Review Article

A brief history of Mastitis and its treatment with Bacteriophage against *Staphylococcus aureus*

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Abstract

The secretion of liquid from mammary glands is termed as milk. During the initial stages of life, it is the only source of food for mammals. Man has learned to domesticate animals for milk purposes; these animals are buffaloes, goats, cows, and camels. Besides its nutritional availability, consumers are also at risk of diseases due to antimicrobial residues and zoonotic pathogens. Raw milk and its derivatives favor the growth of many microorganisms, considered as the significant source of Staphylococcal infection in man. The 5% of Staphylococcal outbreaks in Europe were due to milk and other dairy products. In the last few years, multidrug-resistant Livestock-Associated Methicillin-Resistant *Staphylococcus aureus* (LA-MRSA) has been reported worldwide. Its increase is a significant concern from a public health perspective. Phage therapy proved to be successful for the treatment of bacterial infections in animal models and human patients. TEM126 can be used and selected for phage therapy as an antitherapeutic agent against *Staphylococcus aureus* infections. This review mainly focuses on the source of mastitis infection and their consequences and also discusses their treatment with bacteriophages.

Keywords: Mastitis; Milk; Phage therapy; *Staphylococcus aureus*

Introduction

Mastitis can be referred to as “The Inflammation of the mammary gland” It may be moderate or very severe. The presence of mastitis can change the texture of milk and microbiological contents. There will be a significant loss by this disease in this herd. The loss will be due to a reduction in milk production. In simple words, mass milk should be discarded. A primary microorganism that is involved in the

outcome of mastitis is “Algae” mycoplasma, yeast, and bacteria. The bacterial pathogens involved in this disease are (*Staphylococcus aureus*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Streptococcus agalactia*, and *Escherichia coli*). The most common causative agent of mastitis in caprine and other dairy animals is *Staphylococcus aureus* [1].

Milk contains all necessary nutrients and energy materials for the proper growth of

infants. After India and China, Pakistan is ranked third worldwide to produce the goat [poor man's cow]. Goat milk provides the necessary nutrients for rural families and underprivileged areas. In Pakistan, about 71% of milk is produced from buffaloes, 24% by cattle, and 5% by sheep and goat. Total milk production through sheep and goat is almost 17.69 million-tonne in Pakistan. As compared to the milk of sheep and goat, human and cow milk contains less fat and protein. One-liter milk of goat contains 32g of protein and preferably very good for children up to 11 years of age. It includes a high level of isoleucine, cysteine, tyrosine, lysine, valine, and threonine as compared to cow milk. Milk is rich in fats, proteins, carbohydrates, vitamins, and minerals; hence it is a portion of very nutritional food [2]. Besides its healthy availability, consumers are also at risk of diseases due to antimicrobial residues and zoonotic pathogens. Raw milk and its derivatives favor the growth of many microorganisms, considered as the significant source of staphylococcal infection in man. 5% of Staphylococcal outbreaks in Europe were due to milk and other dairy products. The presence of udder infection, contamination during and after milking, and adulteration are factors that can lower the quality of milk. Milking through contaminated milk machines, clothes, and hands of milkman can aid the contamination of bacteria. Major organisms involved in infection in the mammary glands are *Staphylococcus aureus*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. *Streptococcus agalactiae* and *Escherichia coli*. [3].

Mastitis is the most frequent infectious disease in dairy cows and is the global problem in dairy herds. It is accountable for a significant loss in dairy products because more than 100 microbial species have been isolated from cows' mammary glands, but a small number of these organisms cause

mastitis. *Staphylococcus aureus* is one of them responsible for the leading cause of mastitis in dairy cows. These organisms are generally called "contagious" or "environmental" based on their reservoir and source and transmission mode. The term contagious means that the infected mammary glands are the primary source of infection, which is transferred to a healthy person by milking, from the equipment of milking, towels, and the milkman [4]. The term "environmental" refers to the mastitis that the organisms present other than the area of mammary glands such as Pasture, bedding, and various surfaces. Successive mud, soil, and moisture contribute as a significant factor in the spreading of these organisms. Successful control of "environmental" mastitis is based on maintaining a dry living and clean area, whereas successful control of "contagious" mastitis is based on minimizing exposure to teats contaminated with pathogens found in the milk of infected cows. As I quoted earlier, *Staphylococcus aureus* is the foremost and frequent contagious pathogen that is causing mastitis. Cows with infection are the primary cause of contamination of *Staphylococcus aureus* in raw milk. In particular, cows with subclinical *Staph. aureus* mastitis infections can shed a large number of *Staph. aureus* organisms in their milk. [5].

