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Dishwashing sponges and brushes: Consumer practices and bacterial growth and survival

Trond Møretrø [a,*], Birgitte Moen [a], Valérie L. Almli [a], Paula Teixeira [b], Vânia B. Ferreira [b], Anette Wold Åsli [a], Charlotte Nilsen [a], Solveig Langsrud [a]

[a] Nofima, Norwegian Institute of Food, Fisheries and Aquaculture Research, N-1430 Ås, Norway
[b] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal

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ABSTRACT

Sponges are frequently used in kitchens and have been shown to harbor large numbers of bacteria, occasionally also pathogens. Less is known about kitchen brushes regarding usage and presence of bacteria. In the present study, the use of sponges and brushes was studied in a survey among 9966 European consumers in ten countries, and growth and survival of bacteria in sponges and brushes were examined in laboratory experiments.

Sponges were the preferred hand-cleaning utensils for washing-up in the majority of countries, while brushes were most frequently used in Denmark and Norway. Consumers mostly change their sponges at regular times, but also sensory cues (looks dirty, smelly, slimy) and usage occurrences such as wiping up meat juices may trigger replacement. Besides cleaning the dishes, over a quarter of the dish brush users also use it to clean a chopping board after soilage from chicken meat juices.

The water uptake and drying rate varied considerably, both between different sponges and between brushes and sponges, where brushes dried fastest. *Campylobacter* survived one day in all sponges and *Salmonella* more than seven days in two of three types of sponges. In the type of sponge that dried slowest, *Salmonella* grew on the first day and was always found in higher levels than in the other sponges. Non-pathogenic bacteria grew in the sponges and reached levels around 9 log CFU/sponge. In brushes all types of bacteria died over time. *Campylobacter* and *Salmonella* were reduced by more than 2.5 log to below the detection limit after one and three days, respectively. Bacteriota studies revealed a tendency for a dominance by Gram-negative bacteria and a shift to high relative prevalence of *Pseudomonas* over time in sponges. Both enumeration by agar plating and bacteriota analysis confirmed that the pathogens were in a minority compared to the other bacteria.

Treatments of sponges and brushes with chlorine, boiling or in the dishwasher were effective to reduce *Salmonella*.

We conclude that brushes are more hygienic than sponges and that their use should be encouraged. Contaminated sponges or brushes should be replaced or cleaned when they may have been in contact with pathogenic microorganisms, e.g. used on raw food spills. Cleaning of sponges and brushes with chlorine, boiling or dishwasher may be a safe alternative to replacing them with new ones.

1. Introduction

Kitchen sponges are commonly used by consumers for washing up and scouring of pans and casseroles, but are also used for cleaning kitchen surfaces, such as sinks, refrigerators and stove-tops (Lagendijk et al., 2008). Brushes are the dominant utensils for manual cleaning of dishes (washing up) in Norway (Røssvoll et al., 2015). In an observational study in UK, several consumers used more than one type of cleaning utensil for washing-up, 29%, 50% and 77% of consumers used brushes, sponges and cloths, respectively (Mattick et al., 2003b). There is lacking information about the use of brushes in other countries.

Sponges collected from consumers can contain high bacterial numbers, in the range of 6–9 log CFU, (Evans and Redmond, 2019; Hilton and Austin, 2000; Ikawa and Rossen, 1999; Rossi et al., 2013). A

* Corresponding author.
E-mail address: trond.moretro@nofima.no (T. Møretrø).

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wide diversity of non-pathogenic and opportunistic pathogenic bacteria as well as viruses, Archeae and Eukaryota have been found in used sponges (Cardinale et al., 2017; Jacksch et al., 2020). Presence of the pathogenic bacteria *Salmonella* and *Campylobacter* have also been reported (Borrusso and Quinlan, 2017; Chaidez and Gerba, 2000; Enriquez et al., 1997a; Mattick et al., 2003b). While sponges are used to remove food soils and reduce bacterial numbers, it may also transfer bacteria, including pathogens, to surfaces (Biranjia-Hurdoyal and Latouche, 2016; Mattick et al., 2003a). Thus, sponges have the potential to act as a reservoir and spread bacteria. There is generally much less information about kitchen brushes than sponges in the scientific literature and therefore it is unclear to which degree they can act as reservoirs for pathogens. In a study among elderly in UK, total counts in the range 3–8 log CFU were found in used brushes, and *Enterobacteriaceae* and *Staphylococcus aureus* were frequently detected (Evans and Redmond, 2019).

Sponges absorb large volumes of water/fluids. Sponges often remain wet after use, creating a potential for bacterial growth, while rapid drying may reduce the growth or kill the bacteria. *Salmonella* has been observed to die off in dry sponges, but may grow in humid kitchen cloths (Cogan et al., 2002; Mattick et al., 2003a). There is limited information in the scientific literature linking parameters such as water uptake and drying ability of sponges to growth and survival of pathogenic bacteria. Brushes will likely have less water uptake than sponges, but more knowledge is needed to reveal how this difference affects bacterial growth and survival.

Cleaning or disinfection of cleaning utensils may be a way to control the bacterial contamination and limit further spreading in the kitchen environment and will also lead to reduce waste as the utensils will have a longer lifetime. There are several studies on cleaning and disinfection of sponges (Ikawa and Rossen, 1999; Park et al., 2006; Sharma et al., 2009), but to our knowledge no studies on kitchen brushes. Addition of hypochlorite or dish washing detergents to sponges have been reported to reduce the bacterial levels by 1.5–5 log CFU (Ikawa and Rossen, 1999; Nielsen et al., 2002; Rusin et al., 1998), but some studies report that the antibacterial effect is quenched by the presence of food soil (Kusumaningrum et al., 2002; Sharma et al., 2009). Sharma et al. (2009), reported that the use of dishwasher or 1 min treatment in microwave oven reduced the bacterial levels on sponges by >5 log or 6 log, respectively. The bactericidal effect of treating sponges in microwave oven and dishwasher has been confirmed in other studies (Ikawa and Rossen, 1999; Park et al., 2006). These studies on cleaning and disinfection of sponges focus on killing of bacteria and not on whether food soil is removed from the sponges. Remaining food soil could allow regrowth of surviving microorganisms if the sponge is humid.

To give risk-reducing advice to consumers, more knowledge is needed about the survival and growth of pathogenic bacteria in kitchen brushes and sponges related to consumers' practices for use and handling of these items. In the present study, we examined usage of sponges and brushes among European consumers and evaluated different types of sponges and brushes for water uptake, drying capability, growth and survival of *Salmonella* and *Campylobacter* as well as other kitchen related bacteria. We also tested the effect of different cleaning methods for these utensils.

2. Materials and methods

2.1. Web based survey

2.1.1. Survey questionnaire

A cross-national survey was conducted in ten European countries including Denmark, France, Hungary, Germany, Greece, Norway, Portugal, Romania, Spain and the United Kingdom. The survey included modules on hygiene and food handling practices for diverse food categories and sociodemographic questions (see (Langsrud et al., 2020; Møretrø et al., 2020)). The present publication reports specifically on a sub-selection of questions related to washing up of dishes and cleaning utensils in the kitchen, with focus on response alternatives concerning sponges and dish brushes (Supplementary Table S1).

