Staphylococcus spp. Isolated from Spiders and their Antimicrobial Sensitivity against Essential Oils

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Abstract
Research on insects and their bacterial communities provides valuable information on potential interaction between the host and their symbiotic bacteria. The aim of the present study was to evaluate antimicrobial activity of essential oils against *Staphylococcus* spp. isolated from spiders. Spiders samples were investigated microbiologically and *Staphylococcus* isolates were confirmed with MALDI TOF MS Biotyper. Antimicrobial activity of *Satureja hortensis L., Pimpinella anisum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Ocimum basilicum* L. essential oils was detected. Altogether five *Staphylococcus* species were identified: *S. capitis, S. epidermidis, S. haemolyticus, S. warneri* and *S. xylosus.* All bacterial strains were resistant of ciprofloxacin, levofloxacin, amikacin and gentamicin. *S. haemoliticus* was the most resistant against essential oils of *Rosmarinus officinalis* L., *Salvia officinalis* L. *S. warneri* and *S. xylosus* were the most sensitive against antimicrobial action of the applied EOs.

Keywords: *Staphylococcus*, antimicrobial activity, spiders.

1. Introduction

The few studies on microorganisms associated with spiders concentrated on the medical importance of spiders as vectors of potentially human pathogenic bacteria were published. The emphasis of the previous studies has been put on the general internal and external bacterial community of the spider [1,2], human serum and blood after presumed spider bites [3] and the presence of pathogenic bacteria associated with venom and fangs [4].

Research on insects and their bacterial communities has been undertaken to gain insights into the potential interaction of the hosts and their symbiotic bacteria [6-8]. Studies have shown that symbiotic bacteria affected the fitness of host or conferred benefits to their hosts in most of the insects and members of *Arachnoidea* [9-11]. Spiders are regarded as natural enemies of agricultural pests, and studies have shown that the genera *Wolbachia*, *Rickettsia*, *Cardinium*, and *Spiroplasma* were found with high incidence in spiders [12-14].
Members of the genus *Staphylococcus* are common colonizers of the skin in mammals and birds [15]. Two main groups are distinguished by their ability to coagulate blood: coagulase-positive staphylococci, with the most important species

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being *Staphylococcus aureus*, and coagulase-negative staphylococci, which comprise most species including *Staphylococcus epidermidis*. Humans are colonized by many different staphylococcal species. Some, such as *S. epidermidis* or *Staphylococcus hominis*, are found on virtually all body parts. Some others have more distinct preferences, such as *Staphylococcus auricularis*, which is found mostly in the ear canal [15]. In general, the largest densities of staphylococci are found in sweat glands and on mucous membranes surrounding body openings.

The essential oils were found to show the antimicrobial effect on *Staphylococcus*, including MRSA and MSSA, and the effect was more expressed than on Gram-negative bacteria [16-18]. The aim of the present study was to evaluate antimicrobial activity of essential oils against *Staphylococcus* spp. isolated from spiders.

2. Materials and methods

Microorganisms

Five strains of staphylococci were tested in this study, including *S. capitis, S. epidermidis, S. haemolyticus*, *S. warneri* and *S. xylosus.* All tested strains were collected from the spiders collected from chicken farm in Slovakia. All bacterial strains of *Staphylococcus* were identified with mass spectrometry MALDI TOF MS Biotyper with score higher than 2.20. The bacterial suspensions were cultured in Muller Hinton broth (MHB, Oxoid, Basingstoke, United Kingdom*)* at 37 °C for 24 h.

Antibiotic susceptibility testing

All isolated bacterial strains of staphylococci were used for antimicrobial susceptibility testing. A amount of 100µl of bacterial suspension in physiological solution with 0.5 McF° density from MHB agar was spread with sterile L-rods onto Mueller Hinton agar (Oxoid, UK). Disc diffusion methods was applied and the following antibiotics discs (Oxoid, UK) were used: ciprofloxacin CPR (10µg), levofloxacin LVX (10µg), amikacin AMK (10µg), gentamicin GEN (10µg), tetracycline TET (10µg) and tigecycline TGC (10µg). Inoculated agars were incubated at 35±2°C for 16-20 h according to the EUCAST [19]. Interpretation of inhibition zones were done in line with the EUCAST breakpoints [20].

Detection of antimicrobial activity with disc diffusion method

Antimicrobial activity of *Satureja hortensis L.*, *Pimpinella anisum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Ocimum basilicum* L. essential oils was detected.

