TSP, a virulent Podovirus can control the growth of *Staphylococcus aureus* till 12 hours

Rabia Tabassum  
University of the Punjab

Iqbal Ahmed Alvi  
Hazara University

Muhammad Asif  
University of the Punjab

Abdul Basit  
University of the Punjab

Shafiq ur Rehman (✉️ shafiq.mmg@pu.edu.pk)  
Punjabi University  🌐 https://orcid.org/0000-0002-1265-3442

**Research Article**

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Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a prevailing nosocomial pathogen that causes a large number of diseases in healthcare and community settings. The MRSA causes infections in different tissues of immunocompromised individuals leading to increased morbidity and mortality. It possesses various virulence mechanisms to show resistance against a lot of beta-lactam antibiotics. To tackle this emerging issue of MRSA, there is an urgent need of antibiotic alternatives and utilizing lytic bacteriophages is one of the best promising therapeutic approaches. In the present study, a lytic bacteriophage TSP was isolated from hospital wastewater against MRSA. Its morphology, physiology, host specificity, burst size and lytic spectrum were determined and complete genome sequence was analyzed. TSP phage efficiently inhibit bacterial growth for up to 12 hours. TSP phage showed broad lytic spectrum against clinical isolates of MRSA (78%) and MSSA (37%). It showed stability at varying temperatures (25ºC, 37ºC) and pH (5–9), while its maximum storage stability was observed at 4ºC. It had short latent period (20min) and high burst size (103 PFU/infected cell). TSP genome sequence and restriction analysis revealed that its genome is linear having 17,987 bp in length with an average GC content of 29.7%. The TSP genome showed 98% similarity to *S. aureus* phages SCH1, SCH11 and vB SauP-436A1. According to comparative genomic analysis and phylogenetic tree analysis, TSP phage can be considered as a member of genus “P68viruses”. The strong lytic activity, broad host range and short latent period along with absence of any lysogenic and toxic genes make TSP a very good candidate for phage therapy against MRSA infections if proved safe during *in vivo* studies.

Introduction

Antibiotic resistance is one of the major global issues that limit effective treatments against infections with multiple drug-resistant bacteria (MDRB). Antibiotic resistance arise due to several reasons that include inappropriate and overuse of antibiotics, DNA mutations, importation of drug-resistant genes among bacteria and changes in the defense strategies of microbes [1]. One of the most important gram positive antimicrobial-resistant pathogen is *Staphylococcus aureus* that causes a large number of clinical infections including skin and soft tissue, infective endocarditis, bacteremia, device-related and respiratory infections both in nosocomial and community settings [2]. The main reason for antibiotic resistance in *S. aureus* is changes in penicillin-binding proteins, the formation of autolysin enzymes and excessive and irrational use of antibiotics in health care settings [3]. The most common antibiotic-resistant group of *S. aureus* is methicillin-resistant *Staphylococcus aureus* (MRSA) which shows resistance to a lot of beta-lactam antibiotics, however these organisms are also getting resistance to aminoglycosides, macrolides, fluoroquinolones, chloramphenicol, and tetracycline as well [4]. The constraints of effective MRSA treatment options with antibiotics frequently leads to the development of chronic infections, which not only leads to increased morbidity and mortality but also prolong hospital stays and higher health care costs as compared to methicillin-sensitive *S. aureus* (MSSA) strains [5]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become a challenging pathogen because it poses a serious threat for hospitals and the community. Therefore, it is of great concern to develop new
strategies that can supplement or replace the utility of existing antibiotics for treatment of MRSA infections.

