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Original Article

Comparison of hand hygiene antimicrobial efficacy: *Melaleuca alternifolia* essential oil *versus* triclosan[1]

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Objective: this study aimed to evaluate the efficacy of hand hygiene performed with two different soap formulations: 0.3% *Melaleuca alternifolia* essential oil *versus* 0.5% triclosan, and to compare them with two reference hygiene procedures: the official methodology procedure (soft soap) *versus* the draft version of the procedure (soft soap + propan-2-ol). Method: using the European EN 1499 method, logarithmic reduction factors were determined for the number of colony forming units of *Escherichia coli* K12 before and after hand hygiene of 15 volunteer subjects, and compared using the one-tailed Wilcoxon test. Results: referring to the soft soap, there was no difference between the performance of soap with 0.3% M. alternifolia and soap containing 0.5% triclosan. The soft soap + propan-2-ol proved to be more effective than the other hand hygiene procedures. Conclusion: studies to verify the therapeutic efficacy of essential oil in hand hygiene can improve adherence to this practice.

Descriptors: Hand Desinfection; Tea Tree Oil; Triclosan; Infection Control; Nursing.

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Introduction

Hand hygiene is the single most ancient and efficient, simple and less costly measure to prevent the spread of healthcare associated infections (HAIs)[1]. Effective hand hygiene is that which, in addition to its primary purpose of microbial reduction by means of sanitizing agents, does not cause a negative impact on skin condition, but preserves it. It is recommended that hand hygiene products are well accepted, well tolerated, and formulated with the guarantee to avoid any possible irritation[2].

Essential oils are highly volatile substances extracted from plants and have active ingredients due to their complex chemical composition. The therapeutic use of essential oils in order to improve the physical, mental or emotional well-being of an individual is called aromatherapy. Although the mechanism of physiological action of aromatherapy is not well established, it is inferred that it produces a stimulus that results in the release of neurotransmitters, such as endorphins and enkephalins, which have an analgesic effect and produce feelings of well-being and relaxation[3]. The number of studies on the antimicrobial effects of essential oils has been increasing in the literature[4], among which the essential oil of *Melaleuca alternifolia* (Tea Tree oil – TTO) has become known for its antiseptic properties. Publications report that this essential oil presents antimicrobial activities, among them antibacterial(5), including the decolonization of methicillin-resistant *Staphylococcus aureus* (MRSA)[6], antifungal[7-8] and antiviral[9] activities, as well as anti-inflammatory effects[10].

With regard to the antibacterial property of TTO, studies have shown this activity on a broad range of Gram-positive and Gram-negative bacteria, fungi and viruses[5]. The effect of TTO on the development of antibiotic resistance in *S. aureus* and *Escherichia coli* has already been examined, indicating that this oil and terpinen-4-ol, its main active component, have little impact on the development of antimicrobial resistance[11]. Australian researchers demonstrated *in vitro* actions of TTO to determine the minimal inhibitory and bactericidal concentrations for several strains of microorganisms present in the microflora of the skin[12]. The results obtained were satisfactory with a concentration of TTO ranging from 0.06% to 5.00% for the vast majority of microorganisms. The minimal inhibitory and bactericidal concentrations were the same for each of the Gram-negatives tested; while for the Gram-positives, they were variable[12]. This study further suggests a possible residual effect against transient microflora and that preparations containing TTO for hand washing should be investigated for their efficacy. These results suggest that the use of potentially antiseptic essential oils, such as TTO, may represent an efficient resource in the practice of hand hygiene, both due to their the antimicrobial action and due to being a natural alternative to the synthetic antiseptics on the market, improving the adherence of the healthcare professionals.

Since the 1980s healthcare professionals have reported damage to the integrity of the skin caused by products recommended by the infection control programs, such as triclosan or chlorhexidine based soaps[13]. The selection of hand hygiene products with good acceptability while simultaneously being effective is a key component for the promotion and increase of the adherence to the practice[14]. Thus, the purpose of this study was to verify whether a liquid soap with a natural active ingredient – 0.3% essential oil of *M. alternifolia* – would be able to provide antimicrobial efficacy and be an alternative to conventional antiseptic soap containing 0.5% triclosan. The non-inferiority hypothesis assumed was that the formulation of a liquid soap containing TTO can surpass the reduction of the microbial load of soap with triclosan.