The Most critical pathogen is causing skin infection, food poisoning, nausea, diarrhea, and cramps in humans. Spreading and the outbreak of disease is due to the production of enterotoxins produced by *Staphylococcus aureus*. Five different classes of enterotoxins produced by staphylococcus abuses have been reported, but their mechanism of precluding food poisoning and virulence is not cleared yet. By direct involvement of virulent Bacteria and by internet contamination, *Staphylococcus aureus* can contaminate the milk [6].

***Staphylococcus aureus* resistance**

Staphylococci are Gram-positive bacteria with a range of diameter 0.5 – 1.5 µm, which can be divided into more than one plane and form grape-like clusters that are why individual cocci characterize them. Genus *Staphylococcus* has 32 species and eight subspecies. The Staphylococci are non-spore-forming, non-motile facultative anaerobes that can be grown by fermentation or aerobic respiration. Generally, they require an organic source of nitrogen, given by 5 to 12 essential amino acids, e.g., valine, arginine, and B vitamins with nicotinamide and thiamine. These bacteria are also resistant to high salt concentration. Staphylococci can be distinguished from streptococci based on catalase and oxidase test because staphylococci are catalase-positive while streptococci are catalase-negative, and again, they have a different composition of the cell wall as compared to Staphylococci. Pathogenic staphylococci can produce coagulase, which can clot the blood [7]. This feature of making coagulase can also distinguish between genus *Staphylococcus*, coagulase-positive strains of *Staphylococcus aureus* (a human pathogen), and *Staphylococcus intermedius* and *Staphylococcus hyicus* (two animal pathogens) and coagulase-negative species such as *Staphylococcus epidermidis*. Other features to distinguished the members of *Staphylococcus* are the formation of colonies on a tangible media because Colonies of a *Staphylococcus aureus* often have a golden color when grown on a tangible medium; that's why these species named as aureus while coagulase-negative [*Staphylococcus epidermidis*] form pale, translucent and white colonies. [8].

Staphylococcus aureus is a commensal, non-motile, non-spore-forming, gram-positive, and normally a member of the human body's normal microbiota, especially in the upper respiratory tract and skin. About 60% of the population is the carrier, while 20% are

normally colonized with this bacterium. *Staphylococcus aureus* can evade the immune response by capsular polysaccharides protein A and leukocyte specific toxins and the ability to grow as a biofilm [9]. The human population's normal microbiota contains 20% to 30% *Staphylococcus aureus* found in nostrils, skin flora, and normal inhabitant of women's lower reproductive tract. Although this bacterium is a normal microbiota member, it can be an opportunistic pathogen and become a common cause of skin infection, abscess, respiratory infection such as sinusitis, and food poisoning [10].

Staphylococcus aureus is involved in several diseases in humans and animals, and pathogenicity is related to different genetic characteristics involved in virulence are Immune evasion, invasive capacity, and antibiotic resistance. *S. aureus* has so much importance as an etiological agent in dairy ruminants because of causing both clinical and subclinical mastitis and is involved in economic losses due to a reduction in milk production and quality. In the last few years, multidrug-resistant livestock-associated methicillin-resistant *S. aureus* [LA-MRSA] has been reported worldwide. Its increase is a great concern from a public health perspective [11].

Due to the production of enterotoxin, *Staphylococcus aureus* is a major human pathogen in causing food poisoning. Previously published studies carried out in Italy revealed that 39% of samples of raw milk and raw milk cheese have *Staphylococcus aureus*, and 21% carried enterotoxin genes. The most popular cheese in Colombia is double cream, and its consumption is more than 30%. It is also fresh cheese and quite similar to mozzarella and other curd cheese, which are traditionally made from raw milk. Research on double crema revealed 18.5% of samples were MRSA and carrying *mecA* gene from 65

isolates of staph. Aureus, which were coagulase positive. Dairy products and milk are often implicated in outbreaks of *Staphylococcus aureus*. *S. aureus* may come from different sources, including raw milk [milk from mastitis cow] biofilms in the processing plant, healthy carriers, and the environment [12].

Staphylococcus aureus is the leading cause of food poisoning with various symptoms, including vomiting, nausea, and abdominal pain. It can cause food poisoning by heat-stable toxins, including enterotoxin and toxic shock syndrome toxin [TSS-1]. *Staphylococcus aureus* is majorly transmitted by contaminated food. E.g., dairy and meat products. It is also tolerant to high salt, and its toxins are also stable at high temperature that threatens food safety [13].

