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Research Article

EFFECTIVENESS OF BACTERIOPHAGE IN THE TREATMENT OF *STAPHYLOCOCCUS AUREUS* WOUND INFECTION IN THE DIABETIC ANIMAL MODEL

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ABSTRACT

The emergence of antibiotic resistance is the most eloquent example of Darwin's principle of evolution that there ever was. It is a war of attrition. It is naive to think that we can win over these invisible bacteria which are great survivors and the biggest threat which the world is facing now. Antibiotics are bedrock of modern medicine. But in the very near future, we're going to have to learn to live without them once again. And it's going to get malicious, unless other modalities of treating these drug resistant bacteria are assessed. One such proposal is treating drug resistant bacteria with bacteriophages. The objective of the present study is to evaluate therapeutic potential of phages specific for Methicillin resistant *Staphylococcus aureus* (MRSA)to resolve wound infection in diabetic rabbit. A significant decrease in infection, period of epithelization and wound contraction was observed in the phage challenged group when compared to antibiotic treated diabetic rabbits and the control group. To conclude the study provide new insights into the biology of the broad host range of *Staphylococcus aureus phage* and indicate that phage has potential for treatment and prevention of infections caused by pathogenic multi drug resistant *Staphylococcus aureus*

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

INTRODUCTION

The World Health Organization (WHO) has recently acknowledged that India has the maximum number of diabetic patients than any other country around 50.8 million (Wild, 2030). This is projected to increase to 80 million by the year 2030. India is thus the 'Diabetic Capital of the World (Tripathy, 1970). Since 1975, there is a steady increase in the prevalence of diabetes mellitus in rural areas of India. The prevalence has increased from 0.6% in 1975 to 2.4% in 1995 (Tripathy, 1970, Khanolkar, 2008). WHO expert committee on diabetes has issued a clarion call to workers around the world to carry out epidemiological survey of diabetes with a view to identify, before it is too late, the cultural, social and other factors which may contribute to diabetes and its complications.

Diabetic foot is one of the most feared complications of diabetes and is the leading cause of hospitalization in diabetic patients. Diabetic foot is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation (Khanolkar, 2008, Singh, 2005). Diabetic patients have a lifetime risk as high as 25% for developing foot ulceration (Alavi, 2007). Diabetic ulcers have 15 to 46 times higher risk of limb amputation than foot ulcers due to other causes (Gadepalli, 2006). Every year more than a million diabetic patients require limb amputation (Tentolouris, 1999).

The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection (Alavi, 2007). *Escherichia coli, Proteus* spp*., Pseudomonas* spp*., Staphylococcus aureus* and *Enterococcus* spp. are the most frequent pathogens contributing to progressive and widespread tissue destruction (Khanolkar, 2008). Diabetic foot infections are often poly-microbial (Shankar, 2005). Methicillin-resistant *Staphylococcus aureus* has been commonly isolated from 10-40% of the diabetic wounds (Eleftheriadou, 2010). The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation (Yoga, 2006). Infection with MDR pathogens is also responsible for the increased duration of hospitalisation, cost of management, morbidity and mortality of the diabetic patients (Gadepalli, 2006).

Antibiotics are considered the most important advance in the history of modern medicine. The impact of the use of antibiotics for reducing human morbidity, mortality and economic losses has been phenomenal. This far outweighs the gains achieved with any other medical advance in the past century. Yet with each passing decade, bacteria that defy not only single but multiple antibiotics and which therefore are difficult to control have become increasingly common. The major threat we now confront is escalating resistance to antibiotics, which jeopardizes much of the progress made in the previous century. Antimicrobial resistance (AMR) is now the most important challenge being faced by humanity in its fight against infectious diseases. The emergence and spread of resistance in several microorganisms have rendered the management of diabetic foot diseases difficult. Failure to discover new antimicrobial agents has further hampered the war against infectious agents. One of such substitute's stems up from an old idea is the bacteriophage therapy. Bacteriophage therapy is a method of antibacterial treatment that harnesses the bacteria-killing properties by harmless viruses (Alisky, 1998, Smith, 1982, VinodKumar, 2008).