The present study aimed to evaluate and compare the efficacy of antimicrobial liquid soap containing 0.3% TTO and liquid soap with 0.5% triclosan in reducing the microbial load present on artificially contaminated hands; and to compare the antimicrobial efficacy of the tested products with those of reference indicated in the methodology, which used the reference soap (soft soap) followed or not by the use of 60% propan-2-ol.

Methods

Design and study site

This double-blind (subject and statistician) crossover study was developed in the Microbiological Testing Laboratory (LEM) of the Department of Medical-Surgical Nursing of the University of São Paulo School of Nursing.

Ethical aspects

The study followed the recommendations of Resolution 196/1996 of the National Health Council regarding research with human beings. The study was initiated after the acceptance of the Research Ethics Committee of the University of São Paulo School of Nursing, and the Research Commission of the same

institution under process No. 1069/2011CEP-EEUSP – SISNEP CAAE – 0082.0.196.000-11.

The criteria for inclusion of the subjects in the sample were: to be between 18 and 55 years of age (since there are changes in the composition of the skin microbiota after age 60); to have had no contact with a residual effect antiseptic within the previous 48 hours; to not present apparent signs of dryness of the hands or injuries, to have clean, short and unpolished nails at the time of data collection, and to be willing to sign the Terms of Free Prior Informed Consent (TFPIC). The criteria for exclusion of the subjects from the sample were: to have prior knowledge regarding allergy to any of the substances to be used in the experiment, to be pregnant, or to refuse to take part in the study.

Study protocol

Before starting the experiment, the negative microbiological control was performed for the soaps containing TTO (Doctornatu® liquid soap from Higinatu, Brazil) and triclosan (Rioderme® soap from Rioquímica, Brazil), following the recommendations of Resolution 481/99 of Anvisa[15]. The analysis methods were in accordance with the ABNT NBR ISO 21149, ABNT NBR ISO 21150, ABNT NBR ISO 22717 and ABNT NBR ISO 22718 standards, which are described in the Brazilian Pharmacopoeia[16]. This step aimed to verify the absence of previous contamination of the products tested.

To seek consistent and plausible evidence, a European methodology was used[17] proposed by the European Committee for Standardization – European Standard in force since 1997 with the addition of some considerations existing in its draft version[18], proposed in April 2011, which is still under evaluation. Such considerations were related to the use of the medium for the strain recovery test using Tryptone Soya Selective Agar – TSSA (DIFCO®, BD®, Sparks, USA) and the reference procedure for hand hygiene that uses soft soap followed by the addition of propan-2-ol.

Prior to the experiment, an appropriate neutralizer for each of the products was validated for the purposes of 1) not presenting any toxic effect on the *Escherichia coli* K12 strain, and 2) presenting neutralizing action on the formulation of the product under test, ensuring that the bactericidal and/or residual bacteriostatic activity of the active antiseptic ingredients were neutralized or suppressed. The neutralizers validated were tryptone soya broth – TSB (DIFCO®, BD®, Sparks, USA) for the 0.3% TTO soap, the propan-2-ol and the soft soap. For the soap containing 0.5% triclosan, D/E Neutralizing Broth (DIFCO®, BD®, Sparks, USA) was validated.

For the experiment, the hands of all the subjects were prepared by a simple wash lasting 60 seconds using 5ml of *soft soap*, then rinsing with mineral water for 15 seconds and drying with a paper towel. All the participants were trained to perform the simple hand hygiene technique immediately before starting the data collection and they performed the procedures under the supervision of a monitor. The fingertips were then immersed in a Petri dish containing 10ml TSB for one minute, to obtain the initial bacterial count value (pre-values). Next, artificial contamination of the hands of volunteers was carried out using a suspension containing $2x10^8$ to $2x10^9$ colony forming units per ml (CFUs/ml) of the test microorganism, in this case, *E. coli* K12 (strain used in this study: ATCC 14948; contamination fluid with $5x10^8$CFU/ml). Both hands of each subject were immersed in the contaminated fluid for five seconds to the metacarpals, with the fingers spread, in a sterile stainless steel bowl. The same suspension was used for all test subjects over a maximum period of three hours after the exposure of the hands of the first subject[17].

