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Research article

Antibacterial effect of *Thymus vulgaris* Essential Oil against Ceftazidime-Resistant *Enterococcus faecalis*

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ABSTRACT

Enterococcus faecalis is an opportunistic pathogen commonly found in the gastrointestinal tract. It is associated with biofilm-related infections and increasing resistance to various antibiotics, including cephalosporins. In this study, eleven *E. faecalis* isolates were obtained from 101 catheter samples collected from patients with severe urinary tract infections (UTIs). All isolates were tested for susceptibility to ceftazidime and *Thymus vulgaris* essential oil using the microdilution method. Minimum inhibitory concentrations (MICs) were determined using microtiter plates. Biofilm formation was quantified with a crystal violet-based spectrophotometric method. The results showed high-level resistance to ceftazidime (MICs: 1000–4000 µg/mL). Conversely, *T. vulgaris* essential oil exhibited strong antibacterial activity, with MICs ranging from 1/40 to 1/640 (v/v). All isolates produced biofilms to varying extents, with isolate Ef1 generating the highest biomass. The study confirmed that *E. faecalis* produces a substantial amount of biofilm and is resistant to ceftazidime. Additionally, the findings highlight the promising antibacterial effects of *T. vulgaris* essential oil against ceftazidime-resistant *E. faecalis*.

Keywords: Biofilm, Ceftazidime, *Enterococcus faecalis*, MDR, *Thymus vulgaris* essential oil.

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1. INTRODUCTION

Enterococcus faecalis is a Gram-positive, facultative anaerobic bacterium. It naturally inhabits the intestinal tracts of both humans and animals [1]. It is an opportunistic pathogen responsible for urinary tract infections (UTIs), bacteremia, endocarditis, wound infections, and device-associated infections, especially on urinary catheter tubes [2]. In recent years, it has been reported that this bacterium exhibits multidrug resistance and can form biofilms, which contribute to persistent infections in various human organs, particularly the urinary tract [3]. Treating *E. faecalis* infections is challenging due to its natural and acquired resistance mechanisms, as well as its ability to adapt to antibiotics in infected areas [4]. Unlike many bacteria, including *E. faecalis*, which naturally show low susceptibility to various antibiotics like cephalosporins, ceftazidime, a third-generation cephalosporin, is commonly used to treat different infections caused by various bacteria, but is ineffective against enterococci [5]. This lack of effectiveness may be due to changes in penicillin-binding proteins that lead to resistance to β-lactam antibiotics, eliminating effective drug targets in *E. faecalis*. Still, ceftazidime resistance has been found in clinical isolates of *E. faecalis* [6]. Resistance to antibiotics has increased in recent years. Therefore, finding new strategies to combat *E. faecalis* has become a crucial priority.

One of the main virulence factors of *E. faecalis* is its ability to form biofilms on both abiotic and biotic surfaces, especially on medical devices like prosthetic valves, catheters, and orthopedic implants. Biofilm formation creates a protected environment that helps bacteria survive in harsh conditions, limits antibiotic penetration, and guards bacteria from the immune system [7]. Thus, infections

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caused by biofilm-forming bacteria are challenging to treat with antibiotics, resulting in a high rate of treatment failure [8].
Herbal natural products have been investigated in recent years as alternatives or complements to conventional antibiotics. Essential oils from medicinal plants have gained interest because of the variability of their bioactive components, broad-spectrum antimicrobial activity, low potential for resistance development, and recorded low toxicity [9]. *Thymus vulgaris* (thyme) essential oil is of particular interest because it contains high concentrations of thymol and carvacrol, two phenolic monoterpenes with well-documented antibacterial and antifungal properties. These compounds inhibit bacterial and fungal cell membranes, disrupt enzyme functions, and modulate quorum sensing, ultimately preventing the formation and growth of biofilms [10]. Several prior studies have examined the antibacterial activity of *T. vulgaris* essential oil against pathogenic Gram-positive bacteria [11]. However, there are very few studies focusing on its activity against ceftazidime-resistant *E. faecalis*. This study aims to evaluate the antibacterial effects of *T. vulgaris* essential oil on ceftazidime-resistant *E. faecalis* isolates, exploring its potential as a natural alternative for managing resistant enterococcal infections.

2. MATERIALS and METHODS

2.1. Bacterial Isolates

The urine catheter samples were collected in a sterile container from inpatients (101 samples) suffering from UTIs (Baghdad Teaching Hospital, Baghdad, Iraq). All procedures involving human participants were conducted in accordance with the ethical standards of the University of Baghdad's ethics committee and the 1964 Declaration of Helsinki and its subsequent amendments, or with comparable ethical standards. The patients did not take the antibiotic 72 h prior to sample collection. The standard previous method was followed in isolation and identification of *E. faecalis*. The VITEK 2 DensiCheck instrument (bio-Mérieux, Marcy-l'Étoile, France) was used to confirm the identification of the isolates. The bacterial isolates were sub-cultured onto the nutrient agar plates for short-term storage [12].