To overcome the problem of antimicrobial resistance, different alternative strategies can be used which include use of bacteriophages, monoclonal antibodies, probiotics and antimicrobial peptides. Among all alternative approaches, bacteriophage therapy is the best alternative approach that can be used to treat multiple drug-resistant *S. aureus* infections. The properties which make bacteriophage therapy the best replacement option include safety, high specificity, and effective lytic activity against bacterial cells [6]. Compared to synthesis of new antibiotics, production of bacteriophage is cheaper and faster and they can easily proliferate at infection site with limited or no side effects [7]. *S. aureus* phages have efficient antimicrobial activity as described in various *in vitro* and *in vivo* studies [8]. The phage SLPW and CSA13 isolated from chicken and fecal sewage of pig farm showed a 90% and 92% lytic spectrum against methicillin resistant *S. aureus* strains. Both phages have a short latent period and high burst size. In addition, CSA13 successfully removed *S. aureus* biofilm [9] while SLPW showed ability to cure MRSA infection in mice [6]. Furthermore, phages S24-1 and S13 had been isolated from sewage and showed 100% and 89% lytic spectra against clinical isolates of *S. aureus* [10] respectively.

The current study describes the detailed characterization of lytic TSP phage against *S. aureus*, isolated from hospital wastewater including virion architecture, thermal and pH stability and complete genome sequence analysis. Host range of TSP was determined against clinical local isolates of MRSA, MSSA, and other non-aureus *Staphylococcus* strains. The complete genome of TSP is thoroughly characterized for gene annotation and determining DNA homology.

**Methods**

**Identification and characterization of bacterial strain**

Different clinical strains of *Staphylococcus aureus* were isolated from various clinical samples (skin, blood, abscess, wound, anterior nares, catheters and pus discharge) and identified by standard cultural, morphological and biochemical methods [11]. Clinical sample was collected from Citi Lab, Lahore Pakistan according to standard method of sample collection. Antibiotic susceptibility pattern was determined by Kirby Bauer's Disk diffusion method on Muller Hinton agar with commercially available cefoxitin (30ug), clindamycin (10ug), erythromycin (15ug), cefotaxime (30ug), oxacillin (5ug), penicillin (6ug), fusidic acid (10ug), vancomycin (30ug), linezolid (30ug) and tigecycline (15ug). The results of antibiotic susceptibility testing were interpreted according to CLSI criteria [12]. Sequencing of 16S rRNA gene was carried out from Macrogen, Korea. The bacterial strains were further confirmed by analyzing the 16S rRNA gene sequence through BLAST ([http://blast.ncbi.nlm.nih.gov](http://blast.ncbi.nlm.nih.gov)) and PCR amplification of mecA gene.

**Isolation of bacteriophage**
Biochemically and genotypically confirmed methicillin-resistant *Staphylococcus aureus* strain MR10 was used as host for isolation of bacteriophage from sewage sample collected from Township wastewater effluent, Lahore, Pakistan according to already reported procedure [13]. The sewage sample was centrifuged (10,000 rpm, 10 minutes) and supernatant was filtered (0.45µm) Subsequently, 25ml of the filtrate was enriched with an equal amount of 2X tryptone soya broth (TSB) containing 10mM CaCl$_2$ and 2ml of fresh bacterial culture (4 hours old), incubated overnight at 37°C with constant shaking (160 rpm). After incubation, 1% chloroform was added, flask left un-shaken for half an hour at 37°C, centrifuged (10,000 rpm, 10 minutes) and supernatant was filtered (0.22µm). The filtrate was assessed for lytic activity by spot test and agar overlay method [14] for determining plaque morphology [15].

**Determination of TSP host range**

Bacteriophage host range was determined by standard spot assay and efficiency of plating as described earlier [16]. A collection of 32 MRSA strains, 8 MSSA, 4 *S. epidermidis* strains and some species of gram negative organisms (*E. coli*, *Klebsiella pneumoniae*, *Serratia marsecense*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Enterobacter cloacae*) were used to measure the host range and EOP of bacteriophage TSP.

