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Classification and Replication Mechanism of *Staphylococcus* Phage

Farajollah Maleki[1], Mohamad Hosein Hadadi[1], Fatemeh Rezaei[1], Hasan Reza Mohamadi[2], Afra Khosravi[1] and Ahmad Nasser[1]*

[1]Clinical Microbiology Research Center, Ilam University of Medical Science, Ilam, Iran
[2]Assistant of Professor, Faculty of Neurosurgeon,
Shahid Beheshti University of Medical Science, Tehran, Iran

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The use of antibiotics is causing high resistance in bacterial cell. One of the major pathogen that cause of dangerous infection is *staphylococcus aureus*, these bacteria can urgently resistance to new generation of antibiotic such methicillin. Hence recognize the bacterial genome and mobile element such lysogenic phage and understanding the pathogenesis of pathogen is important. In this review we investigated the Staphylococcus aureus phages and their role in virulence gene transfer.

Key word: Phage, classification of Phage, staphylococcus Phage

Staphylococcus aureus is a Gram-positive bacterium that causes a serious infection such as, pneumonia[1], bovine mastitis, morbidity and mortality to humans[2]. *S. aureus* are resistant to multiple antibiotics and reports that some had acquired high level to new antibiotic generation such vancomycin[3-4]. *S.aureus* encoded virulence factors such a coagulase[5], accessory gene regulator A (agrA), staphylococcal protein A(spa) and sarA to the development of disease[6]. Staphylococci are classified via different methods, one of the major categories on the basis of coagulase. Coagulase is a virulence factor that causes blood clotting in the proximity of the pathogen bacteria (see figure 1)[7].

Methicillin-resistant *S. aureus* (MRSA) are more challenging because is resistance to commonly used antibacterial agents. The mecA gene is liable for the increased antibiotic resistance of MRSA and encodes PBP2a, which is a penicillin-binding protein with low-binding affinity and which mediates methicillin resistance[8].

Phage

Phages are kind of viruses that attack and destroy of the bacteria. There are at least 12 distinct groups of phages and each phage is specific to its bacterial host. The morphology and genetic material (DNA or RNA) varies according to the phage species. Phage are commonly find in the environment: there are more than 10 [10]phage per liter of surface seawater[9]. phage have been characterized by their host range and the physical characteristics of the free virion, including capsid size, resistance to organic solvents, shape, structure and genome size and type such as single-stranded DNA (ssDNA), ssRNA, double-stranded (dsDNA) and dsRNA [10]. phages are known to augment wherever their bacterial hosts exist[11] but the phage can exist freely outside the bacterial host, however all phages like mostly virus are obligate intracellular parasites and need their host to propagate[12]. The International Committee for Taxonomy of Viruses (ICTV) require phage capsid

* To whom all correspondence should be addressed.
E-mail: a.nasseri27@gmail.com

morphology to be established for their formal classification[10] but the phage may exist as a lysogenic prophage that does not produce mature virions[13]. Bacterial classiffication easily done by examining the con-served 16S ribosomal genes[14] but phage lack ribosomal DNA and there are no conserved gene common to all phage on which to base a classification[10]. According to the ICTV system, bacteriophages are classified into one order, *Caudovirales* which consists of three related families. Phage virions can be tailed, filamentous, polyhedral and pleomorphic and most of them contain dsDNA[15].

Life cycle of bacteriophage

Phages can be categorized into Lytic and Lysogenic phages. This is specified by the events that follow injection of nucleic acid into the bacterial cell. Lytic phages usually lead to the release of phage through the host cell bursts[16]. Lysogenic phages have two pathways: first they have ability to undergo lysis in their host cell, where by their new generation of phage is released into the environment. However, they can establish stable relationships with the host in which lytic genes are not expressed and their genome becomes integrated into the bacterial chromosome, and is replicated along with the host cell DNA[17]. Phage can also constitute major vehicles for horizontal gene transfer[18]. Phage also has a major role in virulence by encoding numerous virulence factors and by their movements within genomes[19].