2.2. Antimicrobial Susceptibility Testing

The microdilution technique was followed to measure the minimum inhibitory concentrations (MICs) of Ceftazidime against eleven isolates of *E. faecalis* (Ef1, Ef2, Ef3, Ef4, Ef5, Ef6, Ef7, Ef8, Ef9, Ef10, and Ef18). Three controls were prepared. 1. Sterile MHB; 2. MHB with bacterial isolates; 3. Different double dilutions of Ceftazidime. The lowest antibiotic concentrations completely inhibit growth, as indicated by the MIC [13, 14].

2.3. Essential Oil Preparation and MIC Testing

Commercial *T. vulgaris* essential oil was used. Stock solutions were prepared in dimethyl sulfoxide. MIC values were determined by broth microdilution in 96-well plates. Twofold dilutions (1/20–1/1280 v/v) were tested. The lowest antibiotic concentrations completely inhibit growth, as indicated by the MIC [13, 14].

2.4. Biofilm Formation Assay

Biofilm formation was assessed using the microtiter plate–crystal violet staining method. The previous method was used to measure the biofilm formation of the eleven *E. faecalis* isolates. The experiment was repeated in triplicate [10]. The isolates that were resistant to Ceftazidime were used in the upcoming experiments. Biofilm production was classified as weak, moderate, or strong [15].

2.5. Statistical analyses

The statistical analysis was conducted, and the graphs were generated utilizing Origin v. 8 software (OriginLab, Northampton, USA). The data were presented as means ± standard error (M ± SE).

3. RESULTS

3.1 Susceptibility to Ceftazidime and *T. vulgaris* Oil

The present study revealed that all isolates were resistant to ceftazidime, with MICs ranging from 1000 to 4000 µg/mL. In contrast, *T. vulgaris* essential oil inhibited all isolates, with MICs ranging from 1/40 to 1/640. The isolate Ef10 showed the highest resistance to *T. vulgaris* essential oil. However, the isolates Ef2, Ef4, and Ef9 show the highest susceptibility to *T. vulgaris* essential oil.

Table 1. Minimum inhibitory concentrations (MICs) of ceftazidime and *T. vulgaris* essential oil against 11 *E. faecalis* isolates.

Isolate	MIC Ceftazidime (µg/mL)	MIC *T. vulgaris* (v/v)
Ef1	4000	1/160
Ef2	4000	1/640
Ef3	4000	1/320
Ef4	2000	1/640
Ef5	1000	1/320
Ef6	2000	1/320
Ef7	4000	1/320
Ef8	2000	1/160
Ef9	2000	1/640
Ef10	1000	1/40
Ef11	4000	1/320

3.2 Biofilm Formation

All isolates demonstrated biofilm formation, with varying intensities. The *E. faecalis* (Ef1) produced the highest level of biofilm, followed by Ef9. The lowest biofilm was produced by Ef8. Three isolates produced strong biofilms, four isolates produced moderate biofilms, and four isolates produced weak biofilms. The isolate was highly resistant to ceftazidime and produced a high amount of biofilm (Ef1), which was used in studying the structure of biofilm using an electron microscope.
The SEM micrograph showed the biofilm matrix formed on the polystyrene surface (Fig. 2). The micrograph supports dense microbial colonization with extracellular matrix coverage. The image showed that the matrix of the biofilm formed by *E. faecalis* appeared as a non-smooth surface, with distinct areas arising, indicating the varied topography of the biofilm matrix.

4. DISCUSSION

Recent studies have revealed a consistent rise in bacterial isolates resistant to antibiotics, especially ceftriaxone, which was once considered an effective drug. The reduced effectiveness of this antibiotic in recent years, particularly against Enterobacteriaceae, highlights the serious threat to public health and the significant challenges it presents. To make matters worse, the current study showed that all Enterobacteriaceae isolates were resistant to this antibiotic and only responded at high concentrations. This serves as a warning about the ongoing battle against pathogenic bacteria. The findings emphasize the need for collective efforts to identify suitable alternatives, with plant extracts being among the most promising options.

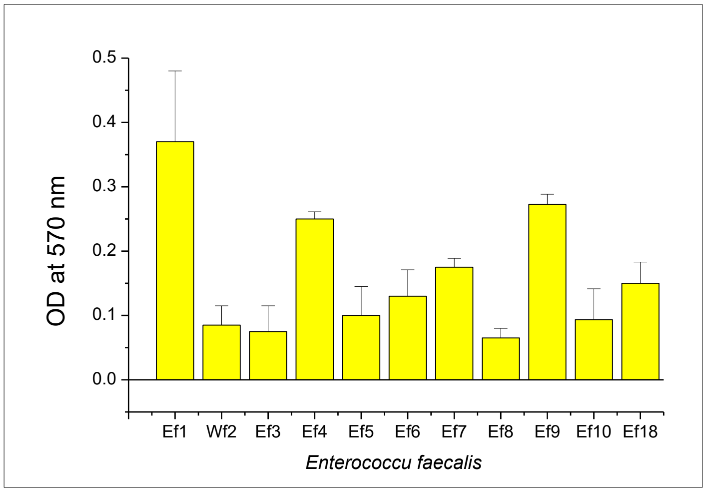

Fig. 1. Biofilm formation of eleven isolates of *E. faecalis*. The microtiter plate-spectrophotometer method was used to measure the biofilm formed by each isolate.

