THERAPEUTIC EFFICACY OF PHAGE LYSATE IN CHRONIC MASTITIS OF FARM ANIMALS

Sanjay Kumar Shukla¹, S. D. Hirpurkar² and Swatantra K. Singh³*

¹Assistant Professor, Department of Microbiology, COVS, Rewa-486001 (MP), India  
²Professor & HOD, Department of Microbiology, COVS, Anjora, Durgh, CG, India  
³Assistant Professor, Department of Pharmacology and Toxicology, COVS, Rewa-486001 (M.P.), India

ABSTRACT
Lytic phages were evaluated for therapeutic activity against antibiotic resistant pathogenic bacteria in chronic mastitis cases. A total of twelve animals affected with chronic mastitis were selected in the clinical trial. Previously these animals were subjected to conventional antibiotic treatment and they were not cured. Therefore phage cocktail were used in these animals. Treatment consisted of 5 ml intra-mammary single infusion of 3 x 10¹² plaque forming unite (PFU)/ml of lytic phage in the experimental animals. Teats were showing gradually reduction in the swelling. Ten animals were showing the complete recovery out of twelve within ten days. Phage therapy is very economic and highly effective as compare to the existing antibiotic therapy.

KEYWORDS: Mastitis, PFU, Phage Cocktail, Phage therapy.

INTRODUCTION

Mastitis is one of the largest production concerns in the dairy industry worldwide and overall frequencies of intramammary infection were estimated to be 48.5% in a survey conducted in the United States [1]. Among the mastitis pathogens, Staphylococcus aureus is considered an agent of major concern due to the low cure rate of S. aureus infections by antibiotic treatment. The natural ability of pathogens to develop resistance to every antibiotic is not only a threat to animal health but also leads to accumulation of antibiotic residues in livestock products which creates a hazardous threat to the human population. The emergence of resistance in the pathogenic bacteria against the currently available antimicrobial agents has become a critical problem in modern medicine. Phages have been extensively used to prevent and treat bacterial infections with improved chances of success [4].

MATERIAL AND METHODS
Clinical trial was conducted to observe therapeutic effect of phage lysate on chronic mastitis in the animals at Live Stock Farm (LSF), College of Veterinary Science. & A.H., M.P.P.C.V.V., Jabalpur, Madhya Pradesh, India. A total twelve animals of chronic mastitis were...
selected in the trial. Phage cocktail prepared by using the phage lysate of EHR1, EHR2 and BsHR1 in equal parts with the concentration of $3 \times 10^{12}$ PFU/ml. These lytic phages were recovered from the animal waste of livestock farms.

In the present study bovine affected with chronic mastitis were selected for the clinical trial. Previously these animals were subjected to conventional treatment and animals prior to any phage treatment, they were not cured. Further these animals were subjected to intramammary infusion of phage cocktail for evaluating its efficacy and observation were recorded on day 0, 5th and 10th.

Pus samples were collected from the affected teats and investigated for isolation and identification of pathogenic bacteria associated with mastitis. Bacterial isolates were subjected to antibiotic sensitivity test to know the antibiotic resistant pattern. Antibiotic resistant isolates subjected to phage sensitivity test to know the sensitivity of phage against the pathogenic bacteria. Cocktail was prepared by mixing phage of broad host range. Phage cocktail (5ml) was infused in the teat with the help of disposable syringe after proper cleaning of teat with normal saline. Appropriate follow-up of the case was maintained to avoid the further, contamination of wound. Therefore the status of wound recovery was recorded at regular time interval (0, 5th and 10th days) to observe the recovery in the form of physical appearance and microbiological investigation wound to compare the condition and status of wound before and after the treatment.

RESULTS AND DISCUSSION

The results were recorded in terms of clinical recovery of wound which was assessed by physical appearance and microbiological investigation of wound. The chronic cases are having the multiple infections of various pathogenic microbes which were showing the multiple drug resistance. Cases were recovered completely after the application of phage lysate swelling and discharge were gradually reduced from the teat after the treatment. There was no any discharge from teat there after 5th day of the treatment. Both the parameters were based on the regular observation. Microbiological investigation to find out bacterial load of wound was carried out to besides the physical recovery from wound infection with respect to presence of organisms in the wound after the treatment. There was no organism found at 5th day in any case after the treatment.

In the present study ten cases were cured out of twelve, therefore phage therapy showed 83 percent of efficacy. Phage therapy is very economic and highly effective as compare to the existing antibiotic therapy. The efficacy of phage lysate was found to be 83 percent which was much more as compared to the conventional treatment. The prospects of the phage therapy are
bright particularly in the antibiotic resistance crisis. Thus infusion of lytic phage in single dose proved to be innovative and effective therapy for treatment of chronic mastitis. These findings are similar with the findings of Gill et al.\(^2\) treated bovine mastitis (Staphylococcal mastitis) effectively with phage therapy. However, our findings showed agreement with the findings of Slopek et al.\(^4\), Alisky et al.\(^1\) and Wills et al.\(^5\) reported rate of success of phage therapy as 75-100% against suppurative infections. Thus, clinically phage cocktail can be prescribed in chronic mastitis.

Table:1-Antibiotic sensitivity pattern of pathogen isolated from chronic septic teats.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Name of bacteria</th>
<th>Bacterial isolates found sensitive to the antibiotic discs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Am  T  E  Cf  Cp  Cx  Ac  G  P  K</td>
</tr>
<tr>
<td>1</td>
<td><em>E. coli</em></td>
<td>0    2   0   5   7   0   0   7   0   4</td>
</tr>
<tr>
<td>2</td>
<td><em>Klebsiella spp</em></td>
<td>0    0   8   9   0   0   0   11  0   5</td>
</tr>
<tr>
<td>3</td>
<td><em>P. aeruginosa</em></td>
<td>0    1   0   0   3   0   0   2   0   0</td>
</tr>
<tr>
<td>4</td>
<td><em>Salmonella spp</em></td>
<td>0    1   1   2   2   0   2   4   0   3</td>
</tr>
<tr>
<td>5</td>
<td><em>S. aureus</em></td>
<td>3    0   5   6   2   2   0   0   0   0</td>
</tr>
</tbody>
</table>

Am-Amikacin, T-Teramycin, E-Erythromycin, Cf-Ciprofloxacin, Cp-Cephalaxin, Ac-Amoxiclav, G-Gentamicin, P-Penicillin, K-Kanamycin

CONCLUSIONS

Chronic cases of mastitis in farm animals were showing the multiple infection with the several microbial agent like *Staphylococcus, Pseudomonas and E coli* which did not respond to antibiotic therapy due to the development of multiple drug resistance (MDR) . These MDR microbial organisms respond to the phage lysate. Phage therapy has been developed as an alternative method of therapy against antibiotic resistance organisms to provide practical solution of the crisis of antibiotic resistance and public health problems. It is also is very effective and economic as compare to existing antibiotic therapy. Response to phage treatment in the present clinical trial was highly encouraging. Therefore these agents can be used to treat the chronic cases of mastitis.

ACKNOWLEDGMENT
I am very much thankful to College of Veterinary Science and Animal Husbandry, Jabalpur to providing all the necessary funds and facilities. I also acknowledge with regards to Dr. (Mrs.) E. Joseph, Professor & Head of the Department of Veterinary Microbiology who is very much friendly and her scientific thoughts heaped me with lots of enthusiasm to work.

REFERENCES


For Correspondence
Swatantra Kumar Singh
Email: dr.swatantravet@rediffmail.com