RESEARCH ARTICLE

Characterization of the Antibacterial, Antibiofilm Activities, and Genetic Structure of Endolysin Extracted from *Enterococcus faecalis* Phage

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ABSTRACT

Background: The multidrug-resistant enterococci (MDR), *Enterococcus faecalis*, is considered a major medical problem in recent times and has been reported increasingly worldwide. Scientists applied phage therapy to induce lysis of antibiotic-resistant pathogenic bacteria in various environments. Alternative to conventional antibiotics, the lytic enzymes (endolysins) produced by bacteriophages were employed to lyse MDR bacteria. These enzymes hydrolyze the cell wall peptidoglycan, resulting in the lysis of the host cell.

Objective(s): This study investigated the antibacterial and antibiofilm activities of the extracted endolysin from *E. faecalis* phage that can be used as an alternative approach for the treatment of MDR enterococci in Iraq, together with its phylogenetic relationship.

Materials and Methods: The endolysin was extracted using gel filtration chromatography (Sepadex G100) from *E. faecalis* phage. The elutes with the highest absorbance at 280 nm were tested by spot analysis assay on MDR *E. faecalis* lawns isolated from a patient with urinary tract infection. The endolysin concentration was measured by Bradford protein assay. The microdilution method was used to determine the minimum inhibitory concentration (MIC). Accordingly, the minimum bactericidal concentration (MBC) of the extracted endolysin was verified.The ability of *E. faecalis* isolates to form a biofilm was evaluated by Congo Red Agar (CRA) method. Furthermore, the microtiter plate method was used to quantify the antibiofilm activity of the extracted endolysin. Phage DNA was extracted for sequencing using the automated Sanger method, and further genetic analysis by NCBI-BLAST program was done.

Results: The antibacterial activity of the extracted endolysin was evaluated with a clear decline in bacterial growth.The endolysin concentration was (256 µg/mL) with (>64 µg/mL MIC and (>128 µg/mL) MBC. Black colonies with dry crystalline consistency were developed on CRA after overnight incubation which indicated the ability of *E. faecalis* isolates to form a biofilm. About (90.04%) of the bacterial biofilm was reduced after the incubation ofthe extracted endolysin with *E. faecalis* mature biofilm. Sequencing of the *Endolysin*gene obtained from the conventional PCR method uncovered (1257) base pairs. The amino acids translation showed a total number of (419). The phylogenetic analysis of the extracted *E. faecalis* phage endolysin gene revealed a 100% similarity with *Enterococcus* phage phiEf11 endolysin gene.

Conclusion: The current study discovered the promising ability of the extracted *E. faecalis* phage endolysin to eliminate the growth of MDR *E. faecalis* and reduce its mature biofilm. The easy and inexpensive method to extract this enzymobiotic agent encourages further studies to use it as an alternative antibacterial agent on such MDR bacteria. The phylogenetic study of its genome clarified a complete description of the genetic sequence that could enhance further molecular studies to produce a cloned endolysin.

Keywords: Bacteriophages, Biofilm, Endolysin, *Enterococcus faecalis*, Multidrug resistance bacteria, Phage therapy.

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INTRODUCTION

Enterococcus faecalis is a Gram-positive bacterium that can be present in water, soil, and as a commensal organism in humans and animals' gastrointestinal tract.[1] It is one of the most common sources of nosocomial infections in hospitals. Itis well-known for its ability to develop and transmit a wide range of gene-adaptive traits such as virulence genes and antimicrobial resistance genes, as well as its genomic plasticity and a large number of extrachromosomal elements.[2] In recent years, transferable genes conferring resistance

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to last-resort antibiotics like linezolid, especially *optrA*, have emerged in various regions, particularly in *E. faecalis* strains obtained from various hosts.[3,4] A number of genomic traits, such as bacteriocins, microbial surface proteins, cell wall-anchored proteins, or biofilm-forming proteins support *E. faecalis* ability to colonize and cause infection in a variety of hosts.[5] Infections become more severe and difficult to treat when bacteria develop resistance to all antibiotics available.[6] Bacteriophages, also called phages, are viruses that only infect and reproduce in bacteria. They are extremely diverse in size morphology, in addition genomic organization.[7] Scientists have re-applied phage therapy to cause antibiotic-resistant pathogenic bacteria to lyse in a variety of habitats, including soil, air, and water.[8] The emergence of multidrug-resistant pathogenic microorganisms has heightened awareness of the critical need for alternative antimicrobial strategies. Lytic enzymes (endolysins) released by bacteriophages during lytic infection are one such alternative to a traditional antibiotic.[9] These enzymes hydrolyze the cell wall peptidoglycan during bacteriophage lytic infection, causing lyses of the host cell.[10]

Endolysins from phages are lytic enzymes made by bacteriophages at the end of their replication cycle. They are antimicrobial enzyme with peptidoglycan hydrolase activity.[11] When added exogenously in the form of purified recombinant proteins, these antimicrobial agents can cause gram-positive bacteria to lyse and die quickly.[12] Endolysins are a new class of antimicrobials for the treatment of drug-resistant bacterial infections because of their rapid action, high specificity, and a low degree of bacterial resistance development.[13] Accordingly, this study aimed to assess the antibacterial and antibiofilm activities of *E. faecalis* extracted phage endolysin as a potential enzymobiotic for MDR bacteria. In addition, exploration of the endolysin genetic structure and compare it with other world strains.

MATERIALS AND METHODS

Bacterial Collection, Identification, and Antibioresistant Pattern

An isolate of *E. faecalis* was previously collected from The Teaching Laboratories in the Medical City, Baghdad, Iraq, from Dec. 2019 to Feb. 2020.[14] The specimen was from a patient with urinary tract infections (UTI) and the isolate was symbolized as *E. faecalis* 13 (EF13) after complete manual and automated bacterial identification.VITEK 2 Compact System was used to detect the bacterium to a species level and for antibiotic sensitivity testing. Fifteen antibiotics were tested, which belong to 10 categories. The isolate EF13 was only sensitive to linezolid, tigecycline, vancomycin, and ampicillin. Thus, the isolate was considered as a multidrug-resistant (MDR) bacterium.[15]

Identification of the Isolated Bacteriophage

Characteristics of the Isolated Phage

A highly lytic phage that produced a demonstrably inhibited zone on *E.faecalis* 13 was isolated from sewage by Ahmed and Hafidh.[14] The isolated phage was symbolized as *E.faecalis* 13 phage 3 (EF13P3). The top layer assay was used to determine the specific phage characteristics (size, clarity, shape, and margin) and the phage titer (10^9 PFU/mL).EF13P3 had the following characteristics: one mm in size, semi-clear clarity, round shape, and irregular margin.

Phage Spot Lysis Assay

Phage was tested using the phage spotting technique on a nutrient agar plate. The formation of inhibition zones recommends the presence of lytic phages.[16] Frozen EF13 was subcultured twice on nutrient broth and incubated at 37°C. An overnight cultured bacterium was spread on nutrient agar plates in a sterile swab to form a bacterial lawn. After 10 to 15 minutes, a frozen phage suspension (EF13P3) of 10 µL was applied as spots. All the plates were incubated for overnight at 37°C. The lytic phage was identified for the target bacterium by the formation of the lytic zone of inhibition after overnight incubation.A sterile pasture pipette collected the identified specific lytic phage for EF13. The collected phages were applied in new sterile Eppendorf tubes with one mL SM buffer. Gentle vortex and shaking were done for 20 seconds. Chloroform (1:10 v/ v) had been added to lysate the bacteria and vortexed at room temperature. The mixture was centrifuged at 1000 g for 3 minutes. The supernatants which contained phages were transported to a new sterile Eppendorf tube, then kept at 4°C. The collected EF13P3 was used for further analyses in this study.