2.1.2. Consumer recruitment and data collection and analysis

Recruitment was subcontracted to a professional survey provider administering a large consumer panel worldwide (Dynata). In each country the population sample consisted of private households selected by stratified random sampling based on the Nomenclature of Territorial Units for statistics level 2 (NUTS2) of the respective country (Eurostat, 2020) and the education level of the target respondent. The within-country stratum sample sizes were proportional to the corresponding population stratum sizes. In the present paper, responses from a total of 9966 households across the ten countries are considered (Supplementary Table S2). Respondents consisted of 49.5% males and ranged from 16 to 90 years old (mean: 46.6 years). A bias towards higher education occurred as an artefact of running the survey online, with 50.9% of the respondents declaring a university education. With regard to food safety, four risk groups were represented in the sampled households: pregnant women, families with children under six years of age, diabetics and immunocompromised, and elderly above 65 years of age, wherein 47.1% of the households represented at least one of the four risk groups. Frequencies of self-reported practices are reported in terms of percentages overall and per country, gender or age group. Cochran's Q test was applied to identify significant differences between treatments at a 95% confidence interval.

2.2. Laboratory studies of sponges and brushes

2.2.1. Bacterial strains

The bacterial strains used in this study are listed in Table 1. The strains from Norwegian and Portuguese kitchens were isolated during consumer visits within the SafeConsume project (Skuland et al., 2020). The bacteria were identified by 16S rDNA sequencing and representatives of the dominant bacterial genera identified among isolates were selected.

All strains were maintained in 20% glycerol at −80 °C. For preparation of cultures for addition to cleaning utensils, frozen stocks of *Campylobacter* strains were streaked on mCCDA (Oxoid) and grown at 37 °C under microaerophilic conditions (CampyGen CN0035A, Oxoid), and thereafter the strains were cultured in two steps in Mueller Hinton Broth (Oxoid), incubated at 37 °C with 150 rpm agitation overnight under microaerophilic conditions. *Salmonella* was cultured on Tryptone Soy Agar (TSA; Oxoid) at 37 °C followed by cultivation in 5 ml TSB (Tryptic Soy Broth, Oxoid) at 30 °C with 150 rpm agitation overnight.

Table 1
Bacterial strains used in this study.

Bacteria	Strain no.	Other information	Reference
Kocuria sp.	MF[a] 6951	Kitchen cloth, Norway	Møretrø, unpublished
Moraxella osloensis	MF 6954	Kitchen cloth, Norway	Møretrø, unpublished
Pseudomonas sp.	MF 6945	Cutting board, Norway	Møretrø, unpublished
Staphylococcus sp.	MF 6941	Cutting board, Norway	Møretrø, unpublished
Serratia liquefaciens	MF 7009	Counter top, Portugal	Teixeira, unpublished
Salmonella Infantis	MF 6976	M2016 ETBI 015346/01, Poultry Hungary	(Møretrø et al., 2020)
Salmonella Enteritidis	MF 6974	Hen's egg, Portugal	(Møretrø et al., 2020)
Campylobacter jejuni	MF 6902	NCTC11168, human clinical isolate	
Campylobacter jejuni	MF 6903	DFVF1099, chicken, Denmark	(On, et al., 2006)

[a] Numbering refers to Nofima's strain collection.

Kitchen related bacteria (Table 1) were cultivated on TSA followed by TSB, as for *Salmonella,* but at 30 °C in all steps.

2.2.2. Sponges and brushes

An overview of the characteristics of the three sponges and three brushes tested, is shown in Supplementary Table 3. Photos are shown in Supplementary Fig. S1. Two of the sponges, S21 and S22, contained the antimicrobials silver and quaternary ammonium compounds, respectively. The brushes and sponges were bought in large retailers in Norway and Portugal and may then be regarded as commonly used in kitchens by Norwegian and Portuguese consumers.

2.2.3. Water absorption and drying capacity of new sponges and brushes

The water uptake capacity and water loss during drying of three types of new sponges (no. 13, 21, 22) and three brushes (no. 12, 32, 34) (see Supplementary Table 3) were measured. The utensils were weighed before and after being immersed in water (22 ± 1 °C) for 1 min while being pressed up and down five times, and allowing excess water to drain off for 1 min. Half of the sponges were squeezed once with one hand after the water uptake and weighted. The drying time of brushes (hanging) and sponges (laid with scrub side up on a clotheshorse (in contact with air on all sides)) was determined by weighing after 4.5 (± 0.5 h) and 24 h. The temperature in the room was 20–22 °C and the humidity, which was influenced by outside weather and varied between the replicates, was in the range 15–40% RH.

2.2.4. Survival of bacteria in new sponges and brush over one week

Three types of new unused sponges (no. 13, 21, 22) and one brush (no. 32) were inoculated with a suspension of a mixture of *Salmonella*, *Campylobacter* and bacteria isolated from kitchens (Table 1) and a food soil suspension. The bacteria were grown individually overnight, mixed in equal volumes and added to the food soil suspension in a concentration of about 10^5/ml. The food soil suspension was a mixture of 0.1% poultry soil, 0.1% egg-based soil and 1% lettuce soil, prepared as described previously (Møretrø et al., 2020). The sponges and the brush were inoculated and soiled by immersing them in the bacteria-soil suspension (5×10^4 CFU/ml). Sponges were immersed in 200 ml bacteria-soil suspension (room temperature) pressed up and down five times while immersed, squeezed once, and placed on a steel tray. Brushes were immersed in 100 ml bacterial-soil suspension, pressed up and down five times while immersed, and shaken before hanging to dry. To simulate regular use, soil (no bacteria) was added after 3 and 7 days of storage and water after 1, 2 and 6 days of storage, by the procedure described above. The temperature and humidity in the room were registered during the experiment. Samples for microbial analysis were taken after inoculation and 1, 3 and 7 days of storage. Sponges and brushes (three of each type) were transferred to bags, 50–100 ml buffered peptone water was added to each bag, and microbes suspended by stomaching (sponge) or hand-massaging of the bag (brush) for 60 s. Enumeration of bacteria was performed by spread plating on PCA (total viable count, 30 °C, two days), XLD (*Salmonella*)(Oxoid) and mCCDA (*Campylobacter*). Initial tests showed that the enumeration of *Salmonella* and *Campylobacter* was difficult due to overgrowth of the plates by non-pathogenic bacteria. To handle this, samples on XLD (one day) and mCCDA (two days) were incubated at 42 °C, instead of 37 °C. Controls confirmed growth of the pathogens at 42 °C. The experiment was performed with three technical replicates of each type of sponge and the brush.

2.2.5. Bacteriota analyses

2.2.5.1. DNA extraction. A volume of 40 ml of stomacher suspension (described in chapter 2.2.4) or 5 ml of the bacterial cocktail used for inoculation of sponges and brushes was centrifuged at 13000 ×*g* for 5 min. DNA was extracted from the pellets using the Qiagen DNeasy PowerSoil HTP96 including bead beating using FastPrep-96 (2 × 1600 rpm, 1 min) and following the manufacturers' protocol.

2.2.5.2. Bacteriota sequencing and data processing. For all samples, PCR was performed in triplicates, and paired end sequencing (2 × 150 bp) was performed using the protocol presented in Caporaso et al. (2012). Briefly, the V4 region of the 16S rRNA gene was amplified with region-specific primers (515F (Parada et al., 2016), 806R (Apprill et al., 2015)) that included the Illumina flowcell adapter sequences (Apprill et al., 2015; Parada et al., 2016). The forward amplification primer also contained a twelve base barcode sequence that supports pooling of different samples. Samples were purified with Ampure (Agencourt Bioscience Corporation) and quantified using the Quant-iT Picogreen ds DNA with picogreen before pooling. The sample pool was purified and quantified as described above, diluted to 4 nM and sequenced using the MiSeq Reagent Kit v3 on a MiSeq (Illumina) following the protocol provided by Illumina. In addition to the experimental samples, the MiSeq run also contained a control library made from phiX Control v3, which, in this run, accounted for 10% of reads. The library quantification and sequencing were performed at Nofima. The MiSeq Control Software (MCS) version used was RTA v1.18.54.