Staphylococcus aureus can cause three basic syndromes [1] superficial lesions, e.g., skin abscess, [2] deep-seated and systemic infections, e.g., bacteremia and endocarditis [3]. Toxicemic syndrome, e.g., toxic shock syndrome [TSS] and Staphylococcal scarlet fever [14]. Due to its worldwide spread and massive treatment with antibiotics, *S. aureus* has gain resistance to most of the antibiotics from penicillin to the latest linezolid. These bacteria are called multidrug-resistant bacteria [MDR]. These resistant bacteria are causing serious problems in the medical and veterinary fields [15].

Among the *Staphylococcus* resistance strains, MRSA [methicillin-resistant *Staphylococcus aureus*] VRSA [vancomycin-resistant *Staphylococcus aureus*] is essential because these strains have got resistant to a vast number of infections. Resistant strains of *Staphylococcus aureus*, e.g. [HA-MRSA] hospital-acquired methicillin-resistant *Staphylococcus aureus* was first isolated in 1961 in the hospitals of the United Kingdom. And now it accounts for more than 60% of isolates from hospitals. In 1972 first resistant

staph aureus has been isolated from cow milk; 5.2% of Belgian cows have resistant *Staphylococcus aureus* [MRSA] out of 232 farms. In the previous study, methicillin-resistant *S. aureus* has been isolated from dairy herds had a prevalence of 9.9%. About 10% of the dairy herds in Belgium have mastitis caused by *Staphylococcus aureus* has methicillin-resistant staph. aureus. Previous investigation in Germany revealed that the prevalence of MRSA in large bulk tank milk in 2009 and 2010 was 4.1% and 4.7%, respectively [16]. But these studies were not differentiating between organic and conventional herds. People who are working on farms have a greater chance of carrying resistant *Staphylococcus aureus* into the health care system during the treatment. However, the risk of MRSA through milk consumption is low due to milk's heat treatment before exporting to the market. But this estimation may differ from the consumption of milk, which is present in an open market for the people and products made from raw milk because this raw milk and raw milk products expose the consumers to many viable bacteria. Until now, no reports have been published indicating the spread of livestock-associated methicillin-resistant *Staphylococcus aureus* [LA-MRSA] to humans via milk [17].

In the United States, MDR [multidrug-resistant] organisms cause at least more than 2 million infections and lead to 23,000 deaths every year. Methicillin-resistant *Staphylococcus aureus* is now an MDR organism and is resistant to several antibiotics and is a major concern for the community [1]. As far as MRSA strains' importance, globally, different MRSA strains present on different earth [18]. A very diverse range of MRSA has occurred at different periods, but during the last ten years, a type of MRSA strains [sequence type 22 MRSA IV] have been dominated in the hospitals of Ireland, while ST30-MRSA-IVc and ST8-

MRSA-Iva have been dominated among community-acquired MRSA [CA-MRSA] isolates [37398]. It is responsible for more than 80,000 infections and more than 11,000 related deaths. WHO [world health organization] nominated this MRSA as an organism of worldwide concern. US government has made a national strategy to combat these MDR organisms and promoting alternative therapy [19].

History and epidemiology of Methicillin resistance

In 1959 first-line methicillin was introduced in response to penicillin-resistant strains. But just after two years, in 1961, methicillin resistance strain reports in the hospitals of “United Kingdom” and called Methicillin-Resistant *Staphylococcus aureus* [MRSA]. After its reporting in the United Kingdom, MRSA was also reported from Japan USA, and Australia [20]. Also, many MRSA strains were Multidrug-resistant [MDR] and gained very Importance in public health points of view. Many organisms can cause infection at the surgical site caused by multidrug-resistant *S. aureus* increases the morbidity and mortality rate high cost of treatment and has been observed in the case of MRSA infection [21].

Both the Bacterial genotype and invasiveness of the disease of *S. aureus* have some association with each other. Colonial complexes [ccs] and specific virulence genes [cap 5] were seen in the MRSA strains. B lactam antibacterial resistance and due the virulence gene carried out by USA 300 strain of Community Associated-Methicillin resistant *Staphylococcus aureus* [CA MRSA] CA-MRSA has penicillin-binding protein. Which is linked to less cost of treatment in comparison to other strains [22].

