MICROORGANISMS IN KITCHEN SPONGES

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ABSTRACT

In this study presence of various kinds of microorganisms in kitchen sponges were studied. Additionally effects of regular dish washing liquid on *Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 13311 were investigated under laboratory conditions in kitchen sponges with or without food residue. The sponges involved in daily use in households were samples were analysed for the presence of total mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, *E. coli* and yeast and moulds. Daily application of the dishwashing liquid to the sponges had no effect on the numbers of yeast and molds, pseudomonads, *E. coli*. But the number of Salmonella spp. had decreased. *S. aureus* was not investigated from the house hold sponges during 10 days. Pseudomonads were the dominant microflora in the sponges during 10 days. In six households, the regular dish washing liquid were applied for 2 weeks, and it was shown that neither dishwashing liquid reduced total mesophilic aerobic counts, *E. coli*, pseudomonads or yeast and moulds, except Salmonella spp., however *S. aureus* declined quickly and was not survived. In addition, in this study the rates of pathogens from artificially contaminated sponges were investigated. The test organism *S. typhimurium* decreased below the detection limit within 24 h. With the amount of dishwashing liquid added to the sponges (3% ± 1.5%), *E. coli* decreased during 24 h either with or without commercial sterilized milk (10%). In the presence of milk suspension *E. coli* survived better within two days in the sponges. The test organism *E. coli* decreased below the detection limit after day of 2 in the presence of milk. *E. coli* decreased below the detection limit after day of 1 without milk. The results of this study showed that the regular dish washing liquid was effective in reducing microorganisms in the laboratory test but not in the used kitchen sponges

KEY WORDS: Kitchen sponge, survival, regular dishwashing liquid.

INTRODUCTION

It's known that during the cleaning process of equipment, utensils, sinks, etc. in kitchens, the pre-washing and washing steps are done with the use of sponges to eliminate food residues. As a consequence of this procedure, part of the food residues adheres to the sponge surfaces. These food residues together with the moisture retained in the sponges offer a favorable environment for bacterial growth. Early studies on bacterial contamination in the kitchen were conducted in the late 1960s investigating bacterial load of hand towels and the hygienic conditions of domestic dishcloths and tea towels. Such cloths were heavily contaminated with bacteria and suspected as one of the main vectors for spreading and dissemination of the bacteria in the kitchen (Speirs *et al*., 1995). The current attention on bacterial contamination in the kitchen was started in the late 1970s. Previous studies have suggested that although raw material is probably the main source of contamination in the kitchen, the area surrounding the kitchen could also act as sources of free living populations of bacteria. Sponges and dishcloths have been recognized as potential agents in the spread of microorganisms, and it has been observed that bacteria persist in these vehicles (Josephson *et al*., 1997; Scott and Bloomfield, 1990; Speirs *et al*., 1995). Several studies indicated that various bacteria, including *Escherichia coli*, *Staphylococcus aureus* and Salmonella spp., survive on hands, sponges/cloths, utensils and currency for hours or days after initial contact with the microorganisms (Jiang and Doyle, 1999; Kusumaningrum *et al*., 2002).

Detergents and dish washing liquids are antibacterial products and specifically manufactured for the reduction of bacteria in sponges and cleaning cloths. A lot of antibacterial products, promoted by producers, are specifically manufactured for the reduction of bacteria in cleaning cloths and sponges. Outbreaks of food poisoning frequently occur as a result of improper food preparation in which cross-contamination in combination within adequate storage or cooking was implicated in many instances (Olsen *et al*., 2000) Dishcloths and sponges were recognized as a potential source for spreading microorganisms and it was observed that bacteria persisted in these vehicles (Josephson *et al*., 1997; Rusin *et al*., 1998). Enteric pathogens, such as *E. coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* were isolated, as well as other types of pathogens

(*S. aureus*) and opportunistic pathogens (*Pseudomonas* spp.).

