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Detection of biofilm-related genes in *Streptococcus pyogenes* and the impact of Oregano extract on their expression

Roaa Mohammed Hussan Al-kinani *

Department of Medical Laboratory Techniques, College of Health and Medical Techniques/Kufa, Al Furat Al Awsat Technical University, Iraq.

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Abstract

Objective: This study aimed to evaluate the antibacterial and antibiofilm activities of Oregano extract against clinical isolates of *Streptococcus pyogenes*, and to assess the presence of biofilm-associated virulence genes (*emm*, *scl1*) following treatment.

Materials and Methods: Thirty clinical streptococcal isolates were obtained, and twelve were selected based on pathogenicity and multidrug resistance. Species identification and antimicrobial susceptibility testing were performed using the VITEK-2 compact system. Antibacterial activity of oregano extract (0.1 g/ml) was assessed by the agar diffusion method on Muller-Hinton agar. Biofilm formation inhibition was evaluated, and PCR was employed to detect the presence or absence of *emm* and *scl1* genes.

Results: Oregano extract at 0.1 g/ml inhibited *S. pyogenes* growth and prevented biofilm formation. Molecular analysis showed a reduction or absence of *emm* and *scl1* genes in treated isolates, indicating suppression of biofilm-associated virulence factors.

Conclusion: Oregano extract demonstrates potent antibacterial and antibiofilm properties against *S. pyogenes*, likely mediated through inhibition of key virulence genes, suggesting its potential as a natural therapeutic option for biofilm-associated infections.

Keywords: Oregano; Biofilm; *Streptococcus pyogenes*; *emm* gene; *scl* 1 gene.

1. Introduction

Even though the production of medications made of pure chemicals or biological materials, like antibiotics, has advanced in recent years, researchers have been very interested in medicinal plants because of the effects these substances have on people's health and the rise in microorganisms that are resistant to them, mostly when used continuously and without medical advice (1). Oregano extract is one of the plants in the *Lamiaceae* family that has great pharmacological efficiency due to its high content of the antibacterial arvacrol and the antifungal thymol. Other substances with antioxidant qualities found in Oregano leaves include phenols, rosmarinic acid, ursolic acid, triterpenes, and oleanolic acid (2), Quorum sensing (QS) is impacted by the phenolic chemicals that inhibit biofilm development (3). A biofilm is defined as a clump of bacteria that are adhered to both living and non-living surfaces and encased in extracellular polymers (4) that the bacteria secrete to evade the host's immune system and develop tolerance to various drugs (5). *Streptococci* are Gram-positive, coagulase- and catalase-negative cocci that usually occur in chains or pairs. They are divided into three categories according to their hemolytic pattern on blood agar: gamma-hemolytic (no lysis),

* Corresponding author: Roaa Mohammed Hussan Al-kinani

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alpha-hemolytic (partial lysis resulting in a greenish hue), and beta-hemolytic (full lysis of red blood cells). Group A *streptococci* (*Streptococcus pyogenes*) and group B *streptococci* (*Streptococcus agalactiae*) are the principal representatives of the beta-hemolytic group, it causes skin infections and strep throat were among the serious, potentially fatal illnesses, Cellulitis, tonsillitis, impetigo, and pharyngitis (strep throat) are common infections. Scarlet fever, rheumatic fever, glomerulonephritis, and necrotizing fasciitis, sometimes referred to as "flesh-eating disease," can result from more serious illnesses (6),

The infections caused by *streptococcus pyogenes* are extremely infectious and usually invade the pharynx, anus, and vaginal mucosa. Airborne droplets, direct hand contact with nasal secretions, contact with contaminated surfaces or objects, contact with infected skin lesions, or ingestion of contaminated food are all possible ways for the disease to spread. Collectively Cellulitis and erysipelas are two diseases that can result from *streptococcal* (GAS) strains that invade the skin through wounds or abrasions (7). Additionally, they may infiltrate muscle and fascia, resulting in necrotizing fasciitis or myositis, which frequently follows minor trauma and can lead to toxic shock syndrome (8). Furthermore, *S. pyogenes* can cause septicemia by infecting the uterus and vaginal mucosa. The most frequent risk factor for serious *S. pyogenes* infections is thought to be skin lesions. crowded spaces, like military bases, assisted living facilities, and The most frequent risk factor for serious *S. pyogenes* infections is thought to be skin lesions. Group A *streptococcal* infection epidemics can occur in crowded settings like schools, nursing facilities, and military camps because they make transmission easier (9).

