

Therapeutic Potential of Staphylococcal Bacteriophages for Nasal Decolonization of *Staphylococcus aureus* in Mice

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ABSTRACT

Bacteriophages represent a rich and unique resource of anti-infectives to counter the global problem of antibiotic resistance. In this work, we assessed the bactericidal activity of two virulent staphylococcal phages, K and 44AHJD, against *S. aureus* isolates of clinical significance, and tested their efficacy *in vivo*. The phage cocktail lysed >85% of the clinical isolates tested. Both the phages were purified by ion-exchange column chromatography following propagation in bioreactors. The purity profiles of the ion-exchange purified phages were compared with those of phages purified using cesium chloride density gradient ultracentrifugation, and infectiousness of the purified phages was confirmed by plaque forming assay. The *in vivo* efficacy of a phage cocktail was evaluated in an experimental murine nasal colonization model, which showed that the phage cocktail was efficacious. To our knowledge, this is the first report of phage use in an *in vivo* model of nasal carriage.

Keywords: Bacteriophage K; Nasal Decolonization; Phage 44AHJD; Phage Endotoxins; Phage Purification; *Staphylococcus aureus*

1. Introduction

During the last century, the human pathogen *Staphylococcus aureus* has become the main cause of nosocomial and community-acquired infections worldwide [1]. Both coagulase-negative *Staphylococcus* (CoNS) and coagulase-positive *Staphylococcus* (CoPS) cause serious infections, ranging from localized or systemic abscesses, septicemia, and endocarditis, to septic emboli and fatal sepsis [2]. After the introduction of methicillin in the early 1960s, isolation of multi-resistant *S. aureus* strains was reported [2-4]. Methicillin-resistant *S. aureus* (MRSA) strains are becoming increasingly difficult to combat, mainly because of emerging resistance to all currently used antibiotics. In addition, the ability of staphylococcal species to produce a variety of toxins and to attach to medical devices by producing biofilms has compounded the problem [4,5].

The emergence of MRSA in both hospital and community settings has prompted researchers to try to develop methods for the nasal decolonization of MRSA and methicillin-susceptible *Staphylococcus aureus* (MSSA) in specific patient groups. In the UK, it is recommended that MRSA carriers who are receiving prophylaxis for an

operation should undergo nasal decolonization with mupirocin, the most commonly used antibiotic for Gram-positive bacteria [6]. Because nasal relapses are common within several months [7], and mupirocin resistant *S. aureus* strains have recently been reported [8], alternate treatments are being pursued by various groups.

Squalamine, a water-soluble natural polyaminosterol isolated from the tissues of the dogfish shark (*Squalus acanthias*), has a 10,000-fold higher antimicrobial activity towards *S. aureus* than mupirocin [9]. Interestingly, the use of a less virulent *Staphylococcus* strain (*S. epidermidis*) to block colonization by pathogenic *S. aureus* strains has also been reported [10,11].

The effectiveness of bacteriophages for phage therapy against pathogenic bacteria in both animals and humans is well documented [12]. Because they are present in all environments, including water, soil, and air, and are highly specific and lethal to their target host [13], bacteriophages are attractive therapeutic agents for combatting life-threatening bacterial infections in humans and animals. The safety and efficacy of phages has been supported by extensive clinical use of phages in eastern European countries, including the former Soviet Union [14]. The success of phage therapy against various oral, topical, and systemic bacterial infections without the need

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for genetic manipulation has been well documented [15, 16].

It is generally accepted that virulent phages are more suitable candidates for therapeutic applications than temperate phages. The complications associated with temperate phages, such as super-infection immunity and possibility of integration into the host genome (lysogeny), and the possibility of transfer of genetic material, such as drug-resistance genes during infection, make temperate phages unsuitable for therapeutic purposes. It has been reported that free-living and virulent *S. aureus* phages in the environment are relatively low in numbers compared with phages infecting other bacterial species, although some virulent phages have been found in *S. aureus* [17-19]. Therefore, we examined the potency of two of the broad host range lytic staphylococcal phages, namely K and 44AHJD, belonging to the families *Myoviridae* and *Podoviridae*, respectively. Complete nucleotide sequences for both of these phages have been reported previously [20,21].

