ORIGINAL ARTICLE

CONTENTS

Potential use of bacteriophages for the oral microbiota modulation in periodontal diseases within the context of maxillofacial pathology

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ABSTRACT

Aim: To study the susceptibility of isolates obtained from the oral cavities of individuals with inflammatory periodontal diseases and maxillofacial pathology to bacteriophages.

Materials and Methods: Biological specimens were obtained from the Dental Medical Center and University Clinic of O.O. Bogomolets National Medical University and the National Specialized Children's Hospital OKHMATDYT. Biomaterial was collected from the wound surface using a sterile FLmedical transport system (Italy). Clinical isolates used in the studies included *Escherichia coli, Klebsiella spp., Pseudomonas spp., and Staphylococcus aureus*. We assessed the susceptibility of microorganisms to bacteriophages by administering 0.01 ml of bacteriophage culture to a bacterial culture. A suspension (inoculum) was prepared from a 24-hour culture of microorganisms in sterile saline and then Mueller Hinton agar was inoculated with it. The results were recorded 24 hours post-incubation in a thermostat at 37°C.

The study used bacteriophages from the bacterial test cultures *Klebsiella pneumoniae* DSM30104, *Staphylococcus aureus* DSM 799, *Escherichia coli* DSM 1103, and *Pseudomonas aeruginosa* DSM 50071, provided by NeoProBioCare Ukraine Ltd.

Results: The susceptibility rates of isolates to phages were 40% for *Klebsiella spp*, 73.3% for *S. aureus,* 50.0% for *E. coli*, and 20.0% for *Pseudomonas spp*. Prior research confirms the effectiveness of bacteriophages in oral diseases. This method may be beneficial in controlling opportunistic microorganisms and maintaining the oral microbiota balance.

Conclusions: The application of bacteriophages for inflammatory oral diseases appears promising and warrants further research. Clinical trials and additional scientific investigations are necessary to confirm its safety and effectiveness.

KEY WORDS: antimicrobial action, alternative antimicrobial agents, periodontitis, maxillofacial diseases

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INTRODUCTION

The idea of using viruses for treating human infectious diseases emerged nearly concurrently with the discovery of phages in 1915. However, the application of phages in medicine was suspended after the introduction of antibiotics [1]. Bacteriophages (phages) are viruses that invade bacterial cells and interfere with their metabolism. During the lytic cycle, they induce bacterial lysis. The principal advantages of phage therapy encompass higher strain specificity with minimal effects on the commensal microbiome, the capacity to multiply at the infection site while simultaneously disappearing alongside the target pathogen, the absence of apparent toxicity, relatively simple to isolate and use in genetic engineering, and co-evolving with their bacterial hosts to eliminate drug-resistant strains [2].

Currently, bacteriophage therapy is a promising method for treating infectious dental conditions [1]. Due to the escalating clinical issue of antibiotic resistance, phage therapy for infectious diseases has once more attracted the interest of specialists. Bacteriophages have been suggested as natural antimicrobial agents to combat antibiotic-resistant bacteria associated with human infections [2].

The oral phage population comprises over 2000 species that infect bacteria from the Actinobacteria

(>300 phages), Bacteroidetes (>300 phages), Firmicutes (>1000 phages), Fusobacteria (>200 phages), and Proteobacteria (>700 phages). Bacteriophage activity inhibits relapses of infectious diseases and the chronicity of inflammatory processes. Bacteriophages in oral cells primarily stimulate anti-inflammatory mediators, including IL-6 and IL1RN, to modulate innate and adaptive immunity, thereby indirectly influencing cell signaling and physiological processes [3]. The application of phage therapy in dentistry remains an entirely unsolved issue [4, 5]. Regrettably, the rapidly advancing bacteriophage therapy presents new challenges for clinical implementation, including phage resistance. At the same time, phage resistance develops approximately tenfold more slowly than antibiotic resistance [6]. A viable strategy to reduce antibiotic dosage and prevent antibiotic resistance during treatment includes the combination of phages with antibiotics. Antibiotics and bacteriophages can not only deal with phage resistance but also reduce antibiotic resistance. The function of engineered phages in reducing bacterial pathogenicity is under investigation. Research indicates that alternating the administration of the antibiotic and phage yields superior outcomes compared to simultaneous administration [7].

