the promise of antibiotics in the 1940s, the commercial pursuit of therapeutic phages in the ‘West’ ceased, although not in Eastern Europe (Krueger and Scrabner, 1941).

d’Herelle was invented by the British government to go to India to work on phage therapy of plague at the Haffkine Institute in Bombay. This short visit lead to the later establishment of “The Bacteriophage Inquiry” in India under the Indian Research Fund Association. This project studied the application of phage therapy in India, especially for cholera epidemic that occurred regularly in association with religious festivals and pilgrimages (Summers, 1993).

Since the discovery of spontaneous bacterial lysis by Twort and by d’Herelle, phage therapy has been used extensively with miscellaneous bacterial infections in the areas of ota-laryngology, stomatology, ophthalmology, dermatology, paediatrics, gynaecology, surgery (especially against wound infections), cirology and pulmonology (Chanishvilli et al., 2001).

Kokin (1947) describes application of the mixtures of anaerobic phage and Staphylococcus and streptococcus phage (produced by the IBMV, Tbilisi, Gerogia) for treatment of gas gangrene in soldiers. The mixture was applied to
767 cases and resulted in a death rate of 18.8% compared with 42.2% in the control group of soldiers treated by other methods. Other authors have observed death rates of 19.2% in the group of soldiers treated with the same mixture of phages against 54.2% treated with other medications (Chanishvili et al., 1947).

The WHO (1959) concluded that, with the success of tetracycline therapy, there did not seem any reasons why investigation into phage therapy should continue (Pollitzer, 1959).

This early strong interest in phage therapy paved way for treating a variety of disease, including dysentery, typhoid, paratyphoid fevers, cholera, pyogenic and urinary tract infections (Ackerman and DuBow, 1987).

Witting et al. (1966) carried out bacteriophage therapy on infective childhood asthma and got successful results. Ha (1968) has studied on protection of *Klebsiella pneumonia* – infected in the mouse with phages. In the same year Babalova et al. (1968) prepared dried dysentrage bacteriophage, for the treatment of dysentery.


After independent discovery of phages by Frederic Twort and Felix d'Herelle, the idea of phage therapy and prophylaxis was reassessed more
recently by Smith and Huggins (1977) after the realization that colicins could be used to treat *E. coli* septicemia caused by a colicin-sensitive strain. This was of considerable significance and relevance to development of the use of phage therapy. After two years, Litvinova et al. (1979) tried the efficiency of the use of *E. coli* -Proteus bacteriophage in intestinal dysbacteriosis in premature infants.

Phage therapy of post operative suppurative inflammatory complications in patients with neoplasms were studied by Kochetkova et al. (1989), and reported the efficacy of phages. Therapy depends on the type of pyoinflammatory complications (the results are the best in the management of wound infections), the microflora pattern of the purulent foci (phages are the most effective with a corresponding monoinfection) and characteristics of the therapeutic phages.

Alisky et al. (1998) were conducted a literature review of all Medline citations from 1966-1996 that dealt with the therapeutic use of phage. The polish and soviets administered phage orally, topically or systemically to treat a wide variety of antibiotic resistant pathogens in both adults and children. Infections included suppurative wound infections, gastroenteritis, sepsis, orteomyelitis, dermatitis and pneumonia; pathogen included *Staphylococcus, Streptococcus, Klebsilla, Escherichia, Proteus, Pseudomonas, Shigella* and *Salmonella* sps. Overall, the polish and soviets reported success rates of 80-95% for phage therapy, with rare, reversible gastrointestinal or allergic side
effects. The British end U.S. reports described phages against specific pathogen and improving the bioavailability of phage.

Payne et al. (2000) were reported on the peculiar kinetics of self replicating pharmaceuticals of phage therapy. Sarkar (2002) reported on phages of *Vibrio cholerae* have been of historical interest. The recent emergence of multi-drug resistant pathogenic bacteria is a very serious problem. The phage especially, the cholera bacteriophage may be helpful as therapeutic agents for treating infections.

Joerger (2003) reported the alternatives to antibiotics like baciocins, antimicrobial peptides and bacteriophages. Phages have received renewed attention as possible agents against injecting bacteria. Development of phage therapy is in fast progress and ever continues practice by several investigators across the world. Sandra et al. (2004) reported the effective phage therapy against *E. coli* infections in both *in vitro* and *in vivo* systems. Wills et al. (2005) established experimental bacteriophage protection against *S. aureus* induced abscesses in a rabbit model.

