Elsevier Editorial System(tm) for
Complementary Therapies in Medicine
Manuscript Draft

Manuscript Number:

Title: Air dispersed essential oils for environmental microbiota control in nosocomial hospitalization rooms

Article Type: Full-length article (max 3000 words)

Section/Category: Other

Keywords: essential oils; antimicrobial; disinfection; hospital; environmental.

Corresponding Author: Dr. Giangiacomo Beretta,

Corresponding Author's Institution: University of Milan

First Author: Fabrizio Gelmini

Order of Authors: Fabrizio Gelmini; Luciano Belotti; Sara Vecchi; Cristian Testa; Giangiacomo Beretta

Abstract: Objective. Environmental bacterial contaminant microorganisms are an ongoing problem in hospitals. Essential oil vapours (EO) may help reducing this type of contamination. Aim of this study was to evaluate the efficacy of nebulized selected essential oils (EO) in reducing the microbial contamination in residential health care house rooms.
Design. The study was carried out in a two-story 112-bed tertiary care structure (approximately 1060 m2). Contamination in rooms and corridors was monitored for a total of n=5 months, including a starting baseline sampling and one end-study point, and without combined treatment (standard sanitization alone). Contact slides were collected for microbiological analysis.
Results. Reductions in both bacterial and fungal contamination were observed between rooms cleaned using standard sanitization alone or in combination with essential oils nebulization (average 90% decrease for total count, $P<0.01$; 90% for yeasts and molds, $P<0.05$). Decreases of antibiotic (70%), mucolytic (100%), bronchodilators (100%), and steroidal (67%) and non-steroidal anti-inflammatory drugs (33%) prescriptions were observed, with no adverse effects on patients.
Conclusions. The selected EO composition is effective in reducing both the environmental microbial contamination and pharmaceutical drugs consumption in a nosocomial health care house.
This study demonstrates that aerial EO diffusion combined with standard sanitization procedures, has great potential to reduce the microbial contamination in critical hospital environments such as hospitalization rooms.

Dear Editor,

On behalf of all the coauthors, I submit to your kind attention the manuscript entitled:

“Air dispersed essential oils for environmental microbiota control in nosocomial hospitalization rooms”

to be considered for publication on Complementary Therapies in Medicine.

Sincerely,

Dr Giangiacomo Beretta

Air dispersed essential oils for environmental microbiota control in nosocomial hospitalizationrooms

Fabrizio Gelmini,[a] Luciano Belotti,[b] Sara Vecchi,[a] Cristian Testa,[c] Giangiacomo Beretta[a]#

Affiliations:

Department of Pharmaceutical Sciences, University of Milan, Italy[a], Fondazione "Don Ambrogio Cacciamatta", Iseo, Italy[b], Functional Point S.r.l., Laboratorio di Microbiologia e Virologia, Bergamo, Italy[c].

Running head: Essential oils in hospitalroomsmicrobiota control.

#Address correspondence toGiangiacomo Beretta, giangiacomo.beretta@unimi.it

Keywords: essential oils, antimicrobial, disinfection, hospital, environmental.

ABSTRACT

Objective. Environmental bacterial contaminant microorganisms are an ongoing problem in hospitals. Essential oil vapours (EO) may help reducing this type of contamination. Aim of this study was to evaluate the efficacy of nebulized selected essential oils (EO) in reducing the microbial contamination in residential health care house rooms.

Design. The study was carried out in atwo-story112-bed tertiary care structure (approximately 1060 m^2).Contamination in rooms and corridors was monitored for a total of n=5 months, including a starting baseline sampling and one end-study point, and without combined treatment (standard sanitization alone). Contact slides were collected for microbiological analysis.

Results. Reductions in both bacterial and fungal contamination were observed between rooms cleaned using standard sanitization alone or in combination with essential oils nebulization (average 90% decrease for total count, $P<0.01$; 90% for yeasts and molds, $P<0.05$).Decreases of antibiotic (70%), mucolytic (100%), bronchodilators (100%), and steroidal (67%) and non-steroidal anti-inflammatory drugs (33%) prescriptions were observed, with no adverse effects on patients.

Conclusions. The selected EO composition is effective in reducing both the environmental microbial contamination and pharmaceutical drugs consumption in a nosocomial health care house. This study demonstrates that aerial EO diffusion combined with standard sanitization procedures, has great potential to reduce the microbial contamination in critical hospital environments such as hospitalization rooms.

INTRODUCTION

Appropriate disinfection of environmentally contaminated hospital surfaces can eliminate or reduce the communicable diseases transmission mediated by environmental pathogens. Due to the presence of several multiresistantstrains of pathogenic microbial species among which*Staphylococcus aureus,Clostridium difficile, Candida*sp, *Acinetobacterbaumannii*, and *Pseudomonas aeruginosa*, the use of conventional environmental sanitization strategies (including the use of active oxygen releasers, hypochlorite, quaternary ammonium salts, etc.), may not show the appropriate efficacy to achieve their eradication [1].

Among the strategies alternative or complementary to those involving conventional sanitizing agents, the use of essential oils (EO) represents a new experimental frontier in terms of safety, efficacy and of patients compliance to antimicrobial treatments.