A lot of antibacterial products, promoted by producers, are specifically manufactured for the reduction of bacteria in cleaning cloths and sponges. These include bleach solutions, detergent, and dishwashing liquid. Limited studies have addressed the e9ectiveness of these products to inactivate microorganisms on sponges. Kusumaningrum *et al*. (2002) studied the effect of an antibacterial dishwashing liquid on *E. coli*, *Salmonella Enteritidis*, *S. aureus* and *Bacillus cereus* in a modified suspension test and in used sponges' with and without food residues under laboratory conditions.

There were two objective of this study. One of it was to evaluate, with respect to microbial status, sponges used in 6 house kitchens. The kitchen sponges were monitored for: total mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, *E. coli* and yeast and moulds. In a second phase, the effects of an antibacterial dishwashing liquid on *E. coli* and *Salmonella typhimurium* were investigated in sponges with or without food residues under laboratory conditions.

MATERIALS AND METHODS

Sponges and dish washing liquid. Synthetic yellow sponges with green pads (Balerina, 9:7:3; Turkey) and regular dishwashing liquid were obtained from retail markets. Prior to use, the sponge was washed to remove all trace of preservatives and other chemicals that may have an antibacterial effect with 0.05% dishwashing liquid in hot water (60-65^{o}C). The sponge was placed into a sterile bag with 100 ml sterile cold tap water, and the bag contents were mixed for 60 s. The sponge was removed from the bag, rinsed thoroughly with cold water and squeezed to remove excess liquid. The sponge was then boiled in sterile distilled water for 10 min, excess water was removed and the sponge was dried overnight in an oven. Ten kitchen sponges were used in 6 different houses for two weeks. Microbiological investigation of these sponges for total mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, total coliforms and faecal coliforms and yeast and moulds were performed on days 3, 7 and 14.

Enumeration of total Mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, total coliforms and faecal coliforms and yeast and moulds on used kitchen sponges. The used kitchen sponge samples were analysed for the presence of total mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, *E. coli* and yeast and moulds. The microbiological methods applied are shown in Table 1 (Anonymous, 1982; Anonymous, 1983; Lancette and Tatini, 1992).

Table 1. Enumeration of total mesophilic aerobic bacteria, staphylococci, Pseudomonas, Salmonella, total coliforms and faecal coliforms and yeast and moulds on used kitchen sponges (Anonymous, 1982; Anonymous, 1983; Lancette and Tatini, 1992).

		Incubation conditions		
Microbes	Medium	Temp (^{o}C)	Time	Atmosphere
Total mesophilic aerobic bacteria	Plate Count Agar	30.0	48-72 h	Aerobic
Coliforms	Violet Red Bile Agar	30.0	48 h	Aerobic
Escherichia coli	Violet Red Bile Agar	37	24-48 h	Aerobic
	Endo Agar	37	24 h	Aerobic
Pseudomonads	Pseudomonas Agar with CFC Supplement	25	48 h	Aerobic
Yeasts and Moulds	Sabaroud Dextrose Agar	25	5 days	Aerobic
Staphylococcus aureus	Baird Parker Agar with egg yolk emulsion supplement	37	24-48 h	Aerobic
Salmonella spp.	Phosphate Buffered Saline (PBS)		18-24h	Aerobic
	Selenite Cystine Broth (SC)		18-24h	Aerobic
	MacConkey Agar			
	Salmonella-Shigella Agar (SS Agar)		18-24h	Aerobic
			18-24h	Aerobic

Test microorganisms and growth conditions. *Escherichia coli* ATCC 8739 and *Salmonella typhimurium* ATCC 13311 were used as the test organism. They were obtained from Pharmaceutical Microbiology and Hygiene Laboratory, University of Antwerp, Belgium.

The bacteria were activated in 10 ml brain hearth infusion (BHI) broth (Difco Lab, Detroit Mich.) suspension followed by incubation for 20-24 h at 37°C for *E. coli* and *S. typhimurium*.