However temperate phages have an alternative life-cycle that is absent from the reproduction of lytic bacteriophages, whereby the bacterial host haven the phage genome and then replicates it during bacterial cell division. This phage genome in host's chromosome expresses resistance to infection by the same phage, but not to infection by heterologous phages[20]. During lysogeny, which follows recombination of the phage genome into the bacterial chromosome; most of the phage genes are repressed[21]. In some prophages with low G+C, phage conversion genes proposed virulence and fitness genes[22]. Lysogeny is not a constant state and during bacterial growth phage arises due to spontaneous prophage

Table 1. *Staphylococcus aureus* phages studied and classified

Family	Author- year	Title	Conclusion
Siphoviridae	Aswani VH et al. 2014	Complete Genome Sequence of a Staphylococcus epidermidis Bacteriophage Isolated from the Anterior Nares of Humans [33]	By examining the entire genome of the phage was observed that the phage has an icosahedral capsid and unusually long non-contractile tail.
	Hongying Jia et al. 2013	Complete Genome Sequence of Staphylococcus aureus Siphovirus Phage JS01 [34]	Isolated phage from milk and use the TEM showed that the phage has a long non-contractile tail and an icosahedral head.
Podoviridae	Swift SM et al. 2014	Complete Genome Sequence of Staphylococcus aureus Phage GRCS [35]	By examining the hole genome of the phage suggested that these could be used as phage-therapy.
myoviridae	Leila Kvachadze *et al.* 2014	Evaluation of lytic activity of staphylococcal bacteriophage Sb-1 against freshly isolated clinical pathogens [36]	The Phage Sb-1 has high spectrum of lytic activity against staphylococcus.
	Katrien Vandersteegen *et al*. 2013	Romulus and Remus, Two Phage Isolates Representing a Distinct Clade within the Twortlikevirus Genus, Display Suitable Properties for Phage Therapy Applications [37]	Two phages have a lytic activity against 70% of staphylococcus aureus isolates and both phage shown biofilm-degrading capacity.

induction. The condition that which prophages enter the lytic cycle depend on chemical or physical agents that damage DNA, including oxidants, antibiotics like mitomycin C, and UV radiation can induce prophage entry into the lytic cycle[23]. The first unit of the prophage is the leftward-transcribed integrase/cI region for the maintenance of phage in lysogeny phase. A large rightward-transcribed region encoding proteins for the lytic pathway including replication, head/tail morphogenesis, packaging and lysis functions is followed by a leftward-transcribed region.

Phages have several potential conversion genes, also called cargo[24] and appears to have a set of lysins immediately followed by three leftward-transcribed genes, two of which encode a supposed membrane protein and a lysM domain-containing protein[24]. These proteins can locate on the host cell surface. Expression of these proteins during growth represents phage conversion genes. If the phage has a serine recombinase (ORF1) a repressor (ORF6) and anti-repressor (ORF7) indicating that the phage is temperate[25]. Repressor is a self-assembling dimer also known as the cI protein[26] and binds to DNA in the helix-turn-helix binding motif. cI protein regulates the transcription of the cI protein and the Cro protein. The life cycle of phages is controlled by cI and Cro proteins and phage will remain in the lysogenic state if cI proteins predominate, but can transformed into the lytic cycle if cro proteins predominate. Auto-negative regulation causes a stable minimum concentration of the repressor molecule and, if SOS signals arise, allows for more efficient prophage induction[27]. That mean In the presence of cI proteins, only the cI gene transcribed and in contrast in absence of cI protein the cro gene is transcribed.

Staphylococcus phages

Phages are widespread in *Staphylococcus aureus* genus and have been extensively studied[28]. The phage was firstly used for the typing of clinical *S. aureus* isolates[29]. The majority of S. *aureus* phages known so far are double-stranded DNA belonging to the *Siphoviridae* family of the *Caudovirales* order[30]. Staphylococcal *Siphoviridae* are composed of an icosahedral capsid and a non-contractile tail[31]. *Staphylococcal Podoviridae*, such SAP-2 phage are composed of

Table 2. Toxins and their functional status

Name of gene or toxin	Performance
sak	immune modulator
chp	chemotaxis inhibitory protein CHIP
sea	Enterotoxins (cause diarrhea, arthritis, atopic dermatitis and toxic shock) [40]
Seg, seh,sei	Enterotoxins [41]
Panton-Valentine leukocidin (PVL)	Cytotoxin [42]
eta (exfoliative toxin A)	staphylococcal scalded-skin syndrome [43]