After drying (three minutes) in ambient air, maintaining the hands in a horizontal position and making rotation movements with the fists to avoid the formation of droplets, the initial value of the bacterial count (pre-values) was obtained for each of the subject from the smear of the fingertips of each hand for one minute on the bottom of a Petri plate containing 10ml of TSB. Then, the hygiene reference procedure was performed (with use of 5ml of soft soap for 60 seconds or the use of 5ml of soft soap for 60 seconds, followed by rinsing and the use of 3ml propan-2-ol for 30 seconds, two consecutive times, followed by rinsing) or the procedure with 1.5 ml of the product under test, according to the group in which the subject was randomly inserted, and the post-values were collected. The post-values were obtained from the smear of the fingertips in a Petri dish containing 10 ml of neutralizer specific for the respective products that were used in the hand hygiene.

The sample consisted of 15 volunteer participants, who were randomized into four groups, identified by the Roman numerals I, II, III and IV, who had the sequence of use of the products designated using a Latin square. It should be noted that all the subjects performed the hand hygiene with the four products under evaluation, varying only the order of use. The plates were incubated for 18 to 24 hours at 37°C and then read to determine the number of CFUs. They were

then reincubated for 24 hours to detect the growth of new colonies.

Analysis of the results and statistics

To obtain the data the mean number of colonies of the right and left hands were calculated and the logarithmic reduction factor (RF) was determined by calculating the difference between the final and initial values of *E. coli* CFUs. As indicated in the methodology, the log reduction was calculated for each of the sampling fluid dilution stages using the weighted mean of the number of pre-value and post-value CFUs/ml.

For the test to be valid, the RF of the mean of the log obtained for the test products should be statistically higher than that found for the reference soap, for at least twelve subjects, and the total logarithmic mean of the "pre-values" for the procedures with the reference product. Furthermore, the results of the procedure with test products should be at least 5 log. The data were entered into a Microsoft Excel® spreadsheet and processed using the *R 2.14.1* program for the statistical analysis. Descriptive statistics (mean and standard deviation) were calculated, with the comparison of the groups made using the Wilcoxon paired nonparametric test (the test recommended by the EN 1499 methodology[17]). The level of significance was established as p=0.01 one-tailed. The adjustment of the test is able to detect a difference of 0.5 log with a power of 80 to 90%. To verify if there was a difference between the pre-values and the evaluation of the Latin squared design, ANOVA was applied (significance level of 5%) and to verify the correlation of the contamination of the hands before and after the procedures of hand hygiene, Pearson's Correlation was used.

Results

The results of the microbiological analysis of the test soaps indicated that microbial contamination of products was absent. Of the 15 volunteers who participated in the trial, 11 were female (73.4%) and four male (26.6%). The minimum age was 23 years and maximum 50 years (mean = 31 years; median = 30 years; standard deviation = 7.67). Twelve subjects were registered nurses, two were 4th year Nursing undergraduate students, and one performed administrative activities.

The ANOVA was used to evaluate the effect of the Latin square model (Table 1). It was observed that there was no significant difference for the position that individual occupied in the group (p=0.81) or for the sequence of procedures performed (p=0.31), i.e., there was no column or group effect. However, there were differences in the treatment (p<0.001) which, in this case, was caused exclusively by the different procedures.

Table 1 - ANOVA for the evaluation of the group, column and treatment effects of the Latin Square model. São Paulo, SP, Brazil, 2012

Factor	Degrees of freedom	p-value
Treatment	3	<0.001
Group	3	0.31
Column	3	0.81

The contamination of the hands with the microbial suspension of the study was successful. The ANOVA showed no statistical difference between the pre-values for each of the four products used as treatment (p-value=0.2804; established significance level p-value=0.05). Table 2 shows the correlations between the contamination of the left and right hands of the subjects before (pre-values) and after (post-values) the hand hygiene procedure, by means of Pearson's correlation.

