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The effect of fennel essential oil in combination with antibiotics on *Staphylococcus aureus* strains isolated from carriers

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ABSTRACT

An increase in the number of staphylococcal infections and carriers among medical staff has forced us to seek more and more effective antibacterial agents. Bacteria from the *Staphylococcus* genus possessing different mechanisms of resistance are the cause of nosocomial infections. The objective of our investigations was susceptibility of *S. aureus* strains isolated from nasal vestibule of medical students to fennel essential oil. The GG-MS analysis of fennel essential oil revealed eleven constituents among which a majority of *trans*-anethole (80%) was found. The D-tests showed $iMLS_B$ (80%), $cMLS_B$ and MS_B (10%) resistant phenotypes of *S. aureus*. The *S. aureus* isolates were intermediate to mupirocin (45%). Fennel essential oil increased the inhibition zone around cefoxitin, mupirocin, co-trimoxazole and ciprofloxacin with statistical significance. Our research showed that the fennel essential oil in combination with mupirocin may be considered as a natural alternative in eradication of *S. aureus* with $iMLS_B$, $cMLS_B$, MS_B resistant phenotypes and is able to decrease the growth rate of antibiotic resistance.

1. Introduction

Foeniculum vulgare Mill. (fennel) is a perennial plant belonging to the celery family (*Apiaceae*). It originates from Southern Europe and the Mediterranean, however it is widely cultivated worldwide [1]. Fennel has a wide range of applications, including in the cosmetics industry, and also in medicine. The existing studies have shown that fennel has many antioxidant, anti-inflammatory, antithrombotic, anti-cancer, anti-diabetic, miticidal, antifungal and antibacterial properties [2]. Fennel essential oil (FEO) contains: *trans*-anethole, camphene, fenchone, estragole, limonene, α-pinene, β-pinene, β-myrcene, camphor, 3-carene, α-phellandrene and *cis*-anethole [3].

Staphylococcus aureus is one of the most common etiological agents of infections in both hospitalized patients and outpatients [4]. These infections are mediated by staphyclococcal

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virulence factors and include skin and soft tissue as well as organ and systemic infections. Up to 80% of cases of staphylococcal sepsis is endogenous origin [5]. *S. aureus* can colonize different body sites: nasal vestibule, perineum, gastrointestinal tract, skin. Such a colonization is closely associated with a risk of staphylococcal infection. Staphylococcal infections may also happen as a result of a direct or indirect contact with a carrier (exogenous infection). Frank et al. estimated that approx. 20–30% of healthy adults are permanent nasal carriers, about 50% — transient carriers and 20% is never colonized by *S. aureus* [6]. The *S. aureus* nasal carriage is common in the human population, particularly in medical students having various relationships with patients and medical staff. The widespread use of antibiotics in hospital settings has already led to an increase in the number of staphylococcal strains with methicillin and/or macrolide-lincosamide-streptogramin B (MLS_B) resistance. It is considered that MLS_B antibiotics group is an alternative treatment option for *S. aureus* skin/topical/superficial infections, but the worldwide development of macrolide resistance has now limited the use of these antibiotics. The emergence of MLS_B resistance phenotype varies widely depending on the geograpihic region [7]. In Iran, the inducible MLS_B ($iMLS_B$), constitutive MLS_B ($cMLS_B$) and macrolide-streptogramin B (MS_B) phenotypes were found in 12.6%, 52.6% and 5.3% of 105 *S. aureus* isolated from clinical samples, respectively [7]. In the study performed in Turkey by Debdas and Joshi [8], $iMLS_B$, $cMLS_B$ and MS_B were observed in 18.0%, 23.0% and 48.0% of 379 *S. aureus* isolated from clinical specimens, respectively. Importantly, in Canada, Lavallee et al. [9] reported 64.7% of $iMLS_B$ isolates among methicillin-resistant *S. aureus* (MRSA) strains. Along with an increasing rate of staphylococcal resistance, medical students during their practice become also colonized with multidrug resistant strains [10]. Moreover, the students can carry and transmit resistant *S. aureus* strains and isolates with a diverse virulence as well [11,12]. There is a justified need for nasal screening in order to identify and suppress the carriage of multiple resistant staphylococcal strains. Use of conventional antibiotics to reduce the staphylococcal nasal carriage in patients and health-medical workers is not sufficient because of increasing antibiotic resistance of nasal isolates. Thus antibacterial activity of FEO against resistant phenotypes of *S. aureus* has been tested in the study.

