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RESEARCH ARTICLE
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In vitro and in vivo study for antibacterial activity of endolysin-HEC gel and mixture gel on acne vulgaris caused by multidrug-resistant Staphylococcus aureus bacteria

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ABSTRACT

Background: Acne is named "acne vulgaris" medically, and it is classified as the eighth most frequent illness in the world. Acne is derived from the Greek word "acme," which means "prime of life." It is predominantly a condition of adolescents and may continue into adulthood.

Aim: This study aimed to extract, purify, and prepare endolysin-hydroxyethyl cellulose (HEC) gel from Staphylococcus aureus–specific bacteriophages, evaluate its effectiveness via in vitro antibacterial validation tests, and clinically investigate its ability to target facial multidrug-resistant (MDR) S. aureus when applied topically to participants with moderate-to-severe acne lesions.

Methods: Twenty-three isolates of MDR S. aureus bacteria were obtained from inflammatory acne lesions of human skin by using disposable cotton swabs. Three bacteriophages with specific lytic activity against MDR S. aureus bacteria were isolated by "conventional microbiological methods" and prepared as bacteriophage cocktail, and endolysin was extracted and purified from them. Gel-based formulations, endolysin-HEC and mixture gel from bacteriophage cocktail, were prepared. In vitro validation tests was performed using spot lysis and top layer plaque assays, and antibacterial activities were also assessed. In vivo tests included the topical application of the gel three times daily for 1 week on facial skin of volunteers aged above 25 years with moderate-to-severe acne lesions caused by MDR S. aureus bacteria.

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Results: Gel-based formulations exhibited 100% in vitro lytic spectrum analysis against MDR S. aureus isolates. No allergic reaction was displayed against the therapy when applied topically on the skin (in vivo) of the subjects with moderate-to-severe facial acne lesions caused by MDR S. aureus. It showed excellent results of clinical improvement response, with decrease in inflammatory signs, size, number of comedones, and presence of decolonized MDR S. aureus viable bacterial growth as compared to the untreated acne lesions “control” when cultured microbiologically.

Conclusion: Endolysin-HEC gel and mixture gel therapy have highly significant potential as an alternative strategy for MDR S. aureus infections of acne vulgaris. It offers a real chance for those suffering from chronic acne lesions caused by MDR bacterial infections, and protects teenagers from the overuse of antibiotics, anxiety, and depression.

Keywords: *Chronic renal failure, Renin , Aspartate Amino Transferase Alanine Amino Transferase , Albumin, Globulin, Calcium , Sodium , Potassium .*

INTRODUCTION

Acne is a multifactorial inflammatory disease considered as one of the common causes of hypertrophic scars. There are several therapy approaches that aim to address the underlying reasons. These include topical, oral (systemic), and physical methods such as laser treatment and photodynamic therapy.1 Benzoyl peroxide has a broad-spectrum antibacterial effect, and thus it is currently advised to use topical agents in combination with benzoyl peroxide either as a leave-on or a rinse-off product to reduce the rates of bacterial resistance. Topical agents are used as the first-line therapy for acne disease.2 High levels of antibiotic resistance acquired by Staphylococcus aureus complicate treatment and cause a significant problem. In the past, S. aureus resistance developed within 2 years after the debut of penicillin antibiotic.3 Following the development of the semisynthetic antibiotic methicillin in the 1950s, methicillin-resistant S. aureus (MRSA) was first discovered clinically in 1960.4 Due to the acquisition and insertion of these mobile genetic elements into the chromosomes of the susceptible strains, Staphylococci of resistant strains have emerged, posing a challenge to the medical community in terms of treatment and management of staphylococcal infections.

5 Due to their high mortality and morbidity rates as well as their resistance to all forms of useable penicillin and the majority of other ß-lactam medications, with the exception of ceftobiprole and ceftaroline, they are of great concern. MRSA has now been identified as the significant cause of community-associated bacterial infections, in addition to health-care locations such as hospitals, nursing homes, and persons connected to these environments.6 Since the widespread introduction of community-associated MRSA strains in the 1980s, there has been an increase in community-acquired MRSA infections.7

Tragically, it is predicted that by 2050, bacterial antibiotic resistance would cause 106 annual deaths worldwide, which is more than that of the COVID-19 effect in 2020.8 The nondevelopment of any new class of antibiotics in more than 10 years by the pharmaceutical industry and the continued rise of antibiotic resistance are serious health concerns, and urgent measures should be taken to combat the problems related to antibiotic resistance in medicine, such as by the use of nontraditional agents,9 stepping up research on inhibiting bacterial virulence by blocking quorum sensing, or focusing on systems that confer resistance against antibiotics, such as the multidrug resistance.10