**Determination of in-vitro bacteriolytic activity of TSP**

Bacteriolytic activity of TSP bacteriophage was determined by an already reported method [13]. An overnight bacterial culture (3x 10$^{10}$ cfu) was added into three TSB broth flasks (50ml). TSP bacteriophage was inoculated at MOI-1 and MOI-10 in two flasks and incubated for 24 hours at 37°C in a shaking incubator at 150 rpm. The third flask having only MR10 was incubated under the same conditions which serves as control. The absorbance (OD$_{600}$) of control and test cultures were assessed for 24 hours with an interval of 2 hours. This assay was performed in triplicate.

**Determination of Bacteriophage stability at different temperature and pH**

Storage stability of TSP phage was determined by incubating phage lysate at different temperatures (4, 25, -20 and -80°C) for 1 month as described earlier [17]. Bacteriophage stability at different temperatures (25, 37, 45, 50, and 60°C) and a wide range of pH values (4, 5, 6, 7, 8, 9, and 10) was done according to the previously described procedure [16]. The survival ability of bacteriophage was determined by the double-layer agar technique. Bacteriophage stability experiments were performed by using phage titer 10$^{10}$ pfu/ml. Each assay was performed in triplicate.

**Determination of adsorption assay and one-step growth curve**

In order to determine the time taken by the TSP phage for adsorbing to the host surface, an adsorption assay was performed as described earlier with some modification [18]. Phage adsorption was assayed at MOI of 0.1. Percentages of un-adsorbed phages were determined at every 3-minute interval by taking the
ratio of PFU/ml to the initial PFU/ml at 0 min in the supernatant. Bacteriophage adsorption rate constant was determined by mathematical formula \( K = \frac{(2.3/Bt) \times \log (P_0/P)}{t} \) [19].

In order to determine the different phases of the bacteriophage lytic cycle such as latent period, rise period and burst size, one-step growth curve analysis was performed according to the protocol described previously [15].

**Analysis of TSP Genome**

Phage DNA was extracted from the filtrate by phage hunting protocol previously described [17]. The isolated phage DNA was analyzed through agarose and quantified through Nanodrop. Bacteriophage genomic DNA was sequenced using illumine sequencing technique from the University of Minnesota, Genomic Centre (UMGC). Reads were analyzed, trimmed and assembled by applying CLC genomic workbench 10. After completing the phage genome assembly, suitable restriction enzymes were selected from analysis of draft genome sequence to determine whether the TSP phage genome is circular or linear. The isolated phage DNA was double restricted with Ncol and EcoRI (Thermo scientific) and incubated at 37°C for 6-8 hours. The restriction pattern was analyzed by running on 0.8% agarose gel electrophoresis. Genome annotation was done by using PHASTER (https://phaster.ca) and online RAST server (https://rast.nmpdr.org/). Open reading frames (ORFs) were identified by using Gene Mark and Gene Glimmer (http://opal.biology.gatech.edu/GeneMark/). All the promised open reading frames (ORFs) were confirmed by using online BLASTp (http://www.ncbi.nlm.nih.gov/BLAST). InterProScan Program and Pfam were used for structural domain prediction and motif searches (http://www.ebi.ac.uk/interpro/search/sequence-search). ARNold was used for the detection of potential rho-independent terminators [20]. The tRNA Scan-SE software was applied for prediction of putative tRNAs [21,22]. The molecular weight of proteins was determined using ExPASy tool (https://web.expasy.org/compute_pi/). The genomic map was constructed through Snapgene software (http://www.snapgene.com/). The genome sequence of the methicillin-resistant *S. aureus* phage TSP had been submitted in GenBank under accession no MW286254. Comparative genomic analysis was done by comparing the whole genome sequence of bacteriophage TSP with other phages of Podoviridae, Siphoviridae and Myoviridae family with BLASTN [23]. Complete genome sequences of phages showed homology with TSP genome were obtained from NCBI data base (http://www.ncbi.nlm.nih.gov/genbank/). Alignment of sequences and phylogenetic tree were made in ClustalW [24]. The phylogenetic tree of bacteriophage TSP was formulated utilizing translated amino acid sequences of putative genes that encodes major capsid and DNA polymerase with the maximum likelihood method through MEGA7 [25].