Conducted a study in which they have done characterization of 8 MRSA [Methicillin-resistant *Staphylococcus aureus*] isolates [23]. They have done this study on cheese [doble crema], which are produced in small

Columbia industries from the raw milk of cow. These all isolates have panton-valentine leucocidin gene and mec A gene. Pulse gel electrophoresis [PFGE] was done on seven isolated, and according to this technique, three isolates were closely related according to profiles. Enterotoxin B gene was also present in 3 of them. An antimicrobial susceptibility test was also done on the isolates with the help of different antibiotics. Resistance against oxacillin, cefotaxim ampicillin penicillin was seen. MIC [minimum inhibitory concentration] value of oxacillin was 4 to 8 mg/L. But Humans are at low risk of diseases due to MRSA, which can be transferred through contaminated foods. But we cannot deny MRSA can infect the severity of Infection and worldwide community [24].

Staphylococcus aureus can be transferred through raw milk, and raw milk can be contaminated through cows with subclinical mastitis are the main source of infection because they can introduce a large number of *Staphylococcus aureus* in the milk. This study was conducted by considering *Staphylococcus aureus*'s threat to human and animal health, the outbreak of methicillin-resistant *Staph. aureus* [MRSA] was isolated. Methicillin susceptible staph aureus [MRSA and oxacillin-susceptible *Staph. aureus* [OS-MRSA] was also tested California mastitis test was done on the milk to check the mastitis milk, and samples were collected from the dairy farms [25]. There was a total of 53 % of *Staphylococcus* species isolated (61 out of 115), and out of these 61 isolates, 60 samples were positive for *Staphylococcus aureus*. The remaining one was the *Staphylococcus epidermidis*. After doing all the tests and identification on these isolates, 48.3 % isolates of staph. Aureus had mec A gene MRSA was 23 %, and OS-MRSA was 25 %. MRSA was involved in causing mastitis was 12.2 %. The major and great concern that can threaten the societies is

infection through MRSA and OSMRSA due to these organisms' public health being at risk [26].

Staphylococcus aureus in the human microbiota, but it is cells involved in several diseases and infections in humans and animals, and most importantly, it is causing inflammatory infection in dairy animals. It is involved in both clinic and subclinical diseases. This study is designed to identify the circulation and prevalence of methicillin-resistant *Staphylococcus aureus* [MRSA]. MRSA was isolated from Italian dairy sheep. Milk was collected from build tank milk for the isolation of MRSA. Three people were also used for sampling purposes, which was in close contact with these animals. First isolation was done in 2012, and after two years of first isolation same multidrug-resistant bacteria, especially a strain of MRSA, was identified. 2 out of 556[0.34%] were multi-drug resistant from *Staph. aureus* isolates whereas methicillin-susceptible *Staph. aureus* [MRSA] was detected in the sample [27]. In further studies, two more isolates of MRSA were detected from the udder skin of animals. PFGE analysis was done on MRSA isolates, which have been isolated from udder skin, and analysis revealed that they are closely related [96.3 % similarity]. The prevalence of MRSA on inter farms was very low, but this study highlights the 200 zoonotic possibilities of *Staph aureus* [28].

S. aureus is also involved in biofilm formation, and the formation of biofilm causes recurrent and persistent infection [e.g., osteomyelitis]. To check the c immunogenic proteins involved in *Staphylococcus aureus* biofilm formation and generate the antibody-mediated immune response [29]. Methicillin-resistant *Staphylococcus aureus* [MRSA] was used to infect the rabbit, especially when the tibias of rabbit serum were collected before infection and after infection on different days. [14, 28,

and 42 days] serum was collected waster blot assays were done on the protein involved in biofilm formation in the laboratory. These biofilm proteins were separated with the help of two-dimensional gel electrophoresis [30]. Severe treatment of antibiotics against *Staphylococcus aureus* made its resistance to these antibiotics methicillin-resistant *S. aureus* has come as a major cause of healthcare-associated [HA] and community-associated [HA] infection. *Staphylococcus aureus* can colonize healthy Individuals and is the cause of spreading in a healthy community. If a person is more immunosuppressed or Immuno-compromised or got a skin injury, he is more susceptible to *S. aureus* Infection. Approximately 30% of the human population is causing asymptomatic Infection [31].

After the discovery of antibiotics, it becomes a major source for billions of people. Some organisms emerged as multidrug-resistant [MDR] and caused obstacles in treating the bacterial infection with his passage. One of the prominent microorganisms which have emerged as multidrug-resistant is *Staphylococcus aureus* [32]. The most common resistance is B. lactamase among strains of *Staphylococcus aureus*. Recently Methicillin-resistant *S. aureus* outbreak occurs at hospitals. By proper strategies, we can control the sporadic outbreak of MRSA. Now it is believed that the first line of drugs or treatment will be non-Infective against MRSA shortly. So that is any it's increasing has become a serious threat around the earth [33].