Effect of dishwashing liquid in laboratory sponges. Dishwashing liquid was added to the sponges with the amount of 3% ± 1.5%. Overnight cultures in BHI broth were diluted in saline solution (0.85% NaCl) to final concentrations of about 10^6 cfu/ml. Ten sponges were each contaminated with 10 ml of test suspension of *E. coli* and *S. typhimurium* and in order to generate soiled conditions, commercial sterilized milk (10%) were added other 10 sponges. The sponge samples squeezed with gloved hands to distribute the suspension in the sponges.

All of the sponges were stored at room temperature (20-25 °C, 42± 2% relative humidity) and sampled on 0, 1, 2, 4, 7, and 10th days by suspending the whole sponge in 100 ml of sterile peptone saline solution (0.1% peptone) for 60 s.

Table 2. Enumeration of total Mesophilic aerobic bacteria, *S. aureus*, Pseudomonas, Salmonella, *E. coli* and yeast and moulds on used kitchen sponges.

Organism	n	Day 3	Day 7	Day 10
Total mesophilic aerobic bacteria[a]	6	4.6	5.8	6.9

Artificially contaminated kitchen sponges in laboratory. In uncontaminated used laboratory sponges the microorganisms specified in this study (*E. coli* and *S. typhimurium*) were not found. The total mesophilic aerobic counts were ca. 10^4 CFU/sponge and with the amount of dishwashing liquid added to the sponges (3% ± 1.5%) total mesophilic aerobic counts were ca. 10 CFU/sponge.

In six households, the regular dish washing liquid were applied for 2 weeks, and it was shown that neither dishwashing liquid reduced total mesophilic aerobic counts, *E. coli*, pseudomonads or yeast and moulds except Salmonella spp., however *S. aureus* declined quickly and was not survived.

Enumaration of test microorganisms. Selective agar media were used for the enumeration of test bacteria: Eozin Methylene Blue Agar (EMB, Difco) for *E. coli* and Buffered phosphate solution, Selenite Sistin Broth, Salmonella-Shigella Agar (SS, Difco) for *S. typhimurium*, incubated for 24-48 h at 37°C.

RESULTS

Households' sponges. Microbiological investigations of the sponges for total mesophilic aerobic bacteria, *S. aureus*, Pseudomonas, Salmonella spp., *E. coli* and yeast and moulds were performed on days 3, 7, and 10 by sampling as described in Table 1.

The sponges involved in daily use in households were in contact with the dishwashing liquid at least twice a day. Results from the total mesophilic aerobic bacteria in Table 2 illustrate a significant increase in the number of log CFU/ml. Daily application of the dishwashing liquid to the sponges had no effect on the numbers of yeast and molds, pseudomonads, *E. coli*. But the number of Salmonella spp. had decreased. *S. aureus* was not investigated from the house hold sponges during 10 days. Pseudomonads were the dominant microflora in the sponges during 10 days (Table 2).

In general, the regular dish washing liquid product did not reduce the competitive microorganisms in sponges involved in daily household use except Salmonella spp.

Yeast and moulds	6	3.5	5.4	7.0
Pseudomonads	6	4.5	5.8	7.8
Salmonella spp.	6	6.3	5.8	5.8
E. coli	6	4	5.3	5.3
S. aureus	6	nd[b]	nd	nd

[a]: Mean values (log cfu/ml)
[b]: not detected

In this study the rates of pathogens from artificially contaminated sponges were investigated (Table 3).

The test organism *S. typhimurium* ATCC 13311 decreased below the detection limit within 24 h.

With the amount of dishwashing liquid added to the sponges (3% ± 1.5%), *E. coli* decreased during 24 h either with or without commercial sterilized milk (10%). In the presence of milk suspension *E. coli* survived better within two days in the sponges. The test organism *E. coli* ATCC 8739 decreased below the detection limit

after day of 2 in the presence of milk. *E. coli* decreased below the detection limit after day of 1 without milk.

