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Comparison of the Antimicrobial Effect of Zataria Multiflora Essence and Deconex Surface on Microbial Load of Emergency Ambulances

Ali Jadidi, PhD[1], Behrooz Irannejad, MSc[2], Maryam Mohsenifard, MSc[2], Ali Khanmohamadi Hezave, BSc[1], and Seyed Mohamad Aghaepour, BSc[1]

Abstract

This study aimed to compare the antimicrobial effect of Deconex Surface, a common disinfectant, and *Zataria multiflora* essence on microbial load of Emergency ambulance equipment in Iran. The samples were selected from all the ambulances through convenience sampling. Before the intervention, the cultures were obtained from the contact surfaces and the surfaces were then disinfected with Deconex and *Zataria multiflora*. Then, repeated cultures were obtained from the same surfaces. Next, the samples were immediately sent to a microbiology lab. The obtained data were entered into SPSS 2016 and analyzed using statistical tests. Results showed that disinfecting effect of *Zataria multiflora* is better than Deconex in eliminating certain bacterial species such as *Staphylococcus* and *Bacillus*. So *Zataria multiflora* essence can be used as a surface and hospital equipment disinfectant along with other disinfectant compounds.

Keywords

disinfection, microbial load, Zataria multiflora

What do we Already Know About this Topic?

Nosocomial infections are one of the major challenges of the health system that must be prevented.

How does Your Research Contribute to the Field?

This study aimed to evaluate the effect of thyme essential oil on controlling the microbial load of emergency ambulances and to propose a new method based on complementary medicine to prevent the spread of nosocomial infections.

What Are Your Research's Implications Toward Theory, Practice, or Policy?

According to the findings of this study, the use of thyme essential oil can be as effective as chemical compounds in controlling the microbial load of emergency ambulances, in addition, it does not have many side effects of chemical compounds.

Introduction

Hospital-acquired infections appeared before the origination of hospitals and became a health problem during the miraculous antibiotic era. Due to these infections, not only the costs but also the use of antibiotics increased with an extended hospitalization.[1] This resulted in elevated morbidity and mortality. Currently, in developed countries, these infections occur in about 5–15% of hospitalized cases, and in developing countries, it is estimated to be 25%.[2,3] One of the factors contributing to the spread of nosocomial infections is the contamination of surfaces and equipment in hospitals.[4] Some

[1]Department of Nursing, Arak University of Medical Science, Arak, Iran
[2]Department of Emergency Medicine, Crisis Management and Emergency Medical Services of Markazi Province, Arak University of Medical Sciences, Arak, Iran

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Corresponding Author:
Behrooz Irannejad, Crisis Management and Emergency Medical Services of Markazi Province. Basij square, Arak, Iran.
Email: mohsenimaryam7@gmail.com

studies have shown that contaminated surfaces play an indispensable role in the endemic and epidemic transmission of specific pathogens that cause nosocomial infections.[5,6] The important epidemiological microorganisms surviving on surrounding surfaces near the patients can be transmitted to other people.[3] Many Gram-negative bacteria can survive for months. For example, *Acinetobacter* species can survive on dry surfaces from 3 days to 5 months.[7]

These surfaces are generally divided into 2 categories: high-touch surfaces, which are surfaces repeatedly touched by hands, such as door handles, car keys, bathroom walls, computer desks, and so on, and low-touch surfaces, which are surfaces that are touched less by hands and include the ceiling and floor of the ambulances.[8] In addition to the hospitals, surfaces and equipment inside the ambulances can also grow various sources of microbial pathogens and transmit nosocomial infections to people. Not only patients but also paramedics are exposed to a variety of pathogens that can cause disease.[9] Different studies have shown that infections transmitted to patients through surfaces or equipment of ambulances have vital importance and impose an extra financial cost on healthcare systems. Thus, disinfection of surfaces and equipment in ambulances and hospitals is one of the major ways to control nosocomial infections.[10,11]

Disinfection is the process of eliminating all or most of the pathogenic microorganisms, except bacterial spores, on inanimate surfaces.[12] Today, there are several methods of disinfecting surfaces and equipment. One of these methods is using chemical disinfectants. The use of effective disinfectants is an important factor in preventing nosocomial infections.[13] Among the compounds used for disinfection of medical equipment and surfaces, Deconex is widely used in Iran by medical centers. Deconex Surface, according to its manufacturer's information, is a surface cleaner and disinfectant concentrate, free of aldehyde, environment-friendly and with simultaneous disinfecting and cleaning effect, new and advanced formulation, pleasant smell, maximum efficiency in minimum time and concentration, and no adhesion on the surface.[14] Deconex is used for cleaning and disinfecting floors, walls, tables, beds, baby incubators, hospitals, clinics, dental centers, etc. Its spectrum of activity includes bactericidal, fungicidal, virucidal, and anti-bacillus. It is composed of Didecyl Dimethyl ammonium chloride, N-(3-Aminopropyl)-N-dodecylpropane-1, and 3-diamine, along with surfactant compounds, chelating agents, essence, and dye.[15]

