

Bacteriophage Structure, Classification, Assembly and Phage Therapy

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Current emergence of multidrug resistance and limitations in the development of the new antibiotics has proposed the problem of treating bacterial infections more challenging. This scenario may lead to the fear of failure in treating the multidrug resistant (MDR) bacterial infections and fuelled the uses of bacteriophages as an alternative of the conventional antibiotics in the post antibiotic era. So it is very much essential to know about the details of phage life cycle, assembly of phage complete structure, configuration and function of phage associated proteins etc. Although phages have been discovered a century ago, detailed study about lytic phages are gaining more interest in global fight against MDR bacterial species. This review has highlighted the basic knowledge of bacteriophage with the past and present scenario of several clinical studies targeting the MDR bacterial species. On the other hand it also discussed about the other uses of phages except human clinical trials.

Keywords: Assembly; Bacteriophage; Classification; Multidrug Resistance; Phage Therapy.

The crisis in clinical care imposed by multi-drug resistant (MDR) bacterial species causes a major concern in healthcare. The situation is particularly acute for rising of MDR pathogens for which few antibiotics are using currently (Gibson *et al.* 2019). The mechanisms that are responsible for the evolution of bacteria include interconnected travel, over use of antibiotics, transduction, mutations and conjugations. These suggest that the bacteria are very much flexible to embrace the newly discovered antibiotics within a short period of time.

Fortunately, “Bacteriophage” a natural alternative of chemical antibiotics has achieved an era of interests to overcome this situation (Stearns

2020). The name bacteriophage was derived from ‘bacteria’ and the Greek term ‘Phagein’ which means “eat” came up with Felix d’Herelle (1917). Approximately 1031 bacteriophage particles are present globally and 4-50 % of the produced bacterial populations are killed every day by bacteriophages (Wommack *et al.* 2009). The fact that unlike antibiotics, phages can destroy target bacteria with specificity and has a self-limiting infection in the presence of these targets. This is essential for the application of phages to inhibit specific bacterial growth. It can be a natural, nontoxic and active substitute for antibiotic therapy. Phages multiply exponentially only in the presence of the target host without

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disturbing the host's normal flora. In the view of the massive rise in antibiotic resistance in several clinically important bacterial species, uses of lytic phages are gaining more and more attentions as a therapeutic alternative against infectious diseases. The objective of this review is to explore the fundamental knowledge of phage biology with a brief discussion on the impact of uses on human health.

Biology of the Bacteriophage

Bacteriophages or phages are the natural viral entities that can infect target bacteria and they are supposed to be the most abundant organism on earth. They are classified on the basis of their morphology and the genetic materials. Most of the phages are tailed phage with dsDNA which belongs to the order of Caudovirales. Chromosome of the tailed phages packaged into procapsid by similar DNA translocase molecular motors but the strategy of DNA replication and resulted genome end are not alike (Casjens and Gilcrease 2009). Adsorption of the phages depends on the nature and structure peculiarities of receptor on the host cell surface. Based on the life cycle, phages are mainly categorized into virulent and temperate phages. Virulent phages follow the lytic life cycle. But temperate phages initiate with the lysogenic cycle and occasionally turned into the lytic cycle under specific conditions. Holin and lysine are the two key proteins used by the lytic phages to destroy their host cells.

Bacteriophage Classifications

About 96% of the reported bacteriophages belong to the Caudovirales order, which includes three families: Myoviridae that have contractile tail, Podoviridae that have short tail and Siphoviridae have non-contractile long tail (Kaliniene *et al.* 2017). Filamentous, cubic, and polymorphic phages belong to the same order and are classified into 10 small families which comprise of about 3.6% of the existing bacteriophages. Table 1 shows examples of phage morphologies (Ackermann 2009).