We describe a model for wound infection in rabbit by a strain of MRSA that caused infection on a diabetic foot and protection by a phage.

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 of the institute.

Maintenance of rabbits

Two -three month old healthy male rabbits weighing 2.0-2.5 Kg, bred locally in the 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 ± 2°C), humidity (6 5%) and 10-12 hours of light and dark cycles (Lenzen, 2008). The rabbits were housed individually in cages and free access to food and water.

Induction of Diabetes Mellitus in rabbits

Diabetes is induced by injecting Alloxan hydrate ($C_4H_2N_2O_4.H_2O$ (LOBA CHEMIE PVT LTD, Mumbai), 80mg / kg body weight, subcutaneously in rabbits after 12 hrs of continuous fasting (8). Fasting blood sugar was evaluated by using Glucometer (SD Fine chemicals) after 72 hrs. Rabbits 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 (Mirand, 2009, Jiangpu, 2010, Pincus, 1954).

Bacterial isolate

Staphylococcus aureus strain was isolated from pus of a diabetic foot. Antibiotic susceptibility testing by Kirby-Bauer's (Bauer, 1966) method revealed that the CSV-36 strain was resistant methicillin and to other commonly used drugs. The methicillin resistant *Staphylococcus aureus* was designated as CSV-36 in our nomenclature Bacterial inoculums was prepared by inoculating CSV-36 in nutrient broth, incubating at 37^{o}C overnight followed by repeated centrifugation (10,000rpm for 10 mins) and washing, finally re-suspending in normal saline.

Evaluation of minimum infective dose of MRSA CSV-36

The rabbits were randomly divided into 6 groups of five animals each. The upper lateral left hind limb of the rabbit was shaved and cleaned with spirit. An area of about 225 mm^2 was defined and marked. The circular marked area of the skin was excised with full thickness using a surgical sterile blade and scissors under ketamine anesthesis

Each group of diabetic rabbits received inoculation of 400µl aliquots of bacterial suspension in different densities (10^2-10^7CFU) onto wound. The animals were observed for 48 h. Rabbits 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 (18, 19). 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 (Biswas, 2002). Pus sample were collected and gram stain and culture were done. Blood for blood culture was collected when the animal showed the symptoms of septicaemia**.**

Isolation and purification of phage strains for MRSA CSV-36

The *Staphylococcus aureus* phage was isolated from raw sewage at a municipal sewage treatment plant, Davangere by the method of Smith and Huggins (Smith, 1982, Biswas, 2002). The phage was denoted as Ø SH-56. In-vitro confirmation of phage activity was done as per the standard procedures (Biswas, 2002). Further electron microscopic study was carried out at NIHMANS Bangalore (Bojovazova, 1991, VinodKumar et al., 2011)

Excision wound model for studying therapeutic effect of phage Ø SH-56 in diabetic rabbits

The rabbits were randomly divided into 5 groups of five animals each. The upper lateral right hind limb of the rabbit was shaved and cleaned with spirit. An area of about 225 mm^2 was defined and marked. The circular marked area of the skin was excised with full thickness using a surgical sterile blade and scissors under ketamine anesthesia (Goodson, 1977, Muppayavarmath, 1999)

Groups

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

Group II: Rabbits infected with MRSA CSV-36 and challenged with 3X10^{9}PFU/ml phage Ø SH-56 preparation (local spray) after 48 h of infection

Group III : Rabbits infected with MRSA CSV-36 and treated with antibiotic clindamycin (8mg/kg body weight) after 48 h of infection

Group IV : Rabbits infected with MRSA CSV-36, and not challenged with antibiotics or phages

Group V: Non infected rabbits, Received only phage preparation

Local infection was introduced using 400µl of a 10^7 MRSA CSV-36 bacterial inoculum in group II, group III and group IV.