**Results**

**Characterization of MRSA strain MR10**

Bacterial strain (MR10: accession no. MT272781) isolated from pus discharge was presumptively identified as *S. aureus* based on microscopic examination, biochemical tests, and 16SrRNA sequence
analysis. According to CLSI criteria, the MR10 showed resistance to large number of drugs, however, it was sensitive to vancomycin, linezolid and tigecycline (Supplementary material Table 1). BLAST analysis of its 16s rRNA gene sequence showed 98-99% similarity to \textit{S. aureus} strains. Furthermore, \textit{mecA} gene (310bp) was successfully amplified from its genome (Publication in process). Phenotypically and genotypically confirmed MRSA strain (MR10) was used for isolation of TSP phage.

\textbf{Morphological characterization of TSP bacteriophage}

A novel lytic phage TSP was isolated from hospital wastewater against MR10. TSP phage formed tiny clear, round plaques (1mm) in diameter (Fig. 1.)

\textbf{TSP phage showed broad host range against MRSA strains}

TSP phage showed broad lytic activity against MRSA (24 of 32 strains, 78\%) and MSSA (3 of 8 strains, 37\%). However, it was unable to lyse the tested \textit{S. epidermidis} strains and isolates of gram negative organisms (\textit{E. coli}, \textit{Klebsiella pneumoniae}, \textit{Serratia marsecens}, \textit{Pseudomonas aeruginosa}, \textit{Acinetobacter baumannii} and \textit{Enterobacter cloacae}). The plaque formation ability of TSP phage was observed against 18 isolates of MRSA and 1 isolate of MSSA. The efficiency of plating (EOP) of TSP was grouped into four categories; EOP>0.5 for high production, 0.1<EOP<0.5 for medium production, 0.001<EOP<0.1 for low production, and EOP<0.001 for very low production. The higher EOP values of TSP phage against MR5, MR19 and MR26 suggest that these are more susceptible to phage compared with MR10, while remaining 15/19 isolates have low efficiency of plating as compared to the host strain (Supplementary material Table 2).

\textbf{TSP bacteriophage showed strong bacteriolytic activity till 12 hours post-inoculation}

TSP phage inhibit the bacterial growth for initial 12 hours at MOI-1 and 10 leading to increased bacterial growth after this time in the phage treated mixture but it was still less than growth in the untreated control (Fig 2).

\textbf{TSP bacteriophage highest stability observed at 37\(^{\circ}\)C and varying pH (5-9) while maximum storage stability at 4\(^{\circ}\)C}

To assess the stability of bacteriophage TSP for therapeutic use in the future, its thermal, pH and storage stabilities were analyzed. TSP bacteriophage showed highest stability at temperature 25\(^{\circ}\)C and 37\(^{\circ}\)C, however at high temperature (45\(^{\circ}\)C, 50\(^{\circ}\)C and 60\(^{\circ}\)C) a progressive decrease in phage titer was observed which destroyed phage activity at temperature above 60\(^{\circ}\)C (Fig 3A). The TSP stayed highly active at wide pH range (5 to 9), but under extreme pH (below 5 and above 10) conditions, a marked decrease in phage titer was observed (Fig. 3B). Long term storage stabilities showed that TSP phage was more viable at refrigerator temperature (4\(^{\circ}\)C) as compared to frozen temperatures (-20\(^{\circ}\)C and -80\(^{\circ}\)C). However, TSP phage showed better survival at -80\(^{\circ}\)C (1.95 \times 10^{10}) while a significant reduction in phage titer was observed at -20\(^{\circ}\)C and 25\(^{\circ}\)C (Fig 3C).
**TSP bacteriophage revealed short latent period and higher burst size**

According to phage adsorption assay, almost 99% of phage TSP could adsorb to the host cell surface within 9 min at 25ºC (Figure 5A). Adsorption rate constant of phage calculated within the interval of 3 to 9 minute is $4.3 \times 10^{-12}$ pfu/ml/min. One step growth curve analysis showed short latent period of 20 minutes and average burst size of 103 virions per infected cells (Fig 5B). These results indicated that this phage can rapidly infect the host and replicate.