Endolysin Extraction

E. faecalis (EF13) in 100 mL of Nutrientbroth medium were cultured and incubate dovernight at 37°C.The next day, 250 mL of sterile Nutrientbroth medium were added to the bacterial growth and incubated for another three hours. A 10 mL of the specific phageattiter 1×10^9 PFU/mL were mixed with the bacterium then put directly in ice for 20 minutes. The mixture was centrifuged at 10,000g for 20 minutes, and the sediment was collected. The sediment was suspended in 10 mL of 0.05 Mphosphate buffer saline +5 mg deoxyribonuclease and incubated for 60 minutes at 37°C. EDTA at (0.005 M) was added and centrifuged at10,000 g for 60 minutes then the supernatant was taken. Ammonium sulfate was added to 85% saturation and incubated overnight at 4°C.The nextday, the suspension was centrifuged at 10,000 g for 60 minutes and wasre-suspended in five milliliters of 0.05 M phosphate-buffered saline at pH7.5. Dialysis against 200 mL of 0.05 Mp hosphate buffer saline was done. The buffer was changed every two hours for two times and kept overnight at 4°C. The dialysissolution was added to Sephadex G100 column chromatography (3×20 cm) in 20 mL of 0.05 Mphosphate-buffered salineat pH 7.5 a seluent. One mL of elute was collected in 50 Eppendorf tubes every 5 minutes. Absorbance of each fraction was measured at 280 nm using a spectrophotometer. From each Eppendorf tube, 10 µL of the elute were dropped onto *E. faecalis* lawns to catch the tube with the end olysinlytic activity.

Measurement of the Endolysin Concentration

The endolysin concentration was measured by the Bradford method, the quantitative colorimetric method.[17] The color development was measured at 595 nm. Subsequently,Bovine serum albumin (the standard protein) curve wasdrawn.

The Minimum inhibitory concentration (MIC) of Purified Endolysin on Live Bacterial Cells

The MIC of the endolysin on *E. faecalis* was determined using the broth serial dilution method. The lowest concentration of the antimicrobial agent that will inhibit the visible growth of the microorganism after overnight incubation is known to be the MIC of that antimicrobial.[18]

The Minimum Bactericidal Concentration (MBC)

The lowest concentration of the antimicrobial agent that will prevent the growth of a microorganism after sub-culturing onto an antimicrobial-free medium is known as MBC.[18] The MBC of the endolysin was determined using the tubes that showed no visible growth by the MIC method. Plates with Mueller-Hinton agar (MHA) were inoculated with 100 µL from each tube with no visible growth. All the plates were incubated for 24 hours at 37°C. The MBC was measured as the lowest concentration of endolysin required to kill *E. faecalis*.

Formation and Quantification of the Antibiofilm Activity of Endolysin

Enterococcus biofilm was detected by the Congo red agar (CRA) using the streaking method.[19,20] To evaluate the antimicrobial activity of the extracted endolysin against the mature EF13 biofilm, the microtiter plate method was used.[21]

Phage DNA Extraction

The phage DNA was extracted using 200 µL of the phage stock solutionfollowing the manufacturer's instructions (Viral DNA extraction kit, Bosphore/Spin Kit). The collected phage DNA was stored at (+4°C) for instant use or stored at -20°C for later use.

Phage Genomic DNA Profile

The extracted genomic DNA (1 µL)was checked by using a Nanodrop spectrophotometer which measured DNA concentration (ng/µL) and check the DNA purity by reading the absorbance at (260/280 nm).

Primers

Two set of PCR primers (Ent Endolysin1 and Ent Endolysin 2) were used in this study. The first primer was to sequence the phage endolysin gene, and the second was to detect and confirm the phage endolysin gene. Both were designed by (primer blast NCBI). These primers were provided by (IDT Company, Canada). The primers were re-suspended by dissolving the lyophilized product. About 295 to 349 µL of PCR water (free nuclease) was added to reach 100 picomole per micro later (pmol/µL) as a stock primer suspension. The working primer solution equal to (10 pmol/µL) concentration was prepared by diluting (10 µL) of stock primer with (90 µL) of PCR water and then mixed well by vortex.

Phage DNA proliferation by polymerase Chain Reaction

The phage DNA proliferation was carried out using (Maxime^{TM} PCR PreMix Kit (i-Taq^{TM})) and the method proceeded according to the manufacturer's instructions. Polymerase Chain Reaction master mixcomponents (DNA template 5 µL, Forward primer (10 pmol/µL) 2.5 µL, Master mix 25 µL, PCR water15 µL, Reveres primer (10pmol/µL) 2.5 µL, Total volume 50 µL) were placed in the standard maxime PCR Pre-mixtubes that containing all other elements which were needed for PCR reaction including Taq DNA polymerase, dNTPs, Tris-HCl (pH 9.0), KCl, $MgCl_2$, and loading dye. Then, all the PCR tubes were centrifuged by Exispin at (3000 rpm) for three minutes. Later, the tubes were applied in PCR Thermocycler (ABM Canada).

Polymerase Chain Reaction Thermo cycling Conditions

The PCR tubes were put into the thermocycler and the right PCR cycling program parameters conditions were adjusted according to each primer. The following programs were carried out in 37 cycles for EntEndolysin 1 primer as 95°C for 5 minutes, 95°C for 30 Seconds, 56°C for 30 seconds, 72°C for 30Seconds, and 72°C for 10 minutes then hold at 4°C. The same program was run for EntEndolysin 2 primer, except the annealing step was (54°C for 1min) and the extension step was 72°C for 1.30 minutes. According to Sambrook and Rusell (2013), the PCR products were analyzed using agarose gel electrophoresis.[22] The 100 bp Plus Opti-DNA Marker (100–3000 bp) was used. The PCR products were run in duplicates and compared to the markers.

Phage Genomic Sequencing

The phage DNA sequencing was performed for *Endolysin* gene using NCBI-BLAST and the phylogenetic tree analysis was carried out. The amplification products for Endolysin gene obtained from conventional PCR were sent for sequencing using the automated Sanger DNA sequencing method.[23]

Phylogentic Molecular Analysis

Nucleotides sets were used to obtain the identity score of this isolate with the world's other reference strains by the online program "Basic local alignment search tool (BLAST) at https://blast.ncbi.nlm.nih.gov/Blast.cgi. By comparing nucleotide or protein sequences to sequence databases and a calculation of the statistical significance, this program can find regions of similarity between biological sequences. BLAST program was also used to analyze the phylogenetic relationship of EF13P3 gene with other nearby *E. faecalis* phage genes.