The present study evaluated the antibacterial potential of *T. vulgaris* (thyme) essential oil against Ceftazidime-resistant *Enterococcus faecalis* isolates. The results of the present study showed that all examined isolates were resistant to Ceftazidime, consistent with the growing prevalence of multidrug-resistant *E. faecalis* in clinical and environmental settings. This result underlines the restrictions of β-lactam antibiotics in treating infections caused by resistant *E. faecalis*.

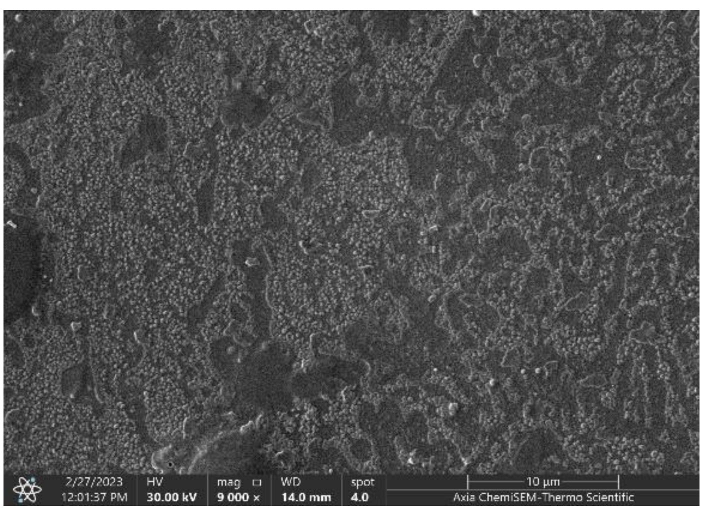

Fig. 2. Biofilm formation of the Ef. 1 that produced the highest level of biofilm. The image showed the rough shape of the biofilm.

Notably, the *T. vulgaris* essential oil showed different antibacterial activity against the Ceftazidime-resistant *Enterococcus faecalis* isolates, highlighting its potential as an alternative or therapeutic agent. The differences in response to this essential oil may result from its complex chemical composition, which includes phenolic compounds such as thymol and carvacrol, known to disrupt bacterial cell membranes, alter permeability, and interfere with bacterial metabolic processes. These mechanisms differ fundamentally from those of antibiotics, which may clarify why this oil remains active against strains resistant to Ceftazidime.

The results of the present study were in line with previous studies that investigated the antimicrobial efficacy of this essential oil against Gram-positive bacteria, including Enterococcus species. The differences in antibacterial effectivity among isolates may reflect intrinsic differences in biofilm formation, membrane composition, or efflux pump activity, which influence susceptibility to natural compounds.

The findings of this study are significant, given the rise in antimicrobial resistance, as they suggest that plant-derived essential oils could be utilized as complementary therapies or as starting points for developing new antibacterial agents. Additionally, using natural products like *T. vulgaris* essential oil may help mitigate the selective pressure that drives antibiotic resistance.

Furthermore, investigations are required to determine the minimum inhibitory concentrations (MICs), cytotoxicity, and synergistic effects with current antibiotics, as well as to discover the antibacterial mechanism of this herbal extract. Moreover, in vivo studies will also be important for interpreting these findings in clinical applications.

5. Conclusion

The present study showed that the eleven *E. faecalis* isolates were resistant to ceftazidime. *T. vulgaris* essential oil exhibited potent activity with MICs ranging from 1/40 to 1/640. The current study reveals that all isolates formed biofilms, but at varying levels. However, it was observed that even low biofilm-forming bacteria were resistant to ceftazidime. These findings support further investigation of *T. vulgaris* essential oil as a natural antibiofilm agent. Thus, the study demonstrates that *T. vulgaris* essential oil has promising antibacterial activity against Ceftazidime-resistant *E. faecalis*, offering a potential alternative strategy for managing infections caused by this bacterium. Its effectiveness emphasizes the importance of exploring natural products as adjunct or novel antimicrobial therapies in the era of antibiotic resistance.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval

This study was approved by the Ethics Committee of the University of Baghdad (CSEC/1124/0103; November 17, 2024). Since it was a retrospective analysis of routinely collected clinical data, individual patient consent was waived in accordance with national ethical guidelines.

CRediT authorship contribution statement

Zukhra Abbasi1: Conceptualization, Formal analysis, Investigation, Supervision, Writing – original draft, Writing-review & editing.

Shams Ihssan Sadiq: Administration, Resources; Methodology, Supervision; Validation; Roles/Writing, Writing–review, Investigation; Project administration; Roles/Writing - original draft; and Writing-review & editing.

Muhammad Obaid: Formal analysis, Investigation, Writing-review & editing.

All authors have read and agreed to the published

Availability of data and materials

Data will be made available on request

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