### 2.3.2 Future prospects

Infectious disease experts have warned that there is now a compelling need to develop totally new classes of antibacterial agents, ones that cannot be resisted by the same genes that render bacteria resistant to antibiotics. Phage therapy represents such a “new” class. It is believed that the impediments like (bacterial debris in the preparations, rapid clearance in the body, etc. can be
overcome, freeing up the phage so that their attributes such as exponential growth, and the ability to mutate against resistant bacteria can be used to great advantage (Carlton, 1999).

Phages have specific properties, which give them advantages as therapeutic agents. They are self-replicating as well as self-limiting. They continue to multiply and penetrate deeper as long as local infection as present. This is in sharp contrast to antibiotics, which decrease in concentration below the site of infection. Phages are lytic against specific bacteria so they can be targeted more specifically than antibiotics which are active against a group of bacteria. Phages do not harm normal intestinal microflora (Park, 2002).

Antibiotics have side effects, which can be serious. But phages have been used in millions of patients without any reported side effects. Phages can be used prophylactically as well as in established infections. The self-perpetuating nature of phages in the presence of susceptible bacteria, makes multiple administrations unnecessary (Berchiera et al., 1991 and Mathur et al., 2003).

Attributes of phages favouring therapeutic response outlined by Carlton (1999) are as under

<table>
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<tr>
<th>The issue</th>
<th>Limitations of antibiotics</th>
<th>Advantages of phages</th>
</tr>
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<tbody>
<tr>
<td>Fate of the “drug” molecule</td>
<td>Metabolic destruction of the molecule, as it works</td>
<td>Exponential growth in numbers, so that the “drug” makes more of itself at the site of infection, where it is needed. “All or nothing” effect”; one phage particle is sufficient to kill a given bacterium</td>
</tr>
<tr>
<td>Concentration of the “drug” required to kill a given bacterium within the</td>
<td>Numberous molecules of the antibiotic are needed to kill a given bacterium. During initiation of therapy (and between doses), the sub-lethal dose that bacteria “see” affords them the opportunity to express</td>
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spectrum resistance genes

Ability to overcome bacterial resistance

Antibiotics are fixed, immutable chemicals that cannot adapt to a bacterial mutation and therefore become obsolete. Bacteria that have resisted them can pass along the resistance trait within and between species.

Phages are “living” organisms that undergo mutations, some of which can overcome bacterial mutations. E.g. mutated phage tail fibers can allow binding to a mutant bacterial receptor, or mutated phage DNA can escape cleavage by mutant bacterial endonucleases.

Spread of bacterial resistance

The antibiotics in use tend to be broad spectrum, thereby provoking resistance in several species and genera of bacteria (in addition to the one targeted)

Although there are some exceptions, phages tend not to cross species boundaries. Thus even though the targeted bacterial species may become resistant to the phage, it is unlikely that other species will

Compared of bacteriophage versus antibiotic therapy as mentioned below Jameel (2003).

Advantages

**Bacteriophages**
High specificity for particular bacterium, thereby reducing the possibilities of secondary infections developing
Repeated administration is unlikely because as long as the target bacterium is present, the phage will be able to reproduce.
Cheap to produce and to date without any observed side-effects.
The receptors to which phages are targeted on the bacterial cell surface are virulence factors, so when bacteria develop phage resistance, they are usually altered, which results in an attenuation of virulence.
Finding a phage which will be active against a bacteria which has developed phage resistance is rapid, taking only a

**Antibiotics**
Active against, wide range of bacteria, thereby avoiding the need to characterize the infective bacterium
matter of days.

<table>
<thead>
<tr>
<th>Disadvantages</th>
<th>Antibiotics</th>
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<tbody>
<tr>
<td><strong>Bacteriophages</strong></td>
<td>Repeated administration is needed and antibiotics therapy is often associated with side-effects such as intestinal problems, secondary infections, e.g., with yeast. Once, a bacteria develops antibiotics resistance, as opposed to phage resistance it remains pathogenic. The development of novel antibiotics (needed for example when bacteria develop resistance) takes on the order of years. Because of a non-specific mode of action, antibiotics also destroy the commensal microflora especially in the intestine, which may lead to intestinal disorders. Expensive to produce.</td>
</tr>
<tr>
<td>Causative agent may need to identified in order to use appropriate phage, unless a phage cocktail-is used</td>
<td></td>
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2.4 BACTERIOPHAGE