Table 3. Effect of dishwashing liquid on the survival of pathogens and their competitive mikroflora (total mesophilic aerobic counts) in used sponges artificially contaminated with (A) *E. coli* ATCC 8739, (B) *E. coli* in 10 % milk-saline solution suspension, (C) *S. typhimurium* ATCC 13311 and (D) *S. typhimurium* in 10 % milk-saline solution suspension.

Days	Group I Artificially contaminated sponges with *E. coli*				Group II Artificially contaminated sponges with *S. typhimurium*			
	A	B	C	D	E	F	G	H
0	1	2.52[a]	<1	1	<1	<1	<1	1
1	1.20	2.76	<1	1	<1	<1	<1	1
2	nd[b]	3	<1	1	<1	<1	nd	<1
4	nd	nd	nd	<1	nd	nd	nd	nd
7	nd	nd	nd	<1	nd	nd	nd	nd
10	nd	nd	nd	nd	nd	nd	nd	nd

[a]: log N (CFU/sponges), A: Sponges with *E. coli,* B: Sponges with *E. coli* and10 % milk-saline solution, C: Total Mesophilic Aerobic Bacteria in sponges, D: Total Mesophilic Aerobic Bacteria in sponges with10 % milk-saline solution, E: Sponges with *S. typhimurium,* F: Sponges with *S. typhimurium* and10 % milk-saline solution, G: Total Mesophilic Aerobic Bacteria in sponges, H: Total Mesophilic Aerobic Bacteria in sponges with10 % milk-saline solution.
[b]nd: not detected

DISCUSSION

Outbreaks of food poisoning frequently occur as a result of improper food preparation in which cross-contamination in combination within adequate storage or cooking was implicated in many instances (Olsen *et al*., 2000). Dishcloths and sponges were recognized as a potential source for spreading microorganisms and it was observed that bacteria persisted in these vehicles (Josephson *et al*., 1997; Rusin *et al*., 1998).

Studies of the domestic environment by Finch *et al.* (1978), Scott *et al.* (1982), Speirs *et al*. (1995), Josephson *et al*. (1997) and Rusin *et al*. (1998) indicate that micro-organisms, including some potentially pathogenic species, are commonly found in all areas of the home environment. The results of these studies indicate that wet sites, such as kitchen sink areas (particularly sink surfaces, draining board, U-tubes), toilets and nappy buckets are most commonly associated with heavy contamination and the occurrence of potentially harmful species. Other wet sites, such as dishcloths and similar cleaning utensils, were also found to be frequently and heavily contaminated. These results suggest that, in the kitchen, although raw food is probably the main source of contamination, the sink, waste trap and surrounding areas can also act as semi-permanent sources or reservoirs which harbour and encourage the establishment of free-living bacterial and fungal populations. Similarly, in the bathroom or toilet, although enteric bacteria probably originate from the toilet or directly from humans, indications are that baths, basins, cleaning cloths and face cloths may form semi-permanent reservoirs of bacteria. These conclusions were further supported by laboratory studies (Scott and Bloomfield 1990, Scott *et al*., 1982) which demonstrated the ability of Gram-negative species, such as *E. coli, Klebsiella* spp. and pseudomonads to grow to substantial numbers in samples of sink U-tube and toilet water, and in contaminated wet cloths. Additionally, although less frequent, potentially harmful organisms are quite often isolated from hand and food contact surfaces in the bathroom and toilet as well as in the kitchen, albeit in low numbers.