Table 2 - Correlations between the mean contamination of the left and right hands of the subjects before (pre-values) and after (post-values) the hand hygiene procedure. São Paulo, SP, Brazil, 2012

Treatment	Moment							
	Pre-value				Post-value			
	Dilution			Mean CFUs	Dilution			Mean CFUs
	10^{-3}	10^{-4}	10^{-5}		10^{0}	10^{-1}	10^{-2}	
Soft Soap	0.981	0.986	0.999	5.63	0.535	0.697	0.598	1.76
Soap with 0.3% TTO	0.774	0.817	0.892	5.98	0.565	0.356	0.792	2.10
Soap with 0.5% triclosan	0.763	0.986	0.934	6.38	0.343	0.805	0.750	2.79
Soft soap + propan-2-ol	0.830	0.917	0.916	6.29	0.359	0.386	0.445	1.40

By applying Pearson's correlation, it was observed that the values were more correlated among themselves in the pre-values (closer to +1 in the dilutions of 10^{-3} to 10^{-5}) indicating that the fluid contaminated both hands similarly, than in the post-values (dilutions from 10^{0} to 10^{-2} – values less close to +1).

Table 3 presents the pre-values (logarithms of *E. coli* present on the hands of the subjects before the hygiene procedure), post-values (logarithms of E. coli present on the hands of the individuals after the hygiene procedure) and the logarithmic reduction factor (RF = pre-value – post-value) for the study subject, after the calculation indicated by the methodology[17], according to the hand hygiene procedure. It can be observed that all the logarithmic means of the pre-values for the four hand hygiene procedures were greater than 5, fitting the aforementioned necessary requirements for the trial to be considered valid (mean = 5.63 for soft soap; 5.98 for the soap containing TTO, 6.38 for the soap containing triclosan; 6.29 for soft soap + propan-2-ol).

Table 3 - Logarithms related to pre- and post-values and logarithmic reduction factor (RF) for each of the hand hygiene procedures for each subject, according to the group. São Paulo, SP, Brazil, 2012

Group	Subject	Soft Soap			TTO			Triclosan			Soft soap + propan-2-ol		
		Pre	Post	RF	Pre	Post	RF	Pre	Post	RF	Pre	Post	RF
I	1	6.31	2.02	4.29	5.88	3.37	2.52	6.26	1.10	5.16	5.48	1.07	4.41
	2	4.56	1.13	3.43	5.36	1.00	4.36	7.29	3.65	3.63	7.18	1.35	5.83
	3	5.02	1.18	3.84	5.25	1.02	4.23	6.00	1.79	4.21	5.44	1.00	4.44
	4	6.25	1.06	5.19	5.68	1.12	4.56	6.11	2.23	3.88	7.42	1.00	6.42
II	5	4.76	1.22	3.54	5.24	1.90	3.34	5.41	2.47	2.94	5.97	1.22	4.76
	6	7.52	4.52	3.00	7.52	4.22	3.30	7.52	4.41	3.10	7.52	2.85	4.67
	7	7.44	3.86	3.57	5.85	1.41	4.44	6.16	1.90	4.26	6.02	1.00	5.02
	8	7.52	3.38	4.14	5.95	1.94	4.01	5.06	1.75	3.31	7.52	1.90	5.61
III	9	5.37	1.13	4.23	6.08	2.36	3.71	7.52	4.44	3.08	4.76	1.06	3.70
	10	7.52	1.06	6.46	7.16	2.78	4.39	7.52	4.23	3.29	6.55	1.00	5.55
	11	6.32	1.00	5.32	3.60	1.04	2.56	5.38	1.35	4.03	4.54	1.00	3.54
	12	3.30	1.18	2.12	6.49	2.18	4.31	7.17	3.56	3.61	6.74	2.00	4.74
IV	13	4.21	1.57	2.65	5.42	1.00	4.42	5.29	1.88	3.41	4.44	1.02	3.42
	14	4.51	1.04	3.47	7.05	3.43	3.61	5.53	3.45	2.08	7.52	2.41	5.10
	15	3.78	1.00	2.78	7.22	2.67	4.55	7.52	3.63	3.89	7.19	1.06	6.13
Mean		5.63	1.76	3.87	5.98	2.10	3.89	6.38	2.79	3.59	6.29	1.40	4.89

The Wilcoxon test was used to verify whether there was a statistically significant difference in the microbial reduction provided by the products, (Tables 4 and 5). Two Wilcoxon tests were performed, one with the soft soap + propan-2-ol reference product (draft version of the reference procedure[18] – Table 4) and the other comparing the test products with the soft soap (official version of the reference procedure[17] – Table 5).