Traditional approaches to periodontal disease management, including dental biofilm control, enhanced oral hygiene, adjunctive gingivitis therapy, mechanical plaque removal, and the application of topical and/or systemic antimicrobials, are recognized as insufficiently effective [8]. Considering the widespread occurrence of antimicrobial-resistant bacteria in the oral cavity, the prevailing viewpoint supports various forms of phage therapy as a supplementary approach to standard periodontal treatment to reduce systemic adverse effects. Bacteriophages that selectively infect bacterial cells are now acknowledged as a novel therapeutic approach for eradicating plaque biofilms in periodontal diseases [9].

Bacteriophage therapy represents an innovative and promising strategy for combating multidrug-resistant bacteria that cause serious dental diseases. Furthermore, investigations into phage therapy for endodontic and periapical infections are underway. The findings indicate that, in contrast to traditional antibiotics, phage therapy may necessitate reduced or more restricted doses, demonstrating effectiveness comparable to or better than standard therapies. The majority of phages isolated to date exhibit considerable specificity for dental pathogens. This benefit reduces the harmful risk to the natural oral microbiota while eliminating the adverse effects associated with chemical antibiotics. Phage therapy presents new opportunities in dentistry, both therapeutically and scientifically, similar to its impact in various medical fields.

AIM

To study the susceptibility of isolates obtained from the oral cavities of individuals with inflammatory periodontal diseases and maxillofacial pathology to bacteriophages.

MATERIALS AND METHODS

Biological specimens were obtained from the Dental Medical Center and University Clinic of O.O. Bogomolets National Medical University and the National Specialized Children's Hospital OKHMATDYT. Biomaterial was collected from the wound surface using a sterile FLmedical transport system (Italy). The study on antimicrobial activity was conducted in the microbiological laboratory of the Department of Genetics, Plant Physiology, and Microbiology at Uzhhorod National University. Clinical isolates used in the studies included Escherichia coli, Klebsiella spp., Pseudomonas spp., and Staphylococcus aureus. We assessed the susceptibility of microorganisms to bacteriophages by administering 0.01 ml of bacteriophage culture to a bacterial culture. A suspension (inoculum) was prepared from a 24-hour culture of microorganisms in sterile saline and then Mueller Hinton agar was inoculated with it. The results were recorded 24 hours post-incubation in a thermostat at 37°C.

The study used bacteriophages from the bacterial test cultures *Klebsiella pneumoniae* DSM30104, *Staphylococcus aureus* DSM 799, *Escherichia coli* DSM 1103, and *Pseudomonas aeruginosa* DSM 50071, provided by NeoProBioCare Ukraine Ltd.

RESULTS

The investigation into the susceptibility of bacterial cultures obtained from patients revealed that *Staphylococcus aureus* exhibited the highest percentage of susceptible isolates (Table 1, Fig. 1). Out of the 30 strains, 22 isolates had susceptibility to the examined bacteriophage, representing 73.3%.

Bacteria of the genus *Klebsiella spp.* demonstrated the lowest susceptibility to bacteriophage. Susceptibility studies of clinical isolates of *Klebsiella pneumoniae* indicated a phage susceptibility rate of 40% (Table 1, Fig. 2). Simultaneously, 20.0% inhibited the growth of the tested clinical cultures (Fig. 2).

Among the *Escherichia coli* isolates studied, 50.0% showed susceptibility to bacteriophages (Table 1, Fig. 3).

Table 1. Susceptibility of clinical isolates from complicated wounds to bacteriophages

Microorganism	Number of strains tested, abs.	Number of susceptible isolates, abs.	Percentage of susceptible isolates, %
Klebsiella spp.	25	10	40.0%
Staphylococcus aureus	30	22	73.3%
Escherichia coli	28	14	50.0 %
Pseudomonas spp.	15	3	20.0 %

