

## ORIGINAL ARTICLE

# Oregano essential oil as an antimicrobial additive to detergent for hand washing and food contact surface cleaning

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**Keywords**

antimicrobials, disinfection, essential oil, food safety, oregano.

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**Abstract****Aims:** To investigate the potential use of oregano essential oil as an antimicrobial agent in liquid soap for hand washing and for food contact surface cleaning.**Methods and Results:** Oregano essential oil (O.E.O.) was emulsified in liquid detergent solution. This was challenge tested against a commercial antimicrobial soap in hand washing trials using natural flora. Soap with O.E.O. was as effective as the commercial antimicrobial soap at reducing aerobic plate count on the hands and more effective than plain soap with no additives. Cloths wetted with soap with O.E.O. were used to clean three different surfaces contaminated with four bacterial pathogens. For three of the four pathogens, the addition of 0.5% v/v O.E.O. to the soap solution enhanced cleaning performance and also reduced bacterial survival on the cloth after cleaning.**Conclusions:** Oregano essential oil (0.5%) is effective as an antimicrobial additive to detergent solutions for hand washing and surface cleaning.**Significance and Impact of Study:** This preliminary study has shown that oregano essential oil is a potential alternative to antimicrobials used in various detergents, such as chloroxylenol and triclosan, which can have adverse environmental and health effects. Further development could lead to a commercial product.**Introduction**

The hands are continually in contact with the environment and therefore can be contaminated by a very wide range of bacterial species, which may then be transferred to foods or food contact surfaces. A study of the hands of 204 homemakers (nonworking parents of preschool children) found 48 different species of Gram-negative bacteria and 12 species of coagulase-negative staphylococci. Most prevalent species were *Pseudomonas fluorescens/putida*, *Staphylococcus warneri*, *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterobacter cloacae*, while the mean mesophilic aerobic count per hand was 5.7 log CFU (Aiello *et al.* 2003). The most frequently isolated bacteria from the hands of food handlers at a Turkish military training hospital were *Staph. aureus*, coagulase-negative staphylococci, *Bacillus subtilis*, other *Bacillus* spp. and diptheroid bacilli (Ayçiçek *et al.* 2004). A study of

50 delicatessen food handlers by Lues and van Tonder (2007) found aerobic plate counts ranging from 'negligible' up to 88 CFU cm<sup>-2</sup> on the palms (the latter figure equates to roughly 4.5 log CFU per hand). A distinction can be drawn between transient and permanent flora, the former being those bacteria that are present at a given moment due to a chance contamination but that will not become established, while the latter are those that have colonized the skin (Teyssou *et al.* 1997).

As bacteria on the hands can potentially contaminate foods and food preparation surfaces, it is desirable to have available an effective antimicrobial soap for hand washing in a domestic and catering environment. Several are available on the market; one example, used as a comparison in these experiments, is Dettol antibacterial hand soap, which contains chloroxylenol as a biocidal agent. This compound can cause contact allergies in some individuals and is toxic if inhaled or ingested

(Magee 1998; Anon. 2006). Another commonly used antimicrobial, triclosan, may be implicated in the development of antibiotic resistance in bacteria (Aiello *et al.* 2007). An alternative antimicrobial ingredient for soap would therefore be beneficial, especially for individuals who are sensitive to other commonly used compounds. Oregano essential oil, more specifically its main active component carvacrol, has a well-established antimicrobial activity and has been tested in a variety of foods as a preservative and to control pathogenic bacteria (Tajkarimi *et al.* 2010). Baydar *et al.* (2004) tested oregano essential oil *in vitro* for inhibitory activity against a total of 15 different bacteria (Gram-positive and Gram-negative) and observed inhibition against all 15 when the oregano E.O. concentration was 2% v/v and against 12 of the 15 strains at 1% v/v concentration. Minimum inhibitory concentrations vary according to several factors, but are of the order of 0.02–0.5% v/v in clean buffer systems according to a review by Burt (2004). The use of essential oils in soap has not been extensively investigated to the knowledge of the authors of the current work. 'Hygienic skin wash' containing tea tree oil is commercially available and was investigated by Messenger *et al.* (2005), who did not find it to be significantly more effective than ordinary soft soap in challenge tests with *Escherichia coli* K12 inoculated onto the subjects' hands. Alcohol-based hand rubs have been shown to have their antimicrobial activity enhanced by the addition of various essential oils, which can act synergistically with other antimicrobial components in the product (United States Patent number US 6,884,763 BT Willard *et al.* 2005; Shintre *et al.* 2006). However, although these are hand cleansing products and are designed to kill the microbial flora of the skin, they are not soap based and are designed to be used without water and are therefore somewhat different to the soap product being examined in the current work.