Among 16 studied staphylococcal phages, 44AHJD is highly virulent because of the high translation efficiency of many of its genes [18], making it a good candidate for a therapeutic anti-bacterial agent. Several phase I studies with bacteriophages have been published [22-24], using phages that have obtained Generally Regarded As Safe (GRAS) status by the US Food and Drug Administration (FDA). In 2009, a US FDA-approved bacteriophage Phase I clinical trial was reported, evaluating a bacteriophage cocktail targeting *S. aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* in venous ulcers [25]. A phage product for controlling *Listeria monocytogenes* in ready-to-eat meat and cheese represents acceptance of phages among regulated antibacterials [26].

In the present study, we purified phages K and 44AHJD by ammonium sulphate precipitation, followed by ion-exchange chromatography. The levels of contaminating host proteins and endotoxins were determined and compared with phages purified by conventional cesium chloride (CsCl) density gradient centrifugation. Purified phages in the form of a cocktail were then evaluated for their *in vivo* efficacy in an experimental *S. aureus* nasal colonization mouse model.

2. Materials and Methods

2.1. Bacterial Strains and Bacteriophages

Eighty-six *S. aureus* isolates, comprising 27 MRSA and 23 MSSA strains collected from hospitals in and around Bangalore, India, and 36 global strains (33 MRSA and 3 MSSA), were used to assess the bactericidal activity of the two virulent staphylococcal phages, K and 44AHJD (GenBank accession numbers AY176327 and AF513032

respectively). Thirty distinct, typed isolates of global representation were obtained from the Public Health Research Institute (PHRI), New Jersey, USA. Phage K (NC07814-02) was obtained from the Health Protection Agency Culture Collections, UK, and phage 44AHJD was a gift from Dr. Udo Blaesi, University of Vienna, Austria. All strains were cultured in Luria-Bertani (LB) broth at 37°C on a rotary shaker (200 rpm), unless otherwise stated. *S. aureus* strain Newman was used in the *in vivo* experiments. Polyclonal antibody for *S. aureus* RN4220 was generated at Raj Biotech, Pune, India.

2.2. Bacteriophage Propagation, Enumeration, and Host Range Determination

Phages K and 44AHJD were amplified in *S. aureus* strains RN4220 and KB600, as described previously [27]. Briefly, the propagating hosts were grown at 37°C in LB broth to an absorbance at 600 nm of ~0.8 and then infected with the respective phages at a MOI of 0.1 and further incubated for 4 h. Phage was harvested following centrifugation of the culture lysate at 3000 × g for 10 min to remove the cell debris. The supernatant was filtered through a 0.2 µm filter and the phage titer was determined. No viable bacteria were detected in the phage preparations. Phage plaques were enumerated and titers were determined using an agar overlay method [28]. The sterile phage solutions were stored at 4°C, and no decrease in the phage titer was observed during the study period, as assessed by plaque assay using suitable indicator cells.

2.3. Phage Purification

Phage K crude lysate was precipitated using solid ammonium sulfate fractionation from 0% - 30% and 30% - 70% ammonium sulfate at room temperature, and then centrifuged at 12,860 × g for 45 min at 4°C. The pellet obtained from the 30% - 70% fraction was dialyzed against 25 mM Tris-Cl pH 7.5 (buffer A) overnight. The dialyzed material was loaded onto a weak anion exchange DEAE cellulose (DE52) column (Whatman Inc., Florham Park, NJ, USA) using a Biologic Duoflo system (Bio-Rad, Hercules, CA, USA) equilibrated with buffer A at a flow rate of 5 mL/min. The column was washed with buffer A until the absorbance of the eluting fractions at 280 nm was zero. The bound phages were recovered by isocratic elution with 0.2 M NaCl in buffer A, dialyzed against buffer A, filter-sterilized through a 0.2 µm filter, and then analyzed by SDS-PAGE followed by silver staining. A similar protocol was followed for phage 44AHJD crude lysate.

Phages were enumerated from all of the chromatographic fractions, and percent phage recoveries were cal-

culated taking the initial material as 100%.