The EOantimicrobial capacity have beenextensively evaluatedby several in vitro studies, which established their inhibitory activity against pathogenic bacteria, fungi, molds and yeasts, using agar layertests,as recently exhaustively reviewed by Lang and Buchbauer and by Seow and colleagues [2,3].

By contrast, Inouye et al. investigated the antimicrobial activity of EOs against pathogens of the respiratory tract by exposure of the culture medium to gaseous components of EO from different botanical origins. These authors found that the growth of *H. influenzae*, *S. pyogenes*, *S. pneumonie*,*S. aureus*, and *E. coli* was inhibited by a wide range of EO concentration in the range 1.56-1600 mg_{EO}/L_{air} (minimal inhibition dose, MID), depending on its botanical origin[4].

On the same line, Hood et al. reported that theEO from *Leptospermum petersonii* was able to inhibit in vitro the growth of the pathogenic fungus *Aspergillus fumigatus* and to eradicate the pathogen trough aerial exposure inlungs of immunosuppressed and infected BALB/c mice [5].

Recently, Laird et al. shown that a vaporized mixture of citrus EO is effective in reducing experimental contaminations from Enterococcus sp. and Staphylococcus aureus on stainless surfaces [6], while Ali and colleagues showed the capacity of gas phase lemon grass EO

components to suppress the mycelial growth and conidial germination of *Colletotrichumgloeosporioides*, the pathogenic agent responsible for anthracnose disease in papaya fruits [7].

These results highlighted the potential of EO volatiles as potent preventive and therapeutically useful tool for the control of environmental pathogens.

However, to the best of our knowledge there are no literature reports on the evaluation of theirefficacy in the control of relevant infective microbial agents in health care houses or hospitals.

Hence, aim of this work was to evaluate the efficacy of volatiles from selected essential oils,in combination with a standard cleaning protocol,in reducing the microbial contamination in the patient rooms of a residential health care house.

The mixture of essential oils was applied in the form of aerosol mixture with water, after nebulization using anultrasound vapour generator to (i) avoid heating of active principle(s), and to (ii) achieve the most uniform distribution of the terpenicantimicrobial agent(s) over the patient room surfaces.The impact of hospital rooms exposure to vaporized EO on the consumption of specific drugs classes have been also evaluated.

Materials and Methods

Location and patients

The study was conducted at the "Don AmbrogioCacciamatta" Foundation, a 114-bed residential health care house in Iseo, Italy. The 2-story building encompasses approximately 1060 m^2. More details about planimetry and patients' number and distribution are reported in the paragraph Experimental design. Patients hospitalized in the control and treated rooms were both age and sex matched.Before entering hospitalization all patients or their legal tutors gave a written consent form.

Essential oil diffusion

The EO mixture was dispersed using commercially available ultrasound vaporisers (mod. Rondo, Gisas.r.l, Romentino, Novara, Italy). The devices were filled daily with water (500 mL) and 100 μL of essential oils mixture, and let working for t=8h during daylight and t=8h during the night, every day throughout all the study period. During daylight hours, room doors were kept open, allowing air diffusion through all the department environments (rooms and corridor).

GC-MS analysis

GC-MS analysis was performed on a Bruker Scion SQ equipped with a single quadrupole and a FactorFour Varian column (VF-5 ms, 30m; 0.25mm i.d., film thickness 0.25μm). The oven temperature was initially set at 60 °C (hold time 3 min), with a gradient from 60 to 120 °C (8.0 °C /min, hold 1 min), then from 120 to 280 °C (4 °C /min, hold 1.5 min) and from 280 to 330 °C (10 °C /min, hold 2 min). The injector temperature was set to 250 °C, and column flow to 1.00 mL/min. Carrier gas was helium 5.5; ionization energy 70 eV; the split/splitless ratio was set to 1:30 after 40

s. Data acquisition was performed in full scan (m/z 50-1200) in EI from 3 to 60 min. EOs were diluted 1:1000 in ethylacetate and injection volume was 1 µl.

Peaks were identified by comparing the retention times with those of authentic standard MS fragmentation patterns, or by matching with those stored in NIST (2011, vers. 2.0) mass spectral database library. The percentage composition of the oils was computed by the normalization method from the GC peak areas, calculated as the mean of three injections for each oil, without correction factors.

For analysis, (A) 1µL of EO mixture was diluted to 1 mL with dichloromethane and 1 µL of this solution injected into the gaschromatograph. (B) To evaluate the aerosol composition, part of the produced dispersion was collected under gentle reduced pressure using an in house built device, bubbled into 10 mL ofdichloromethane, and this step repeated until a sufficient amount of EO components was collected. This mixture was dehydrated by addition of anhydrous sodium sulphate, filtered on paper and 1 mL injected in the same conditions of (A).

Biological Samples Collection and Analysis

Microbial counts were done using Hycheck^{TM} "Total Count" and Hycheck^{TM} "Yeast and Mold" contact slides (Beckton Dickinson, USA). Counts were done according to the manufacturer instructions.

Briefly, each slide was put in contact with the selected surfaces, immediately transferredat T=4°C to the incubator where they were kept for t=120h for yeast and mold assay and t=24h for total microbial count, both at T=37°C, and the number of developed colonies visually counted and recorded. This standardized and conventional protocol allows the detection of the most common environmental microorganisms.