Source: compiled by the authors of this study

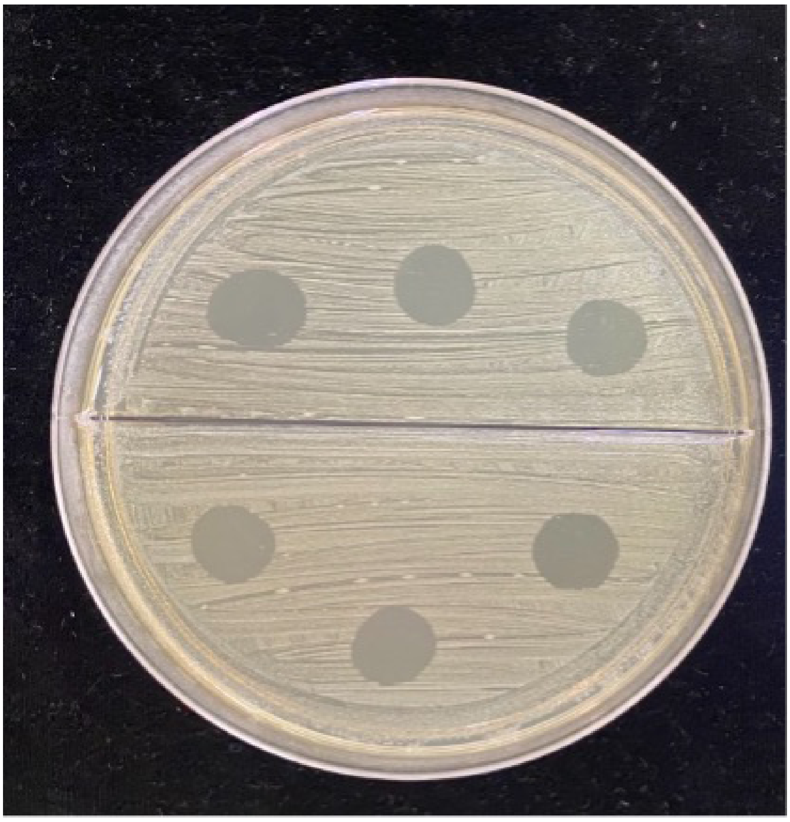

Fig. 1. Susceptibility of a clinical isolate of *Staphylococcus aureus* to a specific bacteriophage
Picture taken by the authors

Research on the susceptibility of clinical isolates of *Pseudomonas aeruginosa* indicated a phage susceptibility rate of 63.3% (Table 1, Fi1. 4).

The majority of isolates from the bacterial species *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* exhibited polyantibiotic resistance. In these circumstances, the application of bacteriophage may be the sole alternative for sanitizing the oral cavity and diminishing the prevalence of antibiotic-resistant microorganisms.

The results regarding the susceptibility of clinical isolates to bacteriophages (Fig. 4) suggest that, if the pathogen is specifically susceptible, bacteriophages may serve as an alternative to antibiotic therapy or significantly enhance the effectiveness of antibiotic treatment.

The examined isolates are composed of opportunistic bacteria, noted for their significant antibiotic resistance (Table 2). It was demonstrated that 25.9% of *Klebsiella spp.*, 20.0% of *Staphylococcus aureus*, 39.3% of *Escherichia coli*, and 40.0% of *Pseudomonas spp*. showed concurrent resistance to three antibiotics. The identified patterns demonstrate the enduring presence of antibiotic-resistant bacteria in the oral cavity, which are challenging to manage and may contribute to complications.

It is noteworthy that some antibiotic-resistant isolates exhibited susceptibility to bacteriophages. Consequently, no discernible patterns in the susceptibility or resistance to bacteriophages were identified among polyantibiotic-resistant isolates.

The identified patterns of strain-specific susceptibility of opportunistic bacterial isolates to antibiotics suggest that the examined bacteriophages may serve as alternatives to antimicrobial therapy or adjuncts to antibiotic treatment.

The data analysis allowed us to determine the susceptibility of isolates from the oral cavities of patients with periodontal diseases and maxillofacial pathology to