Data Analysis

The plots were represented, and the percentages were measured using Microsoft Excel Version (14.0.6023.1000). The bioresistant profile of *E. faecalis* was performed using Vitek 2 system with software version (5.01). Biosystems™ MicrobeBridge™ software platform was used as a Sanger sequencing and fragment analysis software. The software connects the entered data with an online virtual reference

database using 16S sequencing technology. Thus, identify microbial pathogens and compare them globally.

RESULTS

Re-activation of the Isolated Bacteriophage

The previously isolated and identified *E. faecalis* 13 phage 3 (EF13P3) was highly lytic phage and produced demonstrably inhibition zone on *E. faecalis* 13. The phage re-activation was done using the spot lysis assay on EF13 lawn (Figure 1).

Endolysin Extraction

The isolated phage from the spot lysis assay was used to extract endolysin. The enzyme was successfully extracted by using Sephadex G100 column chromatography from *E. faecalis* bacteriophage (EF13P3). The results showed a variable, but approximate peak for the bacteriophage extracted proteins. Suspected proteins were obtained from 12 to 38 fraction number, which is compatible with 0.11 to 0.18 protein absorbance at (280 nm). In addition, the best-obtained protein was with fraction number 26 and 1.8 absorbance the absorbance at 280nm for the purified protein fractions, extracted using gel filtration chromatography G100 Sephadex column (3×20 cm) with 0.05 M phosphate-buffered saline at pH 7.5 as eluent.

Antimicrobial Activity of Endolysin on *E. faecalis*

Antibacterial activity was observed on live bacterial cells. A spot lysis assay was performed to check whether endolysin sample can lyse EF13 by placing a small drop (10 µL) of endolysin on freshly grown lawn of *E. faecalis* on a nutrient agar then incubated at 37°C for 24 hours. The fractions 12–38 which showed the highest absorbance were checked. The best tube containing endolysin with lytic activity was with fraction number,[22] as shown in Figure 2.

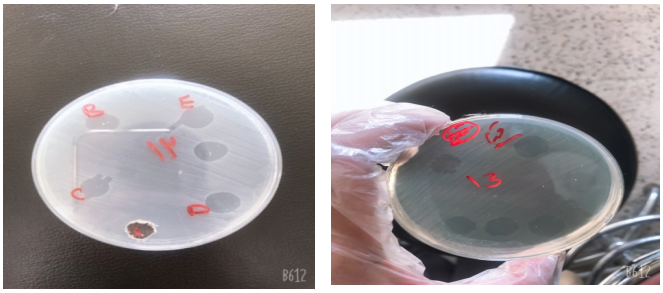

Figure 1: Re-activation of *E.faecalis* specific phage (EF13P3) by the top layer assay.

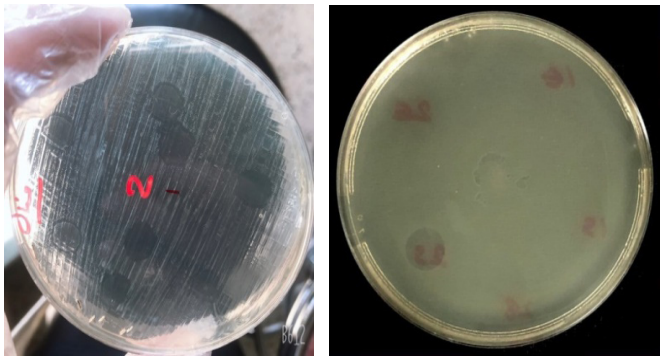

Figure 2: The antibacterial spectrum of the extracted endolysin (fraction number 22) against *E. faecalis* (EF13).

The Concentration of the Extracted Endolysin

In order to measure the MIC of the extracted endolysin in tube number (22), the Bradfrod protein assay was used the concentration of endolysin was (256 µg/mL).

The MIC and the MBC of the P purified Endolysin on Live Bacterial Cells

The MIC was determined as the lowest concentration at which there was no visible growth after overnight incubation. The MIC of the endolysin was >64 µg/mL. In consequence, the MBC, which was the lowest concentration of endolysin that killed *E. faecalis* (EF13) was >128 µg/mL.

Biofilm Formation

After overnight incubation of EF 13 on Congo Red Agar (CRA) black colonies were detected. The ability of the bacterium to form a biofilm is considered positive if such black colonies with a dry crystalline consistency are observed on CRA. Pink colonies were developed after culturing a known *E. faecalis* with no ability to form a biofilm or slime (Figure 3).

The Antibiofilm Activity of the Extracted Endolysin

After proving the ability of EF13 to form a biofilm by the Congo red method, the antibiofilm activity of endolysin was evaluated. A quantitative method using a microtiter plate was used. The antibiofilm activity was measured by reading the absorbance after overnight incubation of the extracted endolysin with the mature biofilm. In addition, the percentage of inhibition of the extracted endolysin was shown to be (90.04%).

Detection and Identification of Phage Endolysin Gene

The extracted phage genome was checked using two set of PCR primers. The first primer (EntEndolysin 1) was to sequence phage endolysin gene and the second (EntEndolysin 2) was for detection and confirmation of the phage endolsyin gene presence. The product size of primer EntEndolysin 1 was (1748 bp), while the product size of primer EntEndolysin 2 was (208 bp), (Figure 4).

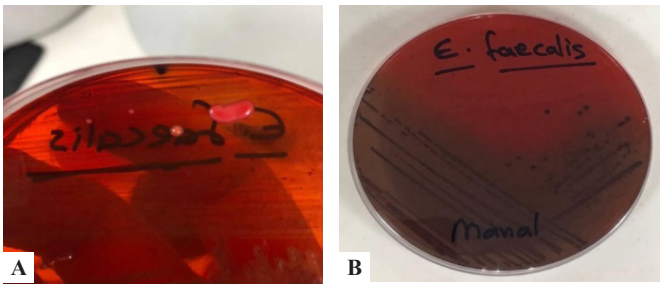

Figure3: A biofilm formation test for EF13 using CRA method. Black colonies with dry crystalline consistency were observed after overnight incubation at 37°C and compared to a known *E. faecalis* with no ability to form a biofilm (pink colonies). A: Non-slime producing *E. faecalis*. B: Slime producing *E. faecalis* (EF13).