Bacteria on the hands, together with those from other sources, can also contaminate food preparation surfaces such as worktops and chopping boards. Food-borne pathogenic bacteria may remain viable on surfaces for several days and can be transferred from surfaces to foods or from one surface to another via cleaning sponges (Kusumaningrum *et al.* 2003). The ease with which bacteria can be removed and/or killed depends on, among other things, the bacterium itself, the surface properties (Gough and Dodd 1998), extent and type of soiling with organic residues (Kuda *et al.* 2008), the time for which the bacterium has been present on the surface and the maturity of the biofilms formed (Nguyen and Yuk 2013; Stojic and Haapasalo 2013), environmental conditions (Nguyen and Yuk 2013) and the sanitizing method used (Bae *et al.* 2012; Koo *et al.* 2013).

In this study, the use of liquid soap containing oregano essential oil as an antimicrobial additive as a hand washing and food contact surface cleaning detergent was investigated.

## Materials and methods

### Bacterial cultures

Bacterial cultures used were *Salmonella enterica* serotype Typhimurium DT193, *E. coli* O157:H7 NCTC 12900 (nontoxigenic), *Staph. aureus* ATCC 6538 and *Listeria monocytogenes* NCTC 10527. Stock cultures were stored on cryogenic storage beads (Pro-Lab Diagnostics, Richmond Hill, ON, Canada) at  $-20^{\circ}\text{C}$  and resuscitated when required by streaking a bead onto plate count agar (PCA; Lab M Ltd., Bury, UK) and incubating at  $37^{\circ}\text{C}$  for 18–24 h. Cultures for the experiments were prepared by inoculating 10 ml tryptone soy broth (TSB; Lab M Ltd.) with an isolated colony from the PCA and incubating at  $37^{\circ}\text{C}$  for 18–20 h.

### Soap solutions

The test soap solution used in all experiments was Cleaner LAN (Loufakis Chemicals SA, Thessaloniki, Greece), a commercial/industrial detergent solution comprised of the sodium salt of alkylbenzenesulfonic acid, sodium lauryl ether sulfate, cocodiethanolamide, ethyl hydroxyl cellulose and NaCl, together with methylchloroisothiazolinone as an in-can preservative. The concentrated detergent solution was diluted 1/5 by volume with distilled water and filter-sterilized prior to use. The commercial antibacterial soap was Dettol antibacterial cream soap (Reckitt Benckiser, Hull, UK), which was used as supplied. Oregano essential oil was provided by Panaroma Ltd., Thessaloniki, Greece, and contained 73.5% w/v carvacrol and 2.1% w/v thymol (data from Panaroma Ltd.).

### Hand washing trial

The hand washing trial was conducted using student and staff volunteers from the Faculty of Food Technology and Nutrition, TEI Thessaloniki and examined the naturally occurring flora of the hands. Soap solutions and controls were tested in pairs on each volunteer, one hand being used for one washing solution and the other hand for the other solution. The pairs used are provided in Table 1 (Results section). The test procedure for each person was as follows. All jewellery was removed from the hands. The hands were examined, and persons with cuts, grazes or other abnormalities were rejected. The person under test rinsed their hands with light rubbing under running

**Table 1** The residual microbial population of the hand after washing once with one of three different soaps or with water only (paired comparisons)

Washing treatment	N	Mean APC per hand (CFU) $\pm$ SD	Paired <i>t</i> -test ( <i>P</i> )
Water	38	2.9 $\pm$ 0.63	<0.01
Commercial antimicrobial soap		2.2 $\pm$ 0.69	
Water	30	3.0 $\pm$ 0.68	<0.01
Soap with oregano E.O.		2.1 $\pm$ 0.79	
Plain soap	30	3.1 $\pm$ 0.79	<0.01
Soap with oregano E.O.		2.5 $\pm$ 0.89	
Commercial antimicrobial soap	30	2.4 $\pm$ 0.68	0.12
Soap with oregano E.O.		2.3 $\pm$ 0.73	