2.4. Purification by CsCl Ultracentrifugation

One liter each of phage K and 44AHJD lysate was centrifuged at $25,000 \times g$ for 2 h at 4°C . The pellet, containing bacteriophage particles, was resuspended in approximately 1 mL of buffer A. CsCl density gradient ultracentrifugation of this phage concentrate was performed following standard methods [29]. The density of CsCl used ranged from $1.81 - 1.27 \text{ g}\cdot\text{L}^{-1}$. The bacteriophages banded at a CsCl density of $1.54 - 1.40 \text{ g}\cdot\text{L}^{-1}$, and were recovered by careful siphoning. This fraction was dialyzed against buffer A to remove CsCl, and then filter-sterilized.

2.5. Protein Analyses and Endotoxin Content of Phage Preparations

Protein content at different stages of phage purification was determined according to the method of Lowry *et al.* (1951) [30], using bovine serum albumin (BSA) as standard. Protein profiles of phage preparations were analyzed by SDS-PAGE on 12% gels and visualized by silver staining.

The endotoxin content of the phage preparations was measured using an Endosafe Rapid LAL reagent kit (Charles River, Wilmington, MA, USA).

2.6. Experimental Murine Nasal Colonization Model

2.6.1. Animals

Healthy 6-week-old BALB/c mice (National Institute of Nutrition, Hyderabad, India) were used in all experiments. Animal experiments were performed at St. John's Medical College and Hospital, Bangalore, India. The experiments were approved by the Institutional Animal Ethics Committee (IAEC) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). St. John's Medical College is registered with CPCSEA (Registration No. 90/1999/CPCSEA dated 28/4/1999).

2.6.2. Evaluation of Commensal Nasal Flora of Mice

The commensal nasal flora of the mice was evaluated by nasal swabbing as described previously [31]. After nasal sampling, the swabs were placed in 150 μL of sterile 0.85% NaCl in microfuge tubes. Tubes were thoroughly vortexed and the supernatant along with the swabs were plated on nutrient agar containing 5% sheep blood. Staphylococcal colonies were identified based on morphology and biochemical characteristics (HiStaph Identification kit, HiMedia, Mumbai, India), and confirmed using an *S. aureus*-specific ELISA.

2.6.3. Determination of *S. aureus* Newman Colonization Rate

Mice were administered chloramphenicol sodium succinate at 0.5 mg/mL in drinking water, beginning at 24 h prior to inoculation of challenge strain, which continued until the end of the study.

Chloramphenicol-resistant *S. aureus* strain Newman was grown at 37°C overnight on Columbia agar containing 2% NaCl to induce capsule formation [32]. The culture was harvested in sterile PBS, then centrifuged at $5800 \times g$ for 10 min and resuspended in sterile PBS at $5 \times 10^7 \text{ CFU}\cdot\mu\text{L}^{-1}$, for nasal inoculation. Groups of mice were anaesthetized by intraperitoneal injection of ketamine ($90 \text{ mg}\cdot\text{kg}^{-1}$ body weight) and xylazine ($9 \text{ mg}\cdot\text{kg}^{-1}$ body weight). Ten microliters of *S. aureus* cell suspension was inoculated into the nares of all animals on day 1. Subsets of mice were euthanized by anesthetic overdose on days 7, 10, and 14 post-inoculation. The nasal tissue was excised and processed for quantitative evaluation of colonization in such a way that the skin around the nares was removed prior to dissection as described by Kiser *et al.* [32]. The suspension was briefly centrifuged to settle the particulate tissue and the supernatant was cultured overnight at 37°C on LB agar containing chloramphenicol ($34 \mu\text{g}/\text{mL}$). The resulting chloramphenicol-resistant colonies were enumerated. Representative colonies from each presumptive *S. aureus* Newman positive animal were purified on LB agar for confirmation by ELISA.

2.6.4. Evaluation of Phage Efficacy *in Vivo*

Three groups of mice ($n = 8$) were used for the study. These were colonized with the challenge strain as described in 3.8. Daily doses of phage cocktail containing 1×10^{10} PFU of phage K and 4×10^{10} PFU of phage 44AHJD in 10 μL of 0.85% NaCl were administered intranasally to the test group on days 5, 6, and 7. The placebo control group was administered 10 μL of 0.85% NaCl. On day 8, the mice were euthanized and nasal tissue was taken for confirmation and enumeration of the test strain, as described above.