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Peptidoglycan hydrolase and endolysin enzymes are ubiquitous with wide host ranges and are not significantly impacted by the development of bacterial resistance. Endolysin enzymes are a potential class of antimicrobial medicines for various pathogenic illnesses due to their ability to lyse gram-positive bacteria in a variety of in vitro applications. Gram-positive bacteria lack the protective outer membrane.11 Endolysins are very promising candidates to be used as new antibacterial agents in human and veterinary medicine because of their specificity, high activity, and limited incidence of resistance.12 This study aimed to extract, purify, and prepare endolysin-hydroxyethyl cellulose (HEC) gel from S. aureus–specific bacteriophages, evaluate its effectiveness via in vitro antibacterial validation tests, and clinically investigate its ability to target facial MDR S. aureus when applied topically on participants with moderate-to-severe acne lesions.

MATERIALS AND METHODS

Bacterial sampling and processing

A total of 23 different S. aureus from patients aged above 20 years who attended the Dermatology Consultant Unit of Al-Yarmouk Teaching Hospital, Baghdad, Iraq and complained of skin lesions, which were diagnosed clinically by the dermatologist as lesions of acne vulgaris. Samples (swabs) were transported to the laboratory within 4 h of collection and were processed immediately. Using standard microbiological techniques, such as hemolysis on blood agar, colony morphology, gram staining, growth and fermentation on mannitol salt agar, and generation of coagulase and catalase enzymes, S. aureus was isolated and identified.13 The VITEC2 system (BioMerieux, France) was used to assess the minimum inhibitory concentration (MIC) of antibacterial drugs against S. aureus. All isolates were screened and identified using the VITEK-2 system in accordance with the manufacturer's instructions. This method of phenotypic diagnosis relies on biochemical processes to identify the isolates.

Isolation of bacteriophages

Bacteriophages were identified from environmental samples, from "sewage water from Al-Bayaa Central Station Baghdad City." These samples were collected in clean "50 mL" tubes wrapped with parafilm and were sent to the lab in an ice bag to be processed the same day. The approach for isolating and propagating primary bacteriophages was followed exactly as described by Missiakas and Schneewind.14 Virulent bacteriophages were screened on nutrient agar using a bacteriophage "spotting test" as a preliminary identification of the virulent bacteriophage's ability to lyse bacterial isolates and generate obvious zones of inhibition.15 Specific lytic bacteriophages to MDR S. aureus were collected using a sterile loop, counted and distinguished using a top layer plaque assay, and then placed in 1.5 mL sterile Eppendrof tubes, and shaked moderately for 5 min at 4°C.

Extraction and purification of endolysin

After incubating in 100 mL of broth containing S. aureus bacteria, aerobically at 37°C for 24 h, the bacterium (1 × 1012) was placed in 1000 mL of broth for 3 h. A total of 300 mL of bacteriophage at titer 1 × 1013 PFU/mL was added for 20 min (MOI 1:10), and the total volume was divided among 50 mL tubes put directly on ice. The tubes were centrifuged at 104 rpm for 15 min, and the sediment was taken and put in 6 mL of 0.05 M phosphate buffer with deoxyribonuclease (5 mg) and incubated for 60 min at 37°C. 0.005 M of ethelyenediamine tetra acetic acid (EDTA) was added, and the supernatant was collected after centrifugation at 104 rpm for 1 h. At 4°C, sodium tetrathionate (0.3 M) was added and stirred for 1 h. The next day, the tubes were centrifuged at 104 rpm for 1 h. The mixture was then resuspended in 5 mL of phosphate buffer (0.05 M, pH 6.1).

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Overnight dialysis was performed against 200 Ml of phosphate buffer saline (PBS) with 2X concentration. at 4°C. Purification was accomplished by using column chromatography with sephadex G.100 in 0.1 M phosphate buffer. They were collected at 10-min intervals in 10 mL plane tubes. The absorbance of each fraction was measured at 280 nm.16 Each portion (10 mL) was pipetted onto bacterial lawns of the appropriate bacterium to determine which plane tube had the endolysin. The concentration of extracted endolysin was determined using the Bradford technique.17 The MIC and minimum bactericidal concentration (MBC) were determined using the broth dilution method,18 which were dependent on bacterial inoculum density of broth media in microtiter plate containing endolysins at doubling dilution levels (0.5, 1, 2, 4, 8, and 16 g/mL). The bacterial stock solution was produced in 3 mL sterile saline (aqueous 0.45–0.50% NaCl, pH 4.5–7) at 0.5 McFarland standards (1 × 107 CFU/mL). The suspension was then transferred (1–5 mL) to all but two wells of a microtiter plate containing doubling dilutions of 100 mL extracted endolysins to be tested (3 extracted endolysin for S. aureus) using a disposable plastic inoculator, with the remaining wells serving as broth and endolysin sterility controls. The ultimate inoculum size per well was 5 × 105 or 5 × 104 CFU. The plates were then incubated aerobically for 24 h at 37°C for S. aureus. The turbidity of the microtiter plate wells was visually evaluated, and the first well in the series with the lowest concentration of endolysin that inhibited visible bacterial growth defined the MIC.