Group I and Group V were non infected rabbits. Group V received only phage Ø SH-56 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 wound surface was done for culture and gram stain till day 20 and phage count was evaluated in group II and group V. Blood culture was done for the animal which showed moribund status. Grading of pus for inflammatory cell was done as per the Clinical microbiologist numerical scale; cellular infiltration were graded as 0 for absence, 1 for rare (occasional presence), 2 for few cells, 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 (Dwajani, 2009).

Phage titer

Samples from the phage treated rabbits were homogenized and then filtered through a 0.22µ pore size membrane (Acrodisk, Pall German Laboratory). The phage titer in the filtrates was determined using a soft agar overlay using MRSA CSV-36 as the host (VinodKumar et al., 2011). The resulting plaques were counted with the ProtoCol plate counter and phage titer was calculated (Bojovazova, 1991).

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 sheets, from which the wound surface area was, evaluated (Dwajani, 2009).

Statistical analysis

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

RESULTS

Diabetic rabbits

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

Minimum infective dose of MRSA CSV-36

Fig-1to Fig 6 depicts lethality of the infective dose of MRSA CSV-36 in rabbit model. Rabbits were inoculated with 10^2 to 10^7 CFU of MRSA CSV-36. Rabbits inoculated with 10^7CFU were moribund with in 48 h [Fig 6]. To evaluate therapeutic utility of phages in rabbit model 10 ^{7}CFU of MRSA CSV-36 was taken as infective dose

Phage strain antibacterial activity

The phage Ø SH-56 was found to form plaques on 74% of the methicillin resistant *Staphylococcus aureus* isolated from diabetic foot infection

Excision wound

Bacteriological evaluation

*Gram stain and culture***:**

The finding of gram stain and culture is depicted in table 1.

In Group I**,** Gram stain and culture did not reveal any bacteria from the swabs collected from day 2 till the end of the experiments. In Group II, Gram stain and culture from the swab collected from day 2 to day 4 revealed gram-positive cocci in clusters along with inflammatory cells in increasing trend and culture revealed growth of MRSA CSV-36. But on day 6 and day 8, inflammatory cells and gram positive cocci gradual decreased and subsequent swabs did not reveal presence of any inflammatory cells or bacteria. While in group III, all the rabbits were moribund at the end of day 2. Clindamycin (8mg/kg body weight) was administered on the day 3rd till day 5. Gram stain and culture of the swab collected on day 4 showed the presence of gram positive cocci and presence of inflammatory cells, but the clinical condition of the rabbits improved after the administration of antibiotic. Swabs on day 6, 8, and 10 showed gradual decrease for the presence of bacteria and inflammatory cells. On day 12th, smear did not reveal any presence of bacteria nor inflammatory cells. In group IV, gram stain from the swab collected on day 2 revealed gram positive cocci in clusters along with few inflammatory cells. Subsequent swabs revealed more neutrophils along with gram positive cocci and culture revealed growth of *Staphylococcus aureus* on 4th, 6th and 8th day. All the rabbits were moribund at the end of 8th day, and died on day 10th. Group V, Non infected wound but wound surface was sprayed with *Staphylococcus aureus* phage Ø SH-56.Gram stain and culture did not reveal any bacteria from the swabs collected on day 2 and the swabs collected till the end of the experiments also did not revealed any sign of infection by phages [Table 1].

Table 1: Quantitative reporting of gram stain of pus from wound of diabetic rabbits.

Groups	Days															
	0		2		4		6		8		10		12		14	
	Gram stain	Pus cells	Gram stain	Pus cells	Gram stain	Pus cells	Gram stain	Pus cells	Gram stain	Pus cells	Gram stain	Pus cells	Gram stain	Pus ells	Gram stain	Pus cells
Group-1	-	0	-	1	-	0	-	0	-	0	-	0	-	0	-	0
Group-2	-	0	+	2	+	4	+	4	+	2	-	0	-	0	-	0
Group-3	-	0	+	4	+	3	+	4	+	3	+	3	-	0	-	0
Group-4	-	0	-+	3	+	2	+	2	+	4			Death			
Group-5	-	0	-	0	-	0	-	0	-	0	-	0	-	0	-	0

Note: Refer materials and method for grading of pus cells / oil immersion field and +/- in gram stain indicates presence of bacteria or absence of bacteria

Table 2: Phage titre at different time intervals in wound sites of diabetic rabbits in Group II and Group V.