Table 4 - Application of the Wilcoxon test on the logarithmic reduction factors (RF) for each one of the test products in relation to the reference procedure with soft soap + propan-2-ol. São Paulo, SP, Brazil, 2012

Product	Mean log		Reduction Factors	Wilcoxon
	Pre-value	Post-value		p-value
Soft Soap	5.63 (1.46)	1.76 (1.17)	3.87 (1.13)	0.0065
0.3% TTO	5.98 (1.01)	2.10 (1.03)	3.89 (0.69)	0.0010
0.5% Triclosan	6.38 (0.95)	2.79 (1.16)	3.59 (0.71)	0.0001
Soft soap + propan-2-ol	6.29 (1.14)	1.40 (0.60)	4.89 (0.91)	-

In Table 4 it can be observed that the performance of the procedure carried out using soft soap + propan-2-ol was superior to the other test products, as the p-value for all products was less than 0.01.

Table 5 - Application of the Wilcoxon test on the logarithmic reduction factors (RF) for each of the test products in relation to the reference procedure with soft soap. São Paulo, SP, Brazil, 2012

Product	Mean log		Reduction Factors	Wilcoxon
	Pre-value	Post-value		p-value
Soft soap + propan-2-ol	6.29 (1.14)	1.40 (0.60)	4.89 (0.91)	0.0065
0.3% TTO	5.98 (1.01)	2.10 (1.03)	3.89 (0.69)	0.2470
0.5% Triclosan	6.38 (0.95)	2.79 (1.16)	3.59 (0.71)	0.2975
Soft Soap	5.63 (1.46)	1.76 (1.17)	3.87 (1.13)	-

It can be verified in Table 5 that when the soft soap is adopted as the reference product, there is no statistically significant difference between it and the performance of the 0.3% TTO soap (p=0.2470) or the 0.5% triclosan soap (p=0.2975). There were significant differences in relation to the soft soap + propan-2-ol, which presented a greater antimicrobial efficacy. For a test product to be considered conforming, following the standardization, its mean RF should be significantly greater than that obtained by the reference procedure[17]. There were no statistically significant differences in any of the Wilcoxon tests performed (Table 4 and 5), the 0.3% TTO and 0.5% triclosan soaps were considered *non-conforming* for the antimicrobial reduction in hand hygiene according to this standard.

Discussion

The results encountered with the performance of the proposed trial showed no significant difference in microbial load after hand hygiene performed with soap containing 0.3% TTO or soap containing 0.5% triclosan. In addition, neither of the products evaluated outperformed the two reference procedures (hand washing with soft soap or soft soap + propan-2-ol). The choice of the concentrations of the antimicrobial agents present in the soaps studied is justified because they are products already circulating in the consumer market, in addition to being registered by Anvisa. This ensured that there was a low health risk for the subjects who were willing to participate in the trial, as well as allowing a check to be made regarding the results obtained by formulations that are already used in the healthcare settings, as in the case of triclosan, and are commercially available for use, as in the case of the soap with TTO. It is emphasized that the commercially available TTO formulation has a concentration well below that considered to be safe for human use without causing allergic effects[19].

The results obtained with the hand hygiene reference procedures demonstrated that the use of soft soap associated with propan-2-ol resulted in a greater log reduction (4.89 log) than the use of soft soap alone (3.87 log), which was expected due to the synergistic action of propan-2-ol. The incorporation of alcohol to the reference procedure of the method, as proposed in the *draft* version[18], is therefore understood to ensure greater methodological rigor.

A logarithmic reduction of CFUs of *E. coli* present on the hands of individuals, provided by soap containing 0.5% triclosan (3.59 log), was higher in this study than that found in another study that assessed hand hygiene with the same antimicrobial agent, however, at a lower concentration of 0.1% (2.8 log)[20]. Furthermore, the present trial confirms the results obtained in other studies, which found that the logarithmic reductions with triclosan were lower compared to other antimicrobial hand hygiene products[2,21].

The results for the soap containing 0.3% TTO showed that there were no significant differences in the efficacy of the hand hygiene performed with soft soap or soap containing TTO or triclosan, (Tables 4 and 5), and therefore these products under test were not endorsed by the methodology. However, it is important to note that, based on the scientific literature, although studies emphasize the potential antimicrobial activity of TTO[5-9,11-12], there is still no standardization determining the minimum effective concentration, time of application, or best way to use TTO. There is evidence that a concentration of 5% in a hygiene formulation, using the same methodology applied in this trial (EN 1499), is possibly effective[22]. Therefore, the development of new studies applying TTO in higher concentrations with hand hygiene products represents a vast field to be explored, subject to a limit of up to 10% of essential oil due to possible allergic reactions[19].