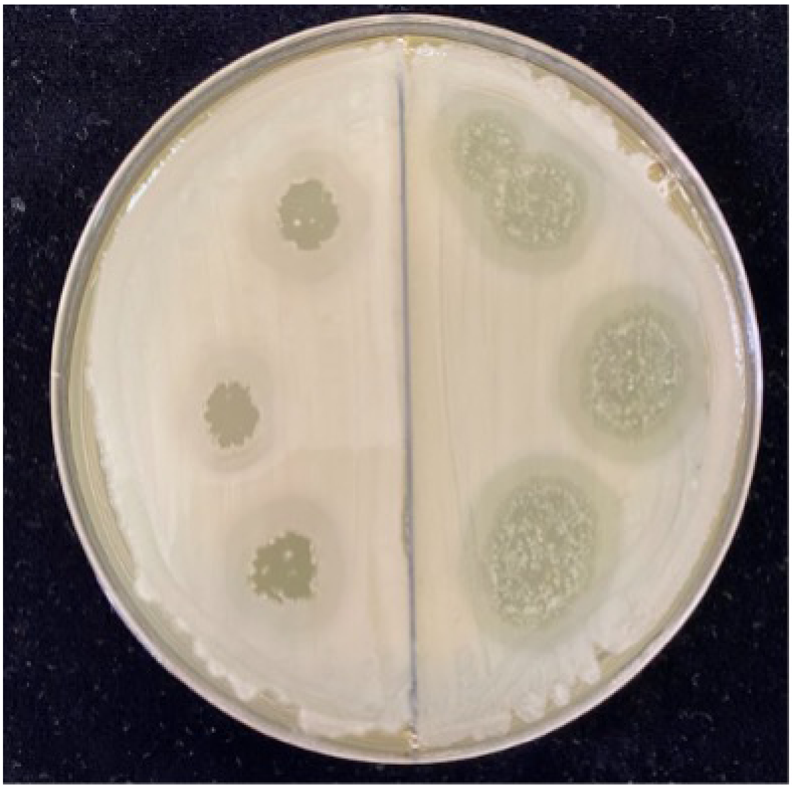

Fig. 2. Susceptibility of a clinical isolate of *Klebsiella pneumoniae* to a specific bacteriophage
Picture taken by the authors

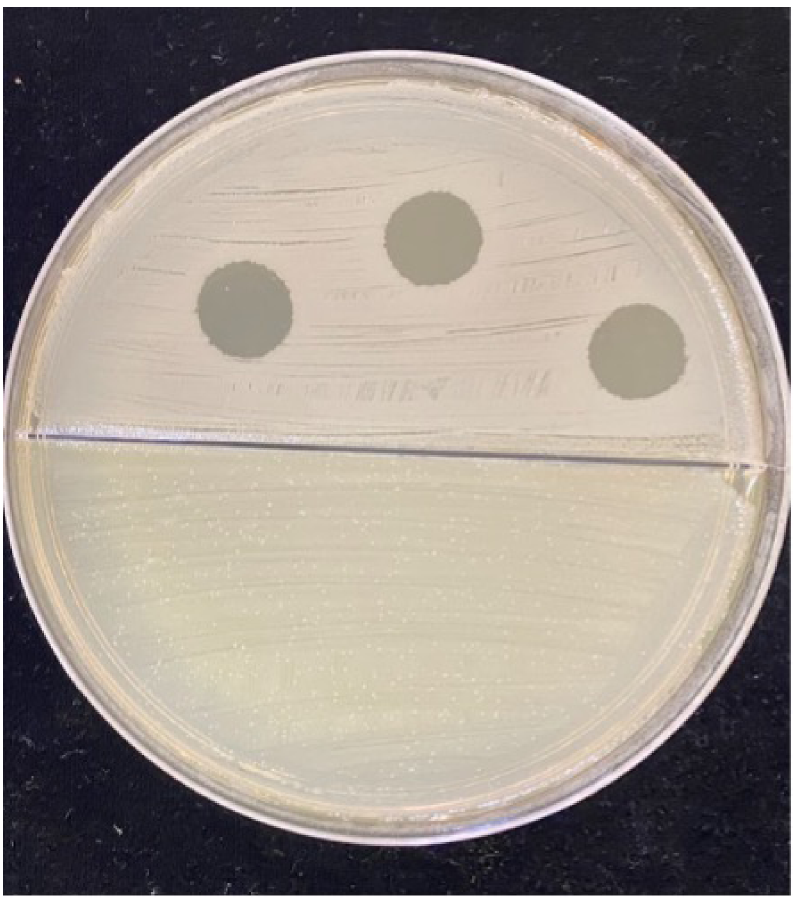

Fig. 3. Susceptibility of a clinical isolate of *Escherichia coli* to a specific bacteriophage
Picture taken by the authors

bacteriophages. Their susceptibility to phages was strain-specific, necessitating the assessment of susceptibility to the phage suspension before initiating treatment.

Prior research confirms the effectiveness of using bacteriophages in the treatment of oral diseases. This approach is effective in combating pathogenic microorganisms and in maintaining the balance of oral microbiota.