Various studies have been conducted on disinfecting efficacy of Deconex. Some articles reported that Deconex has a minor effect or is ineffective on poliovirus type 1, *mycobacterium* bovis, *pseudomonas aeruginosa*, *staphylococcus aureus*, and *mycobacterium tuberculosis*.[16] Also, the Deconex solution has side effects that can be harmful to the healthcare provider and the environment. Moreover, it can irritate the eyes and damage the corneal tissue. Any contact with mucosa irritates mucosal tissue. This solution may also destroy some rubber compounds and artificial coatings in the workplace, including medical equipment, electronic appliances, and office equipment.[17]

In recent years, people are more willing to use herbal compounds as an alternative to chemical medicines. Most researchers believe that the side effects of herbal medicines are less than those of chemical medicines.[18] In this regard, extensive research has been conducted on the use of natural essences. Not only do these essences have antimicrobial properties, but also they have anti-parasite and antitoxic properties, which are related to the types of active ingredients in them.[19]

One of the plants whose essence has been investigated by researchers in recent years is Shiraz thyme, with the scientific name *Zataria multiflora*. Thyme is a strong and durable, greenish-white and aromatic plant with shrubs of about 40 to 80 cm height, numerous stems, and grayish-white or slightly brownish skin which grows in many parts of Iran.[20] The major ingredients of *Zataria multiflora* are Thymol, Carvacrol, Cineol, Cymene, α-Pinene, and Borneol.[21] The antibacterial mechanism of this plant is due to its hydrophobic properties, which cause penetration of its antibacterial substances into phospholipids of bacterial membranes and mitochondria and in turn, disruption and increased permeability of the bacteria's structure and their death.[22] However, many studies on the antimicrobial efficacy of this medicinal plant with other disinfectants have not been performed in clinical settings.

Therefore, due to the lack of information on the efficiency of common disinfectant products, especially herbal ones, to disinfect ambulances, this study aims to compare the disinfecting effect of Deconex Surface and *Zataria multiflora* essence on microbial load of ambulances equipment used in Medical Emergency Services in Iran (Arak).

Materials and Methods

This study was an interventional one and the samples were selected from all the ambulances working in the city of Arak (Iran) through convenience sampling. Sampling surfaces, which were the surfaces mostly touched by patients and personnel in the ambulances, were marked with 3*3 cm stick tape including the surface of the stretcher, the tray under stretcher, the cover of both seats specified to the technician, and the patient's companion, interior and exterior door handles (8 pcs), car steering wheel, car key, oxygen flowmeter, 5 points on the floor of the rear cabin, including 4 corners of the ambulance and one point in the center of the cabin, and 2 points on the floor of the front cabin of the ambulance.

Sampling for cultures was obtained from these 16 marked surfaces once before disinfection and once after each disinfection by Deconex and thyme in 2 media of Blood agar and Mac Conkey. A 1-week break was determined between disinfection with Deconex and that with *Zataria multiflora* essence. As we obtained 32 samples before and 32 samples after the disinfection and each sample was used for 2 media

of Blood agar and Mac Conkey, the total number of cultured samples was 128 (2*32 + 2*32). Sampling was performed at the beginning of each 24-hour shift and the surface was then disinfected using sterile gauze impregnated with 5 ccs of Deconex according to the manufacturer's protocol and sampling was performed again after disinfection. This was repeated at the end of each shift. For *Zataria multiflora*, the sampling was performed similarly with Deconex and then disinfection was performed by the coworker group using 5cc of .1% alcoholic essence of *Zataria multiflora* poured on sterile gauze, and the sampling process was repeated when the solution was dried.

The samples were immediately transferred to the Microbiology Lab of Arak University of Medical Sciences. In the laboratory, first, the tube containing the sample was mixed by vortex mixer and 100 μL of the sample was transferred by sampler to Blood Agar (Merck Co., Germany) and Eosin-Methylene Blue (Merck Co., Germany) media and cultivated by sterile inoculation loop throughout the medium. The plates containing the cultured samples were kept at 37°C for the next 48 hours at the incubator. After incubating, the plates containing the cultured samples (before and after disinfection by disinfectants) were examined and any suspected colonies of pathogenic bacteria were identified by hot staining and routine biochemical tests.

Afterward, the number of pathogenic bacterial colonies identified in the plates containing cultured samples were counted and the number of pathogens per milliliter was determined taking the dilution coefficient into account. If there was any pathogen in the plate after disinfection, the mentioned method was used to count. This method was also used for comparing the colony count of the pathogenic bacteria on all determined surfaces and by both disinfectants used in this study.

Data Analysis

To analyze the data, descriptive tables and chi-square statistical tests were used for determining the difference between the number of positive cultures before and after the disinfection by the 2 methods and the central tendency and dispersion indexes were used to determinate the microbial load. Paired T-test was used to determine the significance of the difference in the number of the microbial count before and after disinfection and the independent T-test was used to determine the significance of the difference between using the 2 disinfectants after disinfection.

Ethical Considerations

The Ethics Committee of the Arak University of Medical Sciences approved this study (code:

IR.ARAKMU.REC.1396.81), and the Vice-Chancellor for Technology and Research of the Arak University of Medical Sciences issued the certificate.