Total microbial assay selectivity: *Enterococcus faecalis*(Gram-positive), *Escherichia coli*(Gram-negative), *Proteus vulgaris*(Gram-negative), *Salmonella typhimurium*, *Staphylococcus*

aureus(Gram-positive). Yeasts and molds assay selectivity: *Candida albicans, Aspergillus niger, Saccharomyces cerevisiae*.

Measurements were done in triplicate and results expressed as mean±standard deviation (colonies/cm^2)

Sanitization

Anionic surfactants for tableware washing; anionic surfactants, nonionic and amphoteric surfactants, polyaminic resin, antifermentation agentsfor hands washing (GruppoDac, Brescia, Italy). Environmental cleaning: two different products containing (i) phosphoric acid (15-25%, ethoxylatedalkylacohol (0-5%) non-ionic surfactants (5%) (Kaltol, Italchim, Bologna, Italy) and (ii) non-ionic surfactants (5-15%), cationic surfactants, 2-butoxyethanol after dilution of 50g in 10L of water (PSC Pavimenti, C.C.I.A.A, Bergamo, Italy).

The EO mixture composition was as follows:*Lavandaangustifolia*24% (w/w), *Melaleucacajeputi*24% (w/w) (Cajeput), *Abiessiberica*20% (w/w), *Mirtocommunis*20% (w/w) and*Pelargonium graveolans* (Geranium bourbon) 12% (w/w).

Experimental design

Nebulizers were placed in n=2 of the n=8 rooms located at the first floor of the residential health care house department. The second floor, with identical planimetry, was used as matching control environment and subjected to standard disinfection and cleaning procedures only.

Sampling was carried out every n=30 days for n=5 months (T_0=baseline, T_1=30^{th} day, T_2=60^{th} day, T_3=90^{th} day, T_4=120^{th} day and T_5=150) in n=5 different random points within the same room (tables andcabinets surfaces) and in corridors (handrails). To evaluate the baseline bacterial charge, and to exclude potential, non-treatment derived antimicrobial effects respectively, at T_0 and during the last 30 days of monitoring (from T_4 to T_5) the vaporizers were not activated.

The EO mixture was diffused as above described, without any modification of the standard daily cleaning protocol comparing to that used in the control sector.

Statistics

Depending on type of comparison, statistical differences were evaluated by one-way ANOVA followed by T-tests on pairwise comparisons with the least square difference (LSD) post hoc adjustment for multiple comparisons, or by student's T-test for comparison of groups alone. Differences were considered significant when $P<0.05$. Computations were performed using the R-commander GUI for R (v. 3.1.3)[8].

RESULTS

Location and patients

The present study was conducted in the hospitalization department of a residential health care house. Control and treatment rooms were those placed at its first floor**(Fig. 1)**.

Each set of n=8 two beds rooms were connected to the same corridor, and aerated independently from the others, and the diffusors placed in n=2 rooms.

All rooms guested the same patients (total number n=32) for the entire duration of the study, from November 2014 to March 2014.

Original and nebulized EO mixture composition

The GC-MS chromatograms reported in **Fig. 2** show that the profile of the EO mixture components recovered from the nebulized EO (upper panel) was almost overlapping that of the same mixture directly diluted in solvent (lower panel). These results demonstrated that the ultrasound nebulization mode allowed incorporating the EO mixture components into the aerosol independently from their individual boiling points and polarity.

Total bacterial, fungi and yeast counts at baseline and postdisinfection

In **Fig. 3**arepresentative photographic representation of the development of bacterial colonies on Hycheck^{TM} "Total Count" contact slidesis shown.

Already at visual inspection, it can be appreciated that the sanitization protocol involving conventional disinfectants alone, had no effect on the presence of bacterial colonies (**Fig. 3a**). Conversely, a dramatic decrease in bacterial growth was evident when the conventional disinfectants were used in combination with vaporized EO (**Fig. 3b**).

The baseline and postdisinfection total bacterial, fungi and yeast counts observed in the selected sampling sites (in room tables, cabinets and handrails in corridors), monitored before treatment

beginning (day 0), during sample treatment (day 30th, 60th, 90th and 120th) and after one month from treatment suspension (from day 121th to day 150th) are reported in **Fig. 4**.

The highest bacterial contaminations were found on table surfaces (tables: 100-200 colonies/cm^2; cabinets: 10-30 colonies/cm^2; handrail: 30-80 colonies/cm^2).

Compared to rooms subjected to standard disinfection only, a stable reduction of microbial charge induced by the exposure to the vaporized EOS mixture was observed in thein room sampling sites until the fourth month of treatment (tables>90%, P<0.001; cabinets surfaces>75%, P<0.001, **Fig. 4**).

One month after suspension of the EOtreatment, the values of microbial contamination in the treated rooms increased again to overlap those measured in control rooms, confirming that the observed antibacterial action was not due to external environmental factor.

By contrast, the bacterial population on the handrails of both treated and control corridors did not show any significant difference in the mean bacterial counts measured throughout the study.This indicated that, very likely, the lower concentration of the EO antimicrobial agents in corridor due to dilution in airreduced their capacity to inhibit the microbial growth.