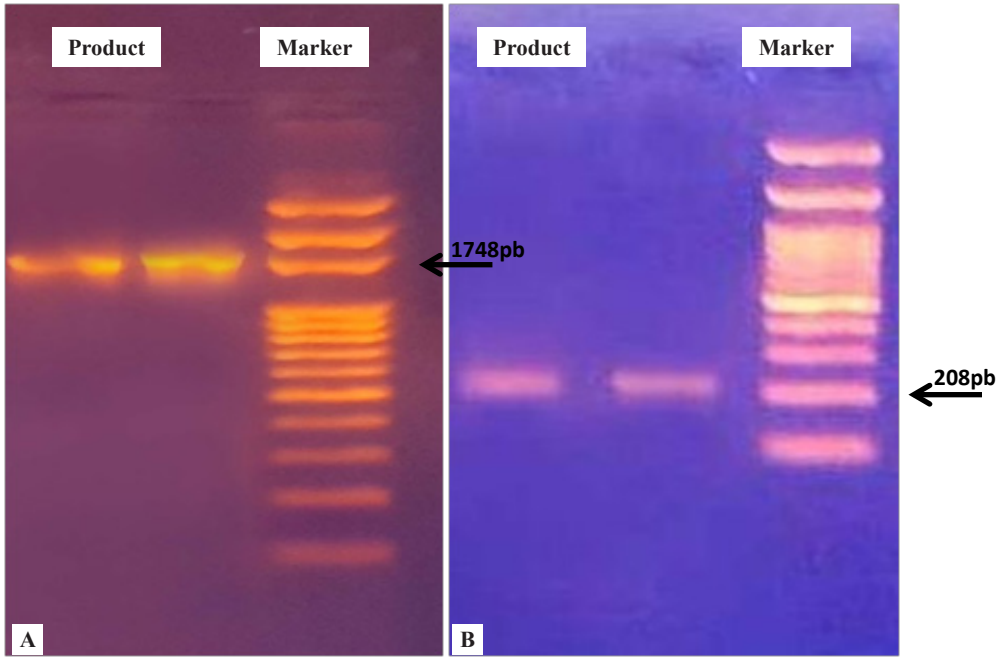

Figure 4: Agarose gel electrophoresis shows duplicates of **(A)** the primer EntEndolysin1 product and **(B)** the primer EntEndolysin 2 product

Table 1: The sequence of *E. feacalis* 13 phage 3 endolysin gene by the automated Sanger DNA sequencing method.

Sequence	*Base pair*
1	atgaaaaagaaaattttagtcggagcgttaatcgctctatttttttatgcctttaaatgta
61	tttgctgctaaaggcgatcaaggtgttgattgggccgtttatcaaggtgaacaaggtcgt
121	tttggttatgcgcatgataaattcgctattgctcaaattggcggctacaatgccagtggt
181	atttacgagcagtatacctataaaacgcaagtagcaagtgccattgctcaaggaaaacga
241	gcacacacctatatttggtatgacacttggggaaacatggacattgcgaaaacaacgatg
301	gattacttcttattacgtattcaaacgcctaaaaattccattgttgctttagactttgag
361	catggcgctagttctgatgtaaacgcaaatacagaaacgattttgtatggtatgcgtcgc
421	atcaaacaagcaggttacacgccaatgtattatagctacaagccttttacgttacaatac
481	gtggactatcagagaattattaaagagtttcctaactctttatggattgctgcctatcct
541	agctatgaggtaacgccagaaccactatatgcttatttcccaagtatggagggcattgga
601	atttggcaatttacgtccacttatattgcaggcggcttagatggtaacgtagatttaaca
661	ggaattacggatagtggttatacagataacaataaaccagaaacggacactccagcaaca
721	gatgcaggtgaagaaattgaaaaaacaccgaattctgatgttaaagtcggcgacacagtt
781	aaagtgaaatttaatgtagatgcatgggcaactggtgaagctattccagattgggtaaaa
841	ggaaacaactacaaagtgcaagaagtaactggaagcagagtattgttagaaggtatcctg
901	tcatggattagcaaaggcgatattgaattattgccagatgcaacaattgttcccgataag
961	caaccagaatctattcacgtagttcaatatggtgaaacattatccagcattgcttaccaa
1021	tacggtactgactatcaaaccttagcttcgctaaatggattggctaatccaaatcttatt
1081	taccctggacaaactttgaaagtaaatcgatcagtagtaagcaatgtttacacagttcaa
1141	tacggtgataatttatcaagtattgcatctaagcttggtacgacataccaagctttagca
1201	caacgaaacaggttaactaatcttaacttgatttatccagggcaaacattaatttat

Phage Endolysin Gene Sequencing

The PCR products (1748 bp) of *Endolysin*gene obtained from conventional PCR was sent for sequencing using the automated Sanger DNA sequencing method. The total number of nucleotides: Adenine (418 A), Cytosine (224 C), Guanine (257 G), and Thymine (358 T) were 1257 (Table 1). The DNA sequence was translated to its amino acid with total number of 419.

The Phylogentic of EF13P3 Gene

After getting the gene sequence of EF13P3, the sequence was compared to other world reference strains of *E. faecalis* phage endolysin using BLAST program. A 1257/1257 (100%) similarities and 0/1257 (0%) gaps were detected with *Enterococcus* phage phiEf11 endolysin. A complete genomic definition was provided by BLAST about *Enterococcus* phage phiEf11 endolysin.

DISCUSSION

Endolysin Antibacterial Activity

Enterococcus faecalis is a Gram-positive, facultative anaerobic, non-motile bacterium that was previously classified as part of the *Streptococcus* Group D system, which lives in the intestines of humans and other mammals.[24,25] It can cause life-threatening infections, especially in the nosocomial (hospital) environment, where the naturally high levels of antibiotic resistance found in *E. faecalis*.[26] *Enterococci* can acquire an extensive variety of resistance mechanisms by mutation or by the acquisition of exogenous genes.[5] *E. faecalis* has been identified as a significant opportunistic pathogen, community-acquired infections. In hospitalized patients with underlying conditions, typical enterococcal infections occur, such as wound, urinary tract infections, and bacteraemia.[27,28]

E. faecalis under investigation was previously isolated from urinary tract infection sample, which was 100% resistant to Cefoxitin, Erythromycin, Amikacin, and Ceftriaxone and 100% sensitive to tigecycline.[14] Thus, it is considered as MDR.[29] This antibioresistant profile was similar to a recent study in Iraq by Al-Naqshbandi *et al.* in 2020, which explored the fact that *E. faecalis* isolates have 100% sensitivity to tigecycline and (100%) resistance to Ceftriaxone and Cefotaxime with 80 to 90% resistant to erythromycin and tetracycline.[30] Exploring the antibioresistant pattern can greatly assist physicians in treating this commonly attributed bacterium in Iraq. These findings were in harmony with other similar studies in Iraq[31] and Egypt.[32] In contrast, a study in Italy by Bertelloni showed that (53%) isolates of *E. faecalis* were classified as MDR and 5.2% strains as possibly extensively drug-resistant (XDR).[33] In addition, the prevalence of the high-level gentamicin-resistant clinical enterococci was rising.[34] All these findings bring to mind the importance of the alternative antibacterial therapy like therapy by phage or its lytic enzyme to eliminate these bacteria.

Bacteriophages are viruses that infect bacteria and can be used as antibiotic alternatives, especially for MDR pathogens.[35] The main two sources for these phages were sewage and wastewater.[36,37,14] Endolysins from bacteriophages are a critical source of peptidoglycan hydrolase, which is induced in antimicrobial enzymes.[38,39] In this study, endolysin was successfully extracted using gel filtration column chromatography from *E. faecalis* bacteriophage (EF13P3). The fractions from numbers (12–38) showed the highest absorbance when measured by the spectrophotometer. The fraction number 22 was the best one to give the lytic activity when tested on the *E. faecalis* lawn and thus considered the tube with endolysin. The concentration of the extracted endolysin was (256 µg/mL) with (>64 µg/mL) MIC and (>128 µg/mL) MBC. The same extracted endolysin was tested previously against (20) MDR *E. faecalis.* It was effective against (90%) of themand was even more effective than the phage alone or the phage cocktail that showed 60% antibacterial activity.[14] The finding agreed with a study that showed the bactericidal of endolysin against *E. faecalis* which can kill 32/36 of diverse *E. faecalis* isolates.[40] Phage endolysins can lyse antibiotic-resistant bacteria. It has proven to be medically superior to antibiotic therapy.[12] The results of this study supported this notion, as phage lysin was shown to be highly efficacious against infections caused by antibiotic-resistant bacteria. This research revealed that bacteriophage lysins from *E. faecalis* can be employed to effectively lower bacterial loads that were multidrug-resistant. The recent investigation validated phage lysins' potential therapeutic relevance in gram-positive bacterial infections as an alternate therapy. As the threat of multidrug-resistant bacteria grows, there is a greater need for new antimicrobial agents. Bacteriophages and their lytic enzymes are naturally occurring agents that can be used to combat the problem.[41]