It is important to emphasize that all the results obtained in the logarithmic reductions were due to the hand hygiene procedures with a duration of 60 seconds, a fact that is not commonplace in healthcare practice. One study verified that, in the care context, healthcare professionals spend 6 to 24 seconds to wash the hands,

and that a realistic expectation would be a duration of 15 seconds[23]. In this case, a product that provides a pleasurable sensation during the procedure may possibly help to increase adherence, especially regarding the time required to adequately perform the technique.

One issue that is included in the acceptability of a product for hand hygiene is the aroma. During the collection, several volunteers made reference to the characteristic smell of the product containing TTO, saying it was very pleasant. This fact was only observed by the monitors, who did not interfere during the exposure of the ideas of the subjects, however, such statements lead to the inference that the pleasant aroma of the TTO can improve the hand hygiene adherence of healthcare professionals. Thus, soap with essential oil could contribute as a "facilitator" for infection control, aiming at the prevention and control of infection within the healthcare services, as suggested by a study that evaluated the impact of strategies to encourage hand hygiene adherence[24].

The selection of a hand hygiene product should be made based on good acceptability and efficacy, since both are factors that contribute to the promotion and increase of the adherence to the practice[2,14]. Although it was not the object of study of this research, the data collection showed that the use of this essential oil contributes to encourage the use of the product due to its pleasant aroma, which is possibly also applicable to the aromas of other essential oils. The stimulation of the olfactory system when using an essential oil is inevitable, therefore it is not possible to separate the aromatherapy effect from the physical effect caused by an oil, which could be exploited in future studies. In this sense, the use of a product containing essential oil in hand hygiene, with therapeutic efficacy associated with the pleasurable experience of its use, can assist in increasing adherence to this essential practice in the prevention and control of HAIs, both by the nursing team and by the multidisciplinary healthcare team.

Conclusions

Regarding the antimicrobial efficacy, there was no difference between hand hygiene performed with soap containing 0.3% TTO and hand hygiene with soap containing 0.5% triclosan. Neither soaps outperformed either of the two reference procedures (soft soap or soft soap + propan-2-ol), although both products are approved by Anvisa. Finally, the hand hygiene reference procedure using soft soap + propan-2-ol, proposed in the draft version, proved to be more effective than the isolated use of soft soap, described in the official methodology.

Final considerations

Studies should be developed to evaluate the antimicrobial efficacy of new formulations of soap with higher concentrations of TTO, within dosages considered safe in the scientific literature (between 5% and 10%). Subsequently, studies should be conducted to verify the acceptability of the product and to compare the hand hygiene adherence between formulations containing the essential oil and those made with products traditionally used in healthcare settings.

References

1. Agência Nacional de Vigilância Sanitária (BR). Higienização das mãos em serviços de saúde [Internet]. [acesso 24 ago 2012]. 2007. Disponível em: http://bvsms.saude.gov.br/bvs/publicacoes/higienizacao_maos.pdf

2. Centers for Disease Control and Prevention (USA). Guideline for hand hygiene in health-care settings: recommendations of the Health Care Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA Hand Hygiene Task Force. 2002 [Internet]. [acesso 27 ago 2012] Disponível em: http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5116a1.htm

3. Potts J. Aromatherapy in nursing practice. Aust Nurs J. 2009;16(11):55.

4. Kunicka-Styczynska A, Sikora M, Kalemba D. Lavender, tea tree and lemon oils as antimicrobials in washing liquids and soft body balms. Int J Cosmetic Sci. 2011;33(1):53–61.

5. Carson CF, Hammer KA, Riley TV. *Melaleuca alternifolia* (Tea Tree) Oil: a review of antimicrobial and other medicinal properties. Clin Microbiol Rev. 2006;19(1):50-62.

6. Dryden MS, Dailly S, Crouch M. A randomized, controlled trial of tea tree topical preparations *versus* a standard topical regimen for the clearance of MRSA colonization. J Hosp Infect. 2004;56(4):283-6.