A suspension of the tested microorganism (0.1 ml of 10^5 cfu/ml) was spread onto Mueller Hinton Agar (MHA, Oxoid, Basingstoke, United Kingdom). Filter paper discs (6 mm in diameter) were impregnated with 15 µl of the essential oils placed on the inoculated agars. The agars were kept at 4°C for 2 h, and later were incubated aerobically at 37°C for 24 h. The diameters of the inhibition zones were measured in mm. All the tests were performed in triplicate.

Determination of minimum inhibitory concentration

The minimum inhibitory concentration (MIC) was tested as described previously by Kačániová et al. [21].

3. Results and discussion

Although several endosymbionts have been reported to be widely distributed in spider species, little information is available about the interaction between of those and the spider hosts. In our study, the microbiota and antibiotic resistance of isolated staphylococci were detected. The results on antibiotic resistance of *Staphylococcus* isolated from spiders are shown Table 1. All bacterial strains were resistant to ciprofloxacin, levofloxacin, amikacin, gentamicin but were sensitive of tetracycline and tigecycline.

Staphylococcus species are some of the most frequently isolated pathogens from community- and healthcare-associated infections, and rapid, reliable identification to the species level is important for guiding therapy, distinguishing true bacteremia from contamination, and identifying potential sources of infection [22,23].

Several studies have shown that the methicillin-resistant staphylococci have started to gain resistance to many widely used antibiotics (quinolone, macrolide group antibiotics, aminoglycosides, tetracycline, trimethoprim-sulphamethoxazole, clindamycin, chloramphenicol) [24-27]. Also our results on

Staphylococcus isolates from spiders show that the resistant staphylococci are widely distributed.

Table 1. Antibiotic susceptibility of *Staphylococcus* isolated from spiders

	Antibiotics used 10 µg					
Bacterial strains	**CPR**	**LVX**	**AMK**	**GEN**	**TET**	**TGC**
Staphylococcus capitis	R	R	R	R	S	S
S. epidermidis	R	R	R	R	S	S
S. haemolyticus	R	R	R	R	S	S
S. warneri	R	R	R	R	S	S
S. xylosus	R	R	R	R	S	S

Legend to antimicrobial drug abbreviation: ciprofloxacin CPR (10µg), levofloxacin LVX (10µg), amikacin AMK (10µg), gentamicin GEN (10µg), tetracycline TET (10µg), tigecycline TGC (10µg)

Many EOs are well known to have antimicrobial properties, so they are considered a promising alternative for disinfection in the food industry. However, not all EOs are highly effective antimicrobials. To prevent the emergence of antimicrobial-resistant strains, antimicrobial treatments using EOs should be based on the rotation and combination of different EOs (or combinations of different active compounds that produce a synergistic lethal effect) or with other biocides. Several EO-based combined treatments have been proposed as an antimicrobial strategy against *S. aureus*. Blends of oregano, basil and bergamot have shown a synergistic bactericidal effect [28], whereas an additive bactericidal effect was detected for mixtures of cinnamon and clove [29]. Combinations of clove and rosemary [30] or thyme and anise [31] have been also tested, but the efficacy did not significantly increase with respect to the application of these EOs individually.

Antimicrobial activity of five essential oils with disc diffusion method and minimal inhibition concentration against *Staphylococcus* are shown in Table 2 and Table 3. *S. haemoliticus* was the most resistant against essential oils of *Rosmarinus officinalis* L., *Salvia officinalis* L. *S. warneri* and *S. xylosus* were the most to antimicrobial activities of all essential oils tested.

Table 2 Antimicrobial activity of EOs against *Staphylococcus* spp. detected with disc diffusion method in mm

	Inhibition zone (mm)				
Bacterial strains	***Satureja hortensis L.***	***Pimpinella anisum L.***	***Rosmarinus officinalis L.***	***Salvia officinalis L.***	***Ocimum basilicum***
Staphylococcus capitis	7.33±1.15	7.67±0.58	5.67±0.58	5.67±0.58	6.33±0.58
S. epidermidis	9.33±0.58	8.33±0.58	7.67±0.58	6.33±0.58	7.33±0.58
S. haemolyticus	4.33±0.58	5.33±0.58	3.66±0.58	3.33±0.58	5.67±0.58
S. warneri	12.33±0.58	11.67±1.53	12.33±2.08	12.00±1.73	11.67±1.53
S. xylosus	11.67±1.15	11.67±1.15	12.00±1.73	11.67±0.58	12.00±1.00

Table 3 Antimicrobial activity of EOs against *Staphylococcus* spp. detected with minimum inhibitory concentration method

