



## Abrogation of *Staphylococcus aureus* Wound Infection by Bacteriophage in Diabetic Rats

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### ABSTRACT

To evaluate therapeutic potential of phages specific for *Staphylococcus aureus* to resolve wound infection in diabetic rats. The objective of the study is for the assessment of wound healing activity, excision wound model was used. Group I was assigned as control, Group II infected with methicillin resistant *Staphylococcus aureus* (MRSA), Group III non infected and received only phage preparation to assess whether phage initiates infection, Group IV infected with MRSA, once the infection was established it was challenged with *Staphylococcus aureus* phages and Group V infected MRSA and treated with antibiotic Clindamycin after 4<sup>th</sup> day of infection. A significant decrease in infection, period of epithelization and wound contraction was observed in the phage challenged group when compared to antibiotic treated diabetic rats and the control group. To conclude the study provide new insights into the biology of the broad host range of *Staphylococcus aureus* phage Ø SH-56 and indicate that phage has potential for treatment and prevention of infections caused by pathogenic staphylococci.

**Keywords:** Methicillin resistant *Staphylococcus aureus*, diabetic rats, bacteriophage.

### INTRODUCTION

Worldwide, diabetic foot lesions are a major medical, social, and economic problem and are the leading cause of hospitalization for patients with diabetes. <sup>[1]</sup> Infectious agents are associated with amputation of the infected foot if not treated promptly. <sup>[2]</sup> *Staphylococcus aureus*, a cause of wound and soft-tissue infection in diabetes, is often resistant to all  $\beta$ -lactam antibiotics. <sup>[3-4]</sup> Several studies found methicillin-resistant *Staphylococcus aureus* in as many as 15–30% of diabetic wounds. <sup>[4-5]</sup> Infection with multidrug-resistant MRSA may increase the duration of hospital stay and cost of management and may cause additional morbidity and mortality. Today, resistance has rendered most of the

original antibiotics obsolete for many infections. The emergence of pathogenic bacteria resistant to most, if not all, currently available antimicrobial agents has become a critical problem in modern medicine, particularly because of the concomitant increase in immuno-suppressed patients. <sup>[6-7]</sup> We describe a model for wound infection in rats by a strain of *S. aureus* that caused infection on a diabetic foot and protection by a phage.

### Objectives

Therapeutic potential of phages specific for methicillin resistant *Staphylococcus aureus* was evaluated for their ability to resolve wound infection in diabetic rats

### MATERIALS AND METHODS

#### Ethical clearance

The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of animals in research, and the protocol was approved by the Institutional Animal ethical Committee, S. S. Institute of

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#### Maintenance of rats

Six month old healthy male Wistar rats weighing 150-200 g, bred locally in the central animal house of S. S. Institute of Medical Sciences and Research Centre, Davangere were selected for the study. They were housed under controlled conditions of temperature ( $23 \pm 2^\circ\text{C}$ ), humidity ( $50 \pm 5\%$ ) and 10-14 hours of light and dark cycles. The animals were housed individually in polypropylene cages containing sterile paddy husk (procured locally) as bedding and free access to food and water (animal chow) *ad libitum* was provided throughout the study.

All the animals maintained on proper diet and were housed in a pathogen-free environment.

#### Induction of Diabetes Mellitus in rats

Diabetes is induced by injecting Alloxan hydrate ( $\text{C}_4\text{H}_2\text{N}_2\text{O}_4 \cdot \text{H}_2\text{O}$  (LOBA CHEMIE PVT LTD, Mumbai), 80mg / kg body weight, subcutaneously in albino rats after 12 h of continuous fasting. [8] Fasting blood sugar was evaluated by using Glucometer (SD Fine chemicals) after 72 h. Rats whose blood glucose levels remained  $<300$  mg/dl for more than one week following the initial injection of alloxan received a second dose of alloxan to maintain a blood glucose level  $>300$  mg/dl for the duration of the study. [9]

#### Bacterial isolate

*Staphylococcus aureus* strain was isolated from pus of a diabetic foot and was designated as CSV-36 in our nomenclature. Antibiotic susceptibility testing by Kirby-Bauer's method revealed that the CSV-36 strain was resistant to methicillin and to other commonly used drugs. Bacterial inoculums was prepared by inoculating MRSA CSV 36 in nutrient broth, incubating at  $37^\circ\text{C}$  overnight followed by repeated centrifugation (10,000rpm for 10 mins) and washing, finally re-suspending in normal saline.