Among the exotoxins produced by *Streptococcus pyogenes* are pyrogenic (erythrogenic) toxins, which cause toxic shock syndrome and the rash associated with scarlet fever. Other virulence factors that aid in tissue invasion and increase the severity of infection include streptokinase, streptodornase, hyaluronidase, and streptolysins (10).

The clinical symptoms induced by *Streptococcus pyogenes* are influenced by a number of virulence variables. The bacteria are shielded from phagocytosis by its hyaluronic acid capsule, and adhesion to host cells is mediated by M protein, lipoteichoic acid, and protein F (11). By binding to complement regulators and fibrinogen, M protein inhibits opsonization, which is another important way it evades the immune response. Notably, M protein is considered the most critical virulence factor for S. pyogenes, as studies have demonstrated that M protein-deficient mutants cannot survive in human blood containing phagocytes (12).

2. Materials and Methods

2.1. Collection and preparation of Oregano Oil extract

Using hard Oregano, an oil cube from the Deer brand in China, different types of Oregano were gathered from the local markets in the governorates of Najaf and Baghdad. In a water bath vibrating at 95 ° C for 15 minutes until it was verified, 3 g of Oregano was dissolved in 30 ml of sterilized Olive oil at 121 ° C and under 15 lb/kg2 of pressure for 15 minutes. To store pure Oregano extract, melt the Oregano completely in Olive oil, put it in a sterile tube, and keep it in the refrigerator at 4 °C until it's used (13).

2.2. Collection of specimens

Twelve specimens in all were taken from swabs, tonsils, wounds, and urinary tract infections in the indoor setting of the Najaf Governorate Hospital between September 2024 and February 2025. The patients' ages ranged from 15 to 35. The samples were processed on nutrient and blood agar media and incubated for the entire night at 37°C. Gram-positive bacteria are identified using common biochemical techniques (oxidase and catalase tests), and the Vitek-2 compact system is also used for diagnosis.

2.3. Detection of the inhibitory activity of Oregano oil towards *Streptococcus pyogenes*

The well diffusion method was used to study the susceptibility of the oregano oil to the bacterial isolates (14). To guarantee that the bacterial implants in the center, the isolated bacteria were evenly distributed over the Muller-Hinton agar medium and allowed to come to room temperature. After that, the center was drilled to a diameter of 8 mm, and 0.1 g/ml of Oregano oil was applied. To make sure that Olive oil had no inhibitory effect on growing colonies, a positive control that contained solely Olive oil was established independently. To enable the extract to permeate the center, let the dishes sit at room temperature for ten minutes. A graduated ruler was used to measure the drill's diameters after it was incubated for 24 hours at 37 °C.

2.4. DNA template preparation by using the boiling method with Tris EDTA-TE buffer

The total DNA of isolates under study was extracted using the Tris EDTA-TE Buffer method (15). After putting the tubes in the cannula for five minutes at 5000 rpm, the bacterial isolates are grown in BHI Broth for twenty-four hours at 37 °C and then disposed of centrally. After dissolving the precipitate in 1 µl of TE Buffer, the tubes were centrifuged centrally for 5 minutes at 5000 rpm and then re-suspended. To make sure all the cells were absorbed, the precipitate was deposited in 100 µl of TE Buffer. The tubes were then immediately discarded for 5 minutes at 5000 rpm after 10 minutes in a 100 cº water bath. then Before using, remove the supernatant from the tube and store it in the freezer at -20 °C.

2.5. Primers used for PCR

As indicated in Table 1, the genes utilized in this investigation are in charge of intercellular adhesion, which is required for *Streptococcus pyogenes* biofilm production. They were acquired from the Korean Alpha DNA Company.

Table 1 The primer used in the current study for PCR amplification

Target Gene	Nucleotide sequences and direction)(5\-------3\		Product size (bp)	Reference
emm	F	5\ GAG GTA AAG CCA ACG CAC TC 3\	151	(10)
emm	R	5\ CCT GTA ACC GCA CCA AGT TT 3\		
scl1	F	5\ GAA CCG CTT GCC ATG TGT TG 3\	483	(11)
scl1	R	5\ GCT TGA CCA TGT TGC GTA ACC 3\		

2.6. PCR amplification procedure

A PCR genotypic investigation was conducted on twelve chosen isolates. By the manufacturer's information (Alpha DNA), the oligonucleotide primers for the *emm* and *scl* 1 genes Table 2 were diluted using TE Buffer to achieve a primary concentration of 100 µl. Amplification was carried out in a PCR thermocycler, and the reaction mixture was made by the manufacturer's recommended protocol (Maxime PCR PreMix kit (i-Taq)). Table 2 below lists the elements of the polymerase chain reaction.