Days	Phage titre (PFU / ml)	
	Group II	Group V
0	--	10^9 PFU of phage was administered locally
2	10^9 PFU of phage was administered locally	No phage grown
4	$1.75x10^{16}$	No phage grown
6	$1.16x10^{11}$	No phage grown
8	$2.1x10^2$	No phage grown
10	<10	No phage grown
12	No phage grown	No phage grown
14	No phage grown	No phage grown

Phage titre

The phage titre was determined in group II and group V. In group II the phage titre was evaluated from day 4 to day 14. While in group V, phage count was evaluated from day 2 to day 14. In group II, phage count was $1.75x10^{16}$ PFU/ ml, $1.16x10^{11}$ PFU/ ml, $2.1x10^2$ PFU/ ml and less than 10 PFU/ ml on the 4th, 6th, 8th, and 10th day respectively. In group V, no phage was cultured on the day 2 onwards (Table 2).

Wound contraction

The mean area of wound and percentage of wound contraction is depicted in table 3 and table 4. The percentage of wound contraction was 4.6±2.2, 11.6±2.2, 16.4±1.4, 20.5±0.9, 27.7±3.0, 44.5±1.1 and 64.0±1.5 as measured on the 4th, 6th, 8th, 10, 14th and 16th day respectively in the group-1 [Table 4]. The wound contraction rate was altered significantly in the different groups on the 4th, 6th, 8th, 10, 14th and 16th day as compared to control group at same time [Table 4]. Apart from this, we noted a positive trend in wound contraction rate in phage and antibiotic treated groups and negative trend of wound contraction in bacterial control group and it was statistically significant on all the days when compared to homogenous groups. However wound contraction rate significantly increased in phage treated group on day 12 compared to non infected group and antibiotic treated group. The percentage of wound contraction in phage challenged group was 2.7±3.5, 9.4±2.3, 14.2±2.9, 19.3±2.3, 32.3±1.8, 45.7±2.2 and 66.0±2.6 on the 4th, 6th, 8th, 10, 14th and 16th day respectively, while in antibiotic challenged group the percentage of wound contraction was -22.7±6.3, -18.3±2.3, -16.9±4.2, -8.2±2.6, 7.5±1.8, 27.7±0.9 and 48.2±2.6 on the same days as mentioned above [Table 4]. A negative trend in wound contraction rate was observed till 10th 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 4]

The mean period of epithelization was found to decrease significantly in phage treated group ($P < 0.001$) when compared to control (Table 3). The duration of epithelization in the antibiotic treated group did not differ significantly when compared to control. The mean ±SEM of the number of days required for epithelialisation in group-1 and group-2 was 21.5±0.99. While in antibiotic challenged group (Group 3) the duration of epithelization period was 25.75±2.5.

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 bacteremia and septic shock and high rates of mortality and morbidity (Sivaraman, 2011). 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 disastrously frequent episode in patients infected with *Staphylococcus aureus* (Anandi, 2004, Singh, 2005). Hence, the development of novel 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 (VinodKumar et al., 2009, Biswas 2002).

Our study shows 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% (Shankar, 2005, Goldstein, 1996, Eleftheriadou, 2010). The risk for MRSA isolation increases in the presence of osteomyelitis, nasal carriage of MRSA, prior use of antibacterials or hospitalization (Nathwani, 2003), larger ulcer size and longer duration of the ulcer (Eleftheriadou, 2010). The need for amputation and surgical debridement increases in patients infected

with MRSA. It is felt that lack of infection control and inappropriate overuse of antibiotics has led to the emergence of MRSA. Growing anti-microbial resistance is now a worldwide issue with MRSA being the most pressing problem (Goldstein, 1996).