Phage Life Cycle

The first stage in infection is the adsorption of the phage to the host cell surface receptor such as lipopolysaccharide (LPS), outer membrane proteins, flagella or fimbriae. This reversible adsorption process is facilitated by tail fibers or tail spikes of phages. Some of these

fibers have enzymatic activity and are able to depolymerize the polysaccharides of the bacterial cell wall (Prokhorov *et al.* 2017). This reversible binding is followed by a base plate structural re-arrangement that triggers the contraction of tail-fibers. Binding becomes irreversible once the tail-fibers contract and bend toward the host cell to inject the virion genome into the cell cytoplasm. The ejection of the phage nucleic acid starts with the penetration of the tail tube into the host cell periplasm. During penetration, the virus uses a variety of lysins and carbohydrate de-polymerases. A transmembrane channel for phage DNA delivery is formed after the tail-fiber fuses with the host cytoplasmic membrane. Phage DNA is then ejected into host cytoplasm through this channel.

Bacteriophages possess several lifecycles; lysogenic or temperate, lytic or virulent, chronic and pseudolysogenic. In case of the lytic lifecycle, after infection, phage rapidly replicates and ends with the release of newly formed phage particles. Lytic infection effects in clear plaques on the host bacterial lawns. Temperate phages possess both lytic and lysogenic life cycle. In the lysogenic life cycle, phage DNA is incorporated into the bacterial chromosome by site-specific recombination or transposition which is called as prophage. In the integrated condition host cell can get new or additional properties upon expression of genes which is known as lysogenic conversion. For example, the cholera and shiga toxins are usually carried out and transported by prophage (Nanda *et al.* 2015). The prophages can be stable for many generations till environmental or physiological conditions trigger to the lytic lifecycle by induction process. As for example controlling of lysogeny and switching to lytic mode in λ phage is mediated by *cl* and *cro* genes (Court *et al.* 2007). Pseudolysogeny is the result of a successful adsorption and genome transfer event of phages followed by non-replicative and non-productive state in adverse condition.

Chronic infection has been detected in some filamentous phages and archaeal viruses. In this case, progeny virions are slowly and continuously released from the cell surface rather than release as single burst phenomenon.

Lysogenic phages incorporate their nucleic acid into the chromosome of the host cell and replicate with it as a unit without destroying the cell.

Phage structure and assembly

Different bacteriophages have different sizes and shapes. Most well studied belong to the dsDNA containing phages. The tail phages have three major parts; a capsid that filled with genome, a tail serve as a pipe for injection of genome to the host cell and a distinct adsorption device at the end of the tail which recognizes the host cell receptor. Assembly of dsDNA phages follow several steps: a. Assembly of prohead which is composed of capsid proteins filled with scaffolding proteins and portal proteins, b. Packaging of DNA, c. Maturation of proheads to mature heads, d. Connection of the neck and tail proteins.

Capsid organization

The mature infective phages have multiple factors including capsid, portal protein, scaffolds, terminase with other essential proteins. The capsids often have the structure of icosahedral symmetry (Tavares 2018). Different copies of capsid protein are usually gathered into icosahedral shells. A minimum 60 copies of the capsid protein of identical environment is necessary to make an icosahedral particle. The number of the copies of the same protein within the independent part of the icosahedral lattice is represented by the triangulation number T . For example if $T=3$, virus has 180 subunits. Total number of viral capsid protein is described by $T \times 60$.

Procapsid formation

The capsid where the DNA to be packaged is called procapsid or prohead. A capsid protein, with the help of scaffold protein forms the correct icosahedral geometry of capsid shell. Local concentration of scaffolding protein are primarily increased by the phage scaffolding protein that results into an entropic sink which promote association of coat proteins (Fane and Prevelige 2003).

In the phages, multi protein connector complex replace the fivefold vertices of the capsid (HTI, head-to-tail interface) comprising of portal protein and head completion protein. Phage genome is kept inside the capsid under high pressure by the control of HTT which serve as valve for closing and opening the channel (Tavares 2018). Portal protein is also involved into the entry and release of DNA along with the attachment of the neck protein to the head. Monomers of the different portal proteins vary in sizes and characterized by four domains:

crow, clip, stem and wing. DNA packaging is facilitated by the clip domain that helps binding to the terminase. This domain is connected to the wing region by a stem that includes basically the outer loops and two α -helices. The wing domain acts as a spine of the wing. Head assembly may be started from the portal tip by polymerization of the capsid and scaffolding proteins.