	Minimum inhibitory concentration (MIC50)				
Bacterial strains	***Satureja hortensis L.***	***Pimpinella anisum L.***	***Rosmarinus officinalis L.***	***Salvia officinalis L.***	***Ocimum basilicum***
Staphylococcus capitis	25.00	25.00	50.00	50.00	50.00
S. epidermidis	25.00	25.00	50.00	50.00	50.00
S. haemolyticus	50.00	50.00	100.00	100.00	50.00
S. warneri	6.25	12.50	6.25	6.25	12.50
S. xylosus	12.50	12.50	6.25	12.50	6.25

Jarrar et al., 2010 found [32] synergistic activity of rosemary in combination with cefuroxime reflected by changes in the MIC values of the MRSAs (FIC index range for synergism, 0.56 to 1.00). Although the level of antibiotic potentiation was low, the potentiation is likely to have been much more pronounced if pure compounds were used.

The antibacterial activities *in vitro* of the aqueous, 50% (v/v) methanol, acetone and petroleum ether extracts of *Pimpinella anisum* (L) fruits were studied by Akhtar et al., 2008. Only aqueous and 50% (v/v) methanol extract exhibited fair antibacterial activity against all the test bacteria - *Staphylococcus aureus* (MTCC 96), *Streptococcus pyogenes* (MTCC 442), *Escherchia coli* (MTCC 723) and *Klebsiella pneumoniae* (MTCC 109) [33].

The essential oil of *S. hortensis* L. showed antifungal activity against phytopathogenic fungi [34] and against food spoilage fungi [35]. The dominant component of this oil is carvacrol, which is capable of inhibiting aflatoxin production by *A. parasiticus* [36] and *A. flavus* in a liquid medium and tomato paste [37,38]. The same authors suggested that the carvacrol could be useful in controlling aflatoxin contamination of susceptible crops. Weak antimicrobial activity of sage, rosemary, eucalyptus, melissa, lavender and thyme essential oils, sage was found against *Staphylococcus aureus*, *Bacillus subtili*s, E. coli and *Pseudomonas aeruginosa* [39-41]. In another study [42], the EOs had significant antibacterial effect against *Staph. aureus* and *B. subtilis* with MIC ranging from 1.25 to 2.5 $\mu l.ml^{-1}$ for *Staph. aureus* and 0.15 to 2.5 $\mu l.ml^{-1}$ for *B. subtilis*..

4. Conclusions

This study confirms that the essential oil of *Satureja hortensis L.*, *Pimpinella anisum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Ocimum basilicum* L. possess moderate antimicrobial effect against staphylococci resistant to ciprofloxacin, levofloxacin, amikacin and gentamicin. *S. haemoliticus* was the most resistant against antimicrobial action of essential oils of *Rosmarinus officinalis* L., *Salvia officinalis* L.. *S. warneri* and *S. xylosus* were the the most susceptible against antimicrobial activities of all essential oils tested.

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References

1. Baxtrom, C., Mongkolpradit, T., Kasimos, J.N., Braune, L.M., Wise, R.D., Common house spiders are not likely vectors of community-acquired methicillin-resistant *Staphylococcus aureus* infections, Journal of Medical Entomology 2006, 43, 962-965

2. Gaver-Wainwright, M.M., Zack, R.S., Foradori, M.J., Lavine, L.C., Misdiagnosis of spider bites: bacterial associates, mechanical pathogen transfer, and hemolytic potential of venom from the Hobo spider, *Tegenaria agrestis* (*Araneae: Agelenidae*), Journal of Medical Entomology 2011, 48, 382-388

3. Mascarelli, P.E., Maggi, R.G., Hopkins, S., Mozayeni, B.R., Trull, C.L., Bradley, J.M., Hegarty, B.C., Breitscgwerdt, E.B., *Bartonella henselae* infection in a family experiencing neurological and neurocognitive abnormalities after woodlouse hunter ,s pider bites, Parasites and Vectors 2013, 6, 98

4. Silvestre, F.G., de Castro, C.S., de Moura, J.F., Giusta, M.S., De Maria, M., Alvares, E.S.S., Lobato, F.C.F. Assis, R.A., Goncalves, L.A., Gubert, I.C., Chavez-Olortegui, C., Kalapothakis, E. Characterization of the venom from the Brazilian Brown Spider *Loxosceles similis* Moenkhaus, 1898 (*Araneae, Sicariidae*). Toxicon, 2005, 46: 927–936.