2.6.5. ELISA and Western Blot Studies

ELISA for detection of *S. aureus* derived proteins and *S. aureus* confirmation was performed using polyclonal antibodies generated for the *S. aureus* host RN4220 cell lysate. Dilutions of the RN4220 host lysate (from 1 ng to 1000 ng protein) served as the antigen, and were used for construction of a standard curve.

For western blot studies, all samples were run on a 12.5% SDS-PAGE gel and then transferred to a Biotrace nitrocellulose blotting membrane (Pall Corporation, Pensacola, FL, USA) and blocked with 3% BSA (in Tris-Cl, pH 8.0, buffered saline with 0.1% Tween 80:1 \times TBST) overnight. Following washing with 1 \times TBST, primary

anti-RN4220 antibody (final concentration: 1:5000) was added, and the membrane washed again with $1 \times$ TBST. Secondary goat anti-rabbit alkaline phosphatase (ALP) conjugate (final concentration: 1:500) was then added. The blot was developed using 5-bromo-4-chloro-3-indolyl-phosphate (BCIP) substrate in conjunction with NBT (nitro blue tetrazolium).

For identification of isolates from the mouse nares, colonies taken from pure isolates from LB agar were suspended in 0.05 M carbonate-bicarbonate buffer pH 9.6, to a cell density of -1×10^9 CFU/mL. Two hundred microliters of this cell suspension were used to coat 96-well plates overnight at 4°C. Wells were washed with TBST and blocked with 200 μ L of 1% BSA in TBST for 1 h at 37°C. After repeated washes with TBST, 100 μ L of rabbit polyclonal anti-RN4220 antisera (1:20,000) was added and plates incubated for 1 h at 37°C. Wells were washed again with TBST prior to addition of 100 μ L of ALP-labeled goat anti-rabbit antibody (1:5000). Plates were incubated for 1 h at 37°C. Following washing of the wells, 100 μ L of substrate (PNPP) was added and plates were incubated for 40 min, after which absorbance was read at 405 nm.

3. Results and Discussion

3.1. Bacteriophage Amplification, Enumeration and Host Range Determination

While phage K was propagated using RN4220 as a propagating host, phage 44AHJD was propagated using KB600 host since we have earlier observed that 44AHJD requires endolysin supplementation for propagation in RN4220 [27]. Both the phages were amplified to a titer of -1×10^{10} PFU/mL in bioreactors using the protocol described earlier [27] and the host range of both the phages were assessed on a panel of *Staphylococcus aureus* isolates that included both MSSA and MRSA.

A total of 86 isolates were tested for phage sensitivity, which included 30 distinct typed *S. aureus* isolates of global representation; six of Community acquired (CA)-

MRSA type strains and 50 clinical isolates from Indian hospitals (data not shown). Nearly 57% of isolates were susceptible to phage K and phage 44AHJD was lytic to 86% of the isolates tested. In case of both phages taken together, 74.4% of isolates were sensitive with plaque formation. In total, 88.3% isolates were susceptible to both phages in combination (**Table 1**).

It has been shown earlier that a phage cocktail significantly reduces the frequency of mutation in bacteria in comparison to the use of a single phage preparation [33]. Therefore, a greater chance of successful treatment of bacterial infections may be expected by combining multiple phages with varied host specificities.

Due to its polyvalent nature, *Staphylococcus* phage K, has been studied in multiple applications, including in the treatment of subclinical bovine mastitis [34] and as an anti-staphylococcal hand-wash solution [35]. Here, we examined phage K alone, and together with 44AHJD as a cocktail, for activity against *S. aureus* isolates that represented highly virulent, antibiotic-resistant, and genetically diverse strains. This established that the therapeutic potential of phages is improved as part of a cocktail, which supports previous literature reports [33,36].

3.2. Phage Purification

The recovery of phage K at various purification steps is summarized in **Table 2**, and the sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) profile of purified phage K is compared with the cesium chloride-purified phage K in **Figure 1**.

The total recovered phage titer following ammonium sulfate precipitation was 32%, with an almost eight-fold reduction in the total protein content.