Endolysin Antibiofilm Activity

Because of the protective and nutritional benefits of living in a tightly knit community, research over the last few decades has determined that 99.9% of all microorganisms found in natural settings are attached to surfaces, both abiotic and biotic surfaces.[42,43] Bacterial isolates form complex exopolymers that stick to surfaces. As the number of bacteria in a given region grows, this adhering population is referred to as a biofilm.[44] Microbial biofilms have been observed in a submerged form and as a floating mat on liquid surfaces.[45] Bacterial isolates in biofilms are frequently immersed in a self-produced extra-polymeric matrix consisting primarily of proteins, lipids, polysaccharides, and nucleic acids.The extracellular matrix is responsible for the microbial biofilm's cohesion, adhesiveness, stability, and three-dimensional architecture.[46,47] Approximately 80% of the entire microbial infection is caused by the biofilm population that linked to both device- and non-device-associated infections.[48] *E. faecalis* is commonly recovered from chronic periapical or root canal infections associated with failed endodontic therapy. It has been proposed that this is associated with their ability to tolerate prolonged periods of starvation, form biofilms, and acquire antibiotic resistance.[49,50]

The enterococcal phage or phage cocktail have been discovered and used to combat bacterial infections and biofilms such as those generated by *E. faecalis.*[51-53] The results of this study demonstrated the ability of *E. faecalis* (EF13) to form a biofilm by the formation of black colonies with a dry crystalline consistency on Congo red agar plates. These findings were in agreement with other studies that *E. faecalis* is the common pathogen isolated from urinary tract infections and usually

associated with biofilm formation that is responsible for its bioresistant.[54,55] The efficient quantitativemode of investigation formation and evaluation of biofilms the effectiveness antibacterial properties drugs is to utilize microtiter plates. This method is used to measure the reduction before and after phage in biofilm matrices or its product handling.[56] The quantitative analysis in this study, after overnight incubation of the extracted endolysin with the mature biofilm, found that the percentage of inhibition of the extracted endolysin was shown to be 90.04%. There is no recent information that we are aware of or previous study in Iraq on the usage of endolysin as an antibacterial substitute against *E. faecalis* biofilm was found. Hence, this study's findings could be considered a promising outcome to develop a cheap and effective antibiofilm agent against this harmful bacterium.

Many studies worldwide tried to focus on the ability of engineered endolysin or peptidoglycan hydrolases to overcome bacterial biofilms'. For instance, engineered endolysin was used to eliminate biofilms formed by two important pathogens in food and clinical environments, *Staphylococcus* and *Streptococcus* species. The successful strategy for endolysin is by its ability to directly lyse biofilm matrix, which happens due to its diffusion through the extracellular material of pathogens.[57,58] Regarding the elimination of *E. faecalis* biofilms, a study carried out by Zhang *et al.* (2019) disclosed the ability of a geneticallyengineered endolysin to disrupt the biofilm within one hour of incubation.[59] The potential of the bacteria to form a biofilm as a resistant mechanism against currently used antibiotics can be overwhelmed by the usage of an endobiotic agent like phage endolysin. The proficiency of resistant development is very difficult to occur against endolysin. This is due to the endolysin's catalytic activity, which acts on the peptidoglycan layer of the bacterial cell wall.[12] Moreover, the vast majority of known endolysins from phages infecting Gram-positive bacteria contain a well-conserved domain architecture, in which the N-terminal region carries one or two enzymatically active catalytic domains and the C-terminus motifs responsible for cell wall binding.[60]

Genome Comparison

Endolysin, which is an important component for phage burst from within the host bacterium, is encoded by a gene in lytic phage.[61,62] In this study, NCBI-BLAST formatted protein database was searched for top matching phage and prophage genomes to find whether there were any phage or prophage endolysin genes that were similar to the currently isolated *E. faecalis* 13 phage endolysin. After extracting the DNA of E. faecalis 13 phage, the endolysin gene was 1257 nucleotides long. The BLAST analysis and the phylogenetic tree map agreed with Enterococcus phage phiEf11 endolysin gene that waswaspreviously obtained, based on the DNA sequence.

The lysozyme, also known as muramidase, is a basic enzyme that functions as an antibacterial agent normally present in saliva, tears, egg white, and many animal fluids. It is a glycoside hydrolase that cleaves the peptidoglycan layer by catalyzing the hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues.[63] Peptidoglycan is the major component of the gram-positive bacterial cell wall. Hydrolyzing this layer weaken the integrity of bacterial cell walls causing lysis of the bacteria. This fact highlights the importance of the genetic analysis of the current study to explore the nature of EF13P3 endolysin that can be used as an alternative antibacterial agent.

It is clear that in the intestinal ecosystem, the induction of the prophages occurs as a response to environmental cues is emerging as one strategy for enterococcal colonization and control.[64]*Enterococcus* phage phiEf11 was a prophage that induced with mitomycin C from *E. faecalis* root canal isolate. It is a temperate prophage and is part of the Siphoviridae family.[65] Later, the geneticallyengineered *Enterococcus* phage phiEf11 was used to reduce the static biofilm of *E. faecalis* after overnight incubation. Thus, improve phage therapy's efficacy in the treatment of *E. faecalis* root canal infections.[53]

The disadvantage of the phages' limited host ranges is a drawback of phage therapy. Therefore, scientists tried to expand by making phage cocktails with a wider host range; you can expand the phage host range,depending on the fact that resistance to all pathogenic phages is unlikely to emerge simultaneously. Additionally, even if phages or the phage cocktails fail in providing a therapy for *E. faecalis*, their naturally or genetically engineered Endolysins could be a viable alternative to antibiotics in the fight against *E. faecalis*-related illness.