Capsid maturation

The scaffolding domain is cleaved off as soon as the procapsid is assembled, and removed from the capsid to make space for the genome. In lambda phage scaffolding protein exits from the capsid with the interaction of DNA, as lambda phage protease is genetically inactive. Release of scaffolding protein and starting of DNA packaging initiate the head maturation. It involves a large structural transition of prohead which results into the more stable bigger thinner shell head. During maturation, prohead can dissociate into subunits which allow proofreading and connection of misassembled intermediates. Many phages incorporate minor and pilot proteins into the head in the course of the head assembly with low copy numbers which are not essential for structure formation but crucial for virion infectivity. In phage P22 there are three minor proteins which localize in the shaft above the connector (Lander *et al.* 2006) T7 phages have an intricate shaft structure (inner core) which forms the capsid and consists of a protein ring with twelve fold symmetry. The release of terminase after DNA packaging mainly depends on the difference in the core structure.

Capsid assembly completion

After package of the DNA into the head, the terminase complex is exchanged by neck protein and forms a gatekeeper complex together with connector which prevents the leakage of the mature DNA from the head and induces the exit of DNA upon attachment to the host. There are two types of neck proteins in Siphoviridae and Myoviridae phages, each make a ring below the portal protein. In SPP1 and HK97 like phages have the same ring closet structure to the connector but different fold presents in case of phage lambda whereas the second ring have the similar fold in SPP1 and lambda (Cardarelli *et al.* 2010).














Phage DNA packaging

Chromosome of tailed phages packaged into a procapsid by the way of DNA translocation

but the strategy of DNA replication and the nature of packaged DNA ends are not same. Different types of characterized termini are (i) direct repeats with circular permutation at the terminal (ii) cohesive single-stranded ends (iii) direct terminal repeats with exact (non-permuted) long, several thousand base pairs, (iv) direct terminal repeats with exact (non-permuted) short, several hundred base pairs, (v) host DNA sequences at the terminal end, and (vi) terminal proteins bound covalently. Circular or replicating concatamer generates five of the above six types of virion chromosome ends by nucleolytic cleavage. DNA molecules replicate as monomeric linear molecules only in the case

of phage genomes with terminal proteins. Phage-encoded enzyme called “terminase” perform these cleavages that are tightly coupled in all cases to the DNA packaging process. Those packaging mechanisms are followed by two steps- In first event terminase cleaves at pac site (Headful packaging phage) or cos site (cohesive end phages) and the resulted DNA ends threaded into viral procapsid unidirectionally. There after a second nucleolytic cut (headful cleavage) occurred when DNA has filled the procapsid which results in the release of the packaged DNA from concatamer. Then second packaging event take place on that concatamer by inserting the unpackaged concatamer end in to a

Table 1. Classification of Bacteriophages

Family	Morphology	Nucleic acid	Characteristic
<i>Myoviridae</i>		Linear dsDNA	contractile tail, Non-enveloped
<i>Siphoviridae</i>		Linear dsDNA	Long non-contractile tail, Non-enveloped
<i>Podoviridae</i>		Linear dsDNA	Short non-contractile, Non-enveloped tail
<i>Tectiviridae</i>		Linear dsDNA	Isometric , Non-enveloped
<i>Corticoviridae</i>		Circular dsDNA	Isometric, Non-enveloped,
<i>Lipothrixviridae</i>		Linear dsDNA	rod-shaped, Enveloped
<i>Plasmaviridae</i>		Circular dsDNA	Pleomorphic, Enveloped
<i>Rudiviridae</i>		Linear dsDNA	Rod-shaped , Enveloped
<i>Fuselloviridae</i>		Circular dsDNA	lemon shaped , Non-enveloped
<i>Inoviridae</i>		Circular ssDNA	Filamentous, Non-enveloped
<i>Microviridae</i>		Circular ssDNA	Isometric, Non-enveloped
<i>Leviviridae</i>		Linear ssDNA	Isometric, Non-enveloped
<i>Cystoviridae</i>		Segmented dsDNA	Spherical , Enveloped,

new procapsid. As like the first one the second event is terminated. Such unidirectional event is usually two five packaging event long. As per the packaging event the DNA of the tailed phages

should be linear ds DNA because the threaded of the DNA into virion and release out from the virion is mediated by a very narrow portal passage which may not allow two parallel ds DNA simultaneously