Following anion exchange chromatography, the recovery was 31%, with enhanced specific activity (**Table 2**). The SDS-PAGE profile of purified phage K was comparable to the CsCl gradient-purified phage K (**Figure 1**). A recovery rate of almost 23% was achieved for purified phage 44AHJD using the ammonium sulfate

Table 1. Susceptibility of *S. aureus* isolates to phages.

Phage sensitivity	Phage K				Phage 44AHJD				Phage K + Phage 44AHJD			
	Panel A		Panel B		Panel A		Panel B		Panel A		Panel B	
	MRSA (27)	MSSA (23)	MRSA (33)	MSSA (3)	MRSA (27)	MSSA (23)	MRSA (33)	MSSA (3)	MRSA (27)	MSSA (23)	MRSA (33)	MSSA (3)
Susceptible with plaques	3	9	26	3	14	9	30	3	15	14	32	3
Susceptible due to lysis from without*	1	0	7	0	8	8	2	0	7	4	1	0
Resistant	23	14	0	0	5	6	1	0	5	5	0	0

Panel A: *S. aureus* from India-27 MRSA and 23 MSSA category; Panel B: Global *S. aureus* panel including PHRI strains and USA type strains-33 MRSA and 3 MSSA category; *Lysis seen due to lysis-from-without phenomenon due to phages at high MOI (>100).

precipitation/ion-exchange protocol (**Table 3**).

Purified phage particles have two major uses: phage biology studies and therapeutic applications. To date, most phage preparations for therapeutic use have been purified by passing the lysate through filters to remove the host bacteria. While such purification reduces the risk of bacterial infections, it does not remove bacterial endotoxins, which can be harmful to patients. Moreover, cost-effective phage purification methods would be beneficial for large-scale production. Because the available literature describes phage purification using a variety of methods, including cesium chloride gradient ultracentrifugation [37], concentration by pelleting [18], monolithic

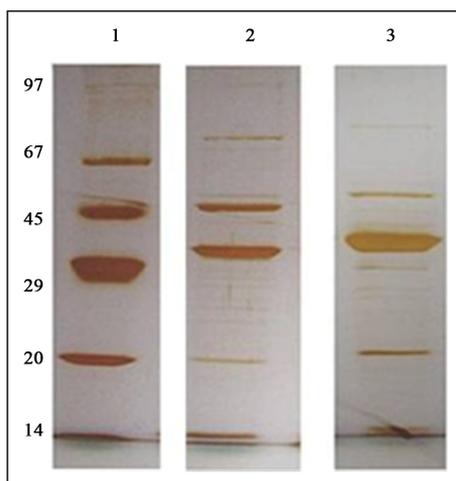


Figure 1. SDS-PAGE profile of purified phage K preparations. Lane 1, molecular weight marker (14 - 97 kDa); lane 2, phage K purified by CsCl gradient density ultracentrifugation; lane 3, phage K purified by ion-exchange chromatography steps.

Table 2. Purification chart of phage K.

Sample	Total volume (mL)	Protein (mg/mL)	Phage titer (PFU/mL)	% yield
1	100	1.2	1.0×10^{10}	100
2	4	4.0	8.0×10^{10}	32
3	14	0.6	2.2×10^{10}	31

Sample 1: Crude phage lysate; Sample 2: 30% - 70% ammonium sulphate fraction; Sample 3: DEAE cellulose purified fraction.

Table 3. Purification chart of phage 44AHJD.

Sample	Total volume (mL)	Protein (mg/mL)	Phage titer (PFU/mL)	% yield
1	500	12.0	3.4×10^{11}	100
2	78	5.3	1.1×10^{12}	51
3	1014	0.4	3.8×10^{10}	23

Sample 1: Crude phage lysate; Sample 2: 30% - 70% ammonium sulphate fraction; Sample 3: DEAE cellulose purified fraction.

columns [38], size exclusion chromatography [39], and anion exchange chromatography [40], the yields achieved vary.

Phages purified by anion exchange methods have been used successfully in a number of human studies [38,41]. Therefore, the systematic approach for phage purification used in the current study would benefit researchers in this field.

