Bacterial Reduction Test on Food Surfaces.

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Introduction

Epidemiological data from Europe, North America, Australia and New Zealand indicate that a substantial proportion of foodborne disease is attributed to improper food preparation practices in consumers' home (Redmond and Griffith, 2003). The failure to effectively remove bacteria from food contact surfaces can have serious implications in the transmission of foodborne disease. In the home a major concern is the transmission of foodborne pathogens by cross-contamination of foods via food contact surfaces, particularly chopping boards, which is found to be one of the top five sites most contaminated with heterotrophic bacteria in the kitchen (Josephson et al., 1997). Other studies also found cross-contamination via domestic hand and food contact surfaces to be a significant contribution to cross-infection and there is a constant risk of microbial transfer from these surfaces (Bloomfield and Scott, 1997; Cogan et al., 1999; Gorman et al., 2002).

Many antibacterial products have been developed to provide fast and effective cleaning to food preparation areas, replacing the traditional two-step detergent and rinse cleaning method. Many of these products such as sprays and wipes are cleaners containing an antibacterial agent and the instructions do not advocate rinsing after their use, despite evidence showing that rinsing is a vital step in the cleaning of domestic food contact surfaces (Cogan et al., 1999; Rusin et al., 1998; Kusumaningrum et al., 2002).

A number of investigations demonstrated effectiveness of antibacterial products on food pathogens. For example, antibacterial dishwashing liquid was effective in reducing pathogens in laboratory suspension test although not in used sponges (Kusumaningrum et al., 2002); cloths treated with quaternary ammonium compounds reduced cross-contamination significantly (Scott and Bloomfield, 1993; Lalla and Dingle, 2004) as did disposable antibacterial-treated cloths (Kusumaningrum et al., 2003). Contrarily, a study by Larson et al. (2004) of two sets of households totalling 1178 persons showed that antibacterial products did not reduce the risk for symptoms of viral infectious diseases, although they may be effective in reducing symptoms of bacterial diseases at the home. The random use of antibacterial agents has been shown to have little effect. Rusin et al. (1998) and Josephson et al. (1997) showed that even effective antimicrobial agents will not reduce levels of bacteria if not incorporated into an appropriate cleaning regime. In addition, the public may not be as vigilant with the cleaning and decontamination of food contact surfaces as they could be (Jay et al., 1999). The speed and relative ease of cleaning provided by antibacterial sprays and wipes could be favoured above that of the proven detergent rinse method of cleaning. It is therefore important to establish how effective such products are at inhibiting bacteria and protecting the public from cross-contamination.

The cutting board is of interest as it poses constant risk of infection in the domestic environment (Rusin et al., 1998). This study investigates the effectiveness of domestic antibacterial wipes and sprays in preventing cross-contamination in a household using a single cutting board initially for the preparation of raw meat followed some time later by the preparation of high-risk ready to eat food. The condition under investigation is the products effectiveness when used up to 2 h after the preparation of contaminated food but immediately before the preparation of ready to eat food.

Experimental Description of Surface Decontamination Using Lotus Spray, Lysol, Odoba, 3% Hydrogen peroxide, and vinegar/lemon juice/baking soda mixture.

Sterile laboratory top and wood and plastic food cutting board surfaces were divided into one inch squares and inoculated with either *E. coli* or Listeria sp. Six squares were used for each organism and 3 of the six were experimental and tested with either Lotus spray, Lysol, Odoban, 3% hydrogen peroxide, or vinegar/lemon juice/baking soda mixtue. The remaining 3 squares were tested as controls and were exposed using sterile phosphate buffer.

Three of squares were inoculated with the bacteria and measured after inoculation, to determine the initial bacterial number applied to the squares. Experimental squares were exposed to Lotus spray (1.25 ppm ozone) at the 15 minutes of Lotus treatment time. Lysol was aersoled, vinegar/lemon juice mixture was poured and Odoban 3% hydrogen peroxide was sprayed on to their respective squares and after 15-30 seconds of exposure, sterile quick swabs were then carefully wiped across the surfaces to remove any excess solution. Sterile quick swabs then brushed over the treatments squares and tested for bacterial levels using aerobic bacterial Petrifilm. Petrifilm after inoculation was incubated at 37C for 24 and 48 hours and bacterial number was determined by counting the colony forming units per 1-inch square. Control squares were tested in an identical fashion.