Table 2. Susceptibility of clinical isolates from complicated wounds to bacteriophages

Microorganisms	Number of strains tested, abs.	Number of isolates resistant to class III antibiotics, abs.	Percentage of isolates resistant to class III antibiotics, %
Klebsiella spp.	25	7	25.9%
Staphylococcus aureus	30	6	20.0%
Escherichia coli	28	11	39.3 %
Pseudomonas spp.	15	6	40.0 %

Source: compiled by the authors of this study

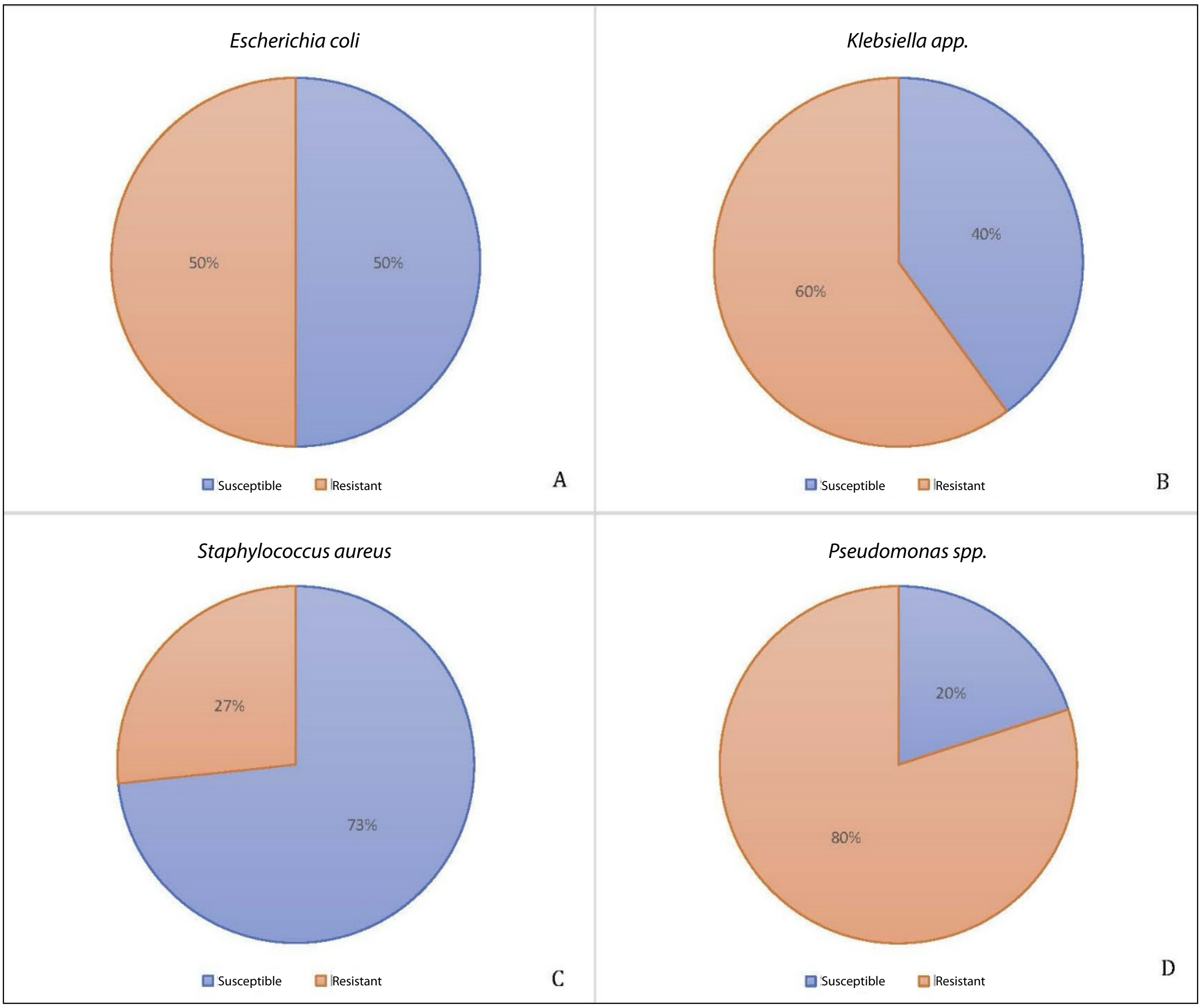

Fig. 4. Percentage of bacteriophage-susceptible and -resistant clinical isolates from the oral cavity
Picture taken by the authors

DISCUSSION

The literature review suggests individual studies regarding the use of bacteriophages in oral diseases of microbial etiology.

Biofilms are found in almost all infectious diseases that threaten oral health, such as caries, periodontitis, gingivitis, endodontic infections, and peri-implantitis. Steier et al. (2019) examined the structure of bacteriophages and their action on bacteria within biofilms, emphasizing the potential use of bacteriophages in dentistry [10].