Table 3: Time of assessment of effect of Bacteriophage on excision wound parameters in diabetic rabbits infected with MRSA

		Study Groups						
Time of Assessment (Days)	**Parameter**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**p* Value, Sig**	**Pairwise Comparison****
0	Mean (mm)	**166.0**	**166.0**	**166.0**	**166.0**	**166.0**	-	-
	SD	0.0	0.0	0.0	0.0	0.0		
2	Mean (mm)	**171.2**	**178.0**	**174.4**	**177.6**	**168.8**	P=0.001, HS	1&2, 1&4, 2&5, 4&5
	SD	2.5	3.2	4.1	3.8	3.0		
4	Mean (mm)	**158.4**	**162.0**	**208.0**	**210.0**	**163.2**	P<0.001 ,HS	1&3, 1&4, 2&3, 2&4, 3&5, 4&5
	SD	3.6	5.1	7.6	4.7	2.3		
6	Mean (mm)	**146.8**	**152.0**	**198.0**	**211.6**	**155.2**	P<0.001 ,HS	1&3, 1&4, 1&5, 2&3, 2&4,3&4, 3&5, 4&5
	SD	3.6	3.2	4.2	4.1	4.1		
8	Mean (mm)	**138.8**	**142.4**	**194.0**	**206.4**	**144.8**	P<0.001 ,HS	1&3, 1&4, 2&3, 2&4, 3&4, 3&5, 4&5
	SD	2.3	4.8	6.9	2.6	4.1		
10	Mean (mm)	**132.0**	**134.0**	**179.6**	**-**	**140.2**	P<0.001 ,HS	1&3, 1&5, 2&3, 3&5
	SD	1.4	3.7	4.3	-	4.0		
12	Mean (mm)	**120.0**	**112.4**	**153.6**	**-**	**124.4**	P<0.001 ,HS	1&2, 1&3, 2&3, 2&5, 3&5
	SD	4.9	3.0	3.0	-	3.6		
14	Mean (mm)	**92.2**	**90.2**	**120.0**	**-**	**96.0**	P<0.001 ,HS	1&3, 2&3, 2&5, 3&5
	SD	1.8	3.6	1.4	-	2.8		
16	Mean (mm)	**59.8**	**56.4**	**86.0**	**-**	**62.0**	P<0.001 ,HS	1&3, 2&3, 3&5
	SD	2.5	4.3	4.2	-	1.4		
18	Mean (mm)	**34.0**	**28.0**	**42.4**	**-**	**30.0**	P=0.001, HS	1&3, 2&3, 3&5
	SD	3.7	3.7	6.1	-	3.7		
20	Mean (mm)	**0.4**	**0.4**	**17.6**	**-**	**6.2**	P<0.001 ,HS	1&3, 1&5, 2&3, 2&5, 3&5
	SD	0.9	0.9	0.9	-	3.6		

*** Oneway ANOVA test, ** Tukey's Post Hoc test**

Table 4: Effect of bacteriophage on percentage reduction in the wound area among diabetic rabbits infected with MRSA .