Drugs for nosocomial related pathologies

In **Table 1** are reported the data regarding medical prescriptions, and the relative total days of treatment, issued to treat the pathologies (mainly infections and airways/respiratory problems) arisen in the patients during their hospitalization (n=120 days).

As expected, antibiotics were the most prescribed drug class, with a total number of n=26 prescriptions generatinga total of 102 days of pharmacological treatment. Antibiotics were followed by mucolytic drugs (n=14 prescriptions, 84 days of treatment), bronchodilators (n=7 prescriptions, 52 days of treatment), NSAIDs (n=3 prescriptions, 7 days of treatment) and corticosteroids (n=6 prescriptions, 3 days of treatment).

In all cases, areduction in both number of prescriptions and days of treatment was observed for the patients in the EO treated branch of the department with an overall total decrease of 80% and 86% respectively.

DISCUSSION

In this study, we evaluated the disinfectant efficacy of an EO mixture against microbial organisms present in the hospitalisation department of a health care house.

For its dispersion into the room environment, we choose an ultrasound device due to its ability to produce a uniform, constant and more reproducible water/EO aerosol comparing to conventional spraying systems.The results reported in this study clearly showed that during the ultrasound mediated dispersion of the EO mixture, each of its component was incorporated in the aerosol according to its abundance only, and not depending from its polarity or boiling point, allowing a continuous administration of aerosol with constant composition. This situation was completely different from that expectable in case of the thermally induced vaporization, which leads to the gasification of more volatile components first, and then of those endowed with increasing boiling point (more polar and/or with higher molecular weight).

As sampling tool,we choose contact slides. These devices have been previously applied to monitor the contamination from methicillin and vancomycin-resistant microbial agents in in hospital environments[9].

Other previous reported applications of contact slides regarded, for example, the monitor of environmental microbial contamination in the International Space Station, or the estimation of the number of cultivable aerobic bacteria and gram-negative enteric bacilli in poultry feathers[10,11].

Among the environmental Gram positive and Gram negative bacteria detectable using the contact slides, there are several species involved in communicable diseases occurring in hospitals and health care hoses, in particular in those treating elder patients. *Enterococcus faecalis* has been associated to nosocomial infections and identified in teeth root during canal treatment, *Escherichia coli* can be present due to fecal contamination, *Proteus vulgaris* from fecal contamination is a common cause of wound and urinary tract infections especially during long-term hospitalization, while Staphylococcus aureus is a diffused cause of respiratory tract and skin infections.

Salmonella typhimurium, although not generally considered as an environmental contaminant, has been identified as the cause of numerous Salmonella outbreaks in nosocomial hospitals [12].

Regarding yeasts and molds assay selectivity among the detectable species of interest there are*Candida albicans* (known to induce oral und urinary infections), *Aspergillus niger* (the cause of aspergillosis), and *Saccharomyces cerevisiae* (sometimes involved in ulcerative colitis).

Our results show an evident correlation between infection events and inflammatory problems in patients,withthe presence of environmental microorganisms.

The aerial exposure of room surfaces to the EOmixture components induced a dramatic reduction (in some cases near to full eradication) of their microbial contamination and, by consequence, a reduction of infection transmissions with concomitant decrease of drugs administration for their treatment.

The same reduction in drugs consumptionwas observed for all the patients dwelling the rooms in the sector treated with EO, and not only for those dwelling rooms with EO diffusors. This finding stronglysuggested that the air dispersed EOcan exert a direct protective effect on the patients' respiratory tract, not only mediated by the reduction of the bacterial populations.

The protective efficacy in patients dwelling rooms adjacent to those in which diffusors were working, compared to the lack of antibacterial action in the correspondent corridors, can be explained in terms of patient exposure to the cumulative dose of residual, air diluted EOabsorbed by the patients through the physiological respiration process.

Theseresults indicate that the antimicrobial effect can be transferred to distant rooms due to the air circulation occurring during the daily hospital activities (during which rooms doors remain open and patients and staff move within and between the different sectors), in spite of the loss of EOdisinfection efficacy oncontact surfaces due their dilution by environmental air circulation.

However, the clarification of this finding will require further future experimental work, as the microbial monitoring was conducted only in rooms where the diffusors were placed.

The main limitation of the present study due to its experimental design, is that no univocal mechanism of antimicrobial action can be proposed for the marked inhibitory effect of the EO mixture used, so that further work is needed to identify the mechanism(s) of action of its components.

However, the increasing number of detailed studies appearing in the recent scientific literature in the last years, help better understand the multifaceted activity of the numerous components present in EO with variable composition and concentrations.

Previous studies [13]showed that the EO from*Helichrysumitalicum* significantly reduces the multidrug resistance of Enterobacteraerogenes, *E. coli*, *P. aeruginosa*, and *A. baumannii*[13]. Interestingly, the authors also found that combinations of the two most active fractions of the EO,with each other or with phenylalanine arginine β-naphthylamide, yielded synergistic activity. Isolated geraniol, one of the EO components, increased the efficacy of β-lactams, quinolones, and chloramphenicol [13].

Rao et al, showed that carvacrol, a component of EO from several plant species, induces a robust transcriptional response in *S. cerevisiae*, closely resembling that of calcium stress and with genetic responses highly similar to those elicited by rapamycin [14].