Bacteriophages or “phages” are viruses of prokaryotes including eubacteria and archaeabacteria. They were discovered twice at the beginning of the 20th century. Frederick William Twort, a British pathologist in London, described in 1915 the glassy transformation of ‘Micrococcus’ colonies by a transmissible agent. He proposed several explanations. One of which was that, the agent was viral in nature. Felix Hubert d’Herelle, a French Canadian then working at the Pasteur Institute of Paris, observed the lysis of *Shigella* cultures in broth and described it in 1917. Twort did not pursue his discovery, but attempted for decades to propagate vertebrate viruses on inert media. d’Herelle, on the contrary, clearly recognized the viral nature of his agent and devoted the rest of his scientific life to it. He coined the term “bacteriophage”,

41
devised several techniques still in use, postulated the intracellular multiplication of viruses and introduced phage therapy or infectious diseases (Ackermann, 2003). The word “phage” comes from the Greek “phagein”, which means, “to eat”.

2.4.1 Biology and Structure

The history of phages begins with the work of Max Delbruck in the late 1930’s, Nobel Laureate Delbruck, originally a physicist, began studying phages as genetic and biochemical experimental systems. His work led others to focus their studies on the structure, biology and assembly of phages. In the late 1960s, Edgar, Kellenberger, Epstein and collaborators demonstrated that assembly of the T4 phage occurs along specific pathways. Since then many phages have been studied.

Bacteriophages undergo two possible life cycles. These are the lytic (or virulent) and lysogenic. Lytic phage multiply vegetatively and kill the host cell at the end of the growth cycle. Temperate phages which undergo the lysogenic cycle as well as multiplying vegetatively can also persist in a lysogenic state, whereby the phage genotype can exist indefinitely by being inserted in the bacterial chromosome (known as the prophage state) (Thiel, 2004).

Phages undergoing lytic cycles only are virulent. Lytic cycles consist of several steps and show considerable variation according to the type of phage. The general diagrammatic representation of the lytic cycle is as shown under.
Phages encounter bacteria by chance and adsorb to specific receptors, generally located on the cell wall, but also on flagella, pili, capsules, or the plasma membrane. The phage nucleic acid enters the host and the shell remains outside. In phages with contractile tails, the cell wall is degraded by phage enzymes located on the tail tip. The sheath then contracts and the tail core is brought in contact with the plasma membrane. It depends largely on the physiological state of the host and varies between 20 min and 30–40 hr. Phage nucleic acid is transcribed to mRNA using host and/or phage RNA polymerases. The assembly of new phages is called maturation. Phage constituents assemble spontaneously or with the help of specific enzymes (Ackermann and DuBow, 1987). The lytic phages are the most suitable candidates for phage therapy, because they quickly reproduce within and lyse
the bacteria in their host range, growing exponentially in number in the process. Depending on the species and conditions, each “parent” phage can produce on average approximately 200 “daughters” per lytic cycle. If each daughter infects and kills a host bacterium there will be 40,000 progeny at the end of the 2nd cycle; 8 million at the end of the 3rd cycle; 1.6 billion at the end of the 4th cycle; and so on.

The lysogenic cycle on the other hand comprises replication of phage, nucleic acid together with the host genes for several generations with major metabolic consequences for the cell. This is a latent mode of infection and it occurs at a very low frequency. The phage gene in this state may occasionally revert to lytic cycle, leading to release prophages particles. This property is known as lysogens and phage that can develop both lytically and lysogenically are said to be phages (Beneett and Howe, 1998).

All phages have single, linear double stranded DNA (dsDNA) chromosomes stored in a protein coat shell called the capsid or head (see picture). The capsid is built by protein molecules along icosahedrally symmetric arrays to form the distinctive shape of phages. The tail extends from one corner of the capsid and interacts with a single host cell. During infection, the distal end of the tail adsorbs to the exterior of the host cell as phage DNA travels through the tail into the cells (Ackermann and Dubow, 1987).
Antje et al. (1998) assigned the phages to different virus families, species and strains based on morphology, DNA homology and host range. The phenotypic diversity of the 22 bacteriophages was examined by electron microscopy and phages were identified by following morphological criteria outlined as per the International Committee of Taxonomy of Viruses. Morphological studies of 22 phages detected by them revealed that all of the phages had tails and thus belong to the order *Caudovirales*. The icosahedral heads of the phages had diameters between 50.2 and 99.3 nm. The phages could be assigned to three virus families. Eleven of the phages belonged to the family *Myoviridae*, which contains phages that have icosahedral heads and long contractile tails; seven phages were assigned to the family *Siphoviridae* which contains phages that have icosahedral heads and
long flexible tails and four phages, which had icosahedral heads and short tails, belonged to the family *Podoviridae*. Phages belonging to 3 different families and their electron micrographs illustrated by them are as under.