The sequences were processed in QIIME2 (qiime2–2019.1). Briefly, the data was: Demultiplexed using demux, paired ends were joined using vsearch, quality filtered based on a q-score above 30, denoised using deblur, and taxonomy was achieved using classify-sklearn with the Greengenes 16S 13_8 database (Amir et al., 2017; Bokulich et al., 2018; Bolyen et al., 2019; McDonald et al., 2012; Pedregosa et al., 2011). Sequences originating from mitochondria and chloroplast (very few) were filtered out of the final dataset. The taxonomy- and feature table was exported to text files and further processed in Excel. The dominating representative sequences were compared to the sequences of the inoculated stains to confirm the identity of the taxa. The sequences representing *Enterobacteriaceae* were also submitted to BLAST nucleotide search (https://blast.ncbi.nlm.nih.gov) to get more information about possible genera.

2.2.6. Antibacterial activity of sponges

According to labelling, two of the sponges (S21 and S22) contained antimicrobial compounds (silver and quaternary ammonium compounds, respectively) to stop microbial growth and odours. Inhibition of bacterial growth was tested for pieces of sponge (2 × 2 × 0.5 cm) in two types of diffusion assays: A test were the pieces were placed on an TSA plate where bacteria had been streaked in high levels to form a lawn, and a test where pieces of sponges were embedded in TS soft-agar (0.7%) containing a bacterial suspension (approximately 10^6 CFU/ml) (Møretrø et al., 2011). The target bacteria tested individually were the *Staphylococcus*, *Pseudomonas* and the two *Salmonella* strains listed in Table 1. The plates were incubated at 30 °C and inspected for inhibition zones. A control known to create inhibition zones, a cloth placemat containing zinc pyrithione (Dia, Portugal) intended to absorb water from wet dishes, was included in the study, and tested as described for the sponges.

2.2.7. Evaluation of cleaning methods for sponges and brushes

The efficacy of different cleaning methods for eliminating bacteria and reduce regrowth after treatment was evaluated on sponges and brushes inoculated with bacteria and food soils and stored for one week. New unused sponges (S13) and brushes (B32) were inoculated with bacteria (cocktail of five kitchen bacteria, *Salmonella* MF6974 and MF6976 (Table 1)) suspended in food soils as described in chapter 2.2.4, but using 500 ml suspension. The items were stored humid under plastic film, added water (brushes and sponges immersed in 100 and 200 ml water, respectively) after 1, 2 and 6 days. After 3 days, the cleaning utensils were re-soiled (immersed in 100 and 200 ml food soil, for brushes and sponges, respectively). Unsoiled sponges and brushes were included as controls. After 7 days, the cleaning utensils were subjected

to different decontamination treatments, as described in Table 2. The treatments were chosen from the literature (Ikawa and Rossen, 1999; Park et al., 2006; Sharma et al., 2009) and observations made during kitchen visits within the SafeConsume project (Skuland et al., 2020). Three parallel cleaning utensils were subjected to each treatment. After the treatment, the three utensils were handled and analysed in one of the three following ways:

1. Analysed for total viable count and *Salmonella* directly.
2. Added *Salmonella* (about 1000 CFU per cleaning utensil) without food soil to test whether soil remaining after the initial treatment could support growth of *Salmonella*. *Salmonella* culture was washed twice and resuspended in 0.9% NaCl. Sponges and brushes were immersed in 500 ml suspension containing 5×10^4/ml of bacteria and pressed up and down five times. Then sponges were wringed, and brushes shaken before being placed in a plastic bag and stored at room temperature for two days, followed by analyses for total viable count and *Salmonella*.
3. Added heat treated food soil to test if a low number of bacteria (below the detection limit) remained after the initial treatment and could regrow in the presence of soil. Heat treated (115 °C) food soil mix was added in volumes as described for treatment no. 2. The cleaning utensil was placed in a plastic bag and stored at room temperature for two days, followed by analyses for total viable count and *Salmonella*.

3. Results and discussion

3.1. Use of washing-up utensils among European consumers

The survey conducted on 9966 households showed that methods for cleaning of dirty dishes vary considerably between European countries. Consumers use preferably a dishwasher, with 44% overall reporting this as their typical method (Fig. 1). Dishwasher usage varies in correlation with household equipment (Pearson $r = 0.9$): 57% of the households are equipped with a dishwasher, and usage prevalence varies between Norway (69% typical usage for 81% equipped) and Germany (59% usage for 72% equipped) on top, down to UK (31% usage for 47% equipped), Hungary (24% usage, 30% equipped) and Romania (lowest with 10% usage for 15% equipped). If not using a dishwasher, the most frequent practice reported is washing up in a sink or bowl with hot water and detergent, using a sponge. On average for the 10 countries, the use of sponges was 2.5 times higher than the use of dish brushes (36% against 14% for brushes), but there were large variations between countries (Fig. 1). For example, in Portugal the use of sponges was 6 times more frequent than brushes, whereas in Norway the use of brushes was 3 times more frequent than the use of sponges. Only 8% of the households declare using a cloth as their typical utensil for doing the dishes, with highest prevalence in Romania (19%) and lowest in Greece (2%). We also noted that consumers combine cleaning utensils. Thus, of the 36% overall who essentially do the washing up with a sponge, 26% also use a dishwasher, 17% a dish brush and 11% a cloth. Of the 14% overall who essentially do the washing up with a brush, 38% also use a dishwasher, 44% a dish sponge and 16% a cloth.

Further, we investigated how dish sponges and brushes are used among their most frequent users. An analysis focused on dish sponge users specifically ($n = 3578$), shows that 56% also use this utensil for cleaning the kitchen countertop. This behaviour is especially salient in France (74% of the dish sponge users) and weakest in Norway (30%). However, sponges are less utilized for cleaning hazardous soilage such as chicken juices on the countertop (26% of all dish sponge users) or on a chopping board (27% of all dish sponge users). In this case consumers often reported using alternatives such as a cloth or kitchen roll. Further, in-between usage occasions, sponges are mostly kept beside the sink (62%), followed by hanging (16%), left in sink (10%) and left on counter (7%). Hungarian and French consumers most typically reported leaving the sponge beside the sink (79 and 71% of respondents, respectively). Among dish brush users specifically ($n = 1402$), the countertop is typically cleaned with a cloth (51%) or sponge (36%). Twenty-eight percent of all dish brush users use the brush to clean a chopping board after soilage from chicken juices, with highest prevalence at 38% in Hungary. It was not asked for other uses of brushes in the present study, but in a former investigation from Norway, 92% of respondents used brushes for handwashing of dishes, and they reported that they also used the brush for cleaning the vegetable drawer in the refrigerator (25%), trash bins (6%) and pet feeding bowls (2%) (Jacobsen and Lavik, 2011). Differences in the use of sponges and brushes may affect both the frequency and type of contamination, soiling and time for drying between use.