5. Monteiro, C.L.B., Rubel, R., Cogo, L.L., Mangili, O.C., Gremski, W., Isolation and identification of *Clostridium perfringens* in the venom and fangs of *Loxosceles intermedia* (brown spider): enhancement of the dermonecrotic lesion in loxoscelism, Toxicon, 2002, 40, 409-418

6. Bili, M., Cortesero, A.M., Mougel, C., Gauthier, J.P., Ermel, G., Simon, J.C., Outreman, Y., Terrat, S., Mahéo, F., Poinsot, D., Bacterial community diversity harboured by interacting species, PLoS One, 2016

7. Monteiro, C.C., Villegas, L.E.M., Campolina, T.B., Pires, A.C., Miranda, J.C., Pimenta, P.F., Secundino, N.F. Bacterial diversity of the American sand fly *Lutzomyia intermedia* using high-throughput metagenomic sequencing, Parasit Vectors, 2016, 9, 480

8. Fryxell, R.T.T., DeBruyn, J.M. The microbiome of Ehrlichia-infected and uninfected lone star ticks (*Amblyomma americanum*), PLoS One, 2016

9. Gotoh, T., Noda, H., Ito, S., Cardinium symbionts cause cytoplasmic incompatibility in spider mites Heredity 2007, 98, 13-20
10. Brownlie, J.C., Cass, B.N., Riegler, M., Witsenburg, J., Iturbe-Ormaetxe, I., McGraw, E.A., O'Neill, S.L., Evidence for metabolic provisioning by a common invertebrate endosymbiont, *Wolbachia pipientis*, during periods of nutritional stress, PLoS Pathogens, 2009
11. Himler, A.G., Adachi-Hagimori, T., Bergen, J.E., Kozuch, A., Kelly, S.E., Tabashnik, B.E., Chiel, E., Duckworth, V.E., Dennehy, T.J., Zchori-Fein, E., Hunter, M.S. Rapid spread of a bacterial symbiont in an invasive whitefly is driven by fitness benefits and female bias, Science, 2011, 332, 254-256
12. Goodacre, S.L., Martin, O.Y., Thomas, C.F.G., Hewitt, G.M. *Wolbachia* and other endosymbiont infections in spiders, Molecular Ecology, 2006, 15, 517-527
13. Duron, O., Hurst, G.D.D., Hornett, E.A., Josling, J.A., Engelstädter, J., High incidence of the maternally inherited bacterium *Cardinium* in spiders, Molecular Ecology, 2008, 17, 1427-1437
14. Martin, O.Y., Goodacre, S.L., Widespread infections by the bacterial endosymbiont *Cardinium* in arachnids, Journal of Arachnology, 2009, 37, 106-108
15. Otto, M., *Staphylococcus* colonization of the skin and antimicrobial peptides, Expert review of dermatology, 2010, 5(2), 183-195
16. Ugur, A., Varol, Ö., Ceylan, Ö., Antibacterial Activity of *Sideritis curvidens* and *Sideritis lanata* from Turkey, Pharmaceutical Biology, 2005, 43(1), 47-52
17. Alhaj, N., Shamsudin, M., Zamri, H., Abdullah, R, Extraction of essential oil from *Nigella sativa* using supercritic al carbon dioxide: study of antibacterial activity, American Journal of Pharmacology and Toxicology, 2008, 3(4), 225-228
18. Fisher, K., Phillips, C., The mechanism of action of a citrus oil blend against *Enterococcus faecium* and *Enterococcus faecalis*, Journal of Applied Microbiology, 2009, 106(4), 1343-1349
19. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antimicrobial susceptibility testing: Eucast disk diffusion method, version 3.0 from April 2013
20. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters, version 5.0 valid from 2015-01-01
21. Kačániová, M., Terentjeva, M., Vukovic, N., Puchalski, C., Roychoudhury, S., Kunová, S., Kluga A., Tokár, M., Kluz, M., Ivanišová, E., The antioxidant and antimicrobial activity of essential oils against *Pseudomonas* spp. isolated from fish, Saudi Pharmaceutical Journal, 2017, 25(8), 1108-1116
22. Ahlstrand, K., Svensson, L., Persson, U., Tidefelt, B.S., Glycopeptide resistance in coagulase-negative staphylococci isolated in blood cultures from patients with hematological malignancies during three decades, European Journal of Clinical Microbiology and Infection Disease, 2011, 30, 1349-1354
23. Mirrett, S., Weinstein, M.P., Reimer, L.G., Wilson, M.L., Reller L.B., Relevance of the number of positive bottles in determining clinical significance of coagulase-negative staphylococci in blood cultures, Journal of Clinical Microbiology, 2001, 39,3279-3281
24. Drozenova, J., Petras P., Characteristics of coagulase-negative *staphylococci* isolated from hemocultures, Epidemiology Mikrobiology Imunology, 2000, 49, 51-58
25. Huang, S.Y. Tang, R.N. Chen, S.Y., Chung, R.L., Coagulase-negative staphylococcal bacteremia in critically ill children: risk factors and antimicrobial susceptibility, Journal of Microbiology and Immunology Infect, 2003, 36, 51-55
26. Jain, A., Agarwa, J., Bansal, S., Prevalence of methicillin-resistant, coagulase-negative staphylococci in neonatal intensive care units: findings from a tertiary care hospital in India, Journal of Medicinal Microbiology, 2004, 53, 941-944
27. Knauer, A., Fladerer, P., Strempfl C., Effect of hospitalization and antimicrobial therapy on bantimicrobial resistance of colonizing *Staphylococcus epidermidis,* Wien Klin Wochenschr, 2004, 116, 489-494
28. Lv, F., Lianga, H., Yuan, Q., Li, C., *In vitro* antimicrobial effects and mechanism of action of selected plant essential oil combinations against four food-related microorganisms, Food Research International, 2011, 44, 3057-3064
29. Goni, P., Lopez, P., Sanchez, C., Gomez-Lus, R., Becerril, R., Nerın, C., Antimicrobial activity in the vapour phase of a combination of cinnamon and clove essential oils, Food Chemistry, 2009, 116, 982-989
30. Fu, Y., Zu, Y., Chen, L., Shi, X., Wang, Z., Sun, S., Antimicrobial activity of clove and rosemary essential oils alone and in combination, Phytotherapy Research, 2007, 21, 989-994
31. Al-Bayati, F.A., Synergistic antibacterial activity between *Thymus vulgaris* and *Pimpinella anisum* essential oils and methanol extracts, Journal of Ethnopharmacology, 2008, 116, 403-406
32. Jarrar, N., Abu-Hijleh, A.A., Adwan, K., Antibacterial activity of *Rosmarinus officinalis* L. alone and in combination with cefuroxime against methicillin – resistant *Staphylococcus aureus* Asian Pacific Journal of Tropical Medicine (2010), 121-123
33. Akhtar, A., Deshmukh, A.A., Bhonsle, A.V., Kshirsagar, P.M., Koleka, M.A., *In vitro* antibacterial activity of *Pimpinella anisum* fruit extracts against some pathogenic bacteria Veterinary World, 2008, 1(9), 272-274