A. *Myoviridae*  
B. *Siphoviridae*  
C. *Podoviridae*

Electron microscopic images of the somatic coli phages (faecal coli forms) isolated from environmental samples by Duran et al. (2002) were as shown below. The structure (A) of bacteriophage with isometric head and short tail indicates *Myoviridae* and another structure (B) of phage with long straight flexible tail indicate the family *Siphoviridae*. 

A. *Myoviridae*  
B. *Siphoviridae*
Sandra et al. (2004) tested stool samples from pediatric diarrhea patients and environment water samples in Dhaka Bangladesh and sewage from Switzerland, yielded nearly exclusively phages with a contractile tail belonging to family *Myoviridae*. The electron microscopic structure and genomic DNA of the A, B and C are main three selected phages based on their potential lytic activity on pathogenic *E. coli* shown as under. All three phages showed the typical morphology of T4 like phages with 170 kb genome (D) upon pulsed field gelelectrophoresis.
Verthe et al. (2004), examined electron microscopic images and genomic DNA molecules of the phages while studying stability and activity of an *Enterobacter aerogenes*–specific bacteriophage under simulated gastrointestinal conditions. Transmission electron microscopy revealed phage particles having an isometric head (A) with a diameter of approximately 65 nm and a short non-contractile tail. After restriction of phage DNA the size of the major bands (B) were approximately 23,000 bp, 7000 bp and 4000 bp. These morphological properties and the estimated genome size of at least 34 kb correspond to the T7 like phages of the genus *Podoviridae*, family *Podoviridea* and order *Caudovirales*.

![Image A](image1.png)

![Image B](image2.png)

The first global attempt to systematically classify viruses took place at the International Congress of Microbiology held in Moscow in 1966. This meeting established the International Committee of Taxonomy of Viruses (ICTV), whose mission was to develop a universal taxonomic system for all viruses infecting animals, plants, fungi, bacteria, and later, archaea. Beginning
with its first report in 1971, the International Committee of Taxonomy of Viruses has met regularly to update virus definitions and taxonomy guidelines, with the seventh and most recent report published in 2000 (Regenmortel, et al., 2000). Viruses are grouped together by shared characteristics, with subgroups having smaller clusters of shared attributes. For example, the tailed order of phage (Caudovirales) is broken down into three families; phage with long, contractile tails (*Myoviridae*), phage with long, noncontractile tails (*Siphoviridae*), and phage with short tails (*Podoviridae*). The families are further broken down into genus and subgenus by criteria such as genome configuration (linear, circular, supercoiled), host range, and genome size.

The inadequacy of the International Committee of Taxonomy of Viruses classification is evident when one looks closely at the numbers. Of the completed phage genomes currently deposited in GenBank (Benson, et al., 2004), 40% (92 of 228) are unclassified beyond the level of family according to International Committee of Taxonomy of Viruses conventions. Furthermore, an additional 10% (23 of 228) are not even assigned an order and are simply listed as “unclassified bacteriophage”.

It is generally agreed that, future phage classifications must reflect genomic data as a primary component. In bacteria, this is easily accomplished by examining the conserved 16S ribosomal genes. However, phage lack ribosomal DNA and there are no conserved gene or protein sequences common to all phage on which to base a classification (Rohwer and Edward, 2002).
Furthermore, any immediate attempt to reclassify phage based exclusively on comparative genomics may be biased toward the lambdoid phages or those involved in industrial fermentation, since these phages dominate the current assemblage of sequenced genomes. Nonetheless, in the fall of 2002, three separate phage research groups proposed alternative classification schemes as outlined below.

An option proposed by Rohwer and Edwards is based on a “Phage proteome tree”, which is constructed by grouping phage both relative to their near neighbours and in the context of all other phage (Rohwer and Edwards, 2002). This method analyzed the entire predicted proteome for a given phage and the results were then transformed into a distance matrix. A tree was constructed based on relationships between phage proteins. The authors list several anomalies in the International Committee of Taxonomy of Viruses system that could be resolved by the proteome tree, including a reclassification of the P22 phage (*Podoviridae*) mentioned above into the lambdoid-like family *Siphoviridae*. Additionally, the proteome tree moves, which International Committee of Taxonomy of Viruses classifies as *Tectiviridae* due to the presence of a lipid membrane below the capsid, to a subgenus of the family *Podoviridae*.

