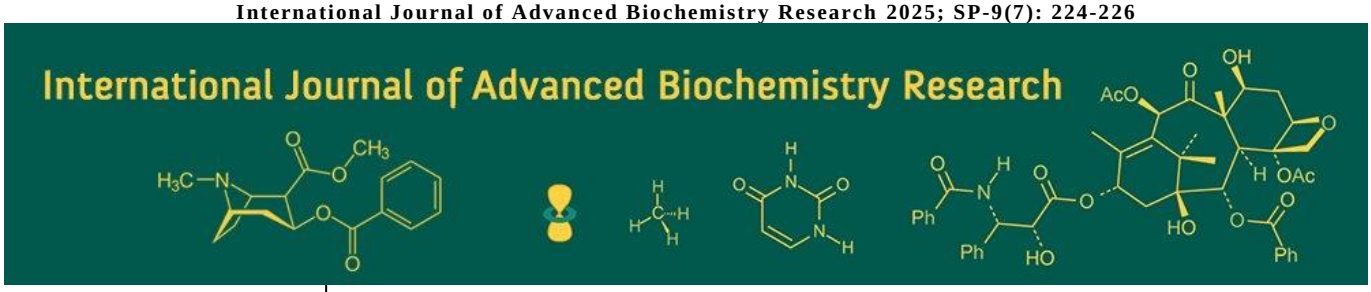

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Evaluation antimicrobial activity of phage cocktail against bacteria isolated from chicken meat and milk samples

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Abstract
Ability of microbes to develop antimicrobial resistance is not only a threat to animal health but also leads to residual effect of antibiotic in livestock products. The aimed of study to investigate the antibacterial efficacy of a bacteriophage against pathogens, specifically *S. aureus* and *E. coli* associated with milk and chicken meat samples. Bacteriophages were isolated from sewage samples of livestock farms by using the DAL method. Four high-quality phage isolates were selected to evaluate their antibacterial effect bacterial pathogens on chicken meat and milk. Total twelve samples were collected from retail shops, consisting of six each of chicken meat and raw milk. Initially the samples were processed for microbial detection to confirm the presence of bacteria (mainly *S. aureus* and *E. coli*. The phage cocktail treatment effectively eliminated *E. coli* from all meat samples but had no effect on *S. aureus*. In milk samples, the phage cocktail reduced the presence of both *E. coli* and *S. aureus* by 33.3%. This study highlights the use of a bacteriophage cocktail against bacterial pathogens.

Keywords: *E. coli*, *S. aureus*, phage and antibacterial activity

Introduction
According to the WHO, diarrheal diseases caused by consuming contaminated foods result in millions of illnesses and hundreds of thousands of deaths annually (Odo, Uchechukwu and Ezemadu, 2021) [6]. Bacteriophages are increasingly recognized as an agent due to their specificity in identifying the causative agents of bacterial diseases, including those in food production settings. Various foodborne pathogens like *Salmonella* species, *L. monocytogenes*, *E. coli*, *Staphylococcus*, and *Shigella* have been targeted by phage bio-preservation products, indicating the need for diverse food preservation strategies (Garvey, 2022) [3]. With some phage therapies already approved by the FDA, the use of bacteriophages as biocontrol agents in food products presents a viable option for combating bacterial infections in animal-derived foods (Jakobsen, *et al.*, 2022) [4]. In the food industry, bacteriophages have emerged as potential biocontrol agents, offering various applications such as pathogen detection, biopreservation and serving as an antibiotic alternative for addressing animal health concerns. Their utilization in food production aims to enhance food quality, prevent foodborne illnesses, and improve production efficiency. Bacteriophages exhibit a unique ability to selectively target and eliminate bacterial pathogens without harming mammalian cells or beneficial microbiota, making them valuable for enhancing food safety (Gildea, Ayariga and Robertson, (2022) [1].

Materials and Methods
Sample collection
About 50 ml of sewage sample was collected and processed for isolation of bacteriophage. Isolation of phage was performed by double agar over lay method as described by Adams. Meat and milk sample were also collected for the isolation of bacteria.

Preparation of bacteriophage lysate

Initially the basal agar plates were prepared by using 10 ml melted agar. Sewage sample were centrifuge at 6000 rpm for 20 min and then filter it with 0.45 syringe filter. Now Filtrate (500 µl) and 6 hrs old bacterial cultures (500 µl) were mixed properly and agitated for 20 min on shaker then mix it to molten agar. Now mixture was spread on basal plates and incubated at 37 °C for overnight, then observed the plaques. Serial dilution of phage lysate was performed for titration to obtain the PFU concentration for antibacterial activity.

Detection of bacteria in milk and meat samples:

The samples (Meat and milk) were initially inoculated in nutrient broth and incubated overnight and then streaked over nutrient agar plates and incubated at 37 °C for 24 hrs. Morphological examination of bacterial growth was on performed for phenotypic identification of bacteria (Quinn, 2004) [10].