N, number of subjects; APC, aerobic plate count; O.E.O., oregano essential oil, 0.5% v/v.

tap water for 30 s to remove superficial contamination. The hands were then dried by shaking in air (without fans etc.). A latex glove was placed on one randomly chosen hand. A 3.0 ml aliquot of either the test solution or the control was pipetted into the palm of the bare hand, which was then used to wash the hands by rubbing the bare and gloved hands together in a manner standardized as much as possible (with particular focus on the palm and insides of the fingers) for a period of 30 s. The hands were then rinsed for 30 s in running tap water, the glove was removed, and the hands were allowed to dry in the air. The hand that was bare while washing was then swab sampled. A fresh glove was placed on the previously bare hand, and the procedure was repeated with the second soap/control solution, so that each of the person's hands was washed with a different washing solution. The choice of hand for each solution and the order of washing were randomly selected for each person.

#### Enumeration of the hand microflora

The hands were sampled for aerobic plate count as follows. A cotton swab (stick type) was moistened in quarter-strength Ringer solution (hereafter referred to as Ringer solution; Lab M Ltd.) containing 0.5% v/v Tween 80 (referred to as Tween Ringer hereafter; Tween 80 from Merck KgaA, Darmstadt, Germany) and used to swab the palm in a regular, repeatable pattern of horizontal and vertical swabs. The swab was then snapped off into a test tube containing 9 ml Tween Ringer. A second moistened swab was used to sample the fingers (palm side and between, not the back) and placed into the same test tube as the previous swab. The tube was then vortex-mixed for 30 s, further serial decimal dilutions were performed as required in plain Ringer solution, and the sample was

inoculated into PCA using a pour plate technique. Plates were incubated at 37°C for 24 h before colonies were enumerated. The 24 h incubation period was chosen following preliminary trials (data not shown) in which further incubation (up to 48 h) was not found to significantly increase the number of colonies, even in samples treated with soap with oregano E.O. or with commercial antimicrobial soap, which might be expected to contain stressed cells. Incubating for only 24 h also eliminated occasional problems caused by overgrowth of colonies. At least 30 subjects were studied for each pair of washing solutions. Raw counts were converted to log CFU per hand. Data were analysed in two ways. Firstly, paired *t*-tests were performed on each pair of washing solutions using Microsoft Excel 2007 to determine whether significant differences existed between the treatments. Secondly, all data from the same treatment were pooled and the pooled data compared using unpaired *t*-tests.

#### Wipe experiments

##### Wipes

Domestic wiping cloths (AquaPur Universal, Lidl Hellas) were cut into 6  $\times$  6 cm squares and sterilized by autoclaving at 121°C for 15 min in a sealed container. Each cloth square was placed in a sterile plastic Petri dish and moistened with 2.5 ml of the test solution, which was distributed over the surface and allowed to disperse within the cloth for a few seconds. This quantity was such that the cloth was wet to the touch but did not drip if lifted.

##### Inactivation of bacteria on the wipes

Overnight cultures of the four test bacteria were prepared as described in the section 'Bacterial cultures' above and diluted 1/10 with Ringer solution. Cloths were prepared with distilled water, plain soap and soap with oregano oil at concentrations of 0.05, 0.2, 0.5 and 1.0% v/v. For each test, 100  $\mu$ l of bacterial suspension was inoculated in a diagonal line across the square wipe. After exactly 2 min, the wipe was transferred to 50 ml Tween Ringer in a plastic sample bag and placed in a laboratory blender (Stomacher 400, Seward Medical, London, UK) on medium speed for 30 s. Further decimal dilutions were carried out in Ringer solution as required, and the diluted samples were plated into PCA using a pour plate technique. Plates were incubated at 37°C for 24 h before colonies were enumerated. All experiments were carried out twice with duplicate samples per experiment, giving a total of four replicates. Raw counts were converted to log CFU per wipe. In cases where no colonies were isolated, an arbitrary value of 2.4 log CFU per wipe was assigned

(0.5× the arithmetic assay detection limit). Statistical significance was determined using analysis of variance with Tukey HSD *post hoc* test (ezANOVA v. 0.98).