By subculturing the last three clear MIC wells into blood agar medium and examining for bacterial growth, the MBC of the extracted endolysins that killed 99.9% of the inoculum was calculated.19

Preparation of gel-based formulation

Gel-based formulations were created by combining the active ingredient purified extracted endolysin (10% v/v) with a gel base composed of 1% transdermal vehicle ingredient HEC as thickening agent, emulsifier, and as a hydrogel-forming substance with the ability to form three-dimensional network structure upon cross-linking, and the subsequent absorption of water dissolved in 10 mL PBS.

HEC and PBS are antioxidant agents, which mean they "inhibit or delay the oxidation of physiologically important molecules."20

Endolysin–hydroxyethyl cellulose and mixture gel formulations

Extracted endolysin was made in 10 mL gel-based formulations (Endolysin Sa1-HEC gel, Endolysin Sa3-HEC gel) for each patient as follows:

To begin with, a gel-forming base of 1% HEC was made by weighing 0.1 g HEC powder and dissolving it in 10 mL PBS with 10% glycerol. The active component, pure extracted endolysin (10%, 5 g/mL) was then progressively added, combined, and homogenized.

The gel mixture was assembled as follows:

To create the combination gel, 1 mL of 10% bacteriophage cocktail at titer (1 × 1010) was progressively combined with 10 mL of endolysin-HEC gel and homogenized gently.

Fusidic acid cream (2%) and Bactroban "Mupirocin" cream (2%), both of which are commercially available as topical antibiotics, were utilized in comparison to the prepared gels (endolysin Sa1-HEC, endolysin Sa3-HEC).

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Gel-based characterizations

Physical characteristics and bioactivity was checked throughout variable conditions to elucidate the effectiveness and the stability of extracted endolysins and bacteriophage cocktail after gel formulations. Each type of these gels was stored at 4°C and 25°C. Visual appearance, "color, consistency," and PH were assessed at weekly intervals.

In vitro lytic activity of endolysin-HEC gels and mixture gels

A plate lytic experiment was used to assess the effectiveness of the endolysin-HEC gel formulation.21 S. aureus culture (1 × 107 CFU) was disseminated over nutritional agar. Antibacterial activity of each gel formulation was determined by pouring 10 mL of gel onto the nutrient agar plates and incubating aerobically for 24 h at 37°C. The plates were checked, and a distinct zone suggested that the gel was effective in killing bacteria. To test the efficacy of the bacteriophage cocktail inside the mixture gel, 100 mL of mixture gel was diluted tenfold in 900 mL of SM buffer. After incubation at 37°C, the titers of effective bacteriophage cocktail (PFU/mL) were assessed, and 10 mL of these dilutions were deposited onto a glass plate with 1 × 107 CFU/mL of bacteria. After incubation, a distinct lytic zone revealed the bacterial lysis activity by bacteriophages.

In vivo topical antibacterial activity of endolysin-HEC gel and mixture gels therapy on MDR S. aureus facial acne vulgaris

IRB 204/3/2 at College of Medicine/Al-Nahrain University accepted the interventional research protocol and subject consent forms. First, skin swabs from facial acne lesions of each participant aged above 25 years, who had previously undergone treatment for at least 3 years with several regimes (topical and systemic) with no response and have not taken any topical or systemic acne vulgaris medication for at least 6 months from the time of study, were taken. Swabs were cultured to evaluate the kind and susceptibility of MDR bacterial infection; safety was tested by applying endolysin-HEC gel and combination gel to intact skin to look for rapid allergic responses. The medication was then applied topically to a 2 cm2 patch of face acne lesions for 30 min three times daily for 1 week, while another patch containing MDR S. aureus was left as a control with no treatment. After 1 week of topical application, the clinical improvement response of the treatment patch was measured by determining the reduction in size, number of acne lesions, and decrease of inflammatory symptoms of face skin with acne vulgaris caused by MDR S. aureus as compared to the control patch. Furthermore, the decolonization in the development of MDR bacterial infection was detected.

RESULTS

The colonies of S. aureus appeared typically in golden yellow color on MSA, and yellow–gray colonies with β-hemolysis on blood agar as shown in Figures 1A, 1B, and 1C.