Percent reductions were determined by comparing the initial bacterial colony forming units with the bacterial colony forming units after being exposed to Lotus ^{TM} water, Lysol ^{TM}, Odoban ^{TM}, 3% hydrogen peroxide, or 1 part vinegar, 1 part lemon juice into two parts water with a pinch of baking soda. Colony forming units for controls and experimental squares was determined, averaged from 5 separate trials and statistical evaluated using a pair t-test.

Table 1. Percent Reduction of *E. coli*, Listeria sp. and *Staphylococcus aureus* On Food Surfaces Exposed for 15 Minutes of exposure Using Lotus spray (0.2-0.4ppm), Lysol, Odoban, 3% Hydrogen peroxide (H_2O_2) and vinegar/lemon juice/baking soda mixture on food surfaces.

	Control	Lotus ^{TM}	Lysol ^{TM}	Odoban ^{TM}	3% H_2O_2	V/L/BS
E. coli	^{a}12,800,000	^{b}1500/99.99	^{b}1400/99.99	^{b}1600/99.99	^{b,c}800/99.999	^{b,c}18,500/99.9
Listeria sp.	^{a}12,800,000	^{b}1340/99.99	^{b}1200/99.99	^{b}1455/99.99	^{b,c}650/99.999	^{b,c}22,000/99.9
Staphylococcus aureus	^{a}12,600,000	^{b}1450/99.99	^{b}1250/99/99	$^{b.c}$1500/99.99	^{b,c}750/99.99	^{b,c}18,200/99.99

All results the means of 5 values are reported as CFU/one inch square and percent reduction. Statistical differences exist between control groups and treatments groups (b) and treatment groups (c).

Ozone Concentration in Lotus ^{TM} Sanitizer Spray.

To measure the ozone concentration in Lotus spray, a protocol using a spectrophotometer was employed in order to avoid any errors in the comparison the two methods. Both methods were applied to the

spectrophotometer and compared. In the tables below you can see the results for both methods.

Table 2.

Ozone Concentration using spectrophotometer Lotus ^{TM} Sanitizer spray			
	Abs	Dilution	Ozone (ppm)
Nebulized	6.6	1	**0.076719577**
half screw turn	3.3	1	**0.164021164**
complete screw turn	2.3	1	**0.190476190**
1.5 screw turns	5.6	2	**0.206349206**
2 screw turns	5.6	2	**0.206349206**

The Table 2 shows the ozone concentration of the Lotus Sanitizer spray using different spray flows. The less concentration is when flow adapter is closed and the flow is complete nebulized, and the highest concentration is reach when the flow adapter is almost open. All samples were taken after sprayed so it's possible that the device produced a higher concentration of ozone, but it loses ozone when is sprayed.

Table 3.

Ozone Concentration using comparison method Lotus ^{TM} Sanitizer spray		
Time (min)	**ppm (close)**	**ppm (bottle open)**
0	1.25	1.5
3	1	1.15
6	1	0.75
9	1	0.65

12	0.9	0.6
15	0.8	0.55

The Table 3 shows the ozone concentration of Lotus ^{TM} Sanitizer spray when the bottle is closed and when the cap is removed (open). By the first minute of the 15-minute treatment, ozone is lost to the environment when is sprayed, and continues slowly to de-gas off into the environment.

CONCLUSIONS:

Concentrations of 0.3 ppm O_3 Lotus ^{TM} Sanitizer spray are able to achieve up to 4 logs of *E. coli*, Listeria and *S. aureus* reduction on food surfaces. Statistical significant reductions (p=0.01) were achieved when comparing untreated controls with the treatments of Lotus ^{TM} Sanitizer spray, Lysol, Odoban, 3% Hydrogen peroxide (H_2O_2) and vinegar/lemon juice/baking soda mixture on food surfaces. Reductions in bacterial number between treatment groups (Lotus ^{TM} Sanitizer spray, Lysol, Odoban, 3% Hydrogen peroxide (H_2O_2) and vinegar/lemon juice/baking soda mixture) on food surfaces were found to be statistically the same.

In conclusion, these experiments demonstrate that *E. coli*, Listeria and *S. aureus* can be reduced equally well (statistically the same, p value of 0.01) using the Lotus ^{TM} Sanitizer spray when compared to a conventionally used chemical sanitizer such as Lysol, Odoban, 3% Hydrogen peroxide (H_2O_2). Vinegar/lemon juice/baking soda mixture was found to be statistically different than the other treatments in bacterial reductions. There were no statistical differences in reductions between species of bacteria and treatments.