7. Hammer KA, Carson CF, Riley TV. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol. 2003;95(4):853-900.

8. Noumi E, Snoussi M, Hajlaoui H, Trabelsi N, Ksouri R, Valentin E, et al. Chemical composition, antioxidant and antifungal potential of *Melaleuca alternifolia* (Tea Tree) and Eucalyptus globulus essential oils against oral Candida species. J Med Plant Res. 2011;5(17):4147-56.

9. Garozzo A, Timpanaro R, Bisignano B, Furneri PM, Bisignano G, Castro A. In vitro antiviral activity of *Melaleuca alternifolia* essential oil. Lett Appl Microbiol. 2009;49(6):806-8.

10. Brand C, Ferrante A, Prager RH, Riley TV, Carson CF, Finlay-Jones JJ, et al. The water soluble components of the essential oil of *Melaleuca alternifolia* (tea tree oil) suppress the production of superoxide by human monocytes, but not neutrophils, activated in vitro. Inflamm Res. 2001;50(4):213-9.

11. Hammer KA, Carson CF, Rileya TV, Rileya TV. Effects of *Melaleuca alternifolia* (tea tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. Antimicrob Agents Chemother. 2012;56(2):909-15.

12. Hammer KA, Carson CF, Riley TV. Susceptibility of transient and commensal skin flora to the essential oil of *Melaleuca alternifolia* (Tea Tree oil). Am J Infect Control. 1996;24(3):186-9.

13. Seitz JC, Newman JL. Factors affecting skin condition in two nursing populations: implications for current handwashing protocols. Am J Infect Control. 1988;16(2):46-53.

14. Larson E, Girard R, Silva CLP, Boyce J, Donaldson L, Pittet D. Skin reactions related to hand hygiene and selection of hand hygiene products. Am J Infect Control. 2006;34(10):627-35.

15. Agência Nacional de Vigilância Sanitária (BR). Resolução nº 481, de 23 de setembro de 1999. Estabelece os parâmetros de controle microbiológico para os produtos de higiene pessoal, cosméticos e perfumes conforme o anexo desta resolução. Diário Oficial da União. 27 set 1999. Brasília; 1999.

16. Farmacopéia brasileira. 5 ed. São Paulo: Atheneu; 2010.

17. European Committee for Standartization. Chemical disinfectants and antiseptics – hygienic handwash – test method and requirements (phase2/step2) [European standard EN 1499]. Brussels, Belgium: Central Secretariat; 1997.

18. European Committee for Standartization. Chemical disinfectants and antiseptics – hygienic handwash – test method and requirements (phase2/step2) [European standard EN 1499]. [Internet]; Brussels, Belgium [acesso 18 abr 2012]. Disponível em: https://www.astandis.at/shopV5/Preview.action;jsessionid=9678A87F9CB216A588CBFF40AFBD5E8A?preview=&dokkey=393356&selectedLocale=en

19. Rutherford T, Nixon R, Tam M, Tate B. Allergy to tea tree oil: retrospective review of 41 cases with positive patch tests over 4.5 years. Australas J Dermatol. 2007;48(2):83-7.

20. Rotter ML. Hand washing and hand disinfection. In: Mayhall CG, editor. Hospital Epidemiology and Infection Control. Baltimore: Williams & Wilkins; 2004. p. 1727-46.

21. World Health Organization.World Alliance for Patient Safety. Forward Programme 2006-2007.[Internet]; Geneva [acesso 28 ago 2012]. Disponível em: http://www.who.int/patientsafety/information_centre/WHO_EIP_HDS_PSP_2006.1.pdf

22. Messager A, Hammer KA, Carson CF, Riley TV. Effectiveness of hand-cleansing formulations containing Tea Tree oil assessed ex vivo on human skin and in vivo with volunteers using European standard EN 1499. J Hosp Infect. 2005;59(3):220-8.

23. Boyce JM, Pittet D. Guideline for hand hygiene in health-care settings. Recommendations of the Healthcare Infection Control Practices Advisory Committee and the HICPAC/SHEA/APIC/IDSA hand hygiene task force. MMWR Recomm Rep. 2002;51(RR–16):1-45 [quiz: CE1–4].

24. Neves ZCP, Tipple AFV, Souza ACS, Pereira MS, Melo DS, Ferreira LR. Hand hygiene: the impact of incentive strategies on adherence among healthcare workers from a newborn intensive care unit. Rev. Latino-Am. Enfermagem. 2006;14(4):546-52.

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