The major resistance mechanism which is adopted by *Staphylococcus aureus* is the change in the target drug, enzyme Inactivation for drug, and trapping of drug *Staphylococcus aureus* has attained mec-element and van A operon by horizontal gene transfer, which increases its resistance. The major efficient drug against MRSA is linezolid because it inhibits protein synthesis.

But sometimes in contrary to linezolid point mutation and other mutations are observed. Resistance mechanism, contrary to the linezolid Cfr gene, is very accountable. But as compared to MRSA, this resistance gene is not transferred among the species [34].

Bacteriophage history

First-time bacteriophages were discovered in 1896 by Earnest Hankin. He was working on malaria and cholera, and he discovered an agent with antibacterial activity, especially against cholera. In the river of India [Jumna and Ganga]. Earnest used excellent porcelain filters to observe the antibacterial element [20]. In 1915 Frederick twort [British Bacteriologist] discovered the Bacteriolytic activity of Bacteriophages. He was a super Intendent of Brown Institute of London. He observed that the small agents or organisms have the ability to lyse and kill the bacterial cell. Just after they discovered, funding has been stopped because of World War I, and their research was unfortunately stopped [35].

About a century ago, in 1917, d'Herelle had given the term Bacteriophages to the viruses that infect or kill the bacteria late he introduced the term phage therapy. These are the organisms [viruses] that lyse the bacterial cells only and do not harm another cell eukaryotic cell. This was the first scientist that used the term phage therapy. Due to its therapeutic action to kill many bacterial cells. And to cure and treat bacterial disease. He used phage therapy experimentally to control dysentery, and after a few hours during this period, he introduced bacteriophages to 12 years old boy, and luckily, he showed a proper recovery from dysentery. In the normal functioning of the patient, there were no side effects of phage therapy [36].

After the discovery and termed introduced by d'Herelle, many companies had struttred phage products production in response lie bacterial disease. But this was not more than just ten years of era. And alexander fleming

discovered the first penicillin in 1928. And the response of completely changed from phage therapy to antibiotics in response to cure the bacterial disease of Europe, especially eastern Europe phage therapy, is still being practiced. Different institutes and Research centers like Eliavia Institute of bacteriophage, Hirschfeld Institute, Poland, and Tbilisi Georgia. As the resistance against antibiotics has been discovered and being discovered bacteriophages are now considered as bioagent to Important control treat Infections [37].

An increase in Staphylococcus aureus resistance against methicillin and vancomycin and different antibiotics and this antibiotic resistance becomes a serious problem for bacterial infections in hospitals and community environments. phage therapy proved to be successful for the treatment of bacterial infections in animal models and human patients. Bacteriophages are the viruses that infect or kill the bacteria. Bacteriophages were discovered by two different scientists, in 1915 by a British pathologist Fredrick twort and in 1917 by Félix d'Hérelle; a Félix d'Hérelle first used bacteriophages as a therapeutic agent against bacterial infection [38]. Besides the successful results, this therapy was not gained as much importance because of less quality, less differentiation, and antibiotics discovery. Antibiotics gained much more importance after their discovery, but soon MDR [multidrug-resistant bacteria] has emerged, and in 1980, phage therapy is gaining importance because it has some advantages over antibiotics. [Viruses 09] Bacteriophage viruses have double-stranded or single-stranded RNA or DNA molecules present inside the protein coat. [39].

International committee on taxonomy of viruses [ICTV] updated that Caudovirales, including Myoviridae, siphoviridae, and podoviridae families, belong staphylococcal phages. Phages belonging to Myoviridae and

Podoviridae are used for combating staphylococcal infection because they are lytic phages [40]. Recent studies also revealed that phages from the Myoviridae family are safe for therapeutic applications. First, phage therapy was done against *Staphylococcus aureus* skin infections. Phages were reported in treating bacterial diseases such as staphylococcal lung infection, staphylococcal skin infection, urinary tract infections, neonatal sepsis, and diabetic foot as a complication of diabetes. Bacteriophages were also very effective against biofilms and suggested as effective biofilm agents [41].

Bacteriophage infection cycle starts with the adsorption of the virus on the surface of bacteria with spikes or tail fibers' help. In gram-negative bacteria, lipopolysaccharides, oligosaccharides, and proteins can be used for the attachment of bacteriophage. Gram-positive bacteria offer a very different site for bacteriophages' attachment to bacteria because it has murine in its cell wall. After the non-reversible adhesion, the bacteriophage's genome must cross the three bacteria barriers to reach the genome of bacteria for the transcription and translation process [42]. First of all, the virus releases enzymes on the bacterial surface by its tail fibers rather than injecting DNA into the bacterial cell. There will be high metabolic activity in bacteria; after getting enough metabolic activities genome starts to replicate. There were two types of bacteriophages, temperate phage, which relies on the bacterial genome, while virulent phage codes on its machinery. In this way, virulent bacteriophages are released from the bacterial cell and will be ready to infect the next bacteria [43].