The aim of the *in vitro* study was to evaluate the effect of FEO on drug antibacterial susceptibility of *S. aureus* isolated from medical students.

2. Materials and methods

2.1. Bacterial strains and culture condition

Twenty *S. aureus* strains, isolated from nasal vestibule of medical students of Pomeranian Medical University (Szczecin) in years 2014–2015, were studied. For each student, nasal specimen was inoculated directly onto Columbia agar with 5% sheep blood and mannitol salt agar (bioMeriéux), and then media incubated at 37°C for 24h. The grown staphylococci were identified based on: colony morphology, Gram-staining, mannitol fermentation, coagulase tube test (Biomed) as well as by Slidex Staph-kit (bioMeriéux). Long-term storage of *S. aureus* strains was at −70°C in tryptic soy broth with glicerol (4:1). Results were compared to *S. aureus* ATCC 29213 reference strain.

2.2. FEO analysis

FEO was purchased from Pollena-Aroma (manufacturer of essential oils). The FEO composition was analysed by gas chromatograph Agilent 6890N with mass selective detector 5973N (GC–MS). 30 μl of FEO was dissolved in 1 ml of acetone (p. a., Chempur) prior to GC–MS analysis. HP-5MSI column (5% phenyl/95% dimethylpolysiloxane), 30 m × 0.25 mm I. D. × 0.25 μm film thickness, was used for the resolution of particular compounds. The column temperature program was as follows: initial temperature 50°C, ramp rate 4°C/min, final temperature 290°C. Helium was used as carrier gas at a flow rate of 1.2 ml/min. The injector temperature was set at 250°C, MS quad: 150°C; MS Source: 230°C. Mass spectra were obtained using electron impact ionization at 70 eV in full scan mode (mass range: 20–500 m/z). The identification of the FEO components was performed by the comparison of their mass spectra with the reference spectra from NIST 02 library. The identity of particular compounds was confirmed on the basis of the retention indices, which were calculated using retention data of n-alkanes and compared with the literature data [13]. For this purpose, standard mixture of C_7-C_{30} n-alkanes (1000 μg/ml in hexane, Supelco) was analyzed in the same chromatographic conditions as the sample of essential oil. The relative contents of particular compounds in FEO were determined as their peak area percentages in total ion chromatogram.

2.3. Antibiotic susceptibility testing of S. aureus

The antimicrobial susceptibility testing (AST) of *S. aureus* to mupirocin — MUP (10 μg), ciprofloxacin — CIP (5 μg) and co-trimoxazole — SXT (1.25/23.75 μg) was determined via disk diffusion method according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [14]. The AST was performed on Mueller-Hinton agar (MHA) inoculated with *S. aureus* in concentration of 1.5×10^8 cfu/ml and antibiotic paper disks delivered by Diag-Med. After incubation period of 18 ± 2h at 35 ± 1°C the clear growth inhibition zones were measured in millimeters and interpreted according to EUCAST recommendations [14]. Identification of MRSA was based on susceptibility testing to cefoxitin — FOX (30 μg). The prevalence of resistance to MLS_B antibiotics was determined by D-test. The D-test used erythromycin — E (15 μg) and clindamycin — CC (2 μg) disks as representatives of MLS_B antibiotics. *S. aureus* strains were considered to have $cMLS_B$, $iMLS_B$ and MS_B resistance phenotypes when interpreted according to Clinical and Laboratory Standards Institute recommendations [15].

2.4. Effect of FEO in combination with antibiotics on S. aureus susceptibility testing

Studies on combined effect of FEO and antibiotics against *S. aureus* were conducted by agar dilution method, developed by