Table 2. Key Features and Deeds of Phage Therapy Centers

Name of center	Country	Main features and deeds
Center for Phage Therapy	Poland	1,500 patients with supportive bacterial infections have been cured by specific bacteriophages, from 1980, routine antibiotic therapy in everyplace has unsuccessful (http://www.iitd.pan.wroc.pl).
Eliava Phage Therapy Centre	Georgia	bacteriophage preparations have been established by a group of eight laboratories for fighting against perilous and antibiotic-resistant superbugs (http://www.phagetherapy.com).
Novomed	Georgia	Not only the local Georgians avail the treatments, but also to foreign patients with chronic wounds and other types of acute infections. (http://www.mrsaphages.com).
Phage Therapy Centre	Georgia	Provides exceptional treatment for patients having bacterial infections and chronic, drug-resistant or not responded to conventional antibiotic therapies (http://www.phagetherapycenter.com).

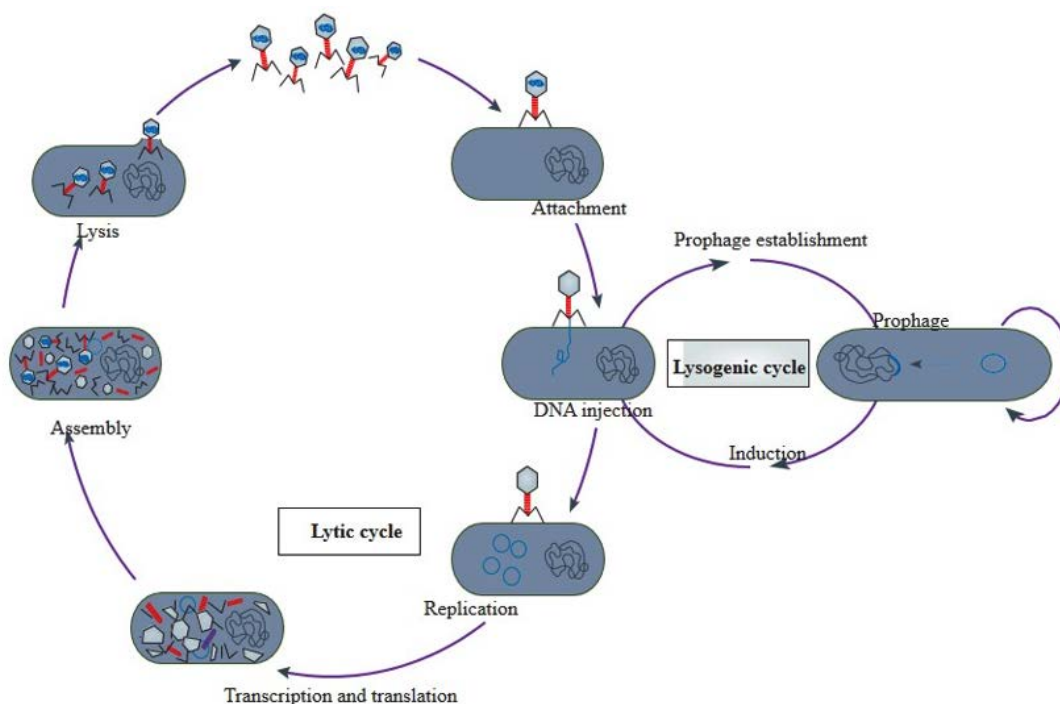


Fig. 1. Phages have two distinct life cycles. These are the lytic cycle and the lysogenic cycle. Lytic phages take over the machinery of the cell to make phage components. They then destroy, or lyse, the cell, releasing new phage particles.