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A. Mannitol Salt agar **B. Blood agar/colonies** **C. Blood agar/hemolysis**

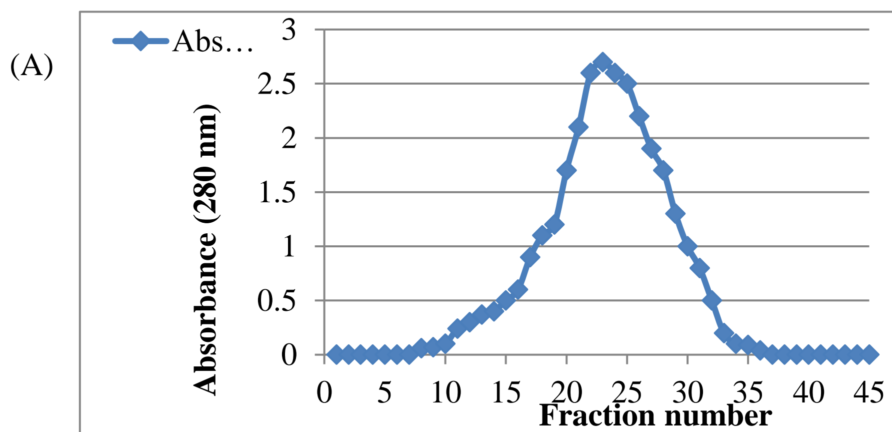
FIG 1. The morphology of Staphylococcus aureus colonies on different media. (A) S. aureus ferment mannitol and turns the medium to yellow color. (B) Yellow–grayish colonies of S. aureus on blood agar. (C) S. aureus surrounded by clear zone of β-hemolysis.

According to the antibiotic susceptibility test results, all S. aureus isolates were identified as sensitive against vancomycin. High antibiotic resistance percentage was observed for oxacillin (100%), ciprofloxacin (95%), piperacillin or tazobactam (91.3%), and gentamicin (69.6%), while moderate resistance was observed for clindamycin (56.5%), rifampicin (52.2%), and erythromycin (49.1%).

In contrast, minimum resistance rates were determined against tetracyclin(26%), chloramphenicol (21.7%), and trimethoprim or sulfamethoxazole (21.7%).

The three specific and lytic virulent bacteriophages for the study were isolated, optimized, and were obtained by using spot lysis assay. The endolysins WERE extracted from bacteriophages specific for S. aureus 1(BP-Sa1), S. aureus 2 (BP-Sa2), and S. aureus 3 (BP-Sa3).

The results of in vitro lytic activity for each extracted endolysin were carried out against MDR bacterial lawn, which revealed clear zones for fraction numbers 17, 11, and 13. So, fraction 17 (OD280:0.9) was extracted endolysin-Sa1, fraction 11 (OD280:0.5) was extracted endolysin-Sa2, and fraction 13 (OD280:0.8) was extracted endolysin-Sa3, as shown in Figures 2A, 2B, and 2C.



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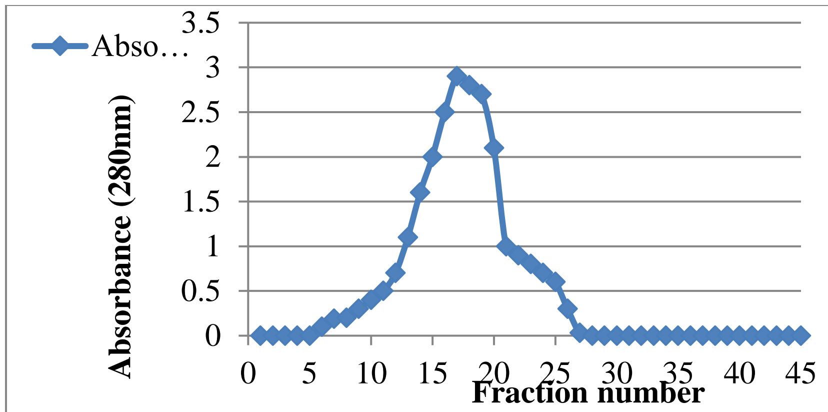
(B)



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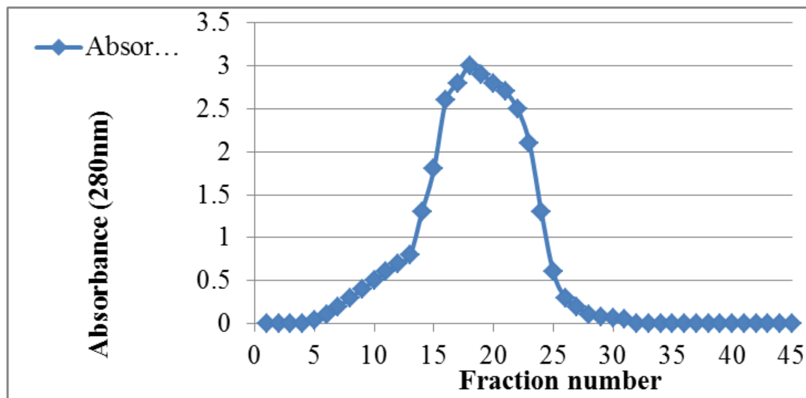

