**Evaluation of in vitro Antibacterial Activity of Phage Lysate against Microbial Pathogen Isolated from Septic Wounds**

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**A B S T R A C T**

In present study, we had isolated the bacteriophages from sewage material of various livestock farms, NDVSU, Jabalpur Madhya Pradesh. The recovered bacteriophages subjected for the identification with electron microscopy. The isolated bacteriophages had shown icosahedral symmetry. Phage had hexagonal head with the size of 52.20 nm in diameter and long tail of 109 nm. They were classified as a member of the Myoviridae family under the order of Caudovirale. These recovered bacteriophages were subjected to spottest to know the antibacterial activity against bacterial pathogens. All the recovered phage lysate showed the antibacterial activity specifically against the *Staphylococcus aureus* isolated from chronic septic wounds. These isolates of bacteriophage had limited host range due to their nature to infect the specific host. There was few isolates (ØVS4 ØVS5 and ØVS9) of bacteriophages were showing the lytic activity against the other microbial organism like *Bacillus* spp and *E. coli*. ØVS4, ØVS5 and ØVS9 three isolates of bacteriophage had broad host range. In vitro antibacterial activity of phage lysates can be used as a potential therapy against multiple drug resistant pathogens in the current era of antibiotic resistance crisis.

**Key words**

In vitro, *S. aureus* Antibacterial activity, Phage lysate, Antibiotic resistance

**Introduction**

Bacteriophages are natural predators of bacteria and found ubiquitously, these organisms are estimated to be present at numbers equivalent to a trillion per grain of sand on earth (Keen, 2015). Bacteriophages that infect *S. aureus*, are of the order Caudovirales (Xia and Wolz, 2014). They are DNA viruses of Siphoviridae and Myoviridae families (Ackermann, 2009). They are obligate intracellular parasites requiring specific bacteria as host cell for their replication (Carlton, 1999). Phages are most widely distributed and diverse entities in the biosphere and ubiquitously present, which can be found in all reservoirs populated by bacterial hosts, such as soil and intestine of animals (McGrath and van Sinderen, 2007). Bacteriophages were co-discovered by Felix d’Herelle and Frederick Twort.

The current resurgence of interest is persisting in light of the growing urgency of the antimicrobial resistance (AMR) crisis, which predicts that AMR will be the leading cause of death by 2050, causing 10 million global deaths per year (O’Neill, 2016).
antibiotic resistance first emerged, novel antibiotics to treat these bacteria were developed. Bacteriophages are potential antibacterial therapeutic agents against such multiple drug resistant pathogens (Burrowes et al., 2011). Also, unlike chemical therapeutic agents, phages are not susceptible to the onset of bacterial resistance because they have the ability to evolve with their host (Sulakvelidze and Kutter, 2005).

The natural ability of pathogens to develop resistance is not only a threat to animal health but also leads to accumulation of antibiotic residues in livestock products. Consumption of these livestock products leads hazardous threat for human population. In vitro antibacterial activity of recovered phage lysates can be used as a potential therapeutic alternate against multiple drug resistant pathogens in the current era of antibiotic resistance crisis.

**Materials and Methods**

**Culture media used during study**

Commercially available ready to use dehydrated media (Hi-Media Laboratories Limited) were used for the preparation of culture media. Instructions given by manufacturers for reconstitution of respective media were followed. The fresh media were prepared, tested for sterility and stored at refrigeration temperature until use.

Following laboratory media were used in the present investigation:

- Basal agar (tryptone broth with 1.5% agar)
- Mannitol salt agar
- Muller-Hinton agar
- Nutrient agar
- Nutrient broth
- Soft agar (tryptone broth with 0.7% agar)
- Tryptone soya broth

**Sewage samples for bacteriophage isolation**

Sewage samples for bacteriophage isolation were collected from livestock farms (cattle, buffalo, pig and goat), N.D.V.S.U., Jabalpur (M.P.). Sewage material consisted of various body excretions from different species of animal’s viz. cattle, buffalo, goat and pig. One hundred fifty samples were collected in sterile tubes from various collection tanks and storage pits of integrated livestock farm complex, Adhartal, N.D.V.S.U., Jabalpur (M.P.).

**Isolation of bacteriophage from sewage samples**

*Staphylococcus aureus* (ATCC 25923) was the host bacterium used for isolation of bacteriophage from sewage samples. Sewage samples isolation were for bacteriophage collected from livestock farms (cattle, buffalo, pig and goat), N.D.V.S.U., Jabalpur (M.P.). Isolation of bacteriophages was done by soft agar overlay method as described by Synnot et al., (2009) with slight modification. The recovered phage isolates were characterized and studied for *in vitro* antibacterial activity

**In-vitro assessment of antimicrobial activity of phage lysate**

Bacteriophage isolates recovered from the sewage samples of various livestock farms were tested to know the antibacterial activity. The bacterial lawn of *S. aureus* was prepared by flooding agar plate with 2 ml trypton soya broth culture. Bacteriophage lysate was spotted onto a lawn, then the plates were incubated at 37°C and formation of plaques was observed at various time intervals at 6, 12, 18 and 24 h. Similar approach was adopted by Iwano et al., (2018).

**Results and Discussion**

Pus samples were collected from the chronic septic wounds infections of animlas. These
collected samples were subjected for the isolation and identification of microbial pathogens associated with wounds. All the chronic septic wounds were infected with multiple pathogens like *S. aureus*, *E coli*, *Bacillus* spp and *Pseudomonas* spp.

Result of bacterial isolation of chronic septic wounds infections indicated that the most of the septic wounds were infected with multiple bacterial pathogens in which *Staphylococcus* spp, *Escherichia* spp, *Pseudomonas* spp and *Bacillus* spp were predominantly associated. Our findings were in confirmation with the findings of Vinodkumar et al., (2008) and Heo et al., (2009).

Further these bacterial isolates were subjected for spot test. The bacterial lawn was prepared by flooding agar plate with 2 ml broth culture. Phage lysate was spotted onto a lawn, then the plates were incubated at 37°C for 24 h and formation of plaques was observed at various time intervals at 6, 12, 18 and 24 h.

Result of present study revealed that all the isolates of bacteriophage were showing the good antibacterial activity against the all *S. aureus* isolates from chronic septic wounds. They are very having narrow host range specifically infecting to *S. aureus*. There were few isolates of phage especially ØVS4, ØVS5 and ØVS9 were showing the antibacterial activity against the other microbial organism like *Bacillus* spp and *E. coli*. Therefore these three isolates of ØVS4, ØVS5 and ØVS9 had broad host range as compare to others isolates. *In vitro* phage lysate had shown good antibacterial activity against *S. aureus*. *In vitro* antibacterial activity of phage lysates exploring the utility of bacteriophage as a potential alternate of antibiotic against multiple drug resistant pathogens which can be helpful to overcome the crisis of antibiotic resistance.

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