34. Boyraz, N., Ozcan M., Inhibition of phytopathogenic fungi by essential oil, hydrosol, ground material and extract of summer savory (*Satureja hortensis* L.) growing wild in Turkey, International Journal of Food Microbiology, 2006, 107, 238-242
35. Adiguzel, A., Ozer, H., Kilic, H., Cetin, B., Screening of antimicrobial activity of essential oil and methanol extract of *Satureja hortensis* on foodborne bacteria and fungi, Czech Journal of Food Science, 2007, 25(2), 81-89
36 Razzaghi-Abyaneh, M., Shams-Ghahfarokhi, M., Yoshinari, T., Rezaee, M.B., Jaimand, K., Nagasawa, H., Sakuda, S., Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*, International Journal of Food Microbiology, 2008, 123, 228-233
37. Omidbeygi, M., Barzegar, M., Hamidi, Z., Naghdibadi, H., Antifungal activity of thyme, summer savory and clove essential oils against *Aspergillus flavus* in liquid medium and tomato paste, Food Control, 2006, 18, 1518-1523
38. Dikbas, N., Kotan, R., Dadasoglu, F., Sahin, F., Control of *Aspergillus flavus* with essential oil and methanol extract of *Satureja hortensis*. International Journal of Food Microbiology, 2008, 124, 179–182.
39. Bosni, T., Softi, D., Gruji-Vasi, J., Antimicrobial activity of some essential oils and major constituents of essential oils, Acta Medica Botanica, 2006, 35(1), 9-14
40. Skovic, M., Tzakaou, O., Pitarokili, D., Couladis, M., Antifungal activities of selected aromatic plants growing wild in Greece, Nahrung,, 2002, 46(5), 317-320
41. Baratta, M.T., Dorman, H.J.D., Deans, S.G., Chemical composition, antimicrobial and antioxidative activity of laurel, sage, rosemary, oregano and coriander essential oils, Journal of Essential Oil Research, 1998, 10, 618-627
42. Miti-Ulafi, D., Vukovi-Gai, B., Kneevi-Vukevi, J., Stankovi, S., Simi, D., Comparative study on the antibacterial activity of volatiles from sage (*Salvia officinalis* L.)", Archives of Biological Sciences,Belgrade, 2005, 57(3), 173-178.