Consumers show different motivations for changing their cleaning utensils (sponge or cloth), relying on time ("I do this at regular times", 65% of the households), on their senses (looks dirty, 37% and when smelly or slimy, 22%) and/or on usage after cleaning juices (22%) or dirt (20%) (Supplementary Fig. S2). There were only small differences between countries (not shown). We also asked for how long the respondents typically use the same sponge or cloth before changing to a new one: 29% of respondents change within 2 days, while 71% change after 3 days or more. Changing the utensil every 2 days was more typically associated to usage (wiping up juices), while changing less frequently was more typically associated to sensory motivations (look, smell or feel). Consumers reporting that they changed at regular times, did not deviate from other consumers with regard to frequency of changing to a fresh utensil (Supplementary Fig. S2). The results corroborate earlier studies. In a survey conducted in Switzerland ($N = 1122$), 52% changed the dish sponge every week or less often, 31% every 4–5 d/once a week, 17% daily/every 2–3 days (Ammann et al., 2019). In an Italian study, all 100 consumers used a sponge for 5 days and longer, and 40% used it for more than 30 days (Marotta et al., 2018).

Looking into gender, age and risk group effects, results showed no notable gender differences in frequency for changing cleaning utensil. However, females more typically changed the utensil at regular times than males (71% against 59%), while the latter relied on their senses more often than females (39% against 35% for "looks dirty"). Further, with increasing age consumers reported more usage of a dishwasher (within equipped households), and lower usage of a dish sponge; increase in change frequency of cleaning utensils and more change based on usage time and less based on sensory cues (Supplementary Fig. S3).

Table 2
Cleaning methods of sponges and brushes.

Treatment	Details
None, new product	Unsoiled new product, untreated control
None, soiled product	Soiled product, untreated control
Washed with dish washing detergent	Sponge and brush immersed and pressed down/ compressed three times in detergent solution (2.5 ml washing up detergent (Zalo, Lilleborg) to 5 l water (43 °C)), followed by rinsing twice in tray with 1.5 l dH_2O (43 °C)[a].
Washed in detergent water + drying	As for no 3. but after rinsing in water the brushes were hanging to dry and the sponges laid in plastic trays until the next day[a]
Microwave treatment	Sponges and brushes were placed in plastic trays in a microwave oven (Wilfa M4–700) and treated at full power (700 W) for 1 min.
Boiling	Sponges added to pot with boiling (100 °C) tap water, kept for 5 min in boiling water.
Chlorine	The sponges and brushes were put in volume of 2 l and 1.5 l chlorine solution (Klorin (Lilleborg) diluted 1:9 in tap water, 4000 ppm hypochlorite) overnight, respectively, followed by rinsing twice in tray with 1.5 l dH_2O (43 °C)[a].
Dish washing machine	Normal program of dishwashing machine (washing 60 °C, rinsing 70 °C, total time 120 min, water usage 8 l) (Senz STD49W15) with dishwashing detergent tablet (Sun Maxpower, Orkla)[a].

[a] Neutralization in 100 ml Dey Engley neutralizing broth (Difco).

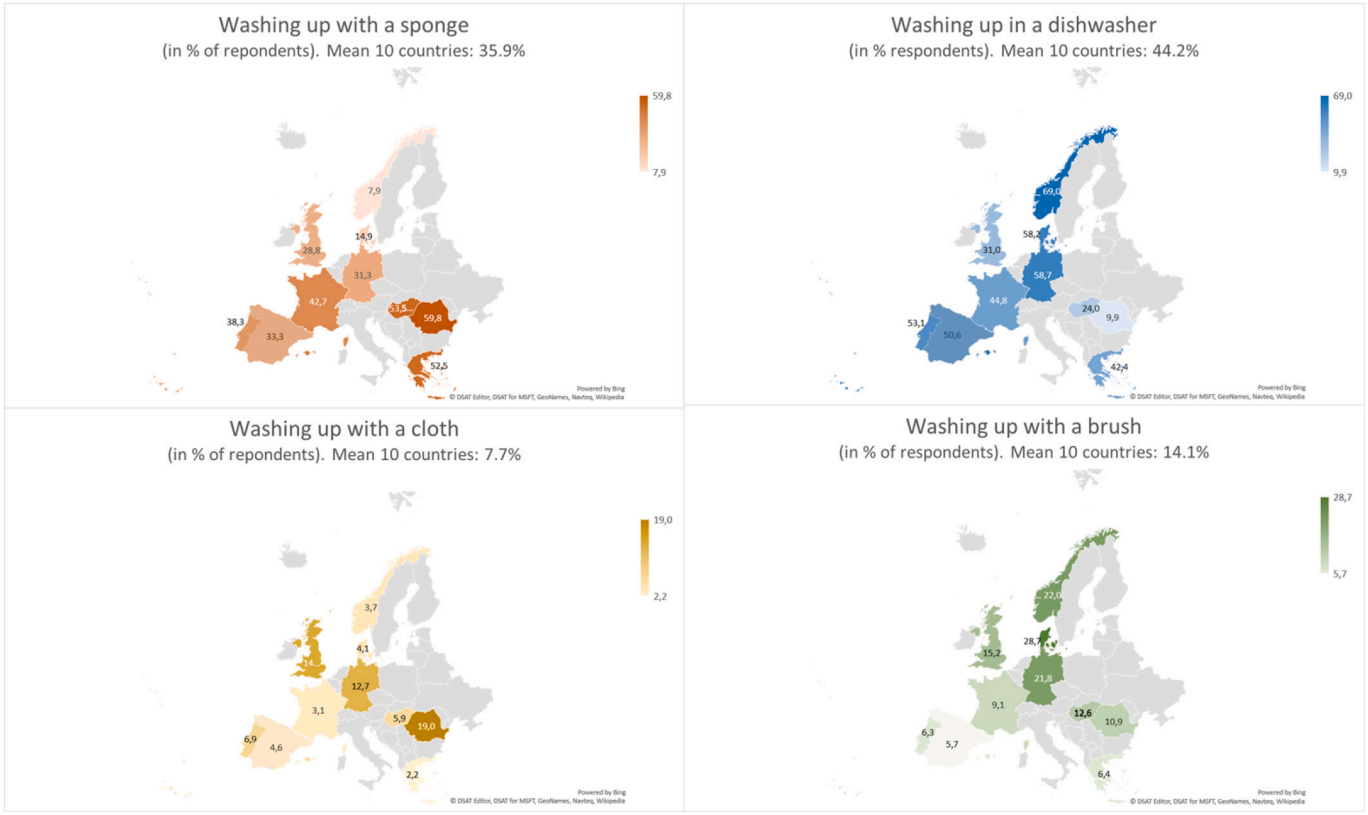

Fig. 1. Choice of cleaning utensil for washing the dishes in 10 European countries, based on a web-based survey with 9966 respondents. Numbers shown are % of respondents. (Original question: "Typically: How do you wash up or clean dirty dishes?")

Interestingly, about 3 to 5% more of the households including individuals at risk (i.e. either pregnancy, young children, diabetes and immunodeficiency, or age over 65 years) responded with higher prevalence of hygienic strategies compared to households without specific risks: more frequent change to a clean or new utensil, decision to change more often based upon time routines or usage and less often based on senses, and more usage of a dishwasher (not shown).