Table 3. Case Study of Phage Therapy

Sr. No	Infection	Complicating Conditions	Antibiotic Resistance	Phage Dose and Application	Duration of Phage Treatment	Outcome
1	<i>Pseudomonas aeruginosa</i> bacteremia	DiGeorge syndrome, congenital heart disease with pacemaker	Meropenem, tobramycin, aztreonam, polymyxin B, colistin, Cephalosporins, and fluoroquinolones	3.5x10 ⁸ PFU delivered intravenously every 6 h.	Initial treatment for 36 hours (six doses total), treatment resumed 11 days later	Blood cultures negative after phage treatment; returned to positive following termination of phage administration Clinical improvement of infection
2	<i>Acinetobacter baumannii</i> infected pseudocyst (3 months)	Necrotizing pancreatitis	Cephalosporins, meropenem, gentamicin, amikacin, trimethoprim-sulfamethoxazole, tetracycline, ciprofloxacin, and colistin Ciprofloxacin	5 x 10 ⁹ PFU delivered intravenously every 6 h	84 days (336 doses total), minocycline added on day 2	four weeks post treatment Cultures negative; no relapse of infection after >2 years
3	<i>Pseudomonas aeruginosa</i> infected aortic graft (3 years)	Aorto-cutaneous fistula	Ciprofloxacin	1 x 10 ⁸ PFU topically delivered on fistula	Single dose	No effect (Only two doses administered prior to expiring)
4	<i>Burkholderia cenocepacia</i>	Cystic fibrosis s/p bilateral lung transplant Post-transplant pneumonia and sepsis	Ceftazidime, Amoxicillin, Tetracycline, Cethromycin, Sulfomethoxazole	One phage treatment comprising "1" phage	2 doses	Negative Blood cultures but not durable
5	<i>Pseudomonas aeruginosa</i>	Left ventricular assist device infection and septicemia Post-surgical knee wound infection	Cephalosporin, Penicillin, Carbapene, Polymyxin B, Tobramycin, Levofloxacin Carbapenem, Cephalospori, Quinolones, Ciprofloxaci, Tigecycline Polymyxin B	One phage treatment comprising "3" phages	6 weeks	Negative blood cultures
6	<i>Klebsiella pneumoniae</i> and <i>Acinetobacter baumannii</i>			<i>K. pneumoniae</i> phage treatment comprising 1 phage <i>A. Baumannii</i> two treatments each comprising 1 phage	2 weeks	
7	<i>Burkholderia dolosa</i>	Cystic fibrosis s/p bilateral lung transplant	Tobramycin, trimethoprim, Ceftazidime, Amoxicillin, Sulfomethoxazole	One phage treatment comprising 1 phage	5 weeks	Negative blood and BAL cultures
8	ESBL <i>E. coli</i>	Bacteremia/ urosepsis s/p kidney transplant	Penicillin, amikacin	One phage treatment comprising 2 phages	2 weeks	Negative blood and urine cultures

Table 4. Clinical trial of Phage Therapy

Trial	Infection	Treatment Group	Phage Dose and Application	Outcome
1	<i>Pseudomonas aeruginosa</i> otitis	12 individuals had received phage cocktail	10 ⁹ PFU was intra-aurally delivered (single dose)	From each group three individuals found to have undetectable levels of <i>P. aeruginosa</i> at the finale of the trial
2	<i>Escherichia coli</i> diarrheal diseases	40 individuals had received phage cocktail M, 39 individuals had received phage cocktail T	1.4 × 10 ⁹ PFU cocktail M or 3.6 × 10 ⁸ PFU cocktail T delivered orally three times per day for 4 days (12 doses) in oral rehydration solution	No substantial difference observed between placebo group and phage treatment group
3	<i>Pseudomonas aeruginosa</i> burn wound infection	12 individuals received a phage cocktail	2 × 10 ⁷ PFU (expected) 200–2,000 PFU (actual) topically applied one time per day upto 7 days (seven doses)	Trial halted, insufficient efficacy; may be due to considerably lower applied dose of phage than estimated
4	Several multidrug-resistant bacteria	157 patients was orally, intrarectally or intravaginally administered to Hirszfeld Institute phage collection	10- 20 ml of phage collection were administered thrice daily for 12 weeks.	No antagonistic events; phage therapy provided good response up to 40% rate
5	<i>Escherichia coli</i> ; <i>Staphylococcus aureus</i> ; <i>Pseudomonas aeruginosa</i>	Intralytix phage cocktail WPP-201 were applied on 65 patients	1 × 10 ⁹ Phage cocktail were topically applied on wound infection once a week for 12 weeks	Different wound healing rates with time (differential wound size reduction over time)