#### To evaluate minimum infective dose of MRSA CSV-36

The rats were randomly divided into 6 groups of five animals each. The back of the each animal was shaved and prepared after washing with spirit. An area of about  $225\text{ mm}^2$  was defined with marker on the depilated back of the rat, in the dorsal interscapular region, 1cm away from the ears of the rats. The circular marked area of the skin was excised with full thickness using a surgical sterile blade and scissors under ketamine anesthesia. Each group of diabetic rats received inoculation of 400 $\mu\text{l}$  aliquots of MRSA CSV-36 suspension in different densities ( $10^7$ - $10^9$ CFU) onto wound. The animals were observed for 100 h. Rats inoculated with bacteria were scored for their state of health on a scale of 5 to 0, based on progressive disease states reflected by several clinical signs. [10] A normal and unremarkable condition was scored at 5; slightly illness defined as lethargy and ruffled fur, was scored as 4; moderate illness defined as severe lethargy ruffled fur and hunched back was scored as 3; severe illness, with the above signs plus exudative accumulation around the site of inoculation, was scored as 2; a moribund state was scored as 1; and death was scored as 0. Two independent observers will observe the result.

Pus sample were be collected and gram stain and culture were done. Blood for blood culture was collected when the animal showed the symptoms of septicemia.

#### Isolation and purification of phage strains for methicillin resistant *Staphylococcus aureus*

The MRSA phage was isolated from raw sewage at a municipal sewage treatment plant, Davangere by the method of Smith and Huggins. [11] Sewage water (50 ml) was collected in sterile conical flask and treated with a few drops of chloroform. To this equal volume of sterile nutrient broth and 1 ml of the 24 h old broth culture of MRSA CSV-36 was added. The sample inoculated with bacterial pathogens was incubated at  $37^\circ\text{C}$  for 12-24 h in shaker water bath. After 12-24 h the lysate was shaken with few drops of chloroform for about 10 min, centrifuged at 10,000 rpm for 10 min and the supernatant was filtered through 0.22 $\mu$  pore size Acrodisc membrane filters (PALL, German Laboratory) to remove the bacteria and subjected to plaque forming unit (PFU) assay using double layer agar method described by Smith and Huggins. [11] Phage preparations to be used therapeutically were passed through a column containing Detoxi- Endotoxin Removing Gel and eluted with pyrogen-free water. The phage was denoted as  $\emptyset$  SH-56.

#### In-vitro confirmation of bacteriophage activity on MRSA CSV-36

The bacterial lawn was prepared on nutrient agar plates employing 1.0 ml of 24 h MRSA CSV 36 culture by flooding and draining out the excess. Wells were dug into the agar by employing a sterile cork borer and the 20  $\mu\text{l}$  phage  $\emptyset$  SH-56 suspension ( $3 \times 10^9$  PFU/ml) was loaded into each of the well. Sterile distilled water served as the control. [11-12] The plates were incubated at  $37^\circ\text{C}$  for 24 h. There after the zone of inhibition, if any, was recorded.

#### Electron microscopic study of Phage $\emptyset$ SH-56

Electron microscopic studies of phage  $\emptyset$  SH-56 isolated were done at NIHMANS, Bangalore. Phage solution was filtered through 0.22 $\mu$  pore size Acrodisc membrane filters (PALL, German Laboratory) to remove the host bacteria. After washing three times with phosphate buffer saline (pH7.0), the retained phage solution was used directly for Electron microscopic study. [12-13]

#### Excision wound model for studying therapeutic effect of phages

The rats were randomly divided into 5 groups of six animals each. The back of the each animal was shaved and prepared after washing with spirit. An area of about  $225\text{ mm}^2$  was defined with marker on the depilated back of the rat, in the dorsal interscapular region, 1cm away from the ears of the animals. The circular marked area was excised with full thickness using a surgical sterile blade and scissors under Ketamine anesthesia. [14-15]