The results of our assessment of the purity of bacteriophages obtained by SDS-PAGE (**Figure 2**) were similar to those reported previously for other phage types [42,43]. It was interesting to note that the SDS-PAGE profiles of the ion-exchange- and cesium chloride-purified phages were very similar.

3.3. ELISA and Western Blot Studies

The cesium chloride phage preparations and the phage cocktail preparation showed host cell contamination of 10 - 100 ng/mL, as determined by ELISA with anti-RN4220 antibodies. The western blot of samples from the CsCl and ion-exchange purified 44AHJD phages showed negligible signals for *S. aureus* host cell contaminants (**Figure 3**, lanes 1 and 7). Interestingly, the $16,000 \times g$ pelleted phage showed a significant amount of *S. aureus* host cell contamination (**Figure 3**, lane 2). Similar results were observed for phage K (data not shown).

The endotoxin levels of the phage cocktail used in our study was in the range as reported by other researchers [22]. Hence, the protocol described here for phage purification could be universally applicable. Production of such a phage cocktail in a facility following good manufacturing practices (GMP) would further reduce the endotoxin content, because it is well known that endotoxins

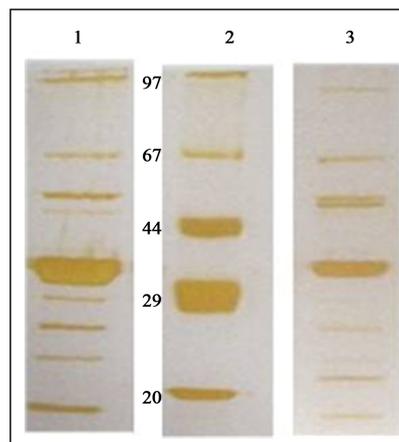


Figure 2. SDS-PAGE profile of purified phage 44AHJD preparations. Lane 1, phage 44AHJD purified by CsCl gradient density ultracentrifugation; lane 2, molecular weight marker (14 - 97 kDa); lane 3, phage 44AHJD purified by ion-exchange chromatography.

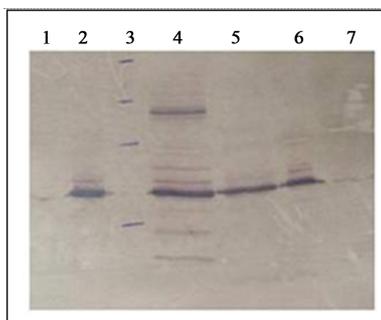


Figure 3. Western blot of phage 44AHJD samples. Lane 1, CsCl purified 44AHJD; lane 2, 16,000 × g pelleted phage; lane 3, marker (14 - 97 kDa); lane 4, *S. aureus* KB600 lysate; lane 5, crude 44AHJD lysate; lane 6, 30% - 70% ammonium sulfate pellet; lane 7, DEAE cellulose (DE52) purified 44AHJD.

that bind to plastic and glass surfaces are efficiently removed by depyrogenation [44].

A recent report on purification of *Staphylococcus* phage VDX-10 showed that >90% of host proteins were removed, which is similar to our observations [38].

3.4. Evaluation of Commensal Nasal Flora

Only coagulase-negative staphylococci (*S. gallinarum*, *S. arlettae*, and *S. equorum*) were found in BALB/c mice used for experimentation. *S. aureus* was not detected in any of the animals (data not shown).

3.5. Determination of *S. aureus* Newman Colonization Rate in Mouse Nares

Of the 24 mice nasally inoculated with *S. aureus* strain Newman, 83.3% were colonized on day 7. On days 10 and 14, 25% (2/8) and 12.5% (1/8) of mice remained colonized, respectively. The carriage rate of *S. aureus* Newman in the currently employed BALB/c mice is similar to reported earlier [32]. Based on this colonization profile, phage cocktail was applied nasally on days 5 - 7 post-inoculation with *S. aureus*.