FIG 2. Purified protein fractions absorbance for (A) Purified protein fractions absorbance for Extracted Endolysin-Sa1; (B) Purified protein fractions absorbance for Extracted Endolysin-Sa2; and (C) Purified protein fractions absorbance for Extracted Endolysin-Sa3: Gel filtration chromatography on Sephadex G 100 column (25 × 3 cm) equilibrated with 0.05 M sodium phosphate buffer pH 7 with 30 mL/h flow rate.

The concentrations and volumes of extracted endolysins were 98 µg/mL, 7 mL; 83µg/mL, 5mL; and 87.2 µg/mL, 6 mL, for endolysin-Sa1, endolysin-Sa2, and endolysin-Sa3, respectively, as shown in Table 1.

TABLE 1. Concentrations and volumes of extracted endolysins.

Type of extracted endolysin	Endolysins concentration µg/mL	Volume (mL)
Endolysin-Sa1	98	7
Endolysin-Sa 2	83	5
Endolysin-Sa 3	87.2	6

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The MIC of the extracted endolysins against each bacterium was measured visually. The MICs of the extracted endolysins against Sa1, Sa2, and Sa3 were 2, 4, and 2 µg/mL, respectively, as shown in Table 2. Lower values of MIC corresponded to greater antibacterial activity of the extracted endolysin as shown in Figure 3. The MBCs of the extracted endolysins were 4, 8, and 4 µg/mL against Sa1, Sa2, and Sa3, respectively, as shown in Table 2.

FIG 3. Microbroth dilution endolysin susceptibility testing. Minimal inhibitory concentration (MIC) tests with Staphylococcus aureus bacteria. The first well where complete inhibition of growth is observed is considered as the MIC for the antimicrobial endolysin. The lowest concentrations of the antimicrobial endolysins are on the first columns of the plate, and the concentration of the antimicrobial endolysins increases in doubling dilutions (0.5, 1, 2, 4, 8, 16 µg/mL). Row 4: first well is the positive control (broth inoculated without lysin), second well is the negative control (uninoculated broth without endolysin), and third well is endolysin control (uninoculated broth with highest concentration of endolysin.

TABLE 2. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracted endolysins against Staphylococcus aureus bacteria.

Extracted Endolysins	Bacteria	MIC (µg/mL)	MBC (µg/mL)
Endolysin Sa1	Sa1	2	4
Endolysin Sa2	Sa2	4	8
Endolysin Sa3	Sa3	2	4

The visual appearance of gel formulations was examined every week, and the results showed that the gel formulations were able to maintain their white color, consistency, and characteristic odor for 30 days. The average pH was 7.1 for the base and the preparations added with the active substances. Assessment of the formulation gels showed that mixture gels retained full lytic capacity and bacteriophage cocktail with high effectiveness at titer (1 × 109 PFU/mL) after 30 days when stored at 4°C as shown in Figure 4, while endolysin-HEC gel retained full lytic capacity by 21 days after formulation when stored at 4°C. The formulation mixture gels of bacteriophage cocktail with endolysin-HEC showed reduced lytic capacity and decreased effectiveness of the bacteriophage cocktail to the titer (1 × 104 PFU/mL) by 21 days, and complete loss (100%) of lytic capacity at 25°C after 30 days, while the formulation gels of endolysin-HEC retained the lytic capacity at 25°C by 14 days and completely lost the lytic activity after 21 days.

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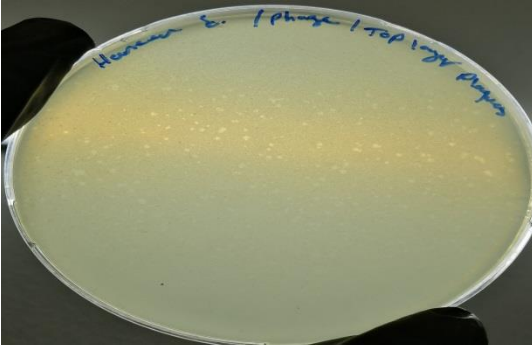

FIG 4. Top layer plaque assay.

The results showed that 10% (5 µg/mL) of endolysin and 1% HEC gel have potential lytic activity against bacterial isolates, which covered approximately 2 cm of the bacterial lawn when compared with lytic activity of commercial antibiotics (Fusidic acid [2%], Bactroban “Mupirocin” cream2) by dripping 10 mL from gel and commercial antibiotics onto bacterial lawn as shown in Figure 5.