#### Groups

**Group I:** Non infected rats, did not receive antibiotic or phage (Control)

**Group II:** Rats infected with MRSA CSV-36 (Bacterial control)

**Group III:** Non infected rats, Received only phage preparation (Phage control)

**Group IV:** Rats Infected with MRSA CSV 36 and challenged with phage  $\emptyset$  SH-56 of dose of  $3 \times 10^9$  PFU/ml, local spray

**Group V:** Rats Infected with MRSA CSV-36 and challenged with antibiotic after 96 h of infection (Clindaycin 8mg/kg body weight)

Local infection was introduced using 400 $\mu\text{l}$  of a  $10^9$  bacterial / ml inoculum of MRSA CSV- 36 in Group II, Group IV and Group V. Group I and Group III were non infected rats.

Group III received only phage to evaluate whether phages initiates any infection, whereas Group I did not receive phages nor antibiotics.

#### Bacteriological evaluation of the wound

Swabs were taken on day 2 to confirm the presence of the MRSA CSV-36 in the pus by doing gram stain. On day 2 onwards sequential sampling of the healing wound surface was done for culture and gram stain and phage count was evaluated in group III and group IV. Blood culture was done for the animal which showed moribund status. Grading of pus for inflammatory cell was done by Clinical Microbiology Proficiency-Testing [CMPT] method.<sup>[15]</sup> Cellular infiltration were graded as 0 for absence, 1 for rare (occasional presence), 2 for few, 3 for moderate and 4 for many.

#### Period of epithelization

Period of epithelization was noted as the number of days after wound healing required for the eschar to fall off leaving no raw wound behind.<sup>[16]</sup>

#### Wound contraction rate

It was noted by following the progressive changes in wound area planimetrically. The size of the wounds was traced on a transparent paper every two days, throughout the monitoring period. The tracing was then transferred to 1 mm 2 graph sheet, from which the wound surface area was evaluated.<sup>[17]</sup> The evaluated surface area was then employed to calculate the percentage of wound contraction by using the following equation

$$= \frac{\text{Initial wound size} - \text{specific day wound size}}{\text{Initial wound size}} \times 100$$

#### Statistical analysis

The results were analyzed using One-way ANOVA followed by Tuckey's *post hoc* test.

## RESULTS

#### Diabetic rats

Alloxane induced diabetic rats showed fasting blood sugar level more than 300 mg/dl and glycosylated haemoglobin level more than 6.5% (48mmol/mol)

#### Lethality of Methicillin resistant *Staphylococcus aureus*

Fig. 1 shows lethality of the minimum infective dose of MRSA CSV-36 in rat model. Rats inoculated MRSA CSV-36 with  $10^9$  CFU established infection and showed significant pus within 48 h. In Fig. 1, each bar indicates the state of health of a single rat, a score of 5 indicates normal health, while 0 indicates death (see the material and methods for full description of the scale). For all other work,  $10^9$  CFU of MRSA CSV-36 was taken as infective dose to evaluate therapeutic utility of phages in rat model.

#### Phage strain antibacterial activity

The phage Ø SH-56 was found to form plaques on 62% of the MRSA isolates and inhibited bacterial growth of an additional 12% of the strains, thus exhibiting an antibacterial effect against 74% of the MRSA strains isolated from diabetic foot infection.

#### Electron microscopy

The phage Ø SH-56 had an icosahedral head, about 70 nm in diameter, and a 100-nm long tail, thus morphologically similar to phages belonging to *Siphoviridae* family

#### Excision wound

#### Gram stain and culture

Grading of gram stain was done as per Clinical Microbiology Proficiency-Testing [CMPT] scale and depicted in Table 1.

#### Group I

Gram stain and culture did not reveal any bacteria from the swabs collected from day 2 till the end of the experiments.

#### Group II (Bacterial control)

Gram stain and culture from the swab collected on day 2 revealed gram positive cocci in clusters along with few inflammatory cells (Table 1). Subsequent swabs on day 4 revealed more neutrophils along with gram positive cocci and culture revealed growth of MRSA CSV-36. Blood culture revealed growth for MRSA CSV-36. All rats died on 6<sup>th</sup> day.

