

Metagenomic Analysis and Pharmacokinetics of Phage Therapy in the Treatment of Bovine Subclinical Mastitis

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Abstract : Metagenomic analysis of milk samples collected from local cattle breed, kankrej (*Bos indicus*), Gir (*Bos indicus*) and Crossbred (*Bos indicus* X *Bos taurus*) cattle harbouring subclinical mastitis was carried out by next-generation sequencing (NGS) 454 GS-FLX technology. Around 56 different species including members of Enterobacteriales, Pseudomonadales, Bacillales and Lactobacillales with varying abundance were detected in infected milk. The interesting presence of bacteriophages against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter* and *Yersinia* species were observed, especially Enterobacteria and *E. coli* phages (0.32%) in Kankrej, Enterobacteria and *Staphylococcus* phages (1.05%) in Gir and *Staphylococcus* phages (2.32%) in crossbred cattle. NGS findings suggest that phages may be involved in imparting natural resistance of the cattle against pathogens. Further infected milk samples were subjected for bacterial isolation. Fourteen different isolates were identified, and DNA was extracted. Genes (Tet-K, Msr-A, and Mec-A) providing antibiotic resistance to the bacteria were screened by Polymerase Chain Reaction and results were validated with traditional antibiotic assay. Total 3 bacteriophages were isolated from nearby environment of the cattle farm. The efficacy of phages was checked against multi-drug resistant bacteria, identified by PCR. In-vivo study was carried out for phage therapy in mammary glands of female rats "Wister albino". Mammary glands were infused with MDR isolates for 3 consecutive days. Recovery was observed in infected rats after intramammary infusion of sterile phage suspension. From day 4th onwards, level of C-reactive protein was significant increases up to day 12th. However, significant reduction was observed between days 12th to 18th post treatment. Bacteriophages have significant potential as antibacterial agents and their ability to replicate exponentially within their hosts and their specificity, make them ideal candidates for more sustainable mastitis control.

Keywords : bacteriophages, c-reactive protein, mastitis, metagenomic analysis

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