#### Removal of bacteria from surfaces

Three surface materials were used: stainless steel, wood and plastic. The latter two were domestic chopping boards (Ikea Hellas Ltd., Athens, Greece), while the stainless steel surface was the underside of a test-tube rack. Surfaces were sanitized with ethanol prior to use. Overnight bacterial cultures were prepared as described in the section 'Bacterial cultures' above and then diluted 1/10 in fresh TSB. A 100 µl aliquot of culture was inoculated onto the test surface, spread around to cover a circular area of diameter approximately 5 cm and allowed to dry for one hour at room temperature. Cloth wipes were prepared as mentioned above using 2.5 ml of sterile distilled water, plain soap or soap with 0.5% v/v oregano essential oil. The wipes were then used to clean the bacteria from the surface using the following technique in order to standardize the wiping procedure and avoid pressure spots. The wipe was held by laying it over the inside of the middle finger of a gloved hand so that it curved around the inside of the finger in a semi-circular shape when viewed edge on. It was held in place by gripping with the two adjacent fingers. The middle finger with the wipe underneath therefore formed a ridge that was the contact surface as the hand was held palm-downwards towards the surface to be cleaned. The other fingers were slightly raised to avoid accidental contact with the surface. The inoculated area was cleaned by wiping three times left-right-left and three times up-down-up, giving a total of 12 passes over the target area. The pressure exerted on the surface was kept as similar as possible both within and between individual inoculated areas. The wipe was then placed in a sample bag with 50 ml Tween Ringer and placed in a laboratory blender on medium speed for 30 s. Further decimal dilutions were carried out in Ringer solution as required. The inoculated area was then swabbed twice with cotton stick swabs moistened with Tween Ringer and exerting a firm pressure. Approximately 20 passes were made with each swab. Both swabs were placed in the same tube with 9 ml Tween Ringer. The tube was vortex-mixed for 30 s before further serial decimal dilutions were performed as required in plain Ringer solution. Diluted samples from the wipes and swabs were inoculated into PCA using a pour plate technique. Plates were incubated at 37°C for 24 h before colonies were enumerated. Experiments were carried out twice with duplicate samples per experiment, giving a total of four replicates. Raw counts were converted to log CFU per wipe or per swab. In cases where no colonies were isolated from a wipe or swab, an arbitrary value of

2.4 log CFU per wipe or 0.65 log CFU per swab was assigned (0.5× the arithmetic assay detection limit). Statistical significance was determined using analysis of variance with Tukey HSD *post hoc* test (ezANOVA v. 0.98).

## Results

### Hand washing study

The results of the hand washing study are presented in Tables 1 and 2. Table 2 shows the pooled data in which all results for a given treatment are combined, and Table 1 shows the same data analysed in the original test pairs. Plain soap did not decrease the number of residual micro-organisms on the hands relative to washing in water, but soap with 0.5% oregano essential oil and the commercial antimicrobial hand soap significantly ( $P < 0.01$ ) reduced the viable count on the hands by 0.7 log CFU per hand relative to water. Commercial antimicrobial soap was not significantly different to the soap with oregano E.O. The paired results showed that the commercial soap decreased the microbial load on the hands by 0.7 log CFU per hand relative to water. Soap with oregano E.O. achieved a 0.9 log CFU per hand reduction relative to water and 0.6 log CFU per hand relative to plain soap. The commercial soap and the soap with oregano E.O. were not significantly different ( $P > 0.01$ ).