#### Group III (Phage control)

Gram stain and culture did not reveal any bacteria from the swabs collected from day 2 and the subsequent swabs collected till the end of the experiments also did not revealed any sign of infection by phages. Few inflammatory cells were observed on day 2, but no bacteria were seen (Table 1).

#### Group IV (Infected and challenged with phage)

Gram stain and culture from the swab collected on day 2 revealed gram positive cocci in clusters along with few inflammatory cells (Grade 1). Swabs collected on day 4 revealed more neutrophils (Grade 4) along with gram positive cocci and culture revealed growth of MRSA CSV-36. Inflammatory cells reduced on day 6<sup>th</sup> (phages were administered on day 4), but gram positive cocci were still present in the smear and culture did not reveal any growth for bacteria. Day 8<sup>th</sup> swab revealed the presence of dead inflammatory cells and no bacteria were seen in the smear (Table 1). Subsequent swabs did not reveal presence of any inflammatory cells or bacteria and culture did not show growth for bacteria

#### Group V (Infected and challenged with antibiotic)

Gram stain and culture revealed the presence of bacteria and inflammatory cells from the swabs collected from day 2. Subsequent swab on day 4 showed the presence of bacteria along with increased inflammatory cells (Grade 4). All the rats were moribund at the end of 4<sup>th</sup> day. Clindamycin (8 mg/kg body weight) was administered on the day 4. Swab collected on 6<sup>th</sup> day showed the presence of bacteria and presence of inflammatory cells (Grade 3) but the clinical condition of the rats improved after the administration of antibiotic (Table 1). On day 8, the clinical conditions of the rats were better compared to 6<sup>th</sup> day and also the number of inflammatory cells (Grade 2) was fewer when compared to 6<sup>th</sup> day. Subsequent swabs on day 10<sup>th</sup> and 12<sup>th</sup> showed absence of inflammatory cells and bacteria (Table 1).

#### Wound contraction

The percentage of wound contraction was 12, 25, 29, 36, 58 and 70 as measured on the 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10, 14<sup>th</sup> and 16<sup>th</sup> day respectively in the control group (Table 2). The wound contraction rate was altered significantly in the entire test group on the 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10, 14<sup>th</sup> and 16<sup>th</sup> day as compared to control group at same time (Table 2). Apart from this, we have also noted a positive trend in wound contraction rate in phage and antibiotic treated groups and negative trend in wound contraction rate in bacterial control group and it is statistically significant on all the days when compared to homogenous groups. However wound contraction rate significantly increased in phage treated group compared to control and antibiotic treated group. The percentage of wound contraction in phage challenged group was 18, 27, 37, 43, 64 and 75 on the 4<sup>th</sup>, 6<sup>th</sup>, 8<sup>th</sup>, 10, 14<sup>th</sup> and 16<sup>th</sup> day

**Table 1: Quantitative reporting of gram stain of pus from wound in diabetic rats**

| Group<br>s  | Days              |                             |                   |                             |                   |                             |                   |                             |                   |                             |                   |                             |                   |                             |                   |                             |
|-------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|-------------------|-----------------------------|
|             | 0                 |                             | 2                 |                             | 4                 |                             | 6                 |                             | 8                 |                             | 10                |                             | 12                |                             | 14                |                             |
|             | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g | Gra<br>m<br>stain | Pus<br>cells<br>gradin<br>g |
| Group<br>-1 | -                 | 0                           | -                 | 1                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           |
| Group<br>-2 | -                 | 0                           | +                 | 1                           | +                 | 3                           | -                 | -                           | -                 | -                           | -                 | -                           | -                 | -                           | -                 | -                           |
| Group<br>-3 | -                 | 0                           | -                 | 1                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           |
| Group<br>-4 | -                 | 0                           | +                 | 1                           | +                 | 4                           | +                 | 2                           | -                 | 0                           | -                 | 0                           | -                 | 0                           | -                 | 0                           |
| Group<br>-5 | -                 | 0                           | +                 | 1                           | +                 | 4                           | +                 | 3                           | +                 | 2                           | +                 | 1                           | -                 | 0                           | -                 | 0                           |

Clinical Microbiology Proficiency-Testing [CMPT]