In addition, recently reportedresults demonstrated the capacity of carvacrol to generate stress in the endoplasmic reticulum of *C. albicans*. These key results suggested that EO components may exert antifungal activitythrough activation of specific signalling pathways downstream of cellular interaction rather than nonspecific modification of membrane properties, this latter the way EO were previously commonly believedto act [15].

Finally, Malic et al., investigating the antimicrobial effects of EO against bacteria associated with urinary catheter infection and cultivated in planktonicalform or as biofilms, found that biofilms were up to 8-fold more tolerant of the test agents [16]. One EO of the components, eugenol, exhibited higher antimicrobial effects against both planktonic cells and biofilms comparing to terpinen, tea tree oil, and cineole.

In conclusion, the results reported in the present study highlight the utility of bioactive volatiles from essential oils in controlling and reducing the hospital contamination from environmental microbes, contributing to reduce infective events, and of related drugs administrations, in patients subjected to long-term hospitalisation periodsand for this reason always at risk to enter in contact with resistant pathogens.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

CONFLICT OF INTEREST STATEMENT

None declared.

CITATIONS

1.**Abreu AC, Tavares RR, Borges A, Mergulhão F.Simões M.**2013.Current and emergent strategies for disinfection of hospital environments. J AntimicrobChemother**68**:2718–2732.

2. **Lang, G., Buchbauer, G.**2012.A review on recent research results (2008–2010) on essential oils as antimicrobials and antifungals. A review.FlavFragr J **27:** 13–19.

3.**Seow, Y.X., Yeo, C.R., Chung, H.L., Yuk, H-G.** 2014. Plant essential oils as active antimicrobial agents. *Critic Rev Food SciNutr***54**: 625–644.

4. **Inouye, S., Takizawa, T., Yamaguchi, H.** 2001 Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J AntimicrobChemother 47, 565–573.

5. **Hood JR, Burton D, Wilkinson JM, Cavanagh HMA.** (2010). Antifungal activity of Leptospermum petersonii oil volatiles against Aspergillus spp. in vitro and in vivo. J AntimicrobChemother**65**: 285–288.

6. **Laird K, Armitage D, Phillips C.** 2012. Reduction of surface contamination and biofilms of Enterococcus sp. and Staphylococcus aureus using a citrus-based vapour. J Hosp Infect **80**: 61–66.

7. **Ali A, Pheng TW, Mustafa MA.**2015. Application of lemongrass oil in vapour phase for the effective control of anthracnose of ‘Sekaki’ papaya. J Appl Microbiol 118: 1456–1464.

[8] Fox, J. 2015. Getting started with the R commander: a basic-statistics graphical user interface to

R. J Statistical Softw**14**:1–42.

9. **Squeri R, Grillom OC, La Fauci, V.** 2012Surveillance and evidence of contamination in hospital environment from meticillin and vancomycin-resistant microbial agents. J Prev Med Hyg**53**: 143–145.

10.**Castro VA, Thrasher AN, Healy M, Ott CM, Pierson DL.** 2004. Microbial Characterization during the Early Habitation of the International Space Station. MicrobEcol**47**: 119–126.

11. **Ichida JM, Krizova L, LeFevre CA, Keener HM, Elwell DL, Burtt EH.** 2001. Bacterial inoculum enhances keratin degradation and biofilm formation in poultry compost. J Microbiol Methods **47**:199–208.

12.**Lee MB, Greig JD.** 2013. Review of nosocomial Salmonella outbreaks: infection control interventions found effective. Public Health **127**: 199-206.

13.**Lorenzi V, Muselli A, Bernardini AF, Berti L, Pagès JM, Amaral L, Bolla JM.**2009. Geraniol restores antibiotic activities against multidrug-resistant isolates from Gram-negative species. Antimicrob Agents Chemother**53**: 2209-2211.

14**Rao A, Zhang Y, Muend S, Rao R.** 2010. Mechanism of antifungal activity of terpenoidphenols resembles calcium stress and inhibition of the TOR Pathway. Antimicrob. Agents Chemother**54**:12 5062-5069.

15.**Chaillot J, Tebbji F, Remmal A, Boone C, Brown GW, Bellaoui M, Sellam A. 2015.** The monoterpene carvacrol generates endoplasmic reticulum stress in the pathogenic fungus Candida albicans. Antimicrob Agents Chemother. doi: 10.1128/AAC.00551-15

[16] **Malic S, Jordan RPC, Waters MGJ, Stickler DJ, Williams DW.** 2014. Biocide Activity against Urinary Catheter Pathogens. Antimicrob Agents Chemother. February **58**: 1192-1194.

Legends to figures

Fig. 1. Hospital planimetry.