### Direct inoculation of antimicrobial wipes

Figure 1 shows the effect of oregano E.O. concentration on the number of pathogenic bacteria surviving 2 min after inoculation onto a wipe (intended for surface cleaning), which was moistened with water or soap solution containing different concentrations (0, 0.05, 0.2, 0.5 and 1.0% v/v) of oregano E.O. *Listeria monocytogenes* was reduced to undetectable levels by the plain soap, while *Staph. aureus* was reduced by around 2 log CFU per wipe

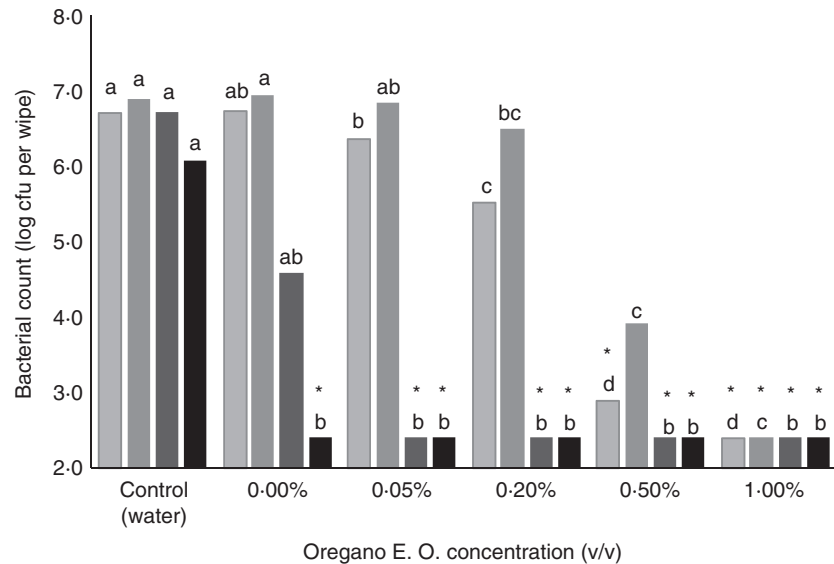
**Table 2** The residual microbial population of the hand after washing once with one of three different soaps or with water only (combined data)

Washing treatment	N	Mean APC per hand (CFU) ± SD
Water only	68	3.0 ± 0.65 <sup>a</sup>
Plain soap	30	3.1 ± 0.79 <sup>a</sup>
Soap with oregano E.O.	90	2.3 ± 0.82 <sup>b</sup>
Commercial antimicrobial soap	68	2.3 ± 0.69 <sup>b</sup>

N, number of subjects; APC, aerobic plate count; O.E.O., oregano essential oil, 0.5% v/v.

Superscript letters indicate significant differences ( $P < 0.01$ ).

**Figure 1** The number of bacteria surviving 2 min after inoculation onto a wipe soaked with water (control) or soap solution containing different concentrations of oregano essential oil (0, 0.05, 0.2, 0.5 and 1.0% v/v). Bars represent mean (of four) counts of *Salmonella* Typhimurium (light grey), *Escherichia coli* O157 (mid grey), *Staphylococcus aureus* (dark grey) and *Listeria monocytogenes* (black). Letters indicate significant ( $P < 0.01$ ) differences between concentrations for the same organism. \*Some or all of the four replicates were below the detection limit of 2.7 log CFU per wipe and were assigned an arbitrary value of 2.4 log CFU per wipe.



by the plain soap (relative to the water control) and was undetectable on the wipe with soap plus 0.05% oregano E.O. *Salmonella* Typhimurium and *E. coli* O157 were unaffected by the plain soap. The *Salmonella* count was reduced by 1 log CFU per wipe at 0.2% oregano E.O. and by nearly 4 log CFU per wipe at 0.5% oregano E.O. *Escherichia coli* was more resistant, being largely unaffected by 0.2% oregano E.O. and reduced by 3 log CFU per wipe at 0.5% oregano E.O. At 1.0% oregano E.O., no pathogens were detected (<2.7 log CFU per wipe).