**The TSP have a linear genome of 18Kb long**

To further determine whether the genome of TSB is linear or circular, we determined the 1 site cutter in the phage genome through Neb cutter and found that restriction through Ncol & EcoRI produce four fragments of 9.1, 5.7, 3 and 0.1 kb sizes, if the genome is linear, as shown in figure S1. Digestion of TSP phage DNA through Ncol & EcoRI produced restriction pattern like the proposed pattern by Neb cutter, which confirm that TSP phage DNA is linear (Fig. 5). Also, the restriction pattern confirms that the isolated phage DNA is pure with no other DNA contamination.

**Genome sequence analysis demonstrates lytic nature of TSP phage**

Whole genome sequencing and annotation showed that TSP phage consists of a double stranded, linear DNA with a genomic length of 17,987 bp and an average GC content of 29.7%. It contains 20 predicted open reading frames (ORFs) and no tRNA gene. According to BLASTn analysis, the complete TSP phage genome sequence showed 98% identity to *S aureus* lytic phages SCH1 (Accession No. KY000084.1), SCH11 (Accession No. KY000085.1) and vB SauP-436A1 (Accession No. MN150710.1) with 94% query coverage. The detailed genomic characterization of TSP phage is given in Supplementary material Table S3. All ORFs presented an ATG start codon. Among all 20 ORFs, 12 had assigned functions while the remaining 8 ORFs were annotated as hypothetical proteins. Annotation and functional analysis of predicted ORFs revealed four functional groups: structural (major capsid and scaffold protein, major and minor tail protein, tail fibers protein, collar proteins, structural protein) host lysis (endolysin, holin and CHAP domain-containing protein), DNA manipulation (single stranded DNA-binding protein, DNA polymerase) and DNA packaging protein. Structural proteins and lysis protein are present on the plus strand while DNA manipulation, DNA packaging and maximum hypothetical proteins are on negative strand. The TSP phage genome consists of 5 potential rho-independent transcription terminators. There were no virulence gene detected in phage TSP genome. The open reading frame ORF7 (Endolysin) was considered to be involved in lytic activity against peptidoglycan of host bacterium. According to Pfam and InterPro Scan analysis, endolysin has two polypeptide domains, one is catalytic domain at N terminus called cysteine, histidine-dependent amidohydrolases/peptidase (CHAP) (pfam05257) and (IPR007921), and other is cell wall binding domain at C terminus named as SH3_5 (pfam08460) and (IPR003646). TSP phage endolysin is located between the structural proteins similar to phage CSA13 and this is the unique characteristic of P68 like viruses.

**TSP phage showed genetic similarity with genus P68virus of family Podoviridae**
The complete genome sequence of TSP phage was assessed for homology for other *S. aureus* phages (Figure 7A). According to BLASTn analysis, TSP phage showed highest similarity (98%) to phage genomes SCH1, SCH11 and vB SauP-436A1 with 94% query coverage. Comparative genomic analysis indicated that TSP genome showed highest homology with the phages of *Podoviridae* family, while it showed a distant relationship to the members of other families. According to BLASTp search, major capsid protein of TSP phage showed 99.9% identity to vB SauP-436A1, SCH1 and S13 with 100% query coverage, while DNA polymerase of TSP phage showed 98.95% identity to SCH1 sequence with 100% query coverage (Fig 7B and 7C).

**Discussion**

*Staphylococcus aureus* is a multi-drug resistant infectious agent responsible for a number of morbidities such as abscesses, skin infections, endocarditis and toxic shock syndrome [26]. Routine antibiotic therapy has been failed to treat infections by MRSA and become a major challenge in the cure of chronic infections. Currently, the exploration of new strategies to supplement existing antibiotic therapy has become a serious objective of research. In the current era of antibiotics resistance, phage therapy is the best possible solution. Our study was aimed to identify the novel virulent bacteriophage against MRSA for controlling the infections of MRSA. A number of studies have been reported on isolation of bacteriophages from sewage water as it is the reservoir of multi drug-resistant bacteria [27].