Fig. 2. GC-MS profile (A) of the original EO mixture diluted in ethylacetate and (B) of the components extracted from the nebulized EO/water aerosol. Major identified compounds:santene (5.37 min), α-pinene (6.87 min), camphene (7.43 min), β-pinene (8.40 min), β-myrcene (8.82 min), cyclofenchene (9.55 min), p-cymene (10.24 min), eucalyptol (10.54 min), β-ocymene (11.10 min), γ-terpinene (11.60 min), terpinolene (12.76 min), β-linalool (13.50 min), isomenthone (16.34 min), endo-borneol (16.68 min), L-terpinen-4-ol (17.05), α-terpineol (17.74 min), β-citronellol (19.16 min), linalyl acetate (20.10 min), trans-geraniol (20.22 min), citronellyl acetate (25.74 min), bornyl acetate (21.63 min), geranyl acetate (25.74 min), β-caryophyllene (27.33 min), α-muurolene (28.25min), β-famesene (28.76 min).

Fig. 3. Representative visual representation of bacterial colonies development on contact slides after exposure to table surfaces, before (left) and after (right) sanitization with conventional disinfectants (a) or with conventional disinfectants in combination with vaporized EO (b).

Fig. 4. Time dependent course of the microbial environmental populations observed throughout the study in control (CTR, continuous line) and treated (EO, dotted line) rooms. EO vaporization was suspended at the 120^{th} day. Asterisks indicate the level of significance of differences between counts overall means in control (CTR) and EO treatment (T) groups (values monitored from 30th to 120^{th} day). *P<0.05, ***P<0.005, n.s. not significant (Student's T-test).

*Manuscript

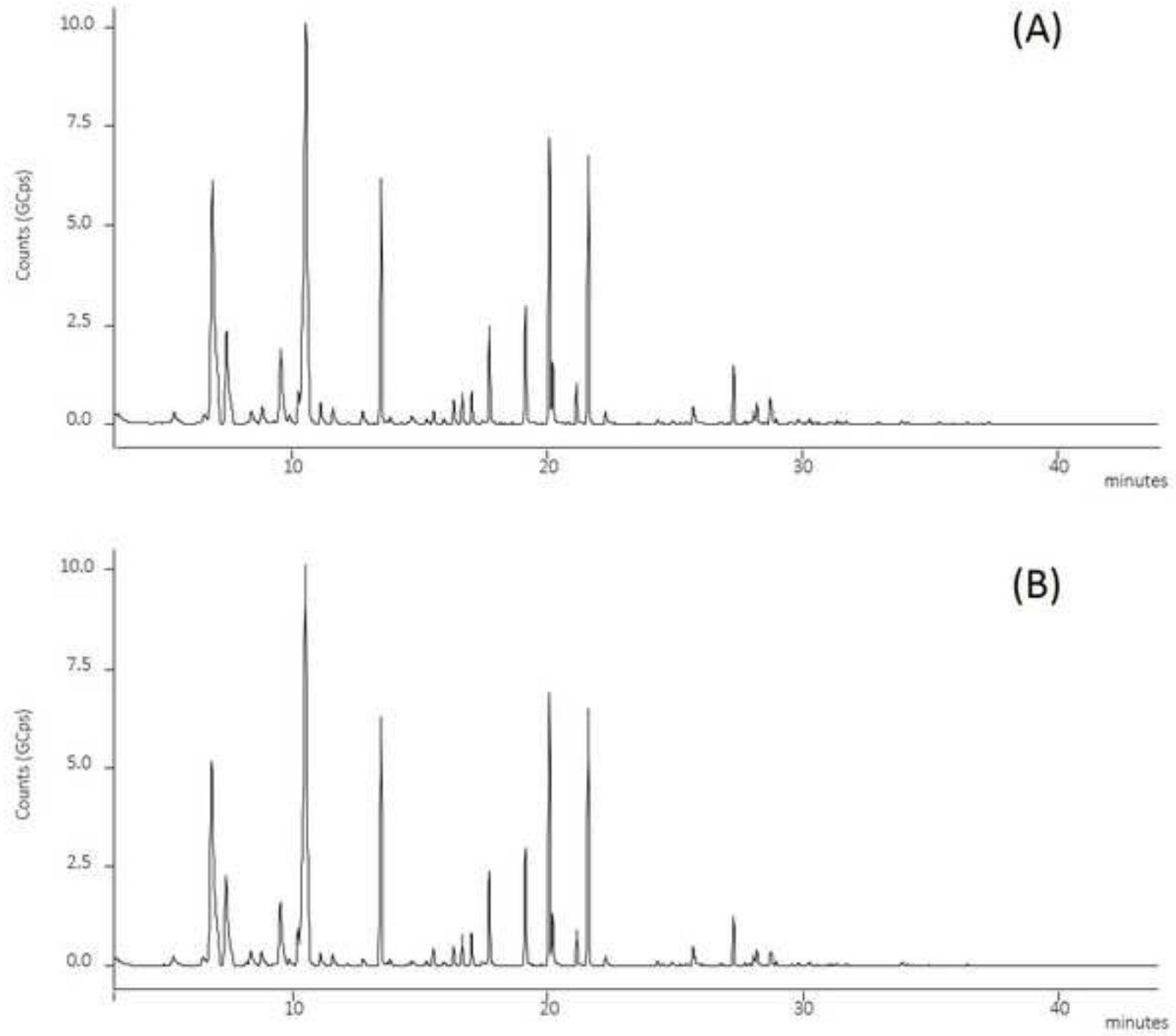
Click here to download high resolution image