Table 2 Components and volumes of PCR mixture

PCR Master Mix	Volume
DNA Template	10 µl
Forward primer	4 µl
Reverse primer	4 µl
Nuclease-free water	2 µl
Total	20 µl

Conditions for PCR amplification, which were set according to this study for the two primers, are shown in Table 3.

Agarose gel electrophoresis was used to examine the amplified PCR product using 1.5% agarose that had been treated with 0.5 µg/ml ethidium bromide for one hour (16). PCR results were evaluated using a DNA ladder (100–1500 bp), visualized by UV light at 336 nm, and captured on a digital camera.

Table 3 Program of thermal cycles to amplify DNA

Gene	Temperature (°C) /Time					Cycle No.
	Initial denaturation	Cycling Condition			Final Extension	
		denaturation	Annealing	Extension		
emm	94/2 min	94 / 1 min	55/1 min	72/2 min	72/7 min	30
emm	94/2 min	94/1 min	60/1 min	72/2 min	72/7 min	30
Scl 1	94/2 min	94/1 min	57.5/1 min	72/2 min	72/7 min	30
Scl 1	94/2 min	94/1 min	60/1 min	72/2 min	72/7 min	30

3. Results and Discussion

In hospitals and other healthcare facilities, the spread of *Streptococcus pyogenes* is a serious concern. Twelve specimens in all demonstrated that the isolates belonged to it based on several biochemical tests. The Vitek-2 compact system was used to conduct another crucial diagnostic test. (17) to verify the diagnosis. The antibacterial action of oregano oil (0.1 g/ml) was detected by the diffusion method, and *Streptococcus pyogen* had a diameter of 40 mm, as seen in Fig. 1.

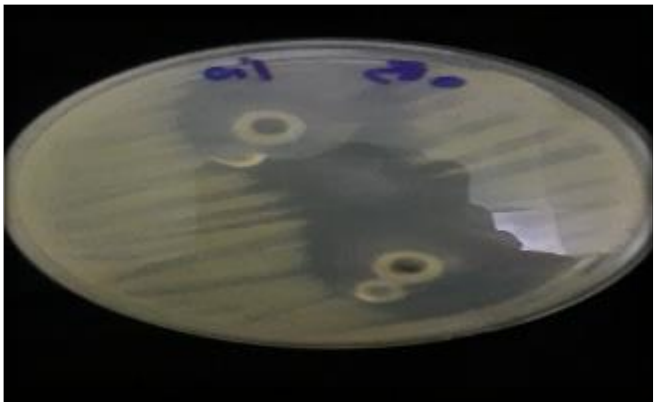

Figure 1 The anti-microbial effect of Oregano oil towards the isolation on Muller Hinton agar at concentrated (0.1 g/ml).

The active components of oil, particularly phenols, are probably what give it its inhibitory effect (18). Along with volatile oils, particularly cineole oil, which makes up 80–90% of the plant's total active ingredients, this chemical also helps to break down bacterial cells and their membranes (19). The PCR approach was then used to select these twelve isolates for the genotypic investigation utilizing two distinct gene sequences that are crucial to the production of biofilms, one of which is the *emm* gene, it is in charge of cell adhesion during biofilm development and is the initial stage of this process. The first one is *emm*, which has an amplified size of 153 bp (20), and *scl* 1 comes in second, with an amplified size of 487 bp. The PCR experiment's outcome showed that the isolates' *S. pyogenes emm* gene had an annealing temperature of 55 cº, but the scl 1 gene had an annealing temperature of 60 cº, as shown in Fig. 2.