Lytic bacteriophages are significantly used in phage therapy because of their ability to lyse the bacterial cell temperate bacteriophages. They rely on the bacterial cell's genome and do not involve in the bacterial cell's lysis

because it consists of altering the bacterial genome. Bacteriophages from Myoviridae and Podoviridae are used against staphylococcal infections because of their lytic nature, while the phages from siphoviridae are temperate phages and usually not used against bacterial infections [44]. The constant threat to bacteria is bacteriophages because of their increasing and much better adaptation to infect bacterial cells. Bacteria have also evolved a particular mechanism or structure to avoid phage infections such as [i] superinfection exclusion system [ii] restriction-modification system [iii] surface receptors and [iv] abortive infection [6]. The majority of Archaea and other bacteria [more than half of all bacterial strains] have an exciting system of clustered regularly interspaced palindromic repeats [CRISPR] and CRISPR-associated sequences [CAS] to counter phage and plasmids. But very interestingly, phages have also evolved the CRISPR-Cas system [*Vibrio cholera* phage] called the ICP! - related phage. These phages were isolated for the ICDDR, B [International Center for Diarrheal Disease Research in Bangladesh. These systems are used against bacteria, which have developed a particular structure as resistance against bacteriophages. Due to their specific lytic activity against pathogenic bacteria, bacteriophages have been suggested to control the infection. Bacteriophage treatment can be the antibacterial source for those bacteria that got resistant to antibiotics such as MRSA. Their treatment has been used for decades in Europe. US Food and Drug Association [FDA] has also permitted bacteriophages for safe food preservation [45].

MRSA's problem has become a significant concern while it is spreading from hospitals out into the community. In Pakistan, its prevalence has been increasing from 5% to 51% only from 1989 to 2003. So, there is a need for bacteriophage therapy. This study's

main objective is to isolate bacteriophages from sewage water against *Staphylococcus aureus* involved in the number of infections [44]. It is thought that there are ten times more bacteriophages than bacteria, which leads to a more diverse and most expanded form of life on earth. Phage therapy has many more advantages as compared to antibiotic treatment. Phages are bactericidal [that kill the bacteria] in nature. More specific as compared to antibiotics. Due to bactericidal, there is a meager chance of survival of bacteria after phage infection. More importantly, phage therapy is economical and versatile because of several antibiotics and its boosting antibacterial spectrum [46].

Phage therapy has much more advantages over antibiotic therapy because phage isolation is [i] fast [ii] relatively simple [iii] inexpensive, [iv] bacterial resistance is ten times slower than against antibiotic [v] remain infective under harsh environment [vi] can multiply even in reduced bacterial growth [vii] require fewer administration [viii] high level of specificity [ix] reduction in harmless of the normal microbiota of humans [x] elimination of side-effects of chemical antibiotics [xi] suitable for use in humans [xii] do not infect eukaryotic cell [xiii] minor and minimal side effects. Besides all these advantages of phage therapy, bacteriophages have some severe challenges as therapeutic agents [1] for intracellular species, e.g., *Salmonella* species, because it is challenging for a phage to counter human cell [2] social immune system can take bacteriophages as a foreign particle and can start to sever immune response against them [3] can genetic material through horizontal gene transfer [4] phage resistance have also been shown in different laboratories [47]. And there are many more disadvantages of phage therapy. Bacteriophage specificity to their host makes them a unique tool against bacterial disease. Their host specificity makes them safe for other body cells, so

bacteriophages have attracted the scientific community's consideration in no time; these bacteriophages act as a novel therapeutic tool to resistant microorganisms [48].

Life cycle and antibacterial activity of bacteriophages

The most severe threat in the world around them whole countries is the development of resistance against antibiotics, especially the resistance of *Staphylococcus aureus* against methicillin and other antibiotics. Bacteria involved in pathogenicity and bacterial infection like MRSA are highly involved in community-associated disease [CA] and Healthcare-associated infection [HA]. Patients which compromised immune systems can have severe complications of skin infection by MRSA. MRSA is also involved in Hospital-acquired diseases, just like ventilator pneumonia. Around 171200 healthcare-associated infections in Europe each year are caused by resistant bacteria. Diagnosis and prognosis again resistant *staph. aureus* is very difficult and worse as compared to methicillin-susceptible *staph. Aureus* in the coming time, humans can face a very serious threat to resistant bacteria against antibiotics until the earth does not identify any other agent or elements that can kill bacteria [31].