Caries-associated problems are challenging to treat with standard methods due to the development of a biofilm of cariogenic bacteria, which protects them and reduces the effectiveness of common antibacterial agents [11]. It is essential to create therapeutic techniques specific to cariogenic bacteria with a low risk of resistance [12]. Bacteriophage therapy is a promising alternative method. Thus, phage SMHBZ8, specifically targeting S. mutans, has been identified as a potential method for preventing and treating dental caries [13]. H. Ben-Zaken et al. (2021) conducted an experimental investigation on human saliva samples. They discovered that SMHBZ8 is a lytic phage capable of infiltrating, regulating, and suppressing the growth of S. mutans biofilm [14].

Enterococcus faecalis (E. faecalis) is known for its notable resistance to numerous antimicrobial agents. [15, 16]. J. Wong et al. (2021) reported that in root canal infections, *E. faecalis* was detected in every canal, and its presence in the biofilm induces inflammation of the pulp tissue, finally resulting in pulp necrosis and the spread of inflammation/infection to the peri-root tissues [17]. In clinical settings, isolated phages have demonstrated remarkable effectiveness when combined with alkaline disinfectants frequently used to deal with endodontic infections [15].

M. El-Telbany et al. (2021) demonstrated a phage-mediated reduction in *E. faecalis* biofilms in ex vivo dental models. The lytic activity of phage vB_ZEFP against *E. faecalis* biofilms confirmed its capability to inhibit the growth of *E. faecalis* in vitro and to prevent E. faecalis root canal infections [18].

The presence of many bacterial types inside the biofilm structure imposes constraints on the strategies employed to combat them [19]. Historically, it has been contended that while bacteriophages may function as effective antibacterial agents, the heterogeneity of the oral microbiota raises concerns over their effectiveness in treating periodontal diseases [20]. At the same time, in the context of the persistence of opportunistic microorganisms within the oral microbiota and dysbacteriosis, characterized by the dominance of microbial associations from the facultative component of the microbiome, the application of bacteriophages is a justified alternative to antibiotics and antiseptics. Kabwe et al. (2019) identified and studied FNU1, a lytic phage that targets *F. nucleatum*, which forms the basis of a polymicrobial biofilm in periodontitis. The introduction of FNU1 into a biofilm containing *F. nucleatum* led to a 70% decrease in the biomass of this bacterium [21]. A. Abdulkareem et al. (2021) highlighted that the use of bacteriophages in periodontal therapy remains restricted to in vitro investigations. Nonetheless, using bacteriophages to combat periodontal pathogens in periodontal diseases seems promising and can effectively deal with the increasing resistance of bacteria to currently available medicines. At the same time, clinical trials to either confirm or refute this concept in periodontal therapy have been practically nonexistent. However, it has been established that, in contrast to broad-spectrum antibiotics, bacteriophages can destroy periodontal biofilms without disrupting the local oral ecology [22].

A restriction of phage therapy is that only a limited fraction of phages is suitable for medicinal purposes. Only 50% of phages isolated from the environment can be used in medicine [23]. Phage resistance has been recorded in human phage treatment, presenting a potential challenge for the practical use of phage therapy [24]. Various encapsulated carriers, such as fibers, hydrogels, and particles, have been used in phage delivery systems, demonstrating the advantage of maintaining their stability. These systems can function alongside other dental materials to treat or prevent infectious diseases, including periodontitis. Therefore, given that periodontitis is a chronic disease caused by several bacteria, G. Pinto et al. (2016) suggested a phage cocktail as an alternative to standard treatment techniques and outlined the steps for producing phage-based products. The authors emphasize that active bacteriophage combinations in cocktail therapy are crucial for preventing the rapid emergence of phage resistance [25].

CONCLUSIONS

1. The research revealed that the susceptibility of isolates to phages was 40% for *Klebsiella spp.*, 73.3% for *S. aureus*, 50.0% for *E. coli*, and 20.0% for *Pseudomonas spp*. This suggests the potential use of phage therapy in protocols for managing periodontal diseases alongside concurrent maxillofacial pathologies. Strain-specific susceptibility to bacteriophages was determined among isolates resistant to class III antibiotics.
2. The specific susceptibility of strains to bacteriophages necessitates the assessment of the isolate's susceptibility to bacteriophage before initiating treatment for inflammatory oral diseases, including periodontal diseases and maxillofacial pathologies.
3. The application of bacteriophages for inflammatory oral diseases appears promising and warrants further research. Clinical trials and additional scientific investigations are necessary to confirm its safety and effectiveness.

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CONFLICT OF INTEREST

The Authors declare no conflict of interest

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