The TSP showed lytic spectrum of ~78% and can be considered a phage with relatively broad host range against numerous MRSA strains. In literature, broad host range phages already reported such as P68 (84%) [28], CSA13 (90%) [9] and SLPW (92%) [6]. TSP phage possess strong bacteriolytic action that is crucial for phage therapy. The strong reduction in bacterial growth was observed till 12 hours similar to phage SA97 [29] at MOI-1 and 10. However in comparison to phage CS1 and DW2, which reduced bacterial growth only for 3 hours [30], TSP possesses longer inhibitory effect. There was no significant difference between ODs of phage treated group at MOI-1 vs 10 (p value: 0.47). However, lower MOI is preferred because it might generate lower immune response when applied in the living system.

Long term stabilities are vital parameter for any phage preparation to be used for phage therapy [31]. TSP phage showed best survival ability and performance at physiological temperature of 37°C which suits it application against MRSA infections. It can withstand the raised temperature till 45°C but became inactivated at 65 °C. These results were similar to phages SA2 and SLPW where high temperature progressively inactivated their activity [32]. It exhibit good pH stability at wide range of pH (5-9), and optimum activity at neutral pH. These results are similar to previous reported studies [6,33]. Tailed phages mostly maintained virion structure and stability under wide range of pH (5-9) [34]. The inactivity of phage below 4 pH indicates that the denaturation of its structural proteins occurs in acidic environment [35]. These characteristics may be helpful in administration of phages in different environment as therapeutic agent. We found that phage present highest storage stability at refrigerator temperature similar to results previously reported [36]. Phage TSP fulfills the ideal parameters of phage therapy that includes short
latency period and high burst size. Our finding confirms that the newly isolated phage TSP is a lytic phage with higher lytic activity similar to *S. aureus* lytic phage SLPW and Stau2 [6,37].

Based on genome length, low G+C content and gene organization, TSP phage is similar to that of well-studied *S. aureus* lytic phages SLPW, VB_SauP_PhiAG01.3, P66, S13, and SCH1 [38-40,6] which were successfully applied for the treatment of *S. aureus* infections. Genes involved in structure, DNA replication, packaging and lysis showed best match with other *Podoviridae* phages listed in supplementary Table S3 [41]. TSP phage also indicated the only characteristics of *S. aureus* *Podoviridae* phages that DNA packaging and DNA polymerase genes present on plus strand while all structural genes located on another strand (Table 2) as described earlier [42]. It possess the DNA polymerase from B type superfamily, which is a unique feature of *Picovirinae* subfamily. The classical lysis cassette composed of holin-endolysin system was absent in TSP similar to other *Podoviruses* [43], as it possess endolysin between genes for viral morphogenesis. [44].

Due to absence of evolutionary marker, whole genome sequence and protein sequences of major capsid and DNA polymerase were used to infer the evolutionary relationship of TSP phage [9]. Comparative genomic analysis and phylogenetic tree analysis of TSP phage showed its close relationship to non-classified *Rosenblumvirus* phages SCH1, SCH111 and vB SauP-436A. TSP taxonomically classified in to *Picovirinae* subfamily and P68 genus because it possess the hallmarks of this subfamily ([39]. The hallmark of *Picovirinae* sub family include small genome size (16-19kb), low G + C content (27-29%) and predicted number of genes (20-22) [45]. *Podoviridae* *S. aureus* phages belongs to the genus “P68Virus”, an extremely well-conserved group with respect to nucleotide, amino acid homology, morphology, lytic lifestyle and genome size [46]. In comparison to *Myoviridae* and *Siphoviridae* phages, Staphylococcal phages that belong to *Podoviridae* family lack diversity and show affiliation to *Rosenblumvirus* genus and subfamily *Picovirinae* (68-like viruses) [47,9]. Comparative genomic analysis and phylogenetic tree based on major capsid and DNA polymerase revealed that the newly isolated phage TSP is similar to member of genus “P68virus”. So, it has been placed in *Picovirinae* subfamily in the family *Podoviridae*.