Various factors are inducing resistance and empowering the MRSA to become high resistance are intracellular adhesion molecules, Polysaccharides, adhesion proteins, the transmission of genes that cause resistance, capsule, Leucocidins, exo-enzymes, panton-valentine leucocidins, and enterotoxins. MRSA is now considered one of the most important and different types of the syndrome in animals and humans. Bacteriophages have very high. Specificity against bacteria-specific bacteriophages is used to cure and treat bacterial infection. Because a specific bacteriophage can kill a particular bacterium. Their high specificity phages are now considered novel

antibacterial elements against the resistant bacteria, e.g., in treating multidrug-resistant bacteria such as MRSA. By using bacteriophages, MRSA can be controlled in different conditions also [49].

Bacteriophages can be completed in their site cycle in two groups [1] Lysogenic phages [2] lytic phages. The virulent phages which are involved in the lysis and killing of bacteria cell are lytic phages. They stop the bacterial cell for further reproduction due to lysis of bacteria cells, and the group of phages is lysogenic second phages that become inactive in bacterial genome these phages are sometimes called prophages. It will only harm the bacterial cell if it converts into a lytic phage and lyse the bacterial cell of we are talking about phage therapy by bacteriophages. Lytic bacteriophages can be very effective against bacterial infection because they are involved in the lysis of bacteria cells [50].

Rasool *et al.* [6] investigated 75 different cultured samples, and eight phages were identified against *Staphylococcus aureus*. All isolates had lytic activity; three of them were seen in 89% of the sample. Two of them have been isolated in milk. Phages stopped *Staphylococcus aureus* in pasteurized and UHT whole fat milk. Less activity has been shown in skimmed milk as compared to fat dairy. Lysis has been observed at 37°C, not at 0°C.

As the bacteriophages have been discovered in the early 1900s. After their discovery, many experiments have been done on it, explaining their innate ability to kill the bacterial cell. This study's primary purpose was to review bacteriophages and their developments in phage therapy to control bacteria in humans and plants as well. They have studied bacteriophages from the marine and land environment and their effect as antimicrobials to prevent harmful bacteria, incredibly resistant bacteria. This review's primary purpose was to explain the topic of

phage therapy, host bacteriophage interaction, a phage derived protein phage therapy, and the use of phage therapy on marine, animal, humans, and plant pathogens [51].

Morphological families of bacteriophage

All the genera of bacteria are associated with bacteriophages. Due to their characteristics, these viruses are divided into different families. These characteristics that differed families include phylogeny, host-ranging chemical sensitive morphogenesis physical sensitive plaque morphology strategies of infection, and the host cell properties and environmental [52].

The international committee on taxonomy of viruses has been recognized only three orders of viruses. These three orders include 214 genera and 61 families. But as the study proceeds, ICTV acknowledged only one order, consisting of 13 families and 31 genera. Bacteriophages infection the total 140 bacterial genera all include archaea and eubacteria etc. Usually, phages have tailed polyhedral, pleomorphic, or filamentous symmetry. There are only a few bacteriophages that include ds RNA SSRNA or single-stranded DNA most of the bacteriophages that include double-stranded DNA their genetic material bacteriophages which have filamentous or pleomorphic symmetry are classified into families. These ten families contained 208 viruses, and about only 3.7% is the bacteriophages among these viruses. Besides, the phages which have tailed symmetry are classified into caudovirales. 96% phages of tailed phages included in phylogenetic are classified into phylogenetic families [53].

Staphylococcus bacteriophages

Most bacteriophages that belong to, siphoviridae family are still unclassified. Only 46 bacteriophages that have lytic activity against *Staphylococcus aureus* are grouped in the *siphoviridae* family. Bacteriophages that were grouped into these

new genera were against *Staphylococcus aureus* and *staphylococcus epidermidis*. *Phieta-like viruses* used particular tail proteins for the bacterial interaction. Similar viruses used DNA processing proteins and DNA packaging proteins for host interaction. These bacteriophages can be used as a phage therapy against staphylococcal infection [53]. A bacteriophage was isolated by [34] and named it TEM126. Observing under an electron microscope observed that the phage has an isomeric head and non-contractile tail. Genomic analysis of bacteriophage TEM126 revealed that the bacteriophage has double-stranded DNA and have 33540bp. By the bioinformatics analysis, 44 ORF's were also analyzed. TEM126 was classified into the *siphoviridae* family based on molecular characterization. TEM126 can be used and selected for phage therapy as an antitherapeutic agent against *Staphylococcus aureus* infections [54].