A group at the Pittsburgh Bacteriophage Institute, suggests that it may be impossible to have a strictly hierarchical taxonomic system given the genetic mosaicism arising from horizontal gene transfer among phage
(Lawarence et al., 2002). In this paradigm, the top levels of taxonomy would still follow the hierarchical Linnean approach, i.e., viruses would be divided into “domains” according to genome type (double stranded DNA, single stranded DNA, Single–stranded RNA, and double–Stranded RNA), with a further partition known as “divisions” to separate defining characteristics such as tailed phage from filamentous phage. However, from this point on, three basic tenets would guide the remainder of the classification. First, members of a group should exhibit similarity in one or more loosely defined cohesion mechanisms. Second, significant sequence data, preferably from whole genomes, should be available for evolutionary assignment to a taxonomic cluster. Third, the groups may be reticulate, i.e., phage may simultaneously belong to several groups based on multiple and/or differing criteria from the first two tenets. This web like design has an inherent flexibility that is not afforded by either phenetic or genetic hierarchical approaches.

Another approach based on comparative genomics of a structural gene module, has been proposed by Proux et al. (2002). Since it is believed that the structural genes are the oldest and most conserved module in dairy phage, dot plots of temperate lactococcal phage were used to observe graded relatedness between DNA and protein sequences as well as similarity in the organization of the structural genes in the absence of sequence relatedness. Whereas, comparative genomics of non-structural genes tended to lump all of the lactococcal phages studied into one species, comparative genomics of the
structural genes delineated four phage species and distinguished two genera based on head morphology. In the end, it may not be such a bad idea that we agree to disagree (Daniel, 2004) with the exception of a few remarkable outlines as noted above.

2.4.2 Sources and Distribution

Bacteriophages or “Phages” are viruses of prokaryotes including eubacteria and archaebacteria. They were discovered and described twice, first in 1915 by the British pathologist Frederick William Twort and then in 1917 by the Canadian bacteriologist Felix Hubert d’Herella working at the Pasteur Institute of Paris. With about 3500 isolates of known morphology, phages constitute the largest of all virus groups. Phages are tailed, cubic, filamentous, or pleomorphic. Tailed phages are far more numerous than other types, are enormously diversified, and must be very old in geological terms.

Phages have been found in over 100 bacterial genera distributed all over the bacterial world; in aerobes and anaerobes, actinomycetes, archaebacteria, cyanobacteria and other phototrophs, endospore formers, appendaged, budding, gliding, and sheathed bacteria, spirochetes, mycoplasmas and chlamydiads. Phage like particles of the podovirus type have even been found in endosymbionts of paramecia. However, tailed phages reported in cultures of green algae and filamentous fungi are probably contaminants. Most phages have been found in a few bacterial groups: enterobacteria (over 650 phages), bacilli, clostridia, lactococci, pseudomonads, staphylococci, and streptococci.
This largely reflects the availability and ease of cultivation of these bacteria and the amount of work invested. About half of phages have been found in cultures of lysogenic bacteria. Tailed phages predominate everywhere except in mycoplasmas. In archaebacteria, they have been found in the genus Halobacterium only and not yet in methanotrophs and extreme thermophiles. *Siphoviridae* are particularly frequent in actinomycetes, coryneforms, lactococci and streptococci. Myoviruses and podoviruses are relatively frequent in enterobacteria, pseudomonads, bacilli, and clostridia. This particular distribution must have phylogenetic reasons.

Except for phages from extreme environments, phage species generally seem to be distributed over the whole earth. This is suggested by electron microscopical observations of rare and characteristical phage morphotypes in different countries and global occurrence of certain lactococcal phage species in dairy plants and of RNA coliphages in sewage. Unfortunately, most data are from developed countries. Sizes of phage populations are difficult to estimate because plaque assays and enrichment and (most) concentration techniques depend on bacterial host; they therefore only detect phages for specific bacteria and environmental conditions. Consequently, phage titers vary considerably – for example, for coliphages between 0 and $10^9/g$ in human feces and between 1 and $10^7/ml$ in domestic sewage. Titers of actinophages in soil vary between 0 and $10^5/g$. 
Several sources have been used by different researchers for the isolation of bacteriophages and to understand their prevalence. Edwert (1980) studied the distribution of phages in raw sewage and treated effluents. Bitton (1987) also studied the distribution of phages raw domestic waste water. Antije et al. (1998) and Yoon (1999) reported distribution or phages in sea water and marine environment respectively.