		Study Groups						
Time of Assessment (Days)	**Parameter (Percentage of wound contraction)**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**p* Value, Sig**	**Pairwise Comparison****
0	Mean	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	-	-
	SD	0.0	0.0	0.0	0.0	0.0		
2	Mean	**-3.1**	**-4.1**	**-7.7**	**-6.8**	**-1.7**	P=0.001, HS	1&2, 1&4, 2&5, 4&5
	SD	1.5	5.8	7.5	2.1	1.8		
4	Mean	**4.6**	**2.7**	**-22.7**	**-26.0**	**1.7**	P<0.001, HS	1&3, 1&4, 2&3, 2&4,3&5, 4&5
	SD	2.2	3.5	6.3	2.5	1.3		
6	Mean	**11.6**	**9.4**	**-18.3**	**-27.0**	**6.5**	P<0.001, HS	1&3, 1&4, 1&5, 2&3, 2&4,3&4, 3&5, 4&5
	SD	2.2	2.3	2.3	2.6	2.5		
8	Mean	**16.4**	**14.2**	**16.9**	**-23.9**	**12.7**	P<0.001, HS	1&3, 1&4, 2&3, 2&4, 3&4, 3&5, 4&5
	SD	1.4	2.9	4.2	1.5	2.6		
10	Mean	**20.5**	**19.3**	**-8.2**	**-**	**15.5**	P<0.001, HS	1&3, 1&5, 2&3, 3&5
	SD	0.9	2.3	2.6	-	2.4		
12	Mean	**27.7**	**32.3**	**7.5**	**-**	**25.1**	P<0.001, HS	1&2, 1&3, 2&3, 2&5, 3&5
	SD	3.0	1.8	1.8	-	2.2		
14	Mean	**44.5**	**45.7**	**27.7**	**-**	**42.2**	P<0.001, HS	1&3, 2&3, 2&5, 3&5
	SD	1.1	2.2	0.9	-	1.7		
16	Mean	**64.0**	**66.0**	**48.2**	**-**	**62.7**	P<0.001, HS	1&3, 2&3, 3&5
	SD	1.5	2.6	2.6	-	0.9		
18	Mean	**79.5**	**83.1**	**74.5**	**-**	**81.9**	P=0.001, HS	1&3, 2&3, 3&5
	SD	2.3	2.3	3.7	-	2.3		
20	Mean	**99.8**	**99.8**	**89.4**	**-**	**96.3**	P<0.001, HS	1&3, 1&5, 2&3, 2&5, 3&5
	SD	0.5	0.9	0.5	-	2.2		

*** Oneway ANOVA test, ** Tukey's Post Hoc test**

Rabbit was selected over the rat because of its large size and chronic wound model could be easily established in rabbit when compared to rats

Wound is a disruption in the continuity of the living tissues (Goodson, 1977). Wound repair or regeneration or sometimes both lead to wound healing. The various phases of wound healing are inflammation, angiogenesis, epithelialisation, collagenation, and wound contraction (Muppayavarmath, 1993). In the present study phages significantly reduced the duration of epithelialisation and increased the percentage of wound contraction

In Group II, Group III and Group IV rats were infected with 10^7 CFU of MRSA CSV - 3 6 . After 2 days, all rabbits in Group II, III and IV that had received bacteria produced abscess. The rate of wound contraction was in negative trend. After day 8th all the rabbits in Group IV died. In the Group V all the rabbits received only phage (Phage control) but no bacteria. After 4 days the rabbits did not produced abscess, indicating phages did not initiate infection in the wound. Even the rate of wound contraction was in positive trend after day 2 and percentage of wound contraction was statically significant when compared with Group II and III indicating phages could have 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 Group IV which contained higher bacteria counts than the abscess of the

groups receiving the phages (Group II) and the group receiving delayed antibiotic treatment (Group III).

In phage challenged group, No bacteria were isolated from the abscess after 6 days, indicating *Staphylococcus aureus* phage SH-56 has cleared all the methicillin resistant *Staphylococcus aureus* present in the abscess. While in antibiotic challenged group, bacteria could be demonstrated in the abscess till the 10 day.

Phages were not cultured from the wound sites of the phage control but were cultured from those of all rabbits that received bacteria and phage. Form group II, more phages were recovered than the administered dose; this, to our awareness, is the first direct substantiation of administered phage multiplying in the tissues infected by gram-positive bacteria. While in the group V the phage was not cultured, indicating that phage specific bacteria is required for propagation of the phages

The discovery of antibiotics in the 20th century was perhaps one of the greatest breakthroughs in human medicine with millions of lives saved ever since. However, the exponential rise of antibiotic resistance in bacterial pathogens causing human infection has rapidly developed into one of the most significant issues in modern medicine. Of major concern is the emergence of strains of bacteria resistant to many commonly available antibiotics, the so-called "Multi-resistant strains" and no new antibiotics in the pipeline. The situation has reached such a decisive point that the World Health Organization (WHO) has cautioned of a return to a "pre-antibiotic era. In the present study we could able to demonstrate phage activities against experimental MRSA infections similar to those that are common in humans. To conclude, our finding, further reinforce our views that phages could be used in a situations where there is no substitutes available for treating multi-resistant strains.

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