Genomic features of *Staphylococcus aureus* phages

Pelletier and coworkers designed a study in which they explained the genomic characteristics of staphylococcal phages. Their research described 27 genomes of *Staphylococcus aureus*, and their phages belong to 3 families the range of genome size was 20kb to 125 kb. *Staphylococcus* genomic phages were distributed into three groups; class I had less than 20kb of genomic size, class II had approximately 40kb of genomic size class III had a genomic size of 125kb. Intermediate genome size was belonging to the siphoviridae family, and the largest belongs to Myoviridae [55].

The genome of staphylococcal bacteriophages that belongs to siphoviridae are aligned and divided into five parts, as shown in fig. segments of DNA that are involved in metabolism are divided into replication, regulation, and functions. Virulence factors which are used for the lysis of bacterial cell are encoded in lysis segment.

The genes involved in the host's genomic integration present on the strands and genes are usually located at all genes [37].

There is much difference while studying the genome of the podoviridae family. From that difference, one is present in small numbers of ORFs. It consists of functional segments that include genes that encode the lysis, capsid genes, and genes for DNA packaging. Suppose we compare the Podoviridae family with the siphoviridae family. Siphoviridae gene segments are not adequately defined because the genes for lysis and genes encode tail overlying to each other in siphoviridae. A gene called a cfr gene produces resistance against lincosamide, oxazolidinones, streptogramin A, and phenicols. This cfr gene is majorly present and associated with the coagulase-negative staphylococci from animals. There are very few bacteria [MRSA] Methicillin-resistant *Staphylococcus aureus* in which the cfr gene is described. They designed a study in which they describe a cfr gene in MRSA isolates, which was PVL (panton-valentine leucocidin) positive. DNA microarray technique was used to screen PVL positive isolates. The DNA microarray technique detects virulence genes, antimicrobial resistance genes, and typing markers. Molecular analysis revealed a conjugated plasmid of 45kb, which is associated with phenicol resistance. A multidrug-resistant CFR positive variant has emerged in MRSA [56].

Phage endolysin

Endolysin are the enzymes which are released by bacteriophages are used for the cleavage of peptidoglycan bond present in the cell wall of bacteria. These endolysin enzymes used for the cleavage of a bacterial cell are produced at the end stage of the lytic cycle. Endolysin enzymes target the peptidoglycan bond, which is highly unique and conserved, so there is a minimal chance of getting resistance against endolysin enzymes. Host bacterial pathogens are

stopped by phage endolysin by the characteristics of cleaving peptidoglycan; these enzymes can be used as an antibacterial against the gram-positive bacterium [57].

Small proteins that are present on the membrane are holin protein. This protein has 34 families and three topological classes. Genome sequences can analyze the holing genes and endolysins, which are present in bacteriophages. Double-stranded DNA bacteriophages need muralytic enzymes and a holin system to lyse an infected bacterial cell. The gene which encodes endolysin is indicated as the 'R' gene, and the gene which encodes the holin system of bacteriophage is marked as the 'S' gene. Both genes are used for the lysis of bacterial cell because 'S' gene increases the permeability, and 'R' gene attacks the cell wall of host bacterial cell, and both leads to the bacterial cell lysis [58, 59].

Conclusion

The community's public health is at risk due to the contamination of different toxic organisms in food. *Staphylococcus aureus* is causing many diseases to humans, including skin diseases and majorly food poisoning due to enterotoxins production. Further, it is getting resistance day by day to different antibiotics. Phage therapy proved to be successful for treating bacterial infections in animal models, and for human patients, TEM126 can be used and selected for phage therapy as an antitherapeutic agent against *Staphylococcus aureus* infections. For the curing of Infection, you must have controlled monitoring patterns. Infect the residues of antibiotics from animals that can be transferred to human beings by consuming milk. This can cause resistance against microorganisms. The use of these bacteriophages soon will be a preventative agent that can use these bacteriophages to kill bacteria, especially *Staphylococcus aureus*, to make milk free from bacteria. These approaches can open the doors to the devastating lives of bacteriophages. So, we

can use these bacteriophages in different fields of life. But it needs a comprehensive study on bacteriophage, which may take some time to use them as a preventative agent.

Authors' contributions

Conceived and designed the experiments: N Zafar & A Aleem, Performed the experiments: F Fatima, S Sohaib & A Liaqat, Analyzed the data: M Sikandar, S Siddiq & H Nasir, Wrote the paper: N Zafar.

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