(for circular chromosome)(Casjens and Gilcrease 2009). Understanding the actual nature of the linear viral DNA, further studies are required.

Cohesive end

Phage λ , HK 97 are the well-studied COS phages. Both ends of the cohesive termini contain 7-19 nucleotides long protruding single strand phage chromosome which are complementary to each other by sequence. DNA ligase anneals the ends to each other and each strand is closed to form covalently – closed circular molecules which serve as template for DNA replication after injection. Sequence specific, staggered cuts in the two DNA strand on a concatamer are made by terminase to generate the right end of the one chromosome and left of the subsequent chromosome to be packaged. Thus all the individuals of the COS phage having cohesive end termini are present on the genome sequence at identical location (Casjens and Gilcrease 2009).

Head full packaging

P1, P22, SPP1, T1 and T4 phages contain circularly permuted and terminally redundant chromosomes which called “Headful packaging” phage. For phage P22, a sequence specific cleavage is took place by terminase at pac site on the replicated concatamer to initiate packaging series when procapsid is filled by DNA (Weigele *et al.* 2005). Upon infection the direct terminal repeats of the end goes for homologous recombination to generate the circular genome template for DNA replication. Cohesive ends are not present at headful packaging phages because it is assumed that, as they are able to ligate to the other blunt ended DNA, the DNA ends are usually blunt.

Short exact repeats

Phages like T3 and T7 have direct double stranded repeats of several hundred base pair long same nucleotide at the termini of every virion chromosome (non-permuted). Duplication of the direct repeat DNA in concert with packaging produces the terminal repeats.

Long exact repeats

The phage like T5 and SP01 has several hundred base pair precise direct terminal repeats at the ends (Wang *et al.* 2005). How these long repeats are generated, the exact mechanism though is not yet cleared fully but phages appear to be only one copy of the terminal between genomes in replication generated concatamers with the

exact short repeats, which suggest that during packaging repeat region is duplicated. The best way to estimate the position of the ends of such molecule on the sequence, restriction mapping are the best way to locate.

Host DNA at ends

Phage Mu replicates their genomes into host DNA by duplicative transposition, which results into the packaging of viral genome that is integrated into host DNA randomly. Terminase recognizes a pac site which is located near one end of the genome and DNA Packaging event is initiated by cutting the nearby host DNA (Groenen and van de Putte 1985).

Covalently bound terminal protein

Phages like ϕ 29 have covalently bound protein at the end of their virion and replicative chromosome. Terminal fragments of these type of DNA shows abnormal slow migration in electrophoresis gel while protease treatment restore the normal movement (Salas *et al.* 1978).

Tail assembly

The organization of the tail among the phages is different like; Podoviridae have very short tail, Siphoviridae have long non-contractile tail and Myoviridae have rigid long contractile tail. After the completion of the head assembly, the tail proteins are sequentially attached to the capsid. In Siphoviridae and Myoviridae phages, the tail is linked to the head via neck protein where in case of T4 phages attachment of the tail is followed by the preassembled fiber (Rossmann *et al.* 2004). Head and tail assembly occurred independently in a strict order of association of protein. There are different mechanisms which control that type of sequential attachment. Conformational switching is one of the most common mechanisms among them. The viral capsid with the T number higher than one showed the conformational switching when several quasivalent conformations are taken by the capsid protein. The initiator complex starts the assembly of tail in Siphoviridae and Myoviridae phages that forms the absorption at the distal end of the phage. six to eight proteins usually forms the long initiator complex in Siphoviridae and Myoviridae respectively. Base plate initiates the polymerization of the cylindrical section of the tail during the tail assembly. The cylinder of the Siphoviridae is composed of multiple tail tube components. Lambda tail tube protein gpV cannot form a tubular

structure without the initiator complex and exist as a monomer. The tail tube protein of Myoviridae phage does not assemble into tubes as like lambda gpV without the base plate.