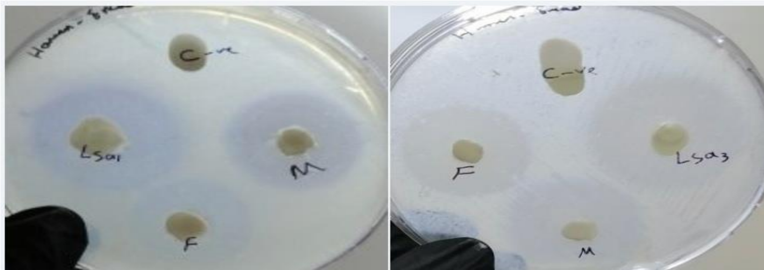

FIG 5. In vitro lytic capacity of endolysin-HEC gel-based formulations and commercial antibiotics against multidrug-resistant bacterial isolates. (A) Endolysin Sa1-HEC (L Sa1) gel, Mupirocin (M) cream, Fusidic acid (F) cream, and PBS-HEC gel as a negative control (C −ve) against MDR. S. aureus isolate; and (B) Endolysin Sa3-HEC (L Sa3) gel, Mupirocin (M) cream, Fusidic acid (F) cream, and PBS-HEC gel as a negative control (C −ve) against MDR. S. aureus isolate.

The mixture gel which was prepared from 10% bacteriophage cocktail at titer (1 × 1010) with endolysin-HEC gel had highly potential lytic activity against bacterial isolates and covered more than 4 cm of bacterial lawn when compared with the lytic activity of endolysin-HEC gel, which reached 2 cm of the bacterial lawn by dripping 10 mL from mixture gel onto nutrient agar as shown in Figure 6.

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FIG 6. In vitro lytic capacity of mixture gel-based formulations and endolysin-HEC gel-based formulations against MDR bacterial isolates. (A) Mixture gel (BPLSa1-HEC) “Bacteriophage cocktail for S. aureus with endolysinSa1-HEC gel,” Endolysin Sa1-HEC (LSa1) gel and PBS-HEC gel as a negative control (C –ve) against MDR S. aureus isolate; (B) Mixture gel (BPLSa3-HEC) “Bacteriophage cocktail for S. aureus with endolysin Sa3-HEC gel (BPLSa3-HEC), Endolysin Sa3-HEC (LSa3) gel and PBS-HEC gel as a negative control (C −ve) against MDR S. aureus isolate.

The results showed that the therapy was a success on comparison of the treated patch of acne lesions on the facial skin of subjects who applied the therapy topically with that of the patch of acne lesions without any treatment. The treatment response was determined by assessing the clinical improvement in the signs of lesions, decolonization of bacterial growth, and absence of any allergic reactions against the therapy as shown in Figure 6.

This interventional study was approved by the Institutional Review Board (IRB) at the College of Medicine, Al-Nahrain University (IRB number 204/3/2, date of approval: January 18, 2022), and all subjects provided their written informed consent.

Subjects who were aged above 25 years with moderate facial acne lesions and confirmed presence of MDR S. aureus in all of them, with a high rate of resistance against many antibiotics, and did not have any treatment for acne lesions for at least 6 months were included as volunteers and were categorized into two groups according to the type of gel-based formulation. Each group had 6 subjects.

Endolysin-HEC gel and mixture gel formulations therapy was applied topically on one or more patches of 2 cm2 of facial skin acne lesions. A patch of facial skin with acne lesions was left “untreated” as control.

The result of this clinical trial showed that the successful in vivo facial topical application of endolysin-HEC gel and mixture gel from bacteriophage cocktail with endolysin-HEC gel therapy was achieved in all subjects when compared with the patches of facial skin acne lesions without any treatment. The response was determined by decolonized bacterial growth and clinical improvement in the signs of facial skin acne lesions. So, there was negative bacterial growth in the subjects with complete response, while there was reduction in the titer of bacterial growth in the subjects with partial response. The results of the clinical investigation showed a decrease in the size and number of comedones (whiteheads/blackheads, papules/pustules, and nodulocystic lesions)

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and inflammatory lesions in all treated patches of subjects compared with the control patch with little variation between the two therapy groups. The group containing subjects with MDR bacterial acne vulgaris lesions, which were treated by a combination of bacteriophage cocktail and endolysin-HEC gel exhibited complete improvement response with 100% negative bacterial growth of facial skin acne lesion swabs in all six subjects. Also, no allergic reactions were observed against the therapy in all treated subjects.