3.2. *Water uptake and drying of sponges and brushes*

As shown in Fig. 2, there was a large variation in water uptake and drying rate among the cleaning utensils. The brushes only absorbed a low volume of water (ca. 5 g) and were dry (< 0.25 g water) after 4.5 h. Compared to the brushes, the sponges absorbed larger volumes of water (approx. 80–180 g). The sponges dried slower, even if they were

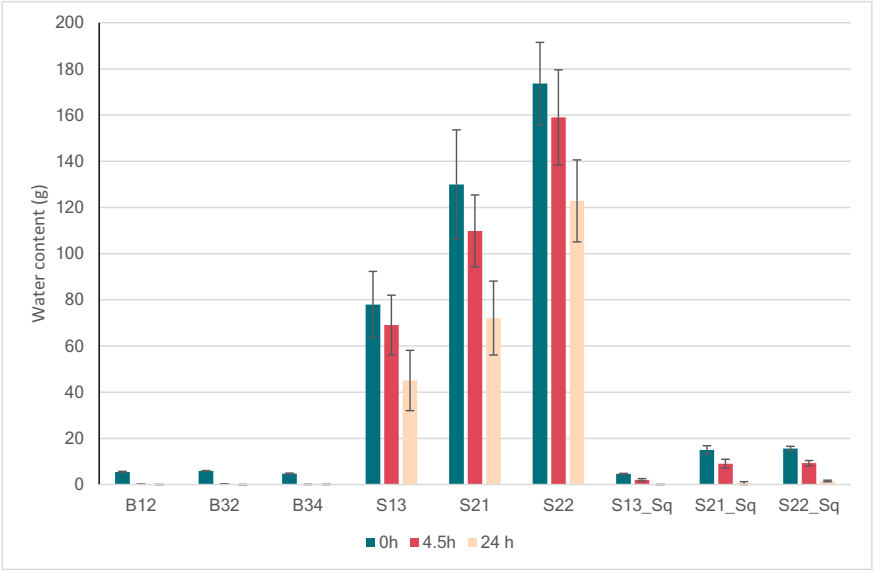

Fig. 2. Water content of sponges (S) and brushes (B) before and after drying for up to 24 h. Brushes and sponges (See Supplementary Table S3 for product description) were immersed in water and left to dry at room temperature. Half of the sponges were hand squeezed (Sq) once after immersion. Mean values and standard deviations for 2–4 replicates are shown.

squeezed once after the initial water uptake before drying. The drying rate varied between the sponges and as expected, large sponges dried slower than small. If not squeezed before storage, all sponges contained relatively high amounts of water (approx. 40–120 g) even after 24 h of storage. Squeezing removed >85% of the water and after 4.5 h of storage, the sponges contained <10 g water. Residual water was only found in the largest sponge (S22) after 24 h (1.5 g). Sponges may be used for several purposes. If considering wiping off surfaces like benchtops, refrigerators etc., there is probably an advantage that sponges absorb water as this will ensure that the cleaning is wet, which is likely to be more effective than dry cleaning. Also, when wiping up wet spills, absorption is advantageous. Brushes does not take up water and are therefore not used for spills but are mostly used for washing up under running water or on utensils immersed in water. The large differences in water uptake and drying rate among and between sponges and brushes may affect survival and growth of bacteria.

3.3. Growth and survival of bacteria in sponges and brushes

The growth and survival of *Campylobacter*, *Salmonella* and kitchen associated bacteria were studied in three types of sponges and in one type of brush. The items tested were previously shown to have different water uptake and drying ability (Fig. 2) and were added water or soil daily except after 4 and 5 days of storage.

There were differences in bacterial survival and growth between the type of cleaning utensil. The lowest bacterial numbers were found in brushes, where a rapid die-off of all types of bacteria was observed (Fig. 3). The rapid die-off corresponds to the fast drying of brush, (as shown in Fig. 2) as no residual water was detected 4.5 h after immersion in water. For sponges, rapid initial growth of bacteria was observed (Fig. 3), and the total bacterial numbers reached around 9 log after 1 to 3 days, and remained stable thereafter. These levels are in line with levels reported in used sponges collected from consumers (Donofrio et al., 2012; Marotta et al., 2018). The levels of *Salmonella* and *Campylobacter* typically decreased over time, and the levels of pathogens in sponges were much lower (0.00001–1%) than the total number of bacteria. Growth of *Salmonella* the first day after inoculation was found in sponge type S22, which according to the drying experiments dried slowly, and *Salmonella* was also detected after 7 days of storage. In average, S22 contained 5.6 g water after 24 h of storage (measured each day during the experiment). In comparison, the two other sponges tested, S13 and S21, contained on average 1.3 and 2.8 g water, respectively. Thus, there are indications that *Salmonella* survived best in the most humid sponge.

Campylobacter died off rapidly, especially in brushes. There was longer survival in sponges, where *Campylobacter* was found after one day of storage, but not at after 3 and 7 days. From other studies it is known that *Campylobacter* is very sensitive to drying (Burgess et al., 2016; Humphrey et al., 2001; Møretrø et al., 2020), compared to *Salmonella* (Burgess et al., 2016; Margas et al., 2014), and this can explain lower survival of *Campylobacter* in dry brushes compared to more humid sponges and also better survival of *Salmonella* than *Campylobacter* in sponges when those dry out over time. Both *Campylobacter* and *Salmonella* have been found in used sponges and cloths collected from consumers (Cogan et al., 1999; Mattick et al., 2003b).

3.4. Bacterial dynamics in sponges and brushes over time

The bacteriota analysis (of the experiment with quantitative bacterial counts shown in Fig. 3) revealed that *Enterobacteriaceae* were dominant after one day of storage of sponges and through the whole storage period for brushes (Fig. 4). Further analysis separated *Enterobacteriaceae* in the two genera that were added: *Serratia* and *Salmonella,* and showed that *Serratia* was the dominating genus. One obvious explanation was that *Serratia* dominated in the inoculum, although the intention was to add all bacteria in equal numbers. The relative