The tail assembly is completed after polymerization of the cylindrical part of the tail by the binding of the terminator proteins. The neck protein then interacts with the terminator proteins and induced the association of head to the tail. Functional and structure comparison of neck concludes that tail completion proteins and tail tube develop from a single ancestral gene. Tail tape measure proteins define the length of the tail.

Assembly completion

Assembly process of Siphoviridae and Myoviridae are completed by the spontaneous joining of head and tail whereas binding of gpw to the neck region forming whiskers prior to head to tail association in case of T4. For the attachment of the preassembled tail fibers along with tail at the proximal part of the base plate whiskers are very much necessary. In most of the Siphoviridae phages distal tail protein (DTP) form an oligomeric ring which is attached to the tail tube (Veesler and Cambillau 2011). Recognition and attachment to receptor binding proteins are served by DTP, sometimes in T5, T4 and other phages tail fibres assisted this interaction. Some of the Gram-negative bacteria specific phages have lysozyme at the end of their tails and digest the peptidoglycan barrier after entry into the periplasm.

Cell Lysis

The last event of the infection cycle is the lysis of the bacterial cells of a lytic phage. The time between the infection and production of the progeny is called the latent period. Optimal time of the lysis depends upon the quality (physiological state) and quantity (density) of the host bacteria. The optimum latent period is shorter under the highest host quality or quantity. Phages have to overcome several host barriers to release the accumulated virion particles in the environment, later which infect the other available host. The cytoplasmic membrane, cell wall and in case of Gram negative bacteria, the outer membrane are the barriers. Phages have evolved several strategies to overcome those barriers. Phages (dsDNA) encode holin protein which forms lesion in the cell membrane. Another phage coded protein endolysin (muralytic enzymes) is able to attack the cell wall as

a result of the disruption of the cell membrane which results in the lysis of the host cell for the release of the virion particles (Catalao *et al.* 2013). Among the well-studied lytic phages the holin-endolysin system is very common but not found in ssDNA or ssRNA phages.

Identification of the Host Cell Surface Receptor

Identification of host cell surface receptor is the crucial for the adsorption which confers the specificity of the host among the phages. Such specificity is dependent on the nature and structural particularities of receptors on bacterial cell surface (Rakhuba *et al.* 2010). Host cell surface of Gram negative bacteria is characterized by complex lipopolysaccharide (LPS) and protein structure. Out of them either LPS or protein served as a receptor. In some cases LPS and protein part both may serve as receptor.

Phage Therapy

Emergence of antimicrobial resistance and limitations in development of new antibiotics is the biggest threat to global health. WHO in 2019 stated that the increasing rate of MDR bacterial species requires serious actions all over government sector and society (Organization 2019). Estimated MDR bacterial species could kill more than 10 million people per year. So in this situation finding out of an alternative to antibiotics is too much essential. Recently Govt. of India has launched a programme "Antimicrobial Resistance Hub" in 2019 to combat those MDR pathogens which give priority on the use of phages as a therapeutic agent. Bacteriophages are the natural predator of the bacteria found ubiquitously in nature which are non-toxic and host specific. The multidrug resistant (MDR) bacterial species that cause clinically relevant bacterial infections can be destroyed by the administration of the bacteriophages. This is called phage therapy.

Although early report of phage therapy, the introduction of antibiotics affected primarily but in the post antibiotic era, well-deserved rebirth is emerging. Some organizations which are devoted to phage therapy are Microbiology and virology (Georgia), Eliava phage therapy centre, Gangagan (India), Institute of immunology and experimental therapy (Poland), Intralytix (USA) and so on.