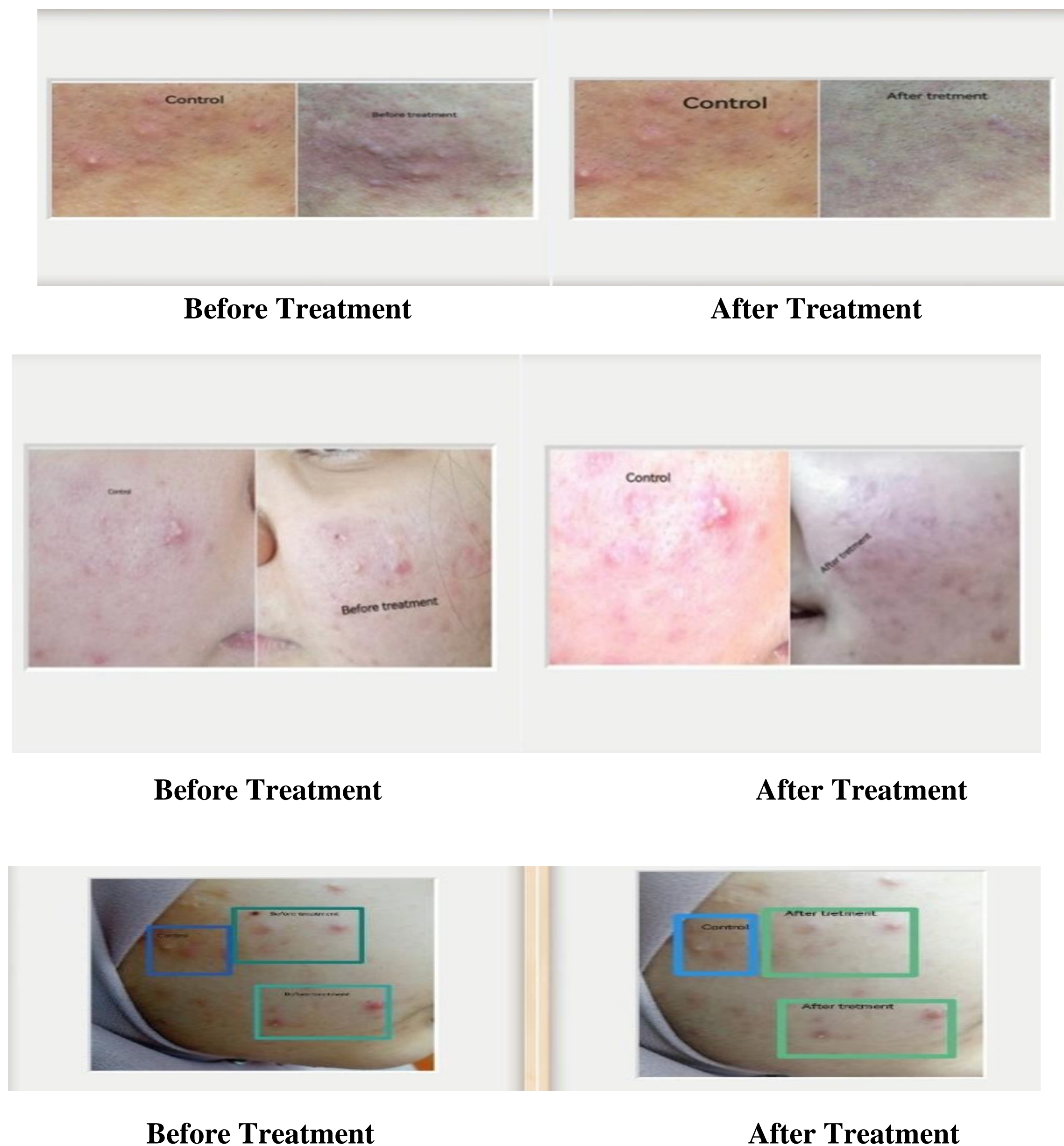

FIGURE 7. Selected subjects treated with endolysin-HEC gel and mixture gel “combination of bacteriophage cocktail with endolysin-HEC gel.”

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DISCUSSION

Clinicians and academicians are now interested in the function of bacteriophages and their extracted endolysins for the treatment of acne vulgaris.21 Bacteriophages with a high safety profile due to their specificity to bacterial strains and established lytic activity were utilized as acne treatment to prevent the possibility of antibiotic resistance gene transfer.22 Humans have utilized phage therapy for a variety of illnesses, with intriguing outcomes.23 A considerable rise in the number of identified staphylococcal bacteriophages has been observed in recent years.24 Another advantage of staphylococcal bacteria is their diversity.25 Even if bacteria evolved bacteriophage resistance, they may still be susceptible to lysins. This implies the development of a unique acne topical medication that targets the bacterial cell wall.26 Bacteriophage extract endolysin therapy is an alternative to antibacterial therapy that has been shown to be medically superior to antibacterial medicines in the treatment of bacterial illnesses.27 The bacteriophage extract endolysins employed in this investigation were effectively synthesized in their soluble forms, with high-yield purity, and extremely effective against MDR S. aureus bacteria. The market now has the first product containing endolysin for use in clinical trials, “Staphefekt, Gladskin brand,” which is intended to treat the early stages of skin infections caused by S. aureus. Acquisition of resistance to endolysins has not been seen so far; this might be due to the fact that their targets in peptidoglycan molecules are required for bacterial viability, and mutations would damage the bacteria.28 Bacteriophage-derived endolysins have a significant advantage over typical antibiotic drugs in that they can kill bacteria inside biofilms even if they are not replicative and are also active against replicative cells.29 Unlike antibiotics, which often have a broad range and lyse many bacteria present in the