Table 3. Classification of phage family of *Staphylococcus aureus* (all data extracted from NCBI with *Staphylococcus aureus* phage keyword)

Phage family	Author-Name of phage- journal
Siphoviridae	Deghorain M_ StB12, StB27, and StB20_ Journal of Bacteriology [46]
	Mariem BJ_ phi7401PVL_BMC Microbiology [47]
	Zhang M_φ7247PVL, φ5967PVL_FEMS Microbiology Letter [48]
	Kim MS_SA11_Journal of Virology [49]
	Diana Gutiérrez_vB_SepiS-phiIPLA5 and vB_SepiS-phiIPLA7_BMC Genomics [50]
	G.E. Christie_80 and 80φ_Virology [51]
Myoviridae	Zelin Cui_JD007_Journal of Virology [52]
	Jingmin Gu_GH15_Journal of Virology [53]
Podoviridae	Tony Kwan_3A and 26 phage_ PNAS[54]
	Son JS_SAP-2_ Appl Microbiol Biotechnol [32]

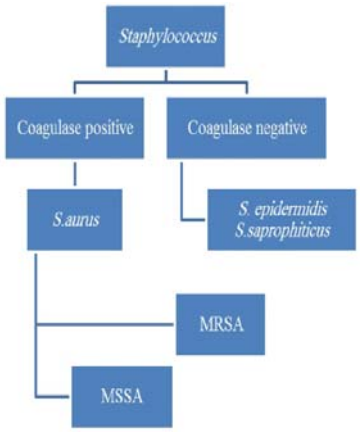

Fig. 1. Differentiation of *S. aureus* based on Coagulase test

a small icosahedral capsid, short, non-flexible and non-contractile tail[32]. Finally *Myoviridae* are characterized by an icosahedral capsid and a long contractile tail.

Virulence factor and phage

Phages encode a some of S. aureus virulence factors and variety of toxins, mainly allowing bacterial cell escaping from host immune system[38] such a widespread immune modulator staphylokinase (sak) responsible for host tissue destruction, the staphylococcal inhibitor of complement SCIN (scn) and chemotaxis inhibitory protein CHIP(chp). Phage also encode a several super-antigen such a: (sea) and (seg), these super-antigens are enterotoxins causing food poisoning, necrotizing fasciitis and toxic shock syndrome[39].(To more information see table 2)

Fig. 2. Modular organization of phage genome, if the suppressor expressed the phage remains in lysogenic phase but if de-repressor activated (SOS response or oxidative stress) phage can be activated and inter the lytic phase

Phages are carrying single virulence factor genes, however some exceptions have been reported[39] and this gene not strictly associated to a specific phage and appear to be transfer horizontally[44]. Virulence genes are often located near the attachment site (att) of the prophage in host chromosome. Phage responsible for the mobilization of *Staphylococcus aureus* Pathogenicity Islands (SaPIs), which encodes major toxin genes such as the toxic shock syndrome toxin 1 and other super-antigens[45]. SaPIs are not mobile by themselves and need the helper phage for moving. The mechanism for induction is the specific interaction of a SaPI repressor and a de-repressor encoded by the helper phage. Different proteins of a helper phage may be involved in induction of different SaPIs.

CONCLUSION

One of the major problems by modern medicine is the spread of antibiotic resistant genes among pathogenic bacteria, as is seen with methicillin resistance in the species *Staphylococcus aureus*. *Staphylococcus aureus* has an extraordinary range of virulence factors that allows it to survive extreme conditions within the human host. This bacterium has a lot of toxins that some of them are transferred by phage. Hence identification of phage types and toxins moved by their phage is important. Phages are the primary vehicles for horizontal gene transfer and mobilization of SaPIs.

Comparison of pathogen *Staphylococcus aureus* with non-toxigenic shown Phage-encoded virulence factors responsible for *S. aureus* pathogenesis are absent in non-*S.aureus*. With identify and classify the phage and how they move and change the genetic of bacterial host, their movements can be investigated. Hence phage can move the virulence factor from pathogen bacteria to non-pathogen bacteria and conversion it to pathogen bacterial cell.

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