Sharp (2001) recorded the distribution of phages in sea water, sewage and also soil. Reanney and Marsh (1973) and Kevin et al. (2003) were also recorded the prevalence of bacteriophages in different soil and sewage.

Bachrach et al. (2003) have isolated bacteriophages from human saliva. Phages for *Enterococcus faecalis* were found in saliva samples. The presence and stability of the *E. faecalis* bacteriophages in human saliva suggests a possible role of these bacteriophages in a oral ecosystem and phage therapy as a way to control oral bacteria might be considered. Hitch et al. (2004) have isolated bacteriophages from the oral cavity. The composition of the oral cavity does not appear to be heavily influenced by interactions between bacteriophages and their hosts and reported bacteriophage for control of oral infections may need to be obtained from other sources.

### 2.5 *IN VITRO* ACTIVITY OF BACTERIOPHAGE

Smith and Huggins (1982) while studying efficacy of phages against pathogenic *E. coli*, isolated 15 phages from specimens of sewage, out of which 9 were identified as specific phages based on the degree of the lytic activity.
(diameter of plaques). The 9 anti *E. coli* phages were much more virulent than the others based on *in vitro* lytic activity. $10^6$–$10^9$ viable particles of the phages were required to lyse broth of *E. coli* seeded with $3 \times 10^8$ viable organisms. 50–120 resistant colonies were also observed within the zone produced by 1 drop of undiluted preparations of few anti *E. coli* phages spotted on a lawn of pathogenic *E. coli*, determining the difference in degree of *in vitro* lytic activity of phages.

Eric et al. (1996) studied effects of sunlight on the viability and structure of bacteriophage in marine aquatic systems. Destruction of virus particles is concluded to be a process separate from loss of infectivity. It is also concluded that strong sunlight affects the viability of bacteriophages in surface waters, with the result that direct counts of virus like particles over estimate the of bacteriophage capable of both infection and replication. However, in deeper waters, where solar radiation is not a significant factor, direct counts should more accurately estimate numbers of viable bacteriophages.

Yoon et al (1999) were suggest that, a novel bacteriophage, designated as VPP97 which infects the of *Vibrio* para haemolytics (halophilic, Gram-negative bacteria) isolated most commonly from marine environments. In this studies characterized phages were almost totally inactivated at 70°C and at pH below 5 or over 10 and the phage treatment appears effective to the infection by *V. parahaemolytics*. 
Carlton (1999) reported that, bacteriophage can be robust antibacterial agents *in vitro*. However, their use as therapeutic agents, during a number of trails from the 1920s to 1950s, was greatly handicapped by a number of factors.

Hazem (2002) reported the effect of temperature pH, UV light, ethanol and chloroform on the growth of thermophilic bacillus phages. Most of the isolated phages were susceptible to above 60°C and inactivated at 103°C. Most phages were resistant to pH range 5-9 and almost all to pH 7-8. Two phages were highly resistant to exposer to UV light for 13 and 20 min. The chloroform or 75% ethanol showed no effect on almost all isolated phages that indicate the possibility of the absence of lipids.

Prior observations of phage host systems *in vitro* have led to the conclusion that, susceptible host cell population must reach a critical density before phage replication can occur. Such a replication threshold or “proliferation threshold” density would have broad implications for the therapeutic use of phage. Kasman et al. (2002) were demonstrated experimentally that, such replication threshold exists and support the threshold in terms of a classical model for the kinetics of colloidal particle interactions in solution.

Phage also were tested for durability under conditions designed to simulate environments possibly encountered during mass phage production, storage, and use against anthrax spore, and also reported phages are sensitive to temperature over 55°C (Walter, 2003).
Feng et al. (2003) studied effects of pH and temperature in the survival of coli phages. MS2 phage survived better in acidic conditions than in an alkaline environment in contrast Qβ phage had a better survival rate in alkaline conditions; than in an acidic environment. The inactivation rates of both coli phages were lowest within the pH range 6-8 and the temperature range 5-35°C. The inactivation rates of both coli phages increased when the pH was decreased to below 6 or increased to above 8. The inactivation rates of both coli phages increased with increasing temperature. Substances or conditions that denature proteins or react chemically with proteins or nucleic acids will inactivate phages. The inactivation of MS2 and Qβ observed in this study could be attributed to reactive radicals and levels of heat stability. Temperature has a major effect on the effectiveness and/or the rate of kill or a given microorganism because it controls the rate of chemical reactions. Thus, as temperature increases, the rate of kill induced by a chemical will also increase. In addition, pH can affect the ionization of chemicals. At extreme pH values, the high concentrations of hydrogen ion and hydroxyl ion present in water are considered to be far greater than the concentration of free reactive radicals and therefore dominate viral inactivation mechanism.