Moreover, the biomass of both vancomycin-sensitive and vancomycin-resistant E. faecalis biofilms was markedly reduced following infection by genetically-modified Enterococcus phage phiEf11.[66] Genomic sequence analysis of Enterococcus phage phiEf11 predicted genes coding for two endolysins: a Cpl-1 type muramidase endolysin (similar to the one extracted in this study) and an N-acetylmuramoyl-l-alanine amidase endolysin also possessing peptidase and acetylglucosaminidase domains.[67] The results of this study demonstrated the potential of the EF13P3 endolysin to reduce *E. faecalis* biofilm matrices were in harmony with a study of Zhang, *et al*. In their study, they found N-acetylmuramoyl-l-alanine amidase endolysin which extracted from Enterococcus phage phiEf11 was able reduce E. faecalis biofilm with one hour of incubation.[59]

CONCLUSION

The significant expansion in the emergence of multi-drug resistant bacteria, particularly, *E. faecalis* increases the need for research to find an alternative antibacterial therapy. This study was a continuous search for the ability of naturally isolated phages as an alternative therapy to antibiotics in the fight against *E. faecalis* infections. The potential of the endolysin extracted from *E. faecalis* bacteriophage was significant to reduce bacterial growth and biofilm in 90.04%. The concentration that needed to inhibit the bacterial growth was (> 64 μg/mL) while the concentration that needed to kill the bacteria was (>128 μg/mL), which focused on the powerful effect of this lytic enzyme to eradicate infections by *E. faecalis.*

The genetic analysis of the extracted endolysin found the relationship between this locally isolated lytic enzyme and other similar enzymes worldwide. It was found to be identical to *Enterococcus* phage phiEf11 endolysin with glycoside hydrolase activity that can cleave the peptidoglycan layer of gram-positive bacteria. Hence, the extracted endolysin can act like the natural lysozyme, which is one of the innate immunity arms of the immune system. These findings emphasize on the importance of the current study and the future aspects of using natural or genetically engineered endolysins to break down gram-positive or gram-negative bacteria and control their resistant infections. Further, in vivo studies arerecommended to explore the potency of a cloned endolysin to eradicate *E. faecalis* infections.

REFERENCES

1. Dale JL, Cagnazzo J, Phan CQ, Barnes AM, Dunny GM. Multiple roles for Enterococcus faecalisglycosyltransferases in biofilm-associated antibiotic resistance, cell envelope integrity, and conjugative transfer. Antimicrobial agents and chemotherapy. 2015;59(7):4094-105. Available from:https://doi.org/10.1128/AAC.00344-315.
2. Clewell DB, Weaver KE, Dunny GM, et al. Extrachromosomal and Mobile Elements in Enterococci: Transmission, Maintenance, and Epidemiology. 2014. In: Gilmore MS, Clewell DB, Ike Y, et al., editors. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection [Internet]. Boston: Massachusetts Eye and Ear Infirmary; 2014; Available from: https://www.ncbi.nlm.nih.gov/books/NBK190430/.
3. Bender JK, Cattoir V, Hegstad K, Sadowy E, Coque TM, Westh H, Hammerum AM, Schaffer K, Burns K, Murchan S, Novais C. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resistance Updates. 2018;40:25-39. Available from:https://doi.org/10.1016/j.drup.2018.10.002.
4. Rello J, Van Engelen TS, Alp E, Calandra T, Cattoir V, Kern WV, Netea MG, Nseir S, Opal SM, van de Veerdonk FL, Wilcox MH. Towards precision medicine in sepsis: a position paper from the European Society of Clinical Microbiology and Infectious Diseases. Clinical Microbiology and Infection. 2018;24(12):1264-1272.Available from:https://doi.org/10.1016/j.cmi.2018.03.011.
5. Gilmore MS, Clewell DB, Ike Y, Shankar N. Enterococci: From Commensals to Leading Causes of Drug Resistant Infection. 2014.Available from: https://pubmed.ncbi.nlm.nih.gov/24649510/.
6. Deshpande LM, Castanheira M, Flamm RK, Mendes RE. Evolving oxazolidinone resistance mechanisms in a worldwide collection of enterococcal clinical isolates: results from the SENTRY Antimicrobial Surveillance Program. Journal of Antimicrobial Chemotherapy. 2018;73(9):2314-2322. Available from: https://doi.org/10.1093/jac/dky188
7. El-Shibiny A, El-Sahhar S. Bacteriophages: the possible solution to treat infections caused by pathogenic bacteria. Canadian journal of microbiology. 2017;63(11):865-879. Available from: https://doi.org/10.1139/cjm-2017-0030.
8. Oechslin F. Resistance development to bacteriophages occurring during bacteriophage therapy. Viruses. 2018;10(7):351.Available from: https://doi.org/10.3390/v10070351.
9. Wang J, Hu B, Xu M, Yan Q, Liu S, Zhu X, Sun Z, Reed E, Ding L, Gong J, Li QQ. Use of bacteriophage in the treatment of experimental animal bacteremia from imipenem-resistant Pseudomonas aeruginosa. International journal of molecular medicine. 2006;17(2):309-317.Available from:https://doi.org/10.3892/ijmm.17.2.309
10. Blasdel BG, Chevallereau A, Monot M, Lavigne R, Debarbieux L. Comparative transcriptomics analyses reveal the conservation of an ancestral infectious strategy in two bacteriophage genera. The ISME journal. 2017;11(9):1988-1996.Available from: https://doi.org/10.1038/ismej.2017.63.
11. Oliveira H, Melo LD, Santos SB, Nóbrega FL, Ferreira EC, Cerca N, Azeredo J, Kluskens LD. Molecular aspects and comparative genomics of bacteriophage endolysins. Journal of virology. 2013;87(8):4558-70.Available from: https://doi.org/10.1128/JVI.03277-12.
12. Schmelcher M, Donovan DM, Loessner MJ. Bacteriophage endolysins as novel antimicrobials. Future microbiology. 2012;7(10):1147-71.Available from: https://doi.org/10.2217/fmb.12.97.
13. Schmelcher M, Tchang VS, Loessner MJ. Domain shuffling and module engineering of Listeria phage endolysins for enhanced lytic activity and binding affinity. Microbial biotechnology. 2011;4(5):651-62.Available from: https://doi.org/10.1111/j.1751-7915.2011.00263.
14. Ahmed, ShH and Hafidh RR. The Isolation of specifically lytic phages along with their extracted endolysins as antibacterial agents to MDR Enterococcus faecalis. Research Journal of Pharmacy and Technology. 2021; 14(9): 4547-54. Available from: https://doi.org/10.52711/0974-360X.2021.00791.
15. Pazoles J, Talbot MK, Alder EA, White AC, Jonas BM, Murray BE, Weinstock GM, Rogers BL. Enterococcus faecalis multi-drug resistance transporters: application for antibiotic discovery. J MolMicrobiolBiotechnol. 2001;3(2):179-184.Available from: https://pubmed.ncbi.nlm.nih.gov/11321571/
16. Yin WJ, Tang H, Wei SH, Al-Jassim MM, Turner J, Yan Y. Band structure engineering of semiconductors for enhanced photoelectrochemical water splitting: The case of TiO 2. Physical Review B. 2010; 82(4):045106.Available from: https://doi.org/10.1103/PhysRevB.82.045106.
17. Jones CG, Hare JD, Compton SJ. Measuring plant protein with the Bradford assay. Journal of chemical ecology. 1989;15(3):979-992.Available from:https://doi.org/10.1007/BF01015193.
18. Andrews, G., Issakidis, C. and Carter, G. Shortfall in mental health service utilisation. The British Journal of Psychiatry.2001; 179(5):417-425.Available from:https://doi.org/10.1192/bjp.179.5.417.
19. Oliveira A, Maria de Lourdes RS. Comparison of methods for the detection of biofilm production in coagulase-negative staphylococci. BMC research notes. 2010;3(1):1-8.Available from:https://doi.org/10.1186/1756-0500-3-260.
20. Mathur T, Singhal S, Khan S, Upadhyay DJ, Fatma T, Rattan A. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. Indian journal of medical microbiology. 2006;24(1):25-29. Available from:https://doi.org/10.1016/S0255-0857(21)02466-X.
21. Mulla SA, Revdiwala S. Assessment of biofilm formation in device-associated clinical bacterial isolates in a tertiary level hospital. Indian Journal of Pathology and Microbiology. 2011;54(3):561. Available from: https://www.ijpmonline.org/text.asp?2011/54/3/561/85093.
22. Palmeira CA, da Silva Rodrigues-Filho LF, de Luna Sales JB, Vallinoto M, Schneider H, Sampaio I. Commercialization of a critically endangered species (largetooth sawfish, Pristisperotteti)

in fish markets of northern Brazil: authenticity by DNA analysis. Food Control. 2013;34(1):249-252. Available from: 10.1016/j.foodcont.2013.04.017.