The *Enterobacteriacae* spp. isolated in the study of Scott *et al*. (1982) included *Klebsiella, Enterobacter, Citrobacter, Proteus* and *E. coli*. A similar pattern was also reported in the studies of Finch *et al.* (1978) and Speirs *et al*. (1995). Although these species are not normally pathogenic to the healthy adult, they must be regarded as indicators of poor hygiene. Other species which were isolated included *Pseudomonas aeroginosa* and *S. aureus*. In a survey of 213 homes, *Listeria* spp. were found in about 47.4% of homes and were recovered from wet sites, such as kitchen sinks, dishcloths and washing up brushes, the refrigerator and the toothbrush (Beumer *et al.* 1996). Speirs *et al.* (1995) isolated *L. monocytogenes* from fridge surfaces in 2.2% of dwellings. *Yersinia enterocolitica* was also isolated

from the sink area in 4.2% of homes, and *Bacillus cereus* from 10.9% of homes.

Household cleaning products containing antibacterial ingredients or without antibacterial ingredients are widely available and popular. Although manufacturers use claims of health benefits to market these products, evidence linking the use of antibacterial products to health outcomes has been lacking. The risk of cross-contamination during regular domestic cleaning is important since kitchen sponges were found to be potential vehicles of pathogens in domestic kitchens (Hilton and Austin, 2000) and pathogens were able to survive in kitchen sponges for at least weeks (Kusumaningrum *et al*., 2002).

Enriquez *et al*. (1997) isolated and identified 23 different bacterial species from 140 sponges, and 13 bacterial species from 56 dishcloths from US homes. The most common bacteria were *Enterobacteriacae* and *Pseudomonas* spp. *Salmonella* spp. were identified in 15% of sponges and 14% of dishcloths. *Pseudomonas* spp. were present in 36% of sponges and 31% of dishcloths. *S. aureus* was present in 20% of sponges and 19% of dishcloths. A UK study also found 84% of dishcloth samples contaminated with *Listeria* spp. (Duggan and Phillips 1998). Hilton and Austin (2000) sampled 100 dishcloths and sponges from domestic kitchens and isolated *S. aureus* from 4% of sponge-type materials, with counts ranging from 10^2 to 4 x 10^4 CFU/ml. The total viable count from all cloth types ranged from 20 to 6 x 10^8 cfu/ml with a mean of 8.5 x 10^7 CFU/ml.

Kusumaningrum *et al*. (2002) studied the effect of an antibacterial dishwashing liquid on *E. coli*, *S. enteritidis*, *S. aureus* and *B. cereus* in a modified suspension test and in used sponges with and without food residues under laboratory conditions. In the suspension tests *S. aureus* and *B. cereus* were susceptible to low concentrations of antibacterial dishwashing liquid (0.5%), whereas *E. coli* and *S. enteritidis* maintained their initial numbers for at least 24 h at 25°C. With higher concentrations (2-4%), all test organisms decreased below the detection limit after 24 h. Over a 24 h period, the antibacterial dishwashing liquid did not significantly reduce these organisms in used sponges in which food residues were present. The antibacterial product did not reduce the competitive microorganisms either. Similar results were found in sponges involved in daily household use. The presence of food residues strongly reduces the product efficacy. This indicates that to determine the efficacy of an antibacterial product and other similar products, treatment under use conditions must be included. Our results about the used household sponges and artificially contaminated kitchen sponges in laboratory were similar to those findings of Kusumaningrum *et al*. (2002).

Our results and those other of studies (Kusumaningrum *et al*., 2002; Hilton and Austin, 2000; Scott and Bloomfield, 1990) suggest that bacterial inactivation in sponges depends on a number of factors and is largely changeable. The number of bacteria increases rapidly under favorable conditions in a used sponge. There is constant risk of contamination transfer from the used surfaces, disposable sponges should be considered for use whenever possible. Reusable sponges should be dried after use or immersed in boiling water for 5 min, an effective means of decontamination (Ikawa and Rossen, 1999).

In this study, the regular dish washing liquid was not shown to be effective in reduction of bacteria in the house hold using. The risk has been considered to be lowered when the surfaces are dry, partly because bacterial growth and survival would be reduced. The presence of food residues strongly reduces the product's efficacy. In the laboratory tests, the regular dish washing liquid was shown to be effective in reduction of bacteria.

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