Sandra et al. (2004) have studied the in vitro and in vivo bacteriolytic activities of E. coli phages. The normal E. coli gut flora of conventional mice was only minimally affected by oral phage application despite the fact that in vitro the majority of the murne intestinal E. coli colonies were susceptible to
the given phage cocktail. Apparently the resident *E. coli* gut flora is physically or physiologically protected against phage, infection.

Verthi et al. (2004) studied on effect of pH, bile salts and pancreatin on bacteriophage in simulated gastro-intestinal *in vitro* conditions. After 1 hr incubation at 34°C at different pH levels, there were no significant differences between the initial phage titer of $6.2 \pm 0.3 \times 10^5$ pfu/ml and the phage concentrations at pH 9, 7, 6 and 4. However, at pH2, the concentration of bacteriophage dropped below the detection limit ($1.0 \times 10^1$ pfu/ml) immediately after the addition of phage.

Richard et al. (2004) were experimentally tested the efficacy of such simple models to predict, qualitatively and quantitatively, the growth of phage and the phage proliferation threshold *in vitro* and explore fully the kinetics of phage therapy. More complex models need to devised and suggest that, it may be necessary to consider and the model interacts between phage growth parameters and bacterial growth parameters.

Tanjil et al. (2004) have screened the 26 phages against *E. coli* and rational producer for selecting an effective cocktail of phage for controlling bacteria were investigated based on the mechanism of phage resistant cell conversion.

Flynn et al. (2004) have studied the exploitation of bacteriophage as biocontrol agents to eliminate the pathogen *E. coli*. Two distinct lytic phages isolated against a human strain of *E. coli* and a cocktail of phages were
evaluated for their ability to lyse the bacterium *in vivo* and *in vitro*. However, bacteriophage–insensitive mutants emerged following the challenge and commonly reverted to phage sensitivity within 50 generations.

### 2.6 IN VIVO EFFICACY OF BACTERIOPHAGE

Smith and Huggins (1982) observed that the anti-K1 phage was very active against *E. coli* (MW strain) than antibiotics. Intramuscular injection of the phage and the pathogen in different region separately also led to prevention of growth of the pathogen and that the phage particles spread to different regions of the body.

Slopek et al. (1984) analyzed the 150 cases of supportive bacterial infections. Positive therapeutic results were obtained in 137 cases (91.3%). The results obtained confirm the great effectiveness of bacteriophages in the treatment of specific infections, spontaneous or postoperative, caused by pyogenic Staphylococci, Klebsiella, Escherichia, Proteus and Pseudomonas.

Analysis of phage therapy results in suppurative bacterial infections were carried out on 273 cases of spontaneous and postoperative septic Staphylococcal infections. The treatment appeared effective in 254 (13.0%) cases. Detailed analysis of the results obtained in particular disease categories revealed that, the Staphylococcal bacteriophages may be efficiently applied in the treatment of suppurative staphylococcal infections resistant to antibiotics (Slopek et al., 1985).
Kilnadze et al. (1986) were used phages for the treatment of children’s hospital salmonellosis. Slopek et al. (1987) studied two healthy volunteers and 56 patients with suppurative bacterial infections and tested for penetration of oral administered phages to the blood circulation system and urinary tract. Kaczowski et al. (1990) used the bacteriophages and antibiotics for prevention of acute post operative emphysema in chronic suppurative lung diseases.

The effectiveness of specific phage therapy was studied on Klebsiella experimental species in non-infired white mice, caused by the intrapentoneal injection of K. pneumonia by Bojovazova et al. (1991). The study revealed that, phages could be detected in the blood and internal organ of the animals within 24 hr irrespective of the route of its administration. One daily inter-peritoneal injection of Klebsiella phage for 15-20 days proved to be the optimum scheme for treatment.