Click here to view linked References

*Manuscript

Click here to download high resolution image

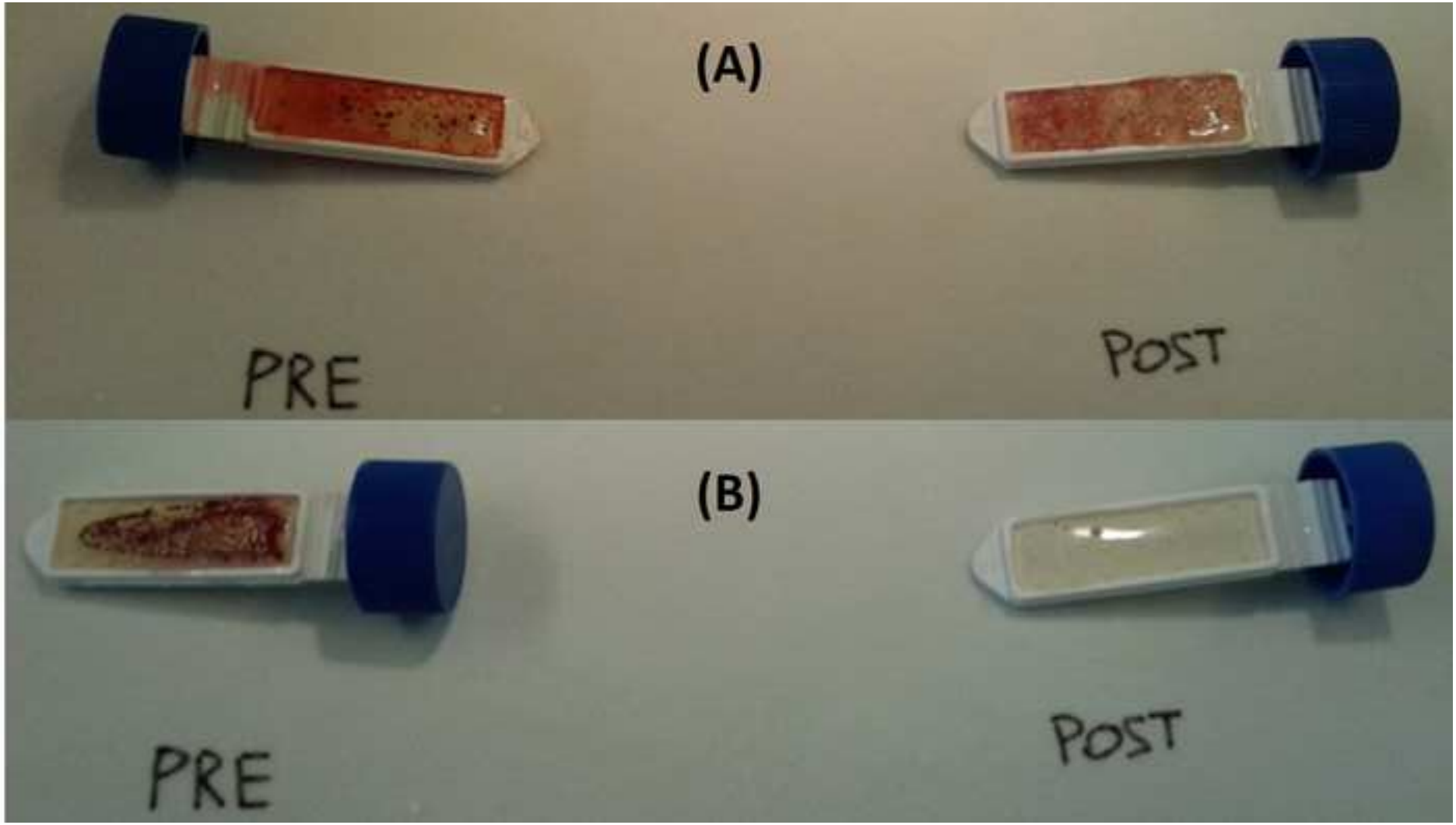
Click here to view linked References



*Manuscript

Click here to download high resolution image

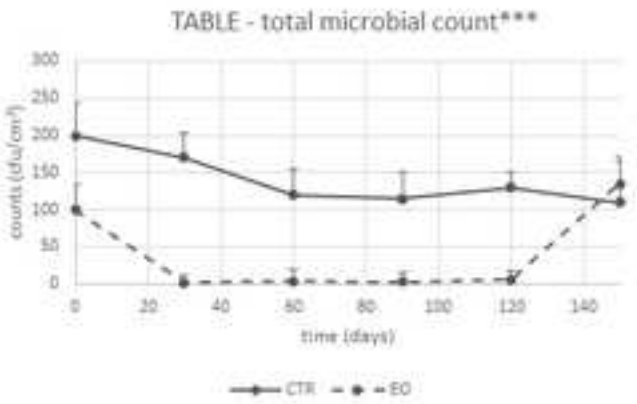
Click here to view linked References

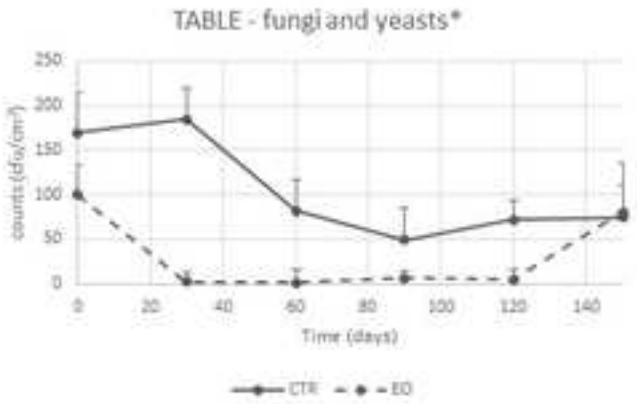


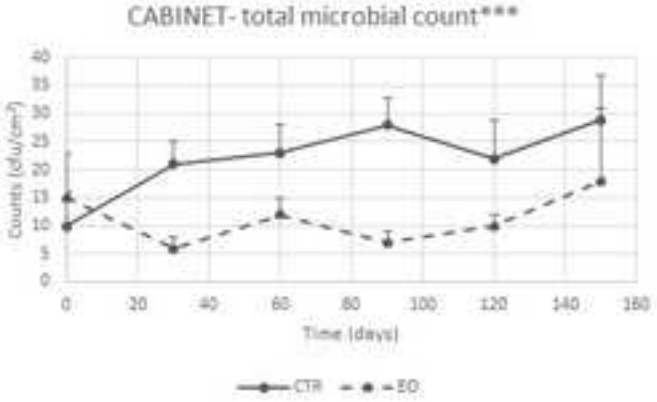
*Manuscript

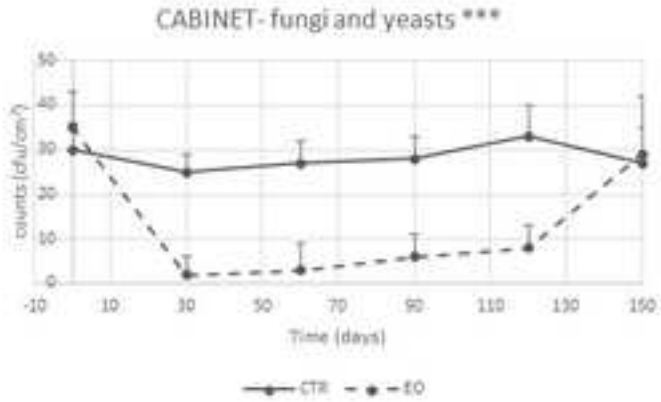
Click here to download high resolution image

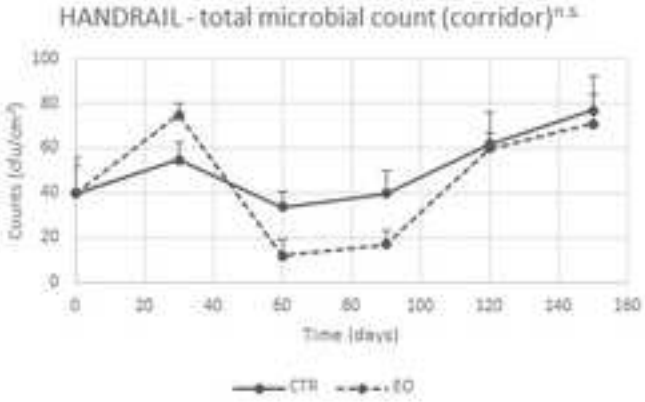
Click here to view linked References











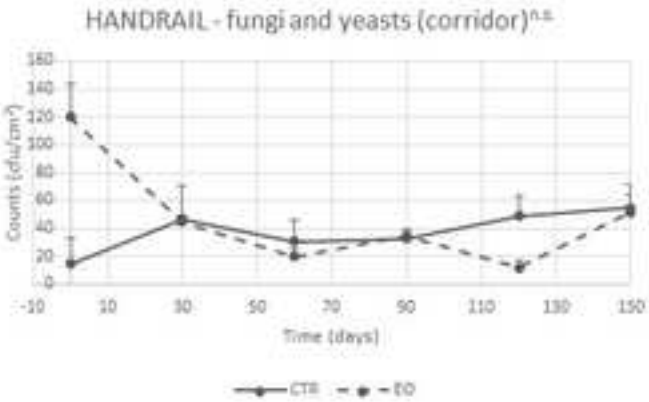


Table 1. Total number of pharmaceutical drugs prescriptions issued for patients in control (CTR) and treated (EO) rooms throughout the study period.Antibiotics: sulphametoxazol+trimetoprim (Bactrim®), amoxicillin, levofloxacin, ceftriaxone; mucolytics: acetylcysistein, bromexin; Bronchodilator: salmeterolxinafoate; NSAIDs: paracetamol, ketoprofen, ibuprofen; corticosteroids: fluticasone, betamethasone, desametasone.

Group:	**CTR**		**EO treated**		**Δ (%, EO vs CTR)**	
Drug class	**Ps**	**days**	**Ps**	**days**	**Ps**	**days**
Antibiotics	20	102	6	25	-70	-76
Mucolytics	14	84	0	0	-100	-100
Bronchodilators	7	52	0	0	-100	-100
NSAIDs	3	7	2	4	-33	-43
Corticosteroids	6	34	2	10	-77	-70
Total	50	275	10	39	-80	-86