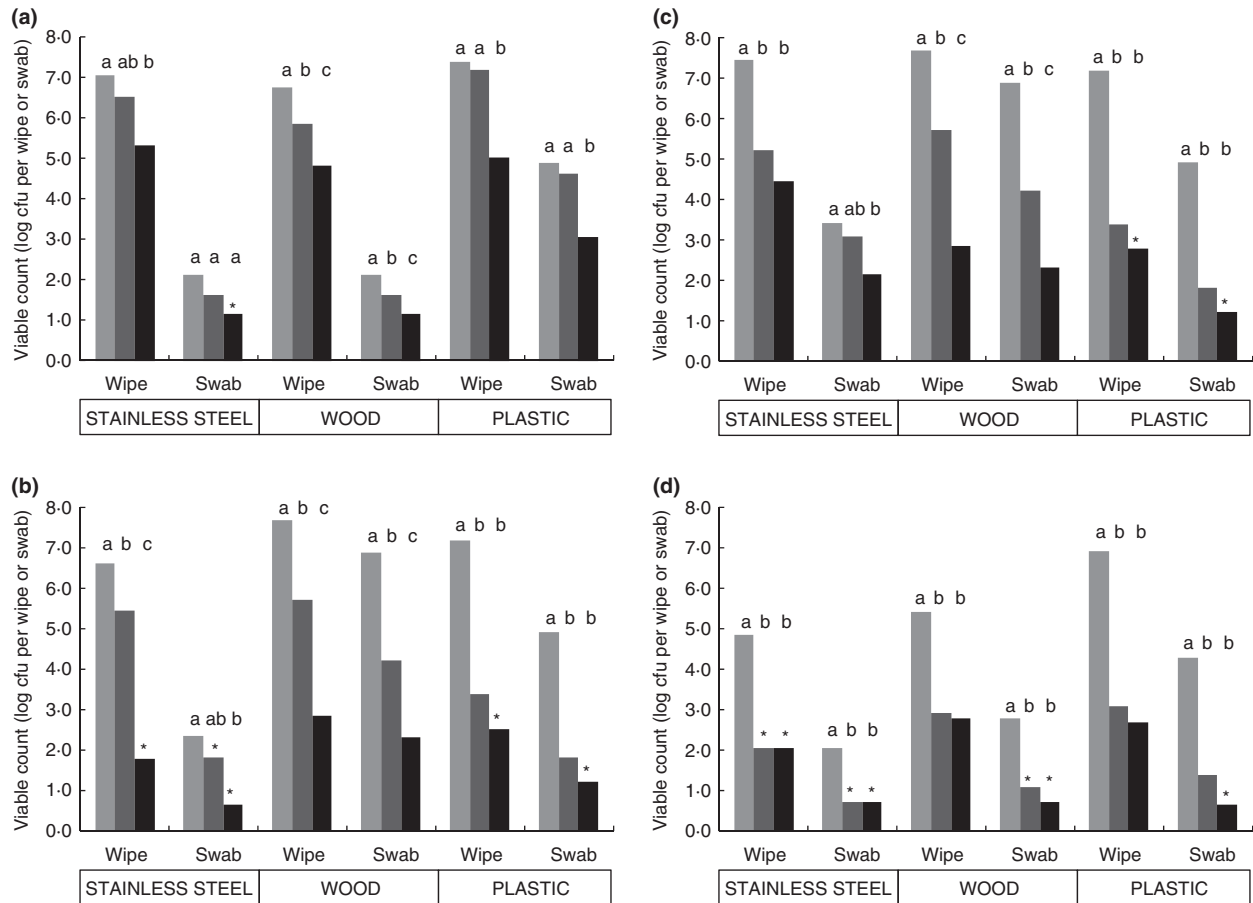
#### Surface decontamination tests

The results of the antimicrobial wipe testing against surface contamination are presented in Figure 2. In these figures, the wipe counts are the bacteria surviving on the wipe immediately after cleaning the surface, and the swab counts are the bacteria surviving on the surface immediately after cleaning. In the experiments conducted with *Salmonella* Typhimurium (Figure 2a), the decontamination characteristics of stainless steel and wood surfaces were very similar. Around 5 log CFU *Salmonella* were removed from the surface by wiping with water alone, leaving around 2 log CFU on the surface. Remaining bacteria were further reduced by the use of plain soap and soap with oregano essential oil: reductions relative to water were around 0.7 and 1.5 log CFU, respectively, on wooden surfaces and slightly smaller (and not statistically significant,  $P > 0.01$ ) on stainless steel. Greater numbers of *Salmonella* were detected on plastic surfaces after wiping: around 5 log CFU with water and plain soap as the cleaning agents, and around 3 log CFU when using soap with oregano E.O. The number of *Salmonella* surviving on the

wipe after cleaning the surface was not significantly different or of marginal significance when comparing water and plain soap, but on wipes moistened with soap with oregano E.O., the number of survivors was significantly lower (1.5–2.5 log CFU per wipe) when compared to water.