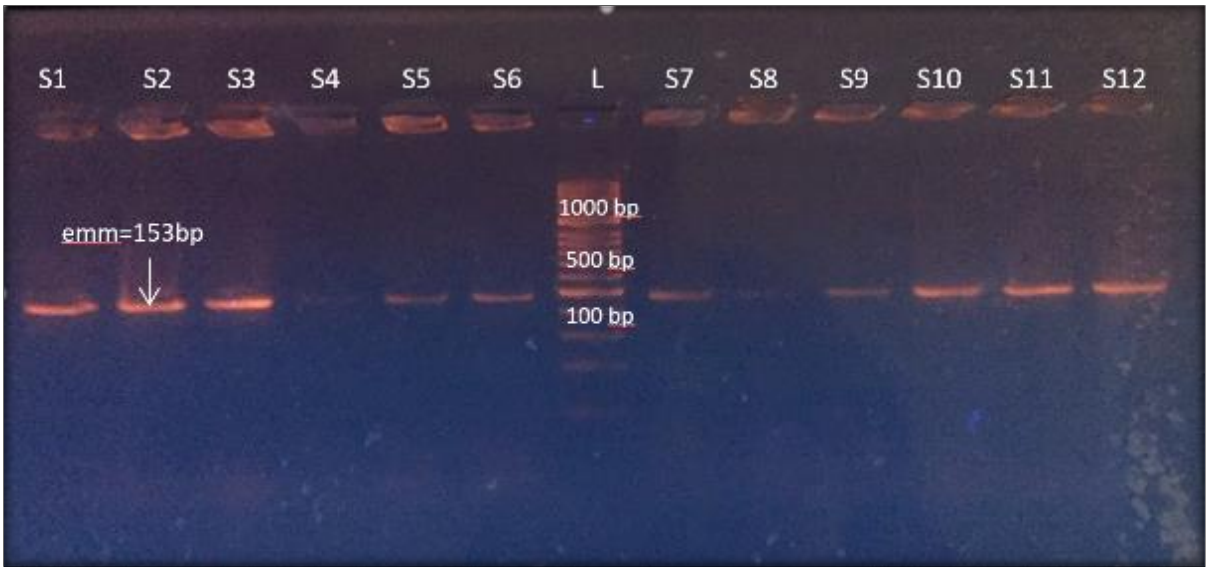

Figure 2 Agarose gel electrophoresis (1.5% agarose) of *emm* gene

However, the results of the *scl* 1 gene indicated that, as shown in Fig. 3, all isolates containing the gene had two annealing temperatures (57.5-60)cº.

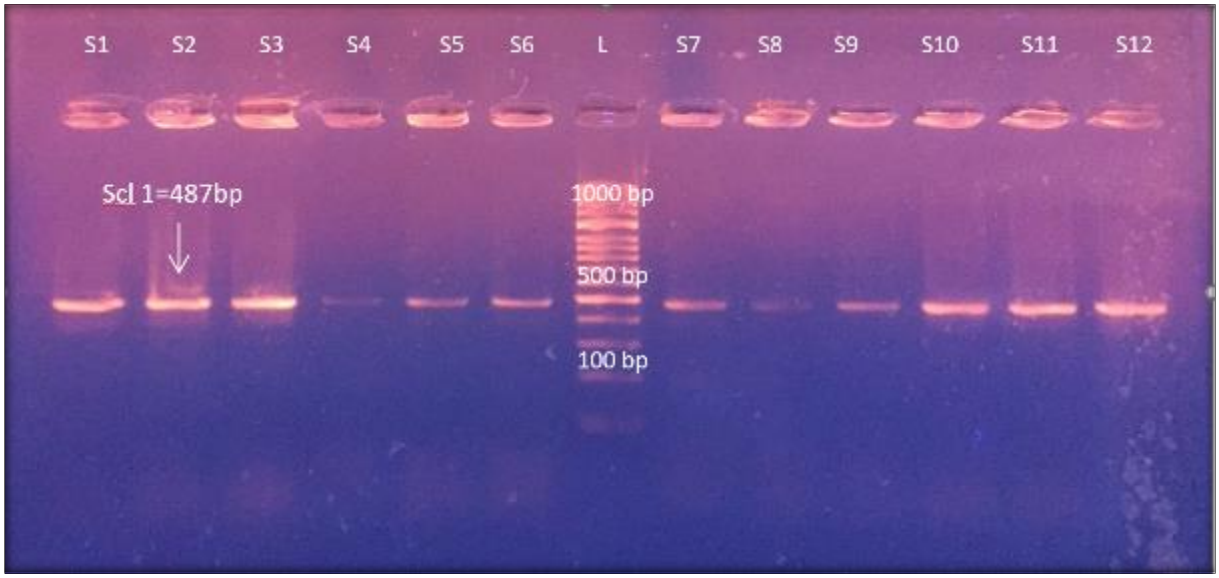

Figure 3 Agarose gel electrophoresis (1.5% agarose) for *scl 1* gene

The sources of sample collection may be the cause of the isolates' presence or lack of virulence genes, as hospital usage of detergents and disinfectants can alter the genetic material that makes up cells and cause some of these genes to be lost (21).

4. Conclusion

Oregano oil exhibited strong antibacterial and antibiofilm activities against *Streptococcus pyogenes*, with a notable inhibition zone and suppression of key virulence genes (*emm* and *scl1*). The findings suggest that its bioactive compounds, particularly phenols, contribute to disrupting bacterial adhesion and biofilm formation. Environmental and clinical factors, including exposure to disinfectants, may influence variations in gene presence. Oregano oil shows promise as a natural alternative for controlling biofilm-associated infections.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

Declaration of generative AI in scientific writing

During the preparation of this work the author did not use any generative AI tool for scientific writing. The author takes full responsibility for the content of the published article.

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