23. Ledergerber C, Dessimoz C. Base-calling for next-generation sequencing platforms. Briefings in bioinformatics. 2011;12(5):489-497.Available from:https://doi.org/10.1093/bib/bbq077.
24. Rice LB, Carias L, Rudin S, Vael C, Goossens H, Konstabel C, Klare I, Nallapareddy SR, Huang W, Murray BE. A potential virulence gene, hylEfm, predominates in Enterococcus faecium of clinical origin. The Journal of infectious diseases. 2003; 187(3):508-512.Available from:https://doi.org/10.1086/367711.
25. ÇALGIN MK, ÇETİNKOL Y. BirÜniversiteHastanesinde 2014-2018 Yılları ID ArasındaÇeşitliKlinikÖrneklerdenİzoleEdilenCitrobacterSuşlarınınAntibiyotikDirenciniDeğerlendirilmesi. ANKEM Dergisi. 2019;33(2):65-69.Available from: https://doi.org/10.5222/ankem.2019.1917
26. Fallah F, Yousefi M, Pourmand MR, Hashemi A, Alam AN, Afshar D. Phenotypic and genotypic study of biofilm formation in Enterococci isolated from urinary tract infections. Microbial pathogenesis. 2017;108:85-90.Available from:https://doi.org/10.1016/j.micpath.2017.05.014
27. Giannakopoulos X, Sakkas H, Ragos V, Tsiambas E, Bozidis P, Evangelou AM, Papadopoulou C, Petrogiannopoulos L, Sofikitis N. Impact of enterococcal urinary tract infections in immunocompromised-neoplastic patients. J BUON. 2019; 24(5):1768-1775.Available from: https://pubmed.ncbi.nlm.nih.gov/31786836/.
28. Contreras GA, Munita JM, Arias CA. Novel strategies for the management of vancomycin-resistant enterococcal infections. Current infectious disease reports. 2019; 21(7):1-8.Available from:https://doi.org/10.1007/s11908-019-0680-y.
29. Peters A, Borzykowski T, Tartari E, Kilpatrick C, Mai SH, Allegranzi B, Pittet D. "Clean care for all-it's in your hands": the May 5th, 2019 World Health Organization SAVE LIVES: Clean Your Hands campaign. Antimicrobial Resistance & Infection Control. 2019;8(1):1-3. Available from:https://doi.org/10.4103/jfmpc.jfmpc_1050_19
30. Al-Naqshbandi AA, Chawsheen MA, Abdulqader HH. Prevalence and antimicrobial susceptibility of bacterial pathogens isolated from urine specimens received in rizgary hospital—Erbil. Journal of infection and public health. 2019;12(3):330-336. Available from: https://doi.org/10.1016/j.jiph.2018.11.005
31. Al-Naqshbandi AA, Chawsheen MA, Abdulqader HH. Prevalence and antimicrobial susceptibility of bacterial pathogens isolated from urine specimens received in rizgary hospital—Erbil. Journal of infection and public health. 2019 ;1;12(3):330-336. Available from: : https://doi.org/10.1016/j.jiph.2018.11.005
32. Esmail MA, Abdulghany HM, Khairy RM. Prevalence of multidrug-resistant enterococcus faecalis in hospital-acquired surgical wound infections and bacteremia: concomitant analysis of antimicrobial resistance genes. Infectious Diseases: Research and Treatment.2019;12:1178633719882929.Availablefrom: https://doi.org/10.1177/1178633719882929.
33. Bertelloni F, Salvadori C, Moni A, Cerri D, Mani P, Ebani VV. Antimicrobial resistance in Enterococcus spp. isolated from laying hens of backyard poultry flocks. Annals of Agricultural and Environmental Medicine. 2015;22(4). Available from: https://doi.org/10.5604/12321966.1185771
34. Dadfarma N., Oskouei M., ImaniFouladi A.A., Farokh P. Studyof AAC(6')IE-APH(2")IA geneinclinicalstrainofEnterococcian didentificationof high-level gentamicinresistantEnterococci. Avicenna journal of clinical medicine (Scientific journal of Hamadan University of medical sciences and health services). 2010; 17(3 (SN 57)): 25-32. Available from: https://www.sid.ir/en/journal/ViewPaper.aspx?id=187843.
35. Tagliaferri TL, Jansen M, Horz HP. Fighting pathogenic bacteria on two fronts: phages and antibiotics as combined strategy. Frontiers in cellular and infection microbiology. 2019;9:22. Available from: https://doi.org/10.3389/fcimb.2019.00022.
36. Kusradze I, Karumidze N, Rigvava S, Dvalidze T, Katsitadze M, Amiranashvili I, Goderdzishvili M. Characterization and testing the efficiency of Acinetobacterbaumannii phage vB-GEC_ Ab-M-G7 as an antibacterial agent. Frontiers in microbiology. 2016;7:1590.Available from: https://doi.org/10.3389/fmicb.2016.01590.
37. Al-Zubidi M, Widziolek M, Court EK, Gains AF, Smith RE, Ansbro K, Alrafaie A, Evans C, Murdoch C, Mesnage S, Douglas CW. Identification of novel bacteriophages with therapeutic potential that target Enterococcus faecalis. Infection and immunity. 2019;87(11):e00512-19.Available from:https://doi.org/10.1128/IAI.00512-19.
38. Fenton M, McAuliffe O, O'Mahony J, Coffey A. Recombinant bacteriophage lysins as antibacterials. Bioengineered bugs. 2010;1(1):9-16.Available from https://doi.org/10.4161/bbug.1.1.9818.
39. Plotka M, Sancho-Vaello E, Dorawa S, Kaczorowska AK, Kozlowski LP, Kaczorowski T, Zeth K. Structure and function of the Ts2631 endolysin of Thermusscotoductus phage vB_Tsc2631 with unique N-terminal extension used for peptidoglycan binding. Scientific reports. 2019;9(1):1-4.Available from:https://doi.org/10.1038/s41598-018-37417-6.
40. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, Jafri W. Asia–Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatology international. 2017;11(4):317-370. Available from:https://doi.org/10.1007/s12072-017-9799-9.
41. Elbreki M, Ross RP, Hill C, O'Mahony J, McAuliffe O, Coffey A. Bacteriophages and Their Derivatives as Biotherapeutic Agents in Disease Prevention and Treatment. Journal of Viruses. 2014; 2014:382539. Available from:https://doi.org/10.1155/2014/382539.
42. Novais SM, DaRocha WD, Calderon-Cortes N, Quesada M. Wood-boring beetles promote ant nest cavities: extended effects of a twig-girdler ecosystem engineer. Basic and Applied Ecology. 2017;24:53-59.Available from:https://doi.org/10.1016/j.baae.2017.09.001.
43. Cowan G, Cranmer K, Gross E, Vitells O. Asymptotic formulae for likelihood-based tests of new physics. The European Physical Journal C. 2011;71(2):1-9.Available from https://doi.org/10.1140/epjc/s10052-011-1554-0.
44. Steinberg N, Kolodkin-Gal I. The matrix reloaded: how sensing the extracellular matrix synchronizes bacterial communities. Journal of bacteriology. 2015;197(13):2092-103.Available from:https://doi.org/10.1128/JB.02516-14.
45. Vasudevan S, Oturan MA. Electrochemistry: as cause and cure in water pollution—an overview. Environmental chemistry letters. 2014;12(1):97-108. Available from:https://doi.org/10.1007/s10311-013-0434-2.
46. Hurlow J, Couch K, Laforet K, Bolton L, Metcalf D, Bowler P. Clinical biofilms: a challenging frontier in wound care. Advances in wound care. 2015;4(5):295-301.Avilable from: http://doi.org/10.1089/wound.2014.0567.