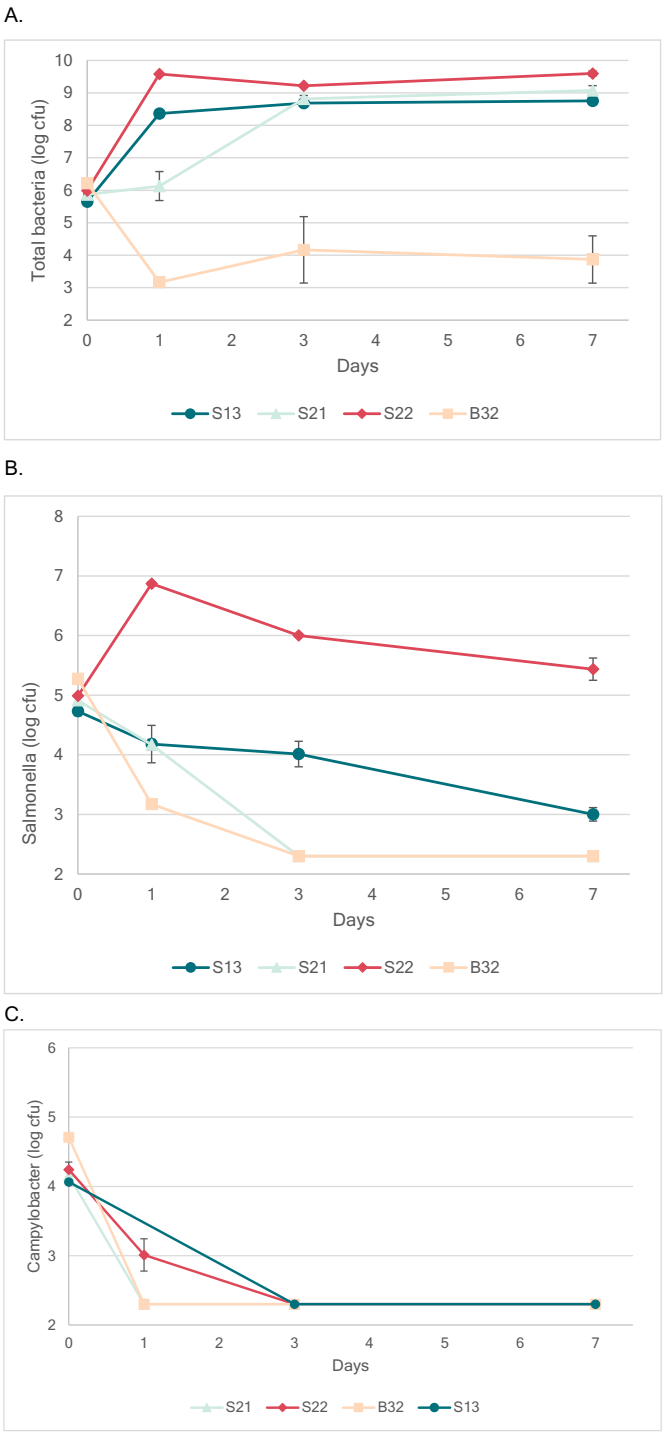

Fig. 3. Total numbers of bacteria (A), *Salmonella* (B) and *Campylobacter* (C) in three types of sponges (S) and one type of brush (B) initially and after 1, 3 and 7 days of storage. The items were inoculated with a suspension of bacteria in food soils, and water or food soil suspension were added at day 1, 2 and 6 and day 3, respectively to simulate usage by consumer. Means of log transformed number bacteria per sponge/brush with standard errors of three technical replicates are shown. Data for S13 at day 1 for *Campylobacter* are missing due to a mistake at the laboratory. Data shown for *Salmonella* for B32 at day 1 is from PCA plates as too high dilution were used for XLD for *Salmonella*. The detection limit was 2.3 log.

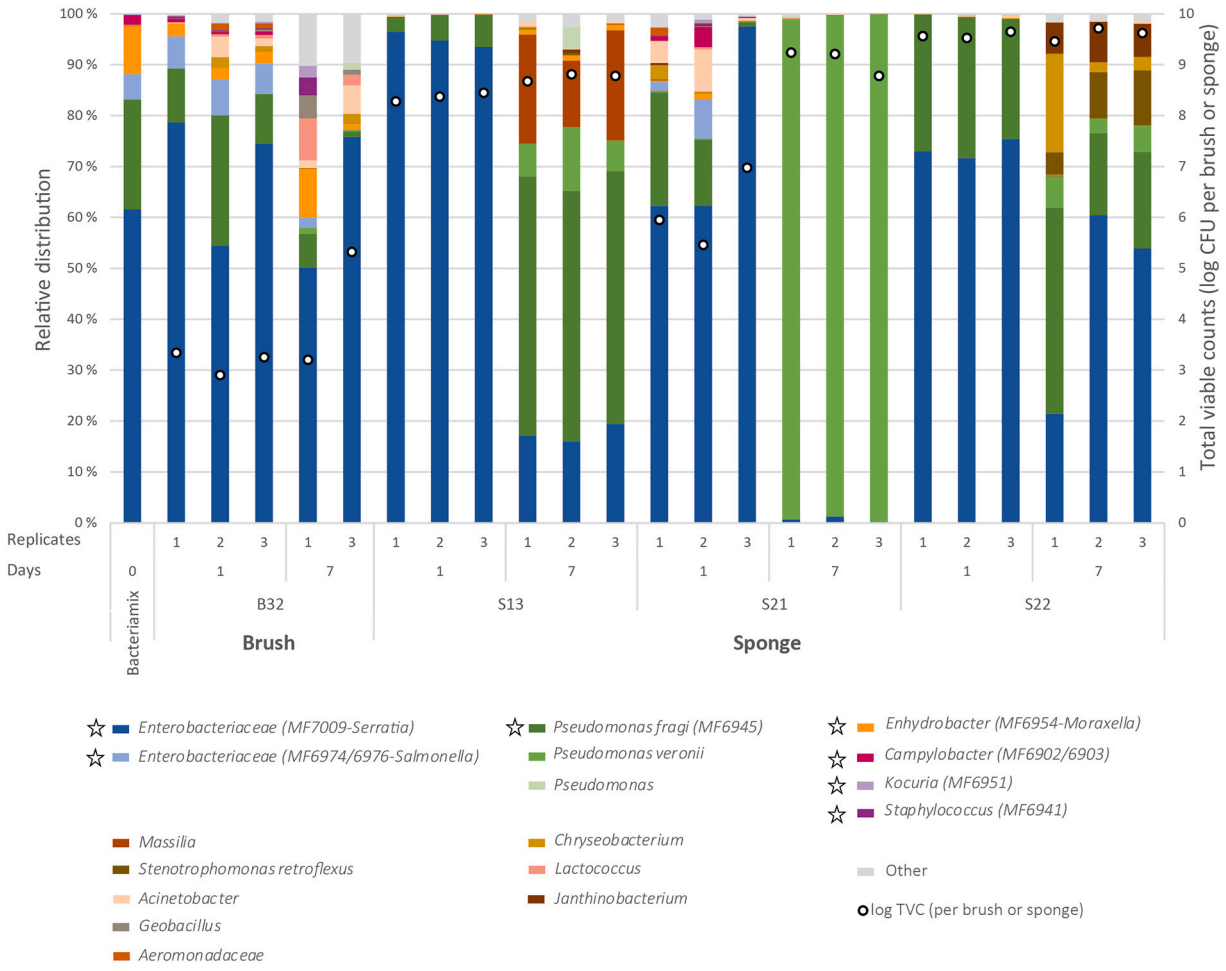

Fig. 4. Relative distribution of bacteria as determined by 16S rDNA-based bacteriota analysis in brushes and three types of sponges added bacteria and food soil and incubated at room temperature. Taxa below an average of 0.15% across all samples is represented as "Other", and multiple representatives of low abundance genera (*Chryseobacterium*, *Janthinobacterium* and *Acinetobacter*) are represented together. The sequence variants corresponding to the deliberately added bacteria are indicated with stars. Total viable count for each sample is indicated with an open circle within the bars, levels relate to the right y-axis. Three technical replicates are shown (numbered 1, 2, 3).

abundance of *Serratia* decreased from d1 to d7 in the sponges, but not in the brush. In sponge S21, *Pseudomonas* was completely dominant at d7, while in S13 *Pseudomonas* dominated together with *Massilia*. In S22, *Pseudomonas* dominated together with genera typically found in the environment (e.g. *Chryseobacterium*, *Stenotrophomonas* and *Janthinobacterium*). An interesting observation is that these environmental bacteria together with *Massilia* in S13 and *P. veronii* in S21 were not part of the bacterial mix added and is likely to have originated from the added food soils (although the new cleaning utensils could not be ruled out as sources of bacteria). The increase in total bacterial count over time in sponges is likely due to high growth of *Pseudomonas*. *Pseudomonas* in general compete well at humid conditions but is sensitive to drying (Møretrø and Langsrud, 2017; Møretrø et al., 2013), and this could explain its higher prevalence in sponges than in brushes. The bacteriota analyses revealed that the pathogenic bacteria were only a small minority of all the bacteria present, confirming the results obtained using selective media for *Salmonella* and *Campylobacter* (shown in Fig. 3).

In initial experiments performed in the same manner, it was revealed after the experiments were completed that *Comamonas* (also from kitchen surface from Portugal) mistakenly had been added instead of *Serratia,* due to a contamination of the inoculated strain. In these experiments, *Comamonas* was prevalent immediately after inoculation and after one day of storage, but it was found in very low numbers after 3 and 7 days, when *Pseudomonas* took over as the dominating genera (Supplementary Fig. S4). The *Pseudomonas* dominance was higher in these experiments, than in the experiments where *Serratia* was added. Regarding the survival of *Salmonella* and *Campylobacter*, as revealed by plate count, the results were similar in the experiments with *Comamonas* and *Serratia* in the inoculum: better survival of *Salmonella* than *Campylobacter* and highest survival of *Salmonella* in sponge 22). When interpreting bacteriota results it must be considered that both living and dead bacteria can be detected, and that differences in copy numbers of 16S rDNA can affect relative abundances.