**Conclusion**

In this study, virulent bacteriophage TSP has been isolated and characterized from sewage water against MRSA. TSP phage showed broad host range, short latency period, and higher burst size. It has strong bacteriolytic activity and capable to resist different conditions of pH and temperature. These are crucial parameters of phage candidates for phage therapy. Whole genome sequencing and annotation along with phylogenetic analysis showed that it’s a member of family *Podoviridae*. Based on morphological, physiological and genomic characteristics, the TSP phage may be a suitable candidate for the eradication of *S aureus* infections in humans after successful animal and clinical trials.

**Declarations**

**Conflict of interest**
The authors declare that they have no conflict of interest.

**Ethical approval**

The article does not contain any studies with human participants and animals performed by any of the authors.

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**Availability of data**

The genome sequence has been submitted to the NCBI GenBank database (accession no. MW286254).

**Authors’ contributions**

R.T. carried out all the experiments and wrote the paper, I.A.I and A.B. performed the genome analysis, M.A. helped in all experiments and S. R. supervised all the experiments and manuscript write up. All authors read and approved the final version of manuscript.

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Figures

Figure 1

(A) represent the bacteriophage TSP on the lawn of MR10. (B) Plaque morphology of TSP phage

Figure 2

Determination of in-vitro bacteriolytic activity of TSP bacteriophage. Phage treated group; Co-culture MRSA strain in logarithmic phase with TSP phage at an MOI-1 and MOI-10, Control group; MRSA culture without phage TSP; OD of control and phage treated groups were measured at 600nm after an interval of
2 hours for up to 24 hours. The results were obtained from three independent experiments and expressed as means of standard deviation.

**Figure 3**

TSP bacteriophage stability assay. (A) Effect of different temperatures (25, 37, 45, 50 and 60°C) on stability of TSP phage (B) TSP phage was treated at wide pH range (4, 5, 6, 7, 8, 9 and 10) for 1 h (C) Storage stability of TSP phage at different temperatures (4, 25, -20 and -80°C) showed maximum survival ability at 4°C. Experiment was performed in thrice and phage titers were expressed in mean ± standard deviation.
Figure 4

(A) TSP phage adsorption kinetics. (B) One step growth curve analysis of bacteriophage TSP infecting MR10 at 37°C. Results were obtained from three independent experiments.
Figure 5

Agarose gel analysis of the TSP phage DNA double digested with Ncol and EcoRI. Lane 1:Restricted TSP phage DNA, Lane 2: Un-restricted TSP phage DNA and Lane M: Lambda phage DNA HindII digested marker (Cat#302005 Bioron).

Figure 6

Linear genome map of TSP phage. The direction of ORFs were depicted via direction of arrows, four functional groups are present in TSP phage genome and genes in each functional group are represented by different colors, structural gene (yellow), regulatory (blue), host lysis (red), DNA packaging (green), while hypothetical ORFs are indicated in purple.
Figure 7

(A) Comparative genomic analysis of S. aureus phages. Whole genome sequences were aligned by Clustal W. Neighbour-joining method was used to construct phylogenetic tree in MEGA 7. The values at the nodes represents the bootstrap support scores as calculated using 1000 replicates. The triangle highlights the novel phage TSP. Phylogenetic analysis of TSP based on amino acid sequence of major capsid (B) and DNA polymerase (C). Phylogenetic trees were constructed with the alignment tool UPGMA.
having bootstrap value of 2000. Pseudomonas phage ZC08 and Streptococcus phage C1 act as an out group. The scale bar represent 0.5 and 0.1 fixed mutations per amino acid position. The dark dot highlights newly isolated phage TSP.

**Supplementary Files**

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