Table 1. Comparison of the Number of Bacterial Colonies before and after the Intervention in 2 Groups.

	Deconex Group	Zataria Group	
Intervention Duration	Mean (SD)	Mean (SD)	*P*-Value
Before intervention	53 863 (2950)	53 863 (2950)	
After intervention	9582 (541)	10 833 (733)	.001*
P-Value	**.001	**.001	

*independent T-test **repeated measures test.

Table 2. Comparison of the Results of Culture of 2 Groups.

	Frequency (percent)		
		After Intervention	
Microorganism Strain	Before Intervention	Zataria Group	Deconex Group
No growth	13 (23.2)	44 (78.6)	45 (80.4)
Staphylococcus	2 (3.6)	1 (1.8)	1 (1.8)
Candida	14 (25)	2 (3.6)	2 (3.6)
E.coli	8 (14.3)	4 (7.1)	3 (5.4)
Staphylococcus epidermis	15 (26.8)	3 (5.4)	4 (7.1)
Enterococcus	1 (1.8)	1 (1.8)	1 (1.8)
Bacillus	1 (1.8)	0	0
Micrococcus	2 (3.6)	1 (1.8)	1 (1.8)

Results

The independent T-test showed that the mean number of bacterial colonies grown in 2 groups did not differ significantly before the intervention ($P > .05$). Also, according to the results of Table 1 obtained from a repeated measures test, it was shown that the intensity of disinfection in both groups was significant ($P < .001$). However, the mean number of colonies that could grow in the medium was slightly higher in the Deconex group than that in the Zataria group (Table 1). According to the results shown in Table 2, *Zataria multiflora* appeared to be more effective in disinfection than Deconex surface. As it can be seen, while the disinfection was performed with *Zataria multiflora*, there was no evidence of any growth of *Staphylococcus* and *Bacillus* colonies. Also, the number of Ecoli colonies was less than that in the Deconex group. However, the growth of *Staphylococcus* colonies was slightly higher than that in the Deconex group. In the rest of the cases, the growth of other bacterial colonies was similar to Deconex Surface (Table 2).

Discussion

This study aimed to compare the antimicrobial effect of Deconex Surface and *Zataria multiflora* essence on microbial load of Emergency ambulance equipment in Iran. Based on the findings, not only disinfecting effect of *Zataria multiflora* essence was not less than Deconex, but also it appeared

to be better than Deconex Surface. A negative culture response was reported in 80.4% of the samples disinfected with thyme essence while only 78.6% of samples had a negative culture response in the Deconex group. In this regard, Isfahanians et al. also showed in their study that Zataria essential oil in low concentrations has long-term antimicrobial effects on the infectious bacteria on the Skin of Rats.[23] In addition, the results of a study by El-Azzouny et al. show that thyme extracts can be used as potential antimicrobial agents against Gram-positive and negative bacteria.[24] Also, Mohammadi et al. (2016) found that *Zataria multiflora* essence has a strong antimicrobial effect on a wide range of pathogens,[25] which confirms the findings of our study.

Another finding of the present study was that *Zataria multiflora* essence appeared to be superior to Deconex Surface at eliminating some bacterial species such as *Staphylococcus* and *Bacillus*. Zendeh et al. Also reported in their study that *Z. multiflora* essential oils had considerable antimicrobial effects against *E. coli*. can reduce the risk of foodborne bacteria and especially *E. coli* in food products.[26] Also, Abramovic et al. reported the antioxidant potential (AOP) and disinfecting effects of *Zataria multiflora*, especially against bacteria such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter* jejunum, which is compatible with our study's findings.[27] According to Osanloo et al. carvacrol and thymol are the main components of ZM which produce antimicrobial effects against fungus, *E. coli*, and *S. aureus* Zomorodian supported the idea of using ZM in mouthwashes and denture cleansers since they show high efficacy in inhibiting microbial strains.[28]

In the present study, only a concentration of .1% *Zataria multiflora* was used and other concentrations were not examined. Another study shows that *Zataria multiflora* extract in 1% and .1% concentrations eliminated C. Albicans and S. Mutans on elastomeric ligatures.[29] The study of Farsizaban and Poorjang also shows that Zataria extract has the most inhibitory effect in the concentration of .62 mg/mL and the highest inhibitory concentration was 1.25 mg/mL.[30]

The disinfection solution should have the maximum disinfecting effect and minimal side effects. In the same way, disinfecting effect of *Zataria multiflora* essence has been challenged by various methods and in many research. With the use of the essential oil of *Zataria multiflora* against bacterial pathogens, a good antimicrobial agent can be obtained without any side effects. It is recommended to study other properties of *Zataria multiflora* essence such as toxicity, tissue penetration, and compatibility with the environments of healthcare centers. Overall, our novel compounds would increase the disinfection efficacy in hospitals and industries, thereby improving the efficiency and minimizing the risk of infections.

Author's note

Behrooz Irannejad, PhD student, is also associated with the Department of Health Economic and Management, School of Public Health, Tehran University of Medical Science, Tehran, Iran.

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Declaration of Conflicting Interests

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ORCID iD

Ali Jadidi https://orcid.org/0000-0001-9870-9779

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