Soothill (1992) tested successfully bacteriophages for Staphylococcus aureus in experimental infections of mice. These studies support the view that, bacteriophages could be useful in the treatment of human infections by antibiotic resistant strains of bacteria.

Perepanova et al. (1995) tried the efficacy of bacteriophage preparations in treating inflammatory urologic disease with bacteriophage preparations (Proteus, Staphylococcus, coliphage, and combined pyobacteriophage). Bacteriophage preparations were used both locally and orally in 46 patients with acute and chronic urogential inflammation. Bacteriophage efficacy
amounted to 84% - 92%. It is inferred that, phage therapy is effective and safe therapeutic modality in the treatment of urinary infection in monotherapy and in combination with antibiotics.

Biswas et al. (1996) showed longevity of bacteriophages in circulating blood, which acts on specific bacteria, whenever they attack.

Gowri Sankar et al. (1998) evaluated the phage therapy using experimental infections in mice. Two common bacterial pathogens namely, *Psuedomonas aeruginosa* and *Streptococcus pyogenes* were isolated from a rural hospital. Test animals (mice) were injected with the bacterial pathogens through the intra-peritoneal route. Their phage partners were also administered subsequently through the same route. Animals protected with phage ingestion exhibited no sign of illness when compared to the unprotected ones. This protection could be attributed to the marked reduction of the pathogen load in the tissues of the animal in response to the presence and persistence of phages.

In recent years, well-controlled animal models have demonstrated that phages can rescue animals from a variety of total infections, while non-controlled clinical reports published in Eastern Europe have shown that phages can be effective in treating drug resistant infections in humans (Carlton, 1999).

Oral administration of specific phage preparations for a period of 5 weeks resulted in complete sterilization of cerebrospinal fluid and unquestionable improvement of child health. Patients with suppurative bacterial infections caused by multidrug-resistant bacteria of different species
were treated with specific bacteriophages by Weber-Dabrowska et al. (2000). Bacteriophage therapy was highly effective and full recovery was noted in 1123 cases (85.91%). In 134 cases, 10.9% transient improvement was observed and only in 50 cases, 3.8% bacteriophage treatment found to be ineffective. The results confirm the high effectiveness of bacteriophage therapy in combating bacterial infections, which do not respond to treatment with the available antibiotics.

Biswas et al. (2002) used bacteriophage therapy for the treatment of colonization of gastrointestinal tract with vancomycin resistant *Enterococcus faecium* (VRE). The phage strain used in the study has lytic activity against a wide range of clinical isolates of VRE. One of the VRE strains was used to induce bacteria in mice by intraperitoneal injecting of 10⁹ cfu. The resulting bacterium was total within 48 hr. A single intraperitoneal injection of 3x10⁸ pfu of the phage strain, administered 45 min after the bacterial challenge, was sufficient to rescue 100% of the animals. The ability of this phage to rescue bacteremia mice was demonstrated to be due to the functional capabilities of the phage and not to a nonspecific immune effect.

Bull et al. (2002) have developed quantitative microbiological procedures that explore the *in vivo* process responsible for the efficacy of phage and antibiotic treatment protocols in experimental infections (the resistance competition assay or RCA) and examined the therapeutic potential of phages *in vitro* (the phage replications assay or PRA).
Matsuzaki et al. (2003) have studied the protective effects of bacteriophages used against experimental *S. aureus* infection in mice. Intraperitonal injections (8x10^8 cells) of *S. aureus*, including methicillin-resistant bacteria, caused bactermia and eventual death in mice. In contrast, subsequent intraperitonal administration of purified phage suppressed of *S. aureus* induced lethality. Inoculation with high dose phage alone produced no adverse effects attributable to the phage. These results uphold the efficacy of phage therapy against pernicious *S. aureus* infections in humans.

Walter (2003) reported that, bacteriophages may help reduce risk from anthrax spores. Dose response studies demonstrated that higher concentrations or mixed *Bacillus anthrax* bacteriophages (3.5x10^8 pfu/ml) inhibited subsequent growth of bacteria when sprayed on *B. anthrax* spores. Phage therapy approach against *E. coli* diarrhea hinger on the *in vivo* phage susceptibility of the injecting pathogenic *E. coli* strains (Sandra et al., 2004). Sandra et al. (2004) were successful in phage therapy approach against *E. coli* diarrhea which hinges on the injected pathogenic *E. coli* strains. Wills et al., (2005) reported successful phage therapy against wound infection caused by *S. aureus* in rabbit.