**Table 2: Effect of Bacteriophage on excision wound parameters in diabetic rats infected with MRSA**

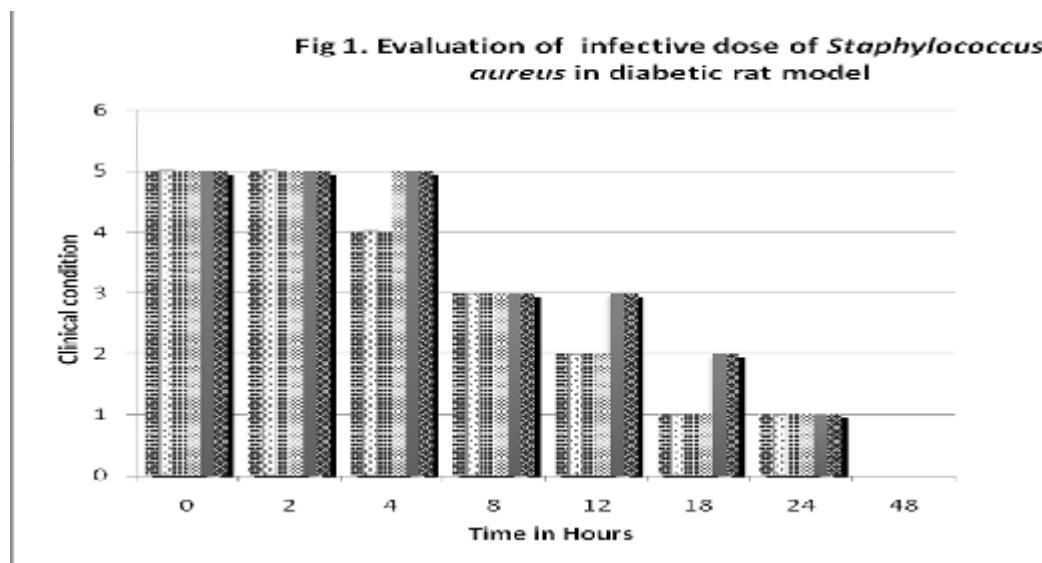
| Time of<br>assessment | Normal |      | Infected |      | Non infected<br>Phage Therapy |      | Infected N<br>Challenged With<br>Phage |      | Infected N<br>Challenged<br>With Antibiotic |      | Statistics  |     | Comparison with<br>control |   |
|-----------------------|--------|------|----------|------|-------------------------------|------|----------------------------------------|------|---------------------------------------------|------|-------------|-----|----------------------------|---|
|                       | Mean   | SD   | Mean     | SD   | Mean                          | SD   | Mean                                   | SD   | Mean                                        | SD   | P*<br>Value | Sig | Homogeneous<br>groups**    |   |
| Day 0                 | 166.0  | 0.0  | 166.3    | 0.9  | 166.0                         | 1.4  | 166.0                                  | 0.0  | 166.0                                       | 0.0  | 0.88        | NS  | -                          | - |
| Day 2                 | 171.6  | 25.3 | 208.8    | 19.0 | 148.3                         | 19.0 | 168.7                                  | 24.5 | 216.2                                       | 11.0 | <0.001      | HS  | 1, 3, 4 & 2, 5             |   |
| Day 4                 | 146.0  | 23.0 | 211.7    | 24.9 | 135.2                         | 21.6 | 136.3                                  | 25.0 | 204.3                                       | 16.5 | <0.001      | HS  | 1, 3, 4 & 2, 5             |   |
| Day 6                 | 124.4  | 14.6 |          |      | 110.3                         | 17.0 | 121.0                                  | 21.1 | 178.8                                       | 15.6 | <0.001      | HS  | 1, 3, 4 & 5                |   |
| Day 8                 | 118.6  | 9.9  |          |      | 104.0                         | 17.2 | 105.3                                  | 18.0 | 143.4                                       | 6.6  | 0.001       | HS  | 1, 3, 4 & 5                |   |
| Day 10                | 105.6  | 15.2 |          |      | 84.3                          | 25.7 | 94.3                                   | 26.6 | 125.6                                       | 11.2 | 0.017       | S   | 1, 3, 4 & 5                |   |
| Day 12                | 85.2   | 14.7 |          |      | 63.7                          | 18.1 | 82.3                                   | 11.2 | 118.6                                       | 30.0 | 0.001       | HS  | 1, 3, 4 & 5                |   |
| Day 14                | 69.6   | 12.3 |          |      | 49.5                          | 7.7  | 59.8                                   | 16.8 | 96.4                                        | 17.6 | <0.001      | HS  | 1, 3, 4 & 5                |   |
| Day 16                | 50.0   | 11.0 |          |      | 29.2                          | 6.6  | 42.0                                   | 23.8 | 74.0                                        | 12.1 | 0.001       | HS  | 1, 3, 4 & 5                |   |
| Day 18                | 23.6   | 7.6  |          |      | 16.8                          | 3.8  | 27.2                                   | 14.8 | 46.2                                        | 6.4  | 0.001       | HS  | 1, 3, 4 & 5                |   |
| Day 20                | 9.0    | 3.2  |          |      | 4.3                           | 1.6  | 11.0                                   | 16.0 | 16.4                                        | 1.7  | 0.14        | NS  | -                          | - |