In a German study, used sponges were found to be heavily colonized by *Acinetobacter*, *Moraxella* and *Chryseobacterium* (Cardinale et al., 2017). In another German study, *Acinetobacter*, *Enhydrobacter*, *Agrobacterium*, *Pseudomonas* and *Chryseobacterium* dominated (Jacksch et al., 2020). These bacteria were also found in the present study, although *Agrobacterium* was only found in very low numbers (< 0.15%). The model system used in the present study seem to favour *Pseudomonas* and *Serratia,* but the reason for this is not known. It should be noted however, that in the present study we tested new sponges in laboratory experiments, while the researchers in the German studies collected used sponges from consumers.

3.5. Antibacterial effect of sponges containing antimicrobial products

The sponges S21 and S22 were claimed by their manufacturers to contain antimicrobial products (silver and quaternary ammonium compound disinfectant, respectively). In agar diffusion tests with

bacteria spread on agar or embedded in soft agar, no growth inhibition zones were observed for *S.* Infantis, *S.* Enteritidis, *Pseudomonas* sp. or *Staphylococcus* sp. (Examples shown in Supplementary Fig. S5). Inhibition zones (about 2 cm) for all bacteria were observed for the absorbent mat containing zinc pyrithione (positive control). In the experiment testing growth/survival of bacteria, the highest growth/survival of total bacteria and *Salmonella* were observed for the S22 sponge containing QAC (Fig. 3). It is likely that high humidity and slow drying were more important parameters for determining growth and survival of bacteria, than the content of QAC. For the S21 sponge containing silver, the growth of total bacteria was slower and the reduction of viable *Salmonella* and *Campylobacter* faster than in the other sponges tested (Fig. 3), however it is difficult to conclude whether this was due to the presence of silver, as no inhibition was observed in the agar diffusion tests. It was also observed that S21 at day seven had a different bacteriota than the other sponges, with absence of *Enterobacteriaceae* and being totally dominated by *Pseudomonas veronii*. To our knowledge there are few other scientific studies examining antimicrobial effects of sponges added antimicrobial compounds. Enriquez et al. (1997b), reported that sponges containing an undisclosed antimicrobial compound had reduced number of total and fecal coliform bacteria in a dish washing five-day model study. In two studies where drops of antimicrobial dishwashing liquids (active compounds not disclosed) were added to conventional sponges, some of the liquids resulted in a 3–5 log reduced total bacterial count in sponges in one of the studies (Nielsen et al., 2002), but no effect on *Salmonella* and total bacterial count was observed in laboratory tests in the other study (Kusumaningrum et al., 2002). In the latter study also no effect on total bacterial count in sponges was found in practical tests in kitchens where antibacterial dishwashing liquid was added daily to sponges (Kusumaningrum et al., 2002). In general for domestic products like cutting boards, counter tops, sinks etc. where antimicrobials are integrated/coated on the

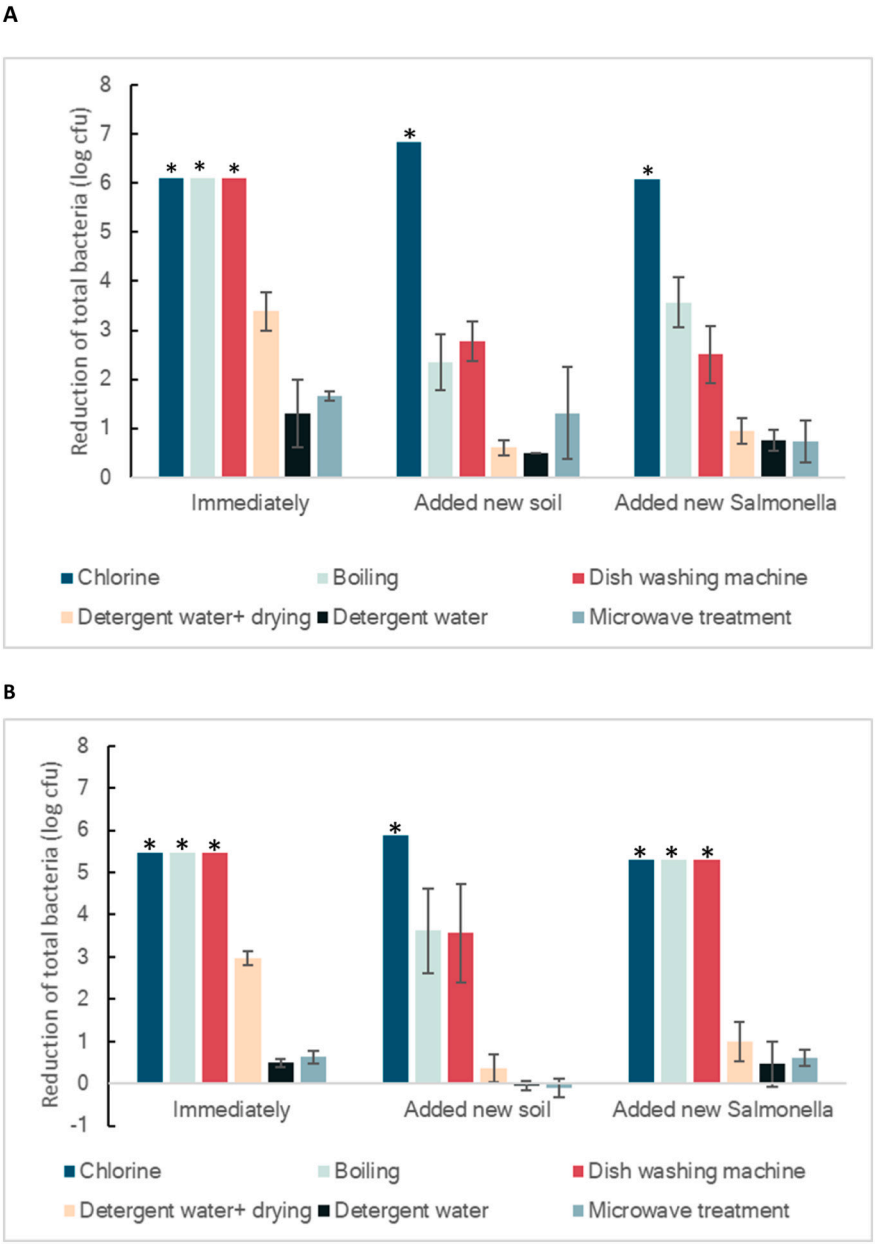

Fig. 5. Effect of treatments of sponges (S13)(A) and brushes (B32)(B) on total bacteria, compared with untreated control, immediately after treatment, and after addition of food soil or *Salmonella* after cleaning treatment follow by humid incubation for 2 d. An asterisk indicates that the bacterial level after treatment was below the detection limit (3 log). Means and standard error of three replicates are shown.

product, their effect on food hygiene are limited (Møretrø and Langsrud, 2011). Even if the added compound has antibacterial properties in vitro, when incorporated in a product there may be challenges like too low concentration, limited availability and declining concentration over time. These factors as well as neutralization in presence of high organic loads, may limit the antibacterial effect under practical use (Møretrø and Langsrud, 2011).