The removal of *E. coli* from surfaces is presented in Figure 2b. On stainless steel, around 2.4 log CFU *E. coli* remained after wiping with water, the count after wiping with plain soap was not significantly lower, but with soap and oregano E.O., no surviving bacteria were detected (<0.95 log CFU). When the wipe was examined after cleaning the stainless steel surface, only 1.8 log CFU viable *E. coli* were detected on the wipe containing soap with oregano E.O., compared with 5.5 and 6.6 log CFU per wipe when the wipe was moistened with plain soap and water, respectively. Viable bacteria remaining on the wooden surface after wiping with water, soap and soap with oregano E.O. were 6.9, 4.2 and 2.3 log CFU, respectively, while those remaining on the plastic surface were 4.9, 1.8 and 1.2 log CFU, respectively. Large variation was observed in the numbers of bacteria detected on the wipe immediately after cleaning the surface. These were in the ranges 6.6–7.7 log CFU, 3.4–5.7 log CFU and 1.8–2.8 log CFU for water, plain soap and soap with oregano E.O., respectively.

Surface decontamination results for *Staph. aureus* are presented in Figure 2c. Surviving bacteria detected on the stainless steel surface after cleaning were 3.4, 3.1 and 2.2 log CFU for water, plain soap and soap with oregano E.O., respectively. Corresponding survivors for the wooden surface were 6.9, 4.2, and 2.3 log CFU and for the plastic surface 4.9, 1.8 and 1.2 log CFU. The numbers of *Staph. aureus* surviving on the wipe after cleaning the



**Figure 2** The removal of bacteria from stainless steel, wood and plastic surfaces using a wipe soaked in water (light grey), plain soap (dark grey) and soap with 0.5% oregano essential oil (black). 'Wipe' represents the bacteria surviving on the wipe immediately after cleaning and 'swab' represents the bacteria remaining on the surface immediately after cleaning. The four graphs represent, with mean starting inoculum  $\pm$  standard deviation in brackets, (a) *Salmonella Typhimurium* ( $7.49 \pm 0.28$  log CFU), (b) *Escherichia coli* O157 ( $7.70 \pm 0.20$  log CFU), (c) *Staphylococcus aureus* ( $7.71 \pm 0.17$  log CFU) and (d) *Listeria monocytogenes* ( $7.04 \pm 0.30$  log CFU). Within the same group (wipe, swab) and material (stainless steel, wood, plastic), letters a-c above the bars indicate significant ( $P < 0.01$ ) differences between treatments. \*Some or all of the four replicates were below the detection limit of 2.7 log CFU per wipe or 0.95 log CFU per swab and were assigned arbitrary values of 2.4 log CFU per wipe or 0.65 log CFU per swab.

surface were in the ranges 7.2–7.7, 3.4–5.7 and 2.8–4.5 log CFU per wipe for water, plain soap and soap with oregano E.O., respectively.

Figure 2d gives the results of the surface decontamination experiments with *L. monocytogenes*. Very few (<1.4 log CFU) viable bacteria were detected on any of the three surfaces after swabbing with either plain soap or soap with oregano E.O., while the numbers of viable *L. monocytogenes* detected on the wipe moistened with either plain soap or soap with oregano E.O. ranged from 2.1 to 3.2 log CFU. There were no significant differences between the plain soap and soap with oregano E.O. *L. monocytogenes* remaining on the surfaces after wiping with water were 4.9, 5.4 and 6.9 log CFU on steel, wood and plastic, respectively.

## Discussion

From the results in Tables 1 and 2, it is clear that the plain soap was ineffective at removing and/or killing bacteria on the hands. It should be noted, however, that all the persons had hands that were visually clean and were briefly rinsed in water before the test washing. Soap could well play an important role in the removal of bacteria in cases where the hands are heavily soiled. Larson and Bobo (1992) also found that nonantimicrobial soap was not effective at reducing bacterial counts on visually clean hands, but small reduction (0.3 log CFU per hand) was observed when soap was used to wash hands that were soiled with blood. Tvedt and Bukholm (2005) examined the efficacy of nonantimicrobial soap on the hands of

hospital staff and found no reduction in bacterial count when the staff washed their hands as they wished, but a 60% reduction (equivalent to around 0.5 log CFU per hand) when a standard washing method was followed. These results illustrate the importance of washing technique as well as the low efficacy of plain soap. In the current work, addition of 0.5% v/v oregano essential oil to the soap rendered it approximately as effective as a commercial antibacterial soap at bacterial inactivation in terms of mesophilic aerobic count reduction. Differences may exist in the composition of the residual flora after washing with the two antibacterial soaps, but this was not investigated.