\*Oneway ANOVA test ; \*\* Tukey's post hoc test

**Table 3: Effect of Bacteriophage on reduction of wound area in diabetic rabbits infected with MRSA**

| Time of<br>assessment | Normal  | Infected | Non infected<br>Phage Therapy | Infected & Challenged<br>With Phage | Infected & Challenged<br>With Antibiotic | P** Value | Sig | Homogeneous<br>groups*** |
|-----------------------|---------|----------|-------------------------------|-------------------------------------|------------------------------------------|-----------|-----|--------------------------|
| Day 2                 | -3±8.4* | -26±9.9  | 11±10.6                       | -2±13.3                             | -30±6.5                                  | <0.001    | HS  | 1, 3, 4 & 2, 5           |
| Day 4                 | 12±9.0  | -27±17.4 | 19±12.3                       | 18±13.5                             | -23±9.4                                  | <0.001    | HS  | 1, 3, 4 & 2, 5           |
| Day 6                 | 25±8.8  |          | 34±10.1                       | 27±12.4                             | -8±8.8                                   | <0.001    | HS  | 1, 3, 4 & 5              |
| Day 8                 | 29±6.0  |          | 37±10.5                       | 37±9.8                              | 11±6.3                                   | 0.001     | HS  | 1, 3, 4 & 5              |
| Day 10                | 36±9.2  |          | 49±13.7                       | 43±14.4                             | 20±11.4                                  | 0.017     | S   | 1, 3, 4 & 5              |
| Day 12                | 49±8.9  |          | 62±9.5                        | 50±6.0                              | 24±18.5                                  | 0.001     | HS  | 1, 3, 4 & 5              |
| Day 14                | 58±7.4  |          | 70±4.0                        | 64±9.1                              | 35±19.0                                  | <0.001    | HS  | 1, 3, 4 & 5              |
| Day 16                | 70±6.7  |          | 82±5.4                        | 75±12.8                             | 46±23.3                                  | 0.001     | HS  | 1, 3, 4 & 5              |
| Day 18                | 86±4.6  |          | 90±5.8                        | 84±8.0                              | 60±29.7                                  | 0.001     | HS  | 1, 3, 4 & 5              |
| Day 20                | 95±1.9  |          | 97±1.0                        | 93±8.8                              | 75±36.9                                  | 0.14      | NS  | -                        |

\* Standard deviation; \*\*Tukey's post hoc test; \*\*\*One way Annova



respectively, where else in antibiotic challenged group the percentage of wound contraction was -23, -8, 11, 20, 35 and 46 on the same days as mentioned above (Table 3). A negative trend in wound contraction rate was observed till 6<sup>th</sup> day in antibiotic treated animals, but a significant positive trend was observed in phage treated group. The rate of wound contraction was highly significant in phage treated group compared to control groups (Table 2).

The mean period of epithelization was found to decrease significantly in phage treated group ( $P < 0.05$ ) when compared to control. The duration of epithelization in the antibiotic treated group did not differ significantly when compared to control. The mean  $\pm$ SEM of the number of days required for epithelialisation in control group was  $23.5 \pm 0.99$ , in phage control group the duration of epithelization period was  $20.75 \pm 0.25$ , in phage challenged group, it was  $22.5 \pm 1.50$  and in antibiotic treated group the epithelization was  $25.25 \pm 0.73$ .