3.6. Cleaning of sponges and brushes

As described previously, used sponges and brushes may contain a high number of bacteria, and at least for sponges in some occasions also pathogens. To reduce the risk, sponges/brushes may be replaced by new items when contaminated. Cleaning of sponges and brushes may be an alternative to replacing them by new items. Observed cleaning methods during kitchen visits as well as other methods described in literature were tested in laboratory studies.

After performing the cleaning routines, some sponges and the brushes were sampled immediately for bacterial enumeration and other sponges were sampled after two days further incubation after adding food soil or *Salmonella*. The purpose of adding the soil was to test if a low number of bacteria (below the detection limit) remained after the initial treatment, and for addition of *Salmonella* the purpose was to test if remaining soil after cleaning treatment could support growth of bacteria including *Salmonella*.

Based on the results for total bacteria (Fig. 5), the cleaning methods tested could be ranked from the most to the least effective as follows: 4000 ppm Chlorine > Dish washing = Boiling > Washing in detergent water + drying > Microwave oven = Washing in detergent water > No washing procedure. The ranking was the same for sponges and brushes and was supported by the results for *Salmonella* (plating on XLD, data not shown). Chlorine eliminated bacteria to below the detection limit and inhibited regrowth. Dish washing machine removed bacteria and most of the soil, but still some soil was left allowing regrowth of bacteria. The cleaning effect may depend on the type of dishwasher and program. It remains to be tested whether economy programs with lower temperatures and water used are effective. New sponges and brushes (no treatment) contained bacteria (not *Salmonella*) that grew when nutrients were added. Cleaning in detergent water followed by drying removed/killed *Salmonella*, but other bacteria remained after the cleaning. Introducing an overnight drying step after detergent cleaning increased the reduction of bacteria. Microwave treatment reduced *Salmonella* and total bacterial count in sponges and brushes, but remaining soil and bacteria lead to regrowth. Boiling killed bacteria to below the detection limit, but remaining soil lead to regrowth of surviving bacteria or growth of *Salmonella* when *Salmonella* was added. Although not tested here, the procedure reported by some consumers with rinsing in water is not likely to be more effective than cleaning with detergent (and water).

The results obtained in the present study were mostly in line with the conclusions from other studies, with some exceptions. Chlorine treatment of sponges has also previously been reported to be effective (Rusin et al., 1998), but in another study only low effect was observed on total bacterial count (Sharma et al., 2009). This may be explained by possible quenching by food soils and low exposure times (<3 min) in the latter study. Sharma et al. (2009), reported high effect (7 log reduction) on total bacteria by use of microwave oven for 1 min at 1300 W. In the present study we found only low effects of microwave treatment on the number of bacteria, maybe because the power level was lower (1 min at 700 W). The effect of different cleaning methods was similar for brushes as for sponges, with several effective methods. We have not found studies on cleaning of kitchen brushes in the literature.

3.7. Food safety impact of contaminated sponges and brushes

As *Salmonella* and *Campylobacter* may be found in sponges, it may be asked how large risk contaminated sponges are for consumers. The most likely scenarios for ingesting pathogens through the use of sponges are the transfer of pathogens a) from sponges to hands and then to mouth either directly or via food or b) from sponges to a surface/equipment and then further transfer to food. As only a fraction of bacteria will be transferred in each step, the direct route from sponge to mouth via hands will potentially transfer the highest number of pathogens.

In eggs, concentrations of *Salmonella* as high as 8–10 log CFU/ml have been reported (Clay and Board, 1991). Thus, in a worst-case scenario, when such spills are wiped up with a sponge, 10 log CFU *Salmonella* may end up in the sponge. Assuming a 1% transfer ratio of bacteria from sponges to hands or surfaces, up to 6–8 log CFU *Salmonella* may end up on hands or surfaces. In such a worst-case scenario both direct transfer from hands to mouth or via food, as well as transfers from a utensil wiped with the sponge to food may lead to a risk of *Salmonella* infection. But it should be noticed that such high levels of *Salmonella* in eggs are not common. The maximum concentration of pathogens in raw poultry are lower than in eggs (European Food Safety Authority, 2010; Huang et al., 2016; Luber, 2009; Luber and Bartelt, 2007; Wang et al., 2013), thus for sponges used for poultry spills only the direct route from sponge to hands to mouth, and not the route from sponge to equipment to food is likely to cause a risk of infection, even in worst-case scenarios. If the food spill is allowed to dry on the surface before it is wiped off, the concentration and transfer of the pathogens, especially *Campylobacter,* will be even lower (Møretrø et al., 2020).

When considering the risk of using contaminated sponges, it is likely less risky to use a sponge with your bare hands than e.g. touching raw poultry with your bare hands, as the concentration of pathogens are likely to be higher on raw poultry than in a sponge used to wipe up e.g. poultry spill, but it cannot be excluded that *Salmonella* may grow to higher concentrations in sponges and lead to increased risk. The risk of ingestion of pathogens through the use of brushes is even lower than for sponges, as the levels of the pathogens will likely be lower in brushes due to die off because of drying. Also, since the brushes have a handle, this reduce the risk of contact with bare hands to the part of the brush which encounters contaminated food spills.

3.8. Advice from food safety authorities regarding cleaning utensils

When checking food safety information from health/food safety authorities in selected European countries as well as from WHO, few recommendations on the use of sponges or brushes were found, as most advice on cleaning utensils focus on cloths (Supplementary Table S4). Consumers are advised to wipe up meat spills with kitchen paper (Denmark and France) or a disposable cloth (Germany). There is generally a focus to clean and change cleaning utensils frequently. The use of sponges is not recommended in Germany or by the WHO. In Germany cleaning brushes in the dishwashing machine is recommended, while in France cleaning/disinfection of sponges by boiling, soaking in diluted bleach or in a microwave-oven, are recommended (Supplementary Table S4).

4. Conclusions and advice to consumers

Advice for use of sponges or brushes from food safety authorities are limited. The present work supports the food safety recommendations for consumers from the WHO to not use sponges for cleaning. A good replacement for dish washing is brushes. The use of brushes is already common in some countries and adopting this practice in other countries should therefore be possible, though it would require efforts from the health authorities, educational institutions and market actors.

In the present study, differences in cleaning practices between countries were found, variations in drying and survival of pathogenic bacteria in new sponges and brushes were observed, and cleaning methods were evaluated. These elements constitute the basis for the following recommendations:

Use brushes rather than sponges for dish washing. It is more hygienic to

use brushes: 1) Brushes dry faster, and there is a lower risk of growth/survival of *Salmonella* and *Campylobacter* in brushes than in sponges; 2) Since brushes have handles, bare hands will not be in contact with the water allowing higher temperatures and thus better cleaning effect; 3) Hands will not be contaminated when using a brush, since pathogens will not be transferred from the brush to bare hands. When using sponges this is a risk; 4) It is easy to keep brushes clean by using a dish washing machine.

Use paper or single-use wipes for raw food spills. Avoid using sponges in high-risk situations such as wiping/cleaning up raw food spills. Using single-use wipes or paper may be alternatives in such situations. If sponges or brushes are used in risky situations they should be cleaned/replaced directly after use, since the pathogens will likely not die off by the time of next usage.

Clean sponges and brushes with chlorine, dish-washing machine or boiling. The most effective methods for cleaning of sponges and brushes are soaking in chlorine (4000 ppm, 16–20 h), cleaning in dish washing machine and boiling.

It should be noted that the research in the present work was performed with new brushes and sponges, and we are currently investigating bacterial levels and the survival of *Salmonella* in brushes and sponges that have been used by consumers.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijfoodmicro.2020.108928.

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