Moreover, a 4 log reductions or 99.99% reduction was achieved. Only 3% hydrogen peroxide achieved a larger log reduction of 5 or a 99.999% reduction when compared to the other treatments.

It should be noted that the use of the Lotus ^{TM} Sanitizer spray does not leave a chemical residuals on the food surfaces like the Lysol, Odoban, 3% Hydrogen peroxide (H_2O_2) and vinegar/lemon juice/baking soda mixture.

NOTE:

A Lotus mixture cleaning composition with ozone and water gives good antimicrobial performance for use on and around food contact surfaces. The food safe mixture is preferably food safe and of low toxicological concern for use on animal, human and food contact surfaces. The Lotus mixture can be used directly and used on a wipe or other substrate, and unlike traditional chemical cleaners requires no rinsing or removal from the surface following application and cleaning.

Bloomfield and Scott, 1997 S.F. Bloomfield and E. Scott, Cross-contamination and infection in the domestic environment and the role of chemical disinfectants, *J. Appl. Microbiol.* **83** (1997), pp. 1–9.

Cogan et al., 1999 T.A. Cogan, S.F. Bloomfield and T.J. Humphrey, The effectiveness of hygiene procedures for the prevention of cross-contamination from chicken carcasses in the domestic kitchen, *Lett. Appl. Microbiol.* **29** (1999), pp. 354–358.

Gorman et al., 2002 R. Gorman, S. Bloomfield and C.C. Adley, A study of cross-contamination of food-borne pathogens in the domestic kitchen in the Republic of Ireland, *Int. J. Food. Microbiol.* **76** (2002), pp. 143–150.

Jay et al., 1999 S.L. Jay, D. Comar and L.D. Govenlock, A video study of Australian domestic food-handling practices, *J. Food Prot.* **62** (1999), pp. 1285–1296.

Josephson et al., 1997 K.L. Josephson, J.R. Rubino and I.L. Pepper, Characterisation and quantification of bacterial pathogens and indicator organisms in household kitchens with and without the use of a disinfectant cleaner, *J. Appl. Microbiol.* **83** (1997), pp. 737–750.

Kusumaningrum et al., 2002 H.D. Kusumaningrum, M.M. van Putten, F.M. Rombouts and R.R. Beumer, Effects of antibacterial dishwashing liquid on food borne pathogens and competitive microorganisms in kitchen sponges, *J. Food Prot.* **65** (2002), pp. 61–65.

Kusumaningrum et al., 2003 H.D. Kusumaningrum, R. Paltinaite, A.J. Koomen, W.C. Hazeleger, F.M. Rombouts and R.R. Beumer, Tolerance of *Slamonella enteritidis* and *Staphylococcus aureus* to surface cleaning and household bleach, *J. Food Prot.* **66** (2003), pp. 2289–2295.

Lalla and Dingle, 2004 F. Lalla and P. Dingle, The efficacy of cleaning products on food industry surfaces, *J. Environ. Health* **67** (2004), pp. 17–21.

Larson et al., 2004 E.L. Larson, S.X. Lin, C. Gomez-Pichardo and P. Della-Latta, Effect of antibacterial home cleaning and handwashing products on infectious disease symptoms, *Ann. Intern. Med.* **140** (2004), pp. 321–329.

Redmond and Griffith, 2003 E.C. Redmond and C.J. Griffith, Consumer food handling in the home: a review of food safety studies, *J. Food Prot.* **66** (2003), pp. 130–161.

Rusin et al., 1998 P. Rusin, P. Orosz-Coughlin and C. Gerba, Reduction of faecal coliform, coliform and heterotrophic plate count bacteria in the household kitchen and bathroom by disinfection with hypochlorite cleaner, *J. Appl. Microbiol.* **85** (1998), pp. 819–828

Rusin et al., 2002 P. Rusin, S. Maxwell and C. Gerba, Comparative surface-to-hand and fingertip-to-mouth transfer efficiency of gram-positive bacteria, gram-negative bacteria and phage, *J. Appl. Microbiol.* **95** (2002), pp. 585–592.

Scott and Bloomfield, 1993 E. Scott and S.F. Bloomfield, An 'in-use' study of the relationship between bacterial contamination of food preparation surfaces and cleaning cloths, *Lett. Appl. Microbiol.* **16** (1993), pp. 173–177.