Rapid killing of bacteria on wipes used for surface contamination is desirable to prevent contamination being spread from surface to surface by the wipe itself (Mattick *et al.* 2003). The investigation of the effect of oregano E.O. concentration on the survival of pathogens on soap-moistened wipes (Figure 1) clearly shows the difference in sensitivity between the Gram-positive and Gram-negative pathogens. *Listeria monocytogenes* was rendered undetectable by the soap itself, probably at least in part due to the presence of the methylchloroisothiazolinone preservative, while *Staph. aureus* was not detected after 2 min in contact with soap with 0.05% oregano E.O. In contrast, 0.5% oregano E.O. was required to cause a major decrease in the viability of the two Gram-negative pathogens. Although low numbers of pathogens survived contact with the 0.5% oregano E.O. soap and none were detected after exposure to 1.0%, the former concentration was chosen for further experiments as the latter was considered to be unrealistically high from an economic standpoint.

The results of the surface decontamination experiments (Figure 2) demonstrate that the mechanical action of the wiping removes a large proportion of the bacteria, as evidenced by the decreases in bacterial counts on the surface and count on the wipe after wiping with plain water. Koo *et al.* (2013) investigated the cleaning efficacy of a variety of cleaning cloths moistened with water and found that the reduction in *L. monocytogenes* on a stainless steel surface after wiping ranged from 0.9 to 2.6 log CFU cm<sup>-2</sup> from a total inoculum of around 5.2 log CFU cm<sup>-2</sup>. The corresponding reduction observed in the current study was somewhat larger, of the order of 5.0 log CFU from a starting inoculum of around 7.0 log CFU. However, as cloths, surfaces and the manner of wiping (pressure, speed) all vary, differences in the results are to be expected. The extent of removal of bacteria was influenced by the type of surface, stainless steel being easier to decontaminate than the wood or plastic. This is most likely to be due to the topography of the surface –

the stainless steel was polished, while the wood and plastic had a rougher texture.

In many cases, the use of plain soap on the wipe significantly decreased the number of bacteria detected on the surface after wiping relative to wipes moistened with water only. This could be due to enhanced removal of the bacteria due to the detergent action of the soap. However, in the majority of cases in which the residual surface counts were reduced, the counts on the wipes themselves were also reduced, suggesting that the differences were due, at least partly, to inactivation of some of the bacteria by the soap. Further reductions were observed with the soap containing 0.5% oregano E.O. For *L. monocytogenes*, no differences were observed between plain soap and soap with oregano E.O., and for *Staph. aureus*, a significant difference was only observed on wood. These results are in agreement with the experiment in which the wipe was directly inoculated (Figure 1), in which the plain soap itself was significantly antimicrobial to the Gram-positive pathogens. The extent to which the oregano E.O. enhances the antibacterial activity of the wiping solution is more evident in the two Gram-negative bacteria. Due to their cell envelope structure, Gram-negative bacteria are often more resistant to inhibitory agents such as surfactants or methylchloroisothiazolinone and so were not inhibited by the plain soap. As oregano E.O. was not tested alone (i.e. without soap), it is not possible to determine whether it acted as the sole effective inhibitor or whether there was an additive or synergistic effect with the soap components.

These experiments have demonstrated that oregano essential oil is a potentially useful antimicrobial additive for hand soaps and could be an alternative to triclosan and chloroxylenol. It is also effective in food contact surface decontaminating solutions. Further experiments are required to investigate two main aspects: firstly, to further characterize the antimicrobial activity of the soap and oregano E.O. system, including activity against other pathogens and under conditions of heavy soiling, and secondly, to further develop the potential product by investigating different soap formulations that may be more effective as antimicrobials and more appropriate from other product technological points of view (such as viscosity and foaming).

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### Conflict of Interest

None declared.

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