Early Phage Therapy

Bacteriophages from human faces was first isolated and used them to kill bacteria

including *Shigella* spp. by Felix d'Herelle and his co-workers in 1917 under laboratory condition and they developed the idea of "phage therapy" (d'Herelle, 1917). In 1921 patients suffering from dysentery at Paris were cured by oral phage applications within one day. In Alexandria patients suffering from bubonic plague were allowed for phage treatment by injection of phages directly to the lymph nodes, the site of infection. In 1927 during cholera epidemic in India the mortality rate of the phage treated patients was reduced to 8% from 68%. Phage therapy was massively implanted on the Soviet soldiers during the winter war between Soviet Union and Russia (1939-1940) against open wound with Staphylococcal or Streptococcal infection which prevent amputation. Staphylococcal or Streptococcal phages with or without variety of phages including Salmonella phage, *Shigella* phage, *E. coli* phage, P.aeruginosaphage were used to treat the wounds which results in the reduction of death rate by Gangrene. There are several organizations which currently use phages as therapeutic agent (Table 2).

Current Phage Therapy

The effects of phage therapy are continuously publishing by the Russia, Poland and Eliava institutes and that helped to keep this approaches popular. The unanswered question of phage therapy is now gradually addressed resulting in the phage therapy gaining more popular and interesting. Phages have been administered topically, orally (tablets or liquid), intravenously and rectally for more than 90 years in Eastern Europe without serious side effects. Phage therapy are now offered by several clinics in different countries to treat diseases (Table 3 and 4) (Duplessis CA *et al.* 2019; Kortright *et al.* 2019). Other Uses of Phages

Apart from the alternative of antibiotics bacteriophages are also being used in different sectors such as livestock and food industries (Polaska and Sokolowska 2019).

Livestock

Salmonella: A cocktail of four phages were used in a boiler chick model to reduce the load of the *Salmonella* spp. which results in the reduction of $1 \log_{10}$ colony forming unit (CFU) after 14 days of administration. Saez *et al.* reported that administration of phages reduce the colonization

and shedding of *Salmonella* in pigs (Saez *et al.* 2011).

Colibacillosis: Spray of two high titer phages reduce the colibacilli associated mortality in poultry (Huff *et al.* 2002).

Campylobacteriosis: Wagenaar *et al.* reports that administration of a phage preparation by oral gavage could protect 10 days old Ross broiler chick and adult chickens from campylobacteriosis caused by *Campylobacter jejuni*. In another report *C. jejuni* count in the ceca and upper intestine of phage treated birds were reduced between 0.5 and 5 \log_{10} CFU/g (Loc Carrillo *et al.* 2005).

Clostridiosis: In a study with 900 birds showed that cocktail of five phages (105 PFU/ml) administration by oral gavage or drinking water against *Clostridium perfringens* infection resulted in a 92% mortality reduction than untreated control group (Miller *et al.* 2010).

CONCLUSION

Recently the emergence of multidrug resistant bacteria is a worldwide problem. Limitations in the development of new antibiotics made the problem more challenging. In this scenario bacteriophages are the promising therapeutic tools alternative to the antibiotics. For that, knowledge about the bacteriophage structure, assembly, phage packaging and phage genetics are very much essential. In this review, this particular aspect has been described in detail. Genetically modified or cocktail/combination of phages are being used to treat the variety of bacterial infections that are resistant to the latest used antibiotics. Several case studies have been carried out where microbiological eradication of the targeted organisms found after the administration of phages. Pathogens of the intensively-reared livestock also have been reduced after the uses of phages. However we cannot conclude that phages are appropriate to clear all the infections and curing of the clinical syndrome. Therefore, more clinical trials with several studies are needed to address the several issues coupled with the phage therapy such as evolutionary strategy, mosaic architecture and involvement of any phage gene in host pathogenesis. Lastly, the promising result of the previous study indicates that phage therapy may be a future remedy against MDR bacterial species.

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Conflicts of interest

Author declares that she have no conflict of interest

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