Evaluation of lytic activity of bacteriophage

Isolated bacterial pathogens were subjected to phage sensitivity test to know the susceptibility of pathogens to prepare the cocktail of phage lysate for better lytic activity. Various phage lysate was used to prepare the phage cocktail. The presence of bacterial pathogens was estimated before and after spraying of phage lysate on food products. Bacteriophages are potentially useful as antimicrobial agents in food, especially cocktails of different bacteriophages which may prevent the development of bacterial resistance. In the present study, raw milk and chicken meat samples were collected from various places in Jabalpur. Study was performed with slight modification as per the method given by Tomat *et al.* (Table 1).

Results

The cocktail of four high quality phage lysates was prepared and applied on milk and meat samples. The lytic activity on bacteria present in food sample was detected by observing the presence of bacterial pathogens before and after the phage application. Twelve samples (06 chicken meats and 06 raw milk) were collected from retail shops of Jabalpur for the isolation of *E. coli* and *S. aureus*.

All the chicken meat (6/6) and milk (6/6) samples exhibited the presence of *E. coli*, while two samples of chicken meat (2/6) and three of milk (3/6) showed the presence of *S. aureus*. Phage cocktail (1ml) was sprayed on chicken meat samples and incubated at different temperatures and duration (37 °C for 24hrs, 25 °C for 24hrs and 4 °C for 6 days). Phage cocktail (1ml) was also applied on milk samples and incubated at 37 °C for 6hrs, 25 °C for 12hrs and 4 °C for 24hrs. After bacteriophage application chicken meat and raw milk samples were subjected for detection of pathogens (*S. aureus* and *E. coli*).

The presence of bacteria was compared with the initial stage of samples before the use of cocktail. Meat and milk samples were treated with phage cocktail to know the biocontrol effect of phage lysate. Out of all six meat samples, none of the sample showed the detection of *E. coli* (0/6), while both the samples demonstrated the presence of *S. aureus* (2/2). Phage cocktail showed a complete elimination of *E. coli* (100%) from meat samples but no effect against *S. aureus* (Table 02). Whereas, from six samples of milk, four samples were found positive for the presence of *E. coli* (4/6) and two for *S. aureus* (2/3). After phage application 33.3% of milk samples showed elimination of *E. coli* and *S. aureus*.

Discussion

Our results align with the results reported by Seo *et al.* 2016 who used bacteriophage BPECO19 against *E. coli* in artificially contaminated fresh meat. They showed the complete elimination of all *E. coli* within 10 min and scaling down of plate count also. These results were also assisted by the results of Lukman *et al.* demonstrated high efficiency, achieving a 99.20% and 99.04% reduction in EPEC or EHEC contamination in meat and Minh *et al.* showed that phages, PBL66-CL1 and PBL116CS6, were effective against 77.7% and 74% of ESBLEC isolates from chicken meat samples. Phage cocktail significantly reduced bacterial counts in broth and raw chicken meat samples. Dewanggana *et al.* 2022 [5] and Poojari *et al.* 2022 [15] also reported the same the phage cocktail was reducing *E. coli* and *Salmonella* on poultry meat demonstrated significant reductions in bacterial counts on chicken meat surfaces within 2 to 6 hrs. Wen *et al.* studied four lytic *S. aureus* phages were isolated from Yangzhou, China sewage. Our finding of phage cocktail on raw milk samples supported by the findings of Gharieb *et al.* who isolated phage and showed effectively control MDR *S. aureus* in milk at both 25 °C and 37 °C. These results align with the results of Duc *et al.* who explored the potency of a phage cocktail against a bacterial cocktail of *S.* Typhimurium, *S.* Enteritidis and *E. coli* O157:H7. The phage cocktail indicated potent antibacterial effects at various temperatures (4 °C, 24 °C and 37 °C).

Conclusion

Food borne disease seen after ingestion of contaminated food. Contamination may due to bacterial pathogens and its toxin which leads the symptoms like diarrhoea, vomiting and gastroenteritis. The most common pathogenic strains that are involved in food borne illnesses are Enterotoxigenic *E. coli*, Enteropathogenic *E. coli* and Enterohemorrhagic *E. coli*. Food handlers or other surfaces like skin of animals, water, soil and processing equipment are usually play a big roll to disseminate *S. aureus* in to food and food products . Bacterial organism from food handlers and other human sources are thought to contributing major role in food poisoning. In our country food borne problems is very is prone due to environmental conditions, nature of food habits, poverty, inadequate basic hygienic and limited awareness of food safety and health. Food and food products can be contaminated at any point of production processing and distribution. Contamination of food samples showed the high presence of *E. coli* than *S. aureus*. Phage cocktail strongly eliminated *E. coli* than *S. aureus* from food samples. Bacteriophage cocktail is very effective against *E. coli* from meat samples while not effective in *S. aureus*. It means bacteriophage cocktail completely eliminated *E. coli* in meat samples. Bacteriophage cocktail is effective against 33.3% of *E. coli* and *S. aureus* in milk samples. Bacteriophage lysate is viable at all temperature and time combinations. Bacteriophage lysate is having good antibacterial effect in broth culture.

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Conflict of Interest
There is no any conflict of interest related to this work

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