human body “some of which are helpful,” endolysins lyse just the disease pathogen with little to no impact on the normal human microbiota. However, bacteriophage endolysins with broad lytic activity have been found in certain circumstances. For example, an enterococcal bacteriophage endolysin has been shown to lyse not just enterococci but also a variety of other gram-positive bacteria such as S. aureus and Streptococcus pyogenes (Group B streptococci), making it one of the most broadly active endolysins discovered.30 Endolysins have various advantages, including their bactericidal property rather than bacteriostatic impact (“many antibiotics are only bacteriostatic”), quick action (“effective in seconds, as opposed to the hours or days needed by other antibiotics”), and safety for human cells. Endolysin seems to be an appealing prospect as an alternative antibacterial agent for treating antibiotic-resistant illnesses for all of these reasons.31 The MIC (2, 4, 2 g/mL) and MBC (4, 8, 4 g/mL) of extracted endolysins (endolysin-Sa1, endolysin-Sa2, and endolysin-Sa3) used in this study reflect that bacteriophage-extracted endolysins have bactericidal effect and a rapid action on MDR bacteria of skin acne lesions. Endolysin enzymes will therefore be of immediate assistance in places where antibiotic-resistant gram-positive infections are a major concern, such as hospitals, nursing homes, and day-care centers.32 The findings of this study was helpful in providing antimicrobial agents (formulation gels of extracted endolysins-HEC gel, and mixture gels “bacteriophage cocktail with endolysin-HEC gel”) that act specifically against S. aureus bacteria. The in vitro validation tests and in vivo topical application experiments show that formulation gel therapy has significant potential as an alternative strategy for acne vulgaris caused by MDR S. aureus bacterial infections.

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The present work is the first to describe the effectiveness of endolysin-HEC gel and a mixture gel of bacteriophages cocktail with endolysin-HEC as a topical treatment against MDR S. aureus infections in vitro and in vivo in Baghdad. HEC was used as the gel-based formulation, and it had no effect on the lytic activity of the extracted endolysins or bacteriophage cocktails. It also functioned as an emulsifier, binder, and thickening for the gel. As a result, this foundation may allow creams to enter the skin into hair follicles and eliminate MDR S. aureus bacteria in acne lesions.33 According to the findings, decolonization of MDR bacteria was an essential element in preventing repeated inflammation of acne lesions and treating acne vulgaris.

So far, the bacteriophage cocktail described has the expected formulation parameters: they are stable at 4°C storage.34 Bacteriophage cocktails retain the same stability and activity at 4°C for more than 3 months, while inside mixture gel formulations "bacteriophage cocktail with endolysin-HEC gel," bacteriophages retain the same efficacy and potential lytic activity for more than 30 days. The endolysin-HEC gel formulations kept the potential lytic activity of extracted endolysins at 4°C for more than 21 days, and the lysin ointment formulation conserved the potential lytic activity of enzyme for more than 15 days at 4°C.35 As a result, it is not unexpected that mupirocin-resistant strains emerge quickly. Endolysins are not in this case. The bactericidal effect of endolysins is independent of active host metabolism. Furthermore, as far as we know, no endolysin resistance has been established.36 The results of in vivo face topical application studies described here show that bacteriophage cocktail treatment with extracted endolysin therapy reduces inflammation produced by MDR S. aureus bacteria in human patients with moderate-to-severe facial acne lesions. With varying degrees of effectiveness, certain clinical double-blind Phases 1 and 2 studies have shown that phage treatment with anti-inflammatory capabilities "can address the principal cause of death in COVID-19," and with high specificity "targeted killing."37–39 Novel therapeutic medicines targeting MDR microorganisms are desperately needed. Bacteriophage cocktail and extracted endolysin therapy may be used instead of antibiotics to treat bacterial infections.40

RECOMMENDATION

Many studies must be conducted to evaluate the effectiveness of endolysin-HEC gel and mixture gel of bacteriophage cocktail with endolysin-HEC gel therapy in the microbiome of human skin.

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CONFLICTS OF INTEREST

The authors declare that there are no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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