47. Rice K, Moraczewski D, Redcay E. Perceived live interaction modulates the developing social brain. Social cognitive and affective neuroscience. 2016;11(9):1354-1362. Available from: https://doi.org/10.1093/scan/nsw060.
48. Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018;4(12):e01067. Available from: https://doi.org/10.1016/j.heliyon.2018.e01067.
49. Gopikrishna V, Kandaswamy D, Jeyavel RK. Comparative evaluation of the antimicrobial efficacy of five endodontic root canal sealers against Enterococcus faecalis and Candida albicans. Journal of Conservative Dentistry. 2006; 9(1):2.Available from:https://doi.org/10.4103/0972-0707.41303
50. Mohamed JA, Huang W, Nallapareddy SR, Teng F, Murray BE. Influence of origin of isolates, especially endocarditis isolates, and various genes on biofilm formation by Enterococcus faecalis. Infection and immunity. 2004;72(6): 3658-3663.Available from: https://doi.org/10.1128/IAI.72.6.3658-3663.2004.
51. Abdulamir AS, Jassim SA, Hafidh RR, Bakar FA. The potential of bacteriophage cocktail in eliminating Methicillin-resistant Staphylococcus aureus biofilms in terms of different extracellular matrices expressed by PIA, ciaA-D and FnBPA genes. Annals of clinical microbiology and antimicrobials. 2015;14(1):1-0. Available from:https://doi.org/10.1186/s12941-015-0106-0.
52. Szafrański SP, Winkel A, Stiesch M. The use of bacteriophages to biocontrol oral biofilms. Journal of biotechnology. 2017;250: 29-44. Available from:https://doi.org/10.1016/j.jbiotec.2017.01.002.
53. Bolocan AS, Upadrasta A, de Almeida Bettio PH, Clooney AG, Draper LA, Ross RP, Hill C. Evaluation of phage therapy in the context of Enterococcus faecalis and its associated diseases. Viruses. 2019;11(4):366.Available from: https://doi.org/10.3390/v11040366.
54. Salem-Bekhit MM, Moussa IM, Elsherbini MM, AlRejaie S. Increasing prevalence of high-level gentamicin resistant enterococci: an emerging clinical problem. African Journal of Microbiology Research. 2011;5(31):5713-5720. Available from: 10.5812/jjm.28923.
55. Al Obaidy AH, Al Mashhady AA, Awad ES, Kadhem AJ. Heavy metals pollution in surface water of Mahrut River, Diyala, Iraq. International Journal of Advanced Research. 2014;2(10):1039-1044. Available from: https://www.journalijar.com/uploads/771_IJAR-4347.pdf
56. Sutherland IW, Hughes KA, Skillman LC, Tait K. The interaction of phage and biofilms. FEMS microbiology letters. 2004;1;232(1):1-6.Available from:https://doi.org/10.1016/S0378-1097(04)00041-2.
57. Ferriol-González C, Domingo-Calap P. Phages for biofilm removal. Antibiotics. 2020;9(5):268.Available from:https://doi.org/10.3390/antibiotics905026.
58. Abdelrahman F, Easwaran M, Daramola OI, Ragab S, Lynch S, Oduselu TJ, Khan FM, Ayobami A, Adnan F, Torrents E, Sanmukh S. Phage-Encoded Endolysins. Antibiotics. 2021;10(2):124. Available from: https://doi.org/10.3390/antibiotics10020124.
59. Zhang H, Buttaro BA, Fouts DE, Sanjari S, Evans BS, Stevens RH. Bacteriophage φEf11 ORF28 endolysin, a multi-functional lytic enzyme with properties distinct from all other identified Enterococcus faecalis phage endolysins. Applied and environmental microbiology.2019;85(13):e00555-19.Available from:https://doi.org/10.1128/AEM.00555-19.
60. Fischetti VA. Bacteriophage lysins as effective antibacterials. Current opinion in microbiology. 2008;11(5): 393-400.Available from:https://doi.org/10.1016/j.mib.2008.09.012.
61. Gondil VS, Harjai K, Chhibber S. Endolysins as emerging alternative therapeutic agents to counter drug-resistant infections. International journal of antimicrobial agents. 2020;1;55(2):105844.Available from:https://doi.org/10.1016/j.ijantimicag.2019.11.001.
62. Stevens MC, Skudlarski P, Pearlson GD, Calhoun VD. Agerelated cognitive gains are mediated by the effects of white matter development on brain network integration. Neuroimage. 2009;48(4):738-746.Available from:https://doi.org/10.1016/j.neuroimage.2009.06.065
63. Mercier C, Domakova E, Tremblay J, Kulakauskas S. Effects of a muramidase on a mixed bacterial community. FEMS microbiology letters. 2000; 187(1):47-52. Available from:https://doi.org/10.1111/j.1574-6968.2000.tb09135.
64. Duerkop BA, Clements CV, Rollins D, Rodrigues JL, Hooper LV. A composite bacteriophage alters colonization by an intestinal commensal bacterium. Proceedings of the National Academy of Sciences. 2012; 109(43):17621-17626.Available from: https://doi.org/10.1073/pnas.1206136109.
65. Stevens RH, Porras OD, Delisle AL. Bacteriophages induced from lysogenic root canal isolates of Enterococcus faecalis. Oral microbiology and immunology. 2009;24(4):278-284.Available from:https://doi.org/10.1111/j.1399-302X.2009.00506.
66. Tinoco JM, Buttaro B, Zhang H, Liss N, Sassone L, Stevens R. Effect of a genetically engineered bacteriophage on Enterococcus faecalis biofilms. Archives of oral biology. 2016;71:80-86. Available from:https://doi.org/10.1016/j.archoralbio.2016.07.001
67. Stevens RH, Ektefaie MR, Fouts DE. The annotated complete DNA sequence of Enterococcus faecalis bacteriophage φEf11 and its comparison with all available phage and predicted prophage genomes. FEMS microbiology letters. 2011; 317(1):9-26.Available from:https://doi.org/10.1111/j.1574-6968.2010.02203.x