## DISCUSSION

*Staphylococcus aureus* plays a prominent role as an etiological agent of serious infections in diabetic foot patients. Foot infection cause a breach in the protective skin barrier and suppress the immune system, rendering the patients highly susceptible to bacterial infection. *Staphylococcus aureus* colonization of diabetic foot and rapid proliferation within the damaged tissues often leads to disseminated infections resulting in bacteraemia and septic shock and high rates of mortality and morbidity. Treatment of such infections is confounded by the innate and acquired resistance of *Staphylococcus aureus* to many antimicrobials. It has been estimated that at least 50% of all deaths caused by diabetic foot are the result of infection and untreatable infections have become a tragically frequent occurrence in patients infected with *Staphylococcus aureus*. Hence, the development of new therapeutic and prophylactic strategies for the control of bacterial infection in diabetic foot patients is needed.

An alternative or supplement to antibiotic therapy, which is currently being re-examined, is the use of bacterial viruses (phage/bacteriophage) to target bacterial infections, i.e. phage therapy

Our study has shown an alarmingly high incidence of MRSA infection from diabetic foot infections. The prevalence rate is found to be 55.6%, which is much higher than most of the reports where it ranged between 19% and 30.2%.<sup>[4-5]</sup> It is felt that lack of infection control and inappropriate overuse of antibiotics has led to the emergence of MRSA. Growing antimicrobial resistance is now a worldwide issue with MRSA being the most pressing problem.

Wound is a disruption in the continuity of the living tissues. Wound repair or regeneration or sometimes both lead to wound healing.<sup>[17]</sup> The various phases of wound healing are inflammation, angiogenesis, epithelialisation, collagenation, wound contraction, etc.<sup>[14, 16]</sup> In the present study phages significantly reduced the duration of epithelialisation and increased the percentage of wound contraction. There are no literatures on use of bacteriophage to study wound healing properties in diabetic rats are available to compare our results. But, there are many literatures available illustrating the utility of bacteriophage as therapeutic agents in many bacterial infections.<sup>[11, 12, 18, 19]</sup>

In Group II, Group IV and Group V rats were infected with  $10^9$  CFU of MRSA CSV-36. After 2 days, all rats in Group II, IV and V that had received bacteria produced abscess. The rate of wound contraction was in negative trend. After 4 days all the rats in Group II died. In the Group III all the rats received only phage (Phage control) but no bacteria. After 4 days the rats did not produce abscess, indicating phages did not initiate infection in the wound. Even the rate of wound contraction was in positive trend and percentage of wound contraction was statically significant when compared with control (Group I) indicating phages enhanced the rate of epithelization. The mean areas and bacterial counts in the abscess (Table 2) increased consistently in the groups which were not challenged with either phages or antibiotic, and the largest abscesses being from the control group (Group II) which contained higher bacteria counts than the abscess of the groups receiving the phages (Group IV) and the group receiving delayed antibiotic treatment (Group V). In phage challenged group, No bacteria were isolated from the abscess after 6 days, indicating *Staphylococcus aureus* phage Ø SH-56 has cleared all the *Staphylococcus aureus* present in the abscess. Where else in antibiotic challenged group, bacteria could be demonstrated in the abscess till the 10 day

In the current study we could able to demonstrate phage prophylaxis against experimental *Staphylococcus aureus* infections in rats similar to those that are common in humans. There was no evidence of general sepsis, and the rats, including phage controls, appeared remarkably well.

The potential of phage therapy has been the subject of several recent reviews and the present study reinforces the view that this potential is worth exploring. The experiments in the present study represent solutions to many of the problems that hindered the prior applications of phage therapy. For example, the relatively narrow host range of most phages which caused many of the early attempts at phage therapy to fail can be overcome by isolating phages that have a broad host range within the species being targeted and today we have number of animal models available by which controlled studies can be carried out.

To conclude, this study has opened a broad research horizon that will enable future researchers to mitigate the use of bacteriophage as an alternative therapeutic agent in multidrug resistant organisms.

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