3.6. Evaluation of Phage Efficacy *in Vivo*

The phage efficacy study involved evaluation of commensal bacterial flora from the mouse nares, then determination of the rate and extent of colonization of nasally inoculated *S. aureus*. Subsequently, efficacy of phage treatment was assessed in *S. aureus*-colonized mice. Phage-effected decolonization was evident in the animals treated with the phage cocktail. Daily doses of phage cocktail administered intranasally on days 5, 6, and 7 fully decolonized all eight animals inoculated with *S. aureus* strain Newman by day 8 while the colonization control group (seven of eight animals) and the group treated with placebo (six of eight animals) remained colonized (**Fig-**

ure 4) during the experimental period. The number of CFU's recovered from the nares of the colonization control group ranged from 1 - 4 CFU/nose while for the placebo treated, the CFU's recovered was in the range of 1 - 39 CFU/nose. No CFU's could be recovered from any of the animals of the phage treated group. The number of CFU's recovered from various groups of our present study correlates well with the literature reports of recovery of 1 - 300 CFU's of *S. aureus* Newman/nose of the BALB/c mice [32].

S. aureus is not a normal commensal organism in mouse nares; therefore establishment of experimental colonization of *S. aureus* in these animals required optimization. We achieved sufficient maintenance of colonization to allow application of phage cocktail and test their efficacy. We found that 80% of mice remained colonized for 7 days in the model reported here. The mice were gradually decolonized of *S. aureus* naturally. Therefore, we chose a phage-treatment window within the 7-day period and ended the study on day 8. This afforded a good contrast between the treated and untreated groups. We observed decolonization of all animals in the treated group, while in the control group, 75% of animals remained colonized on day 8. We believe this study to be the first report of bacteriophage efficacy in a mouse nasal model of *S. aureus* carriage.

It is well established that *S. aureus* colonizes multiple sites in the human body, particularly the anterior nares [45]. Approximately 20% of individuals are persistent carriers since they carry one type of *S. aureus* while a large population (60%) harbor *S. aureus* transiently and 20% of the population never carry any *Staphylococcus* and are called as non-carriers [45]. It has been shown that a substantial proportion of serious nosocomial infections originate from the patient's own flora, and nasal carriage of *S. aureus* is a considerable risk factor for this [45,46]. Hence, elimination of carriage reduces the infection rates

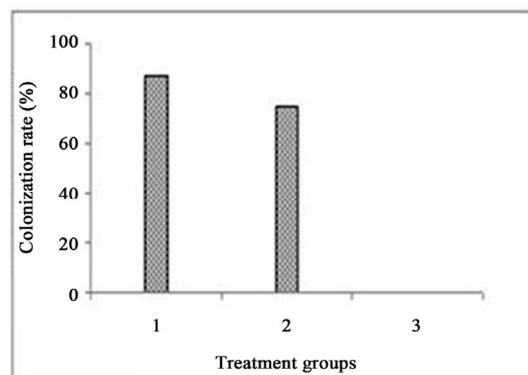


Figure 4. *In vivo* efficacy of phages 44AHJD and K at the end of eight day. Treatment group no. 1: colonization control; treatment group no. 2: placebo treated; treatment group no. 3: phage cocktail treated.

in surgical patients and those on hemodialysis and continuous ambulatory peritoneal dialysis (CAPD) [45]. Higher incidence of such organisms is also reported in the Indian community [47]. There is also compelling genetic evidence that there is a causal relationship between nasal carriage and infective clinical isolates [48]. In light of these observations, it appears that eradication of *S. aureus* during hospitalization would be valuable [49].

As there are no current guidelines on the bacteriophage titer that may be clinically effective against MRSA in the human nose, estimates can be made based on previous related studies of bacteriophage therapy. These include use of bacteriophage titers in respirable powders (10^8 - 10^9 PFU per 100 mg powder) [49], and in bacteriophages targeting *P. aeruginosa* in otitis in humans (10^5 PFU of each of six bacteriophages in 0.2 mL liquid) [50]. Based on these two studies, 10^{10} PFU per mouse nares is likely to be an optimal dose.

Use of bacteriophages for treatment of various bacterial infections including *S. aureus* has been reviewed extensively [51,52]. Although temperate phages of *S. aureus* are more widely known [53,54], due to the potential problems of lysogeny and toxic gene transfer, its therapeutic use is limited [55]. Hence, the present article on efficacy of two lytic bacteriophages of *staphylococcus* namely Phage K and 44AHJD on nasal decolonization of *Staphylococcus aureus* in mice nares is relevant and valuable.

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