Research Article

Microbiological Analysis of used Kitchen Sponges from Selected Areas of the Ernakulum District of Kerala

Mini K Paul*, Swathy Gopinathan

Department of Biosciences, MES College, Marampally, Aluva, Ernakulam, Kerala, INDIA.

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Correspondence:
Dr. Mini K Paul,
Department of Biosciences, Mes College, Marampally, Aluva-7, Ernakulam, Kerala, INDIA.

Email: minikpaul2016@gmail.com

ABSTRACT

The focus of this research was to assess the microbiological contamination of used kitchen sponges as well as the efficacy of natural and chemical disinfectants. Twenty sponges were collected from households in the Ayyampilly area near Vypin in the Ernakulam district of Kerala, India. The total viable bacteria (TVC), faecal coliforms (TCC), and fungus (FC) were then quantified. Two prominent isolates, denoted as KSBT18 and KSBT32, were identified as *Acinetobacter baumannii* and *Staphylococcus caprae*, respectively. Sequencing of the 16S rRNA gene validated the result. The disc diffusion method was used to test the antibiotic sensitivity of isolated bacterial species on Muller-Hinton agar. *Acinetobacter baumannii* KSBT18 were resistant to all antibiotics tested, and *Staphylococus caprae* KSBT32 was found to be resistant to ampicillin, while sensitive to tetracycline and erythromycin. The sponges were disinfected, with both pure natural products (ginger extract, lemon juice and vinegar) and chemical disinfectant (3 % hydrogen peroxide, 0.1% Phenol, and 100% alcohol) for upto15 min. Natural disinfection approaches did not lower bacterial counts, however phenol disinfection (Himedia) demonstrated a larger reduction in total viable bacteria (TC) than the Lysol disinfection method. We found that treating badly polluted kitchen sponges with phenol (0.1 percent) was the most effective way to eliminate bacteria. After 15 min of exposure, bacteria were decreased to 28 CFU/mL, significantly ($P < 0.05$). The majority of households (65%) cleaned to make their homes 'look clean, smell fantastic, and eliminate germs; nevertheless, householders' perceptions of cleanliness did not always reflect microbiological reality. In terms of home hygiene, more investigation and awareness are required.

Key words: *Acinetobacter baumannii, Staphylococcus caprae,* Kitchen sponges, Sanitation, Disinfectants.

INTRODUCTION

An estimated 100 million foodborne illnesses and 120,000 foodborne illness-related fatalities happen annually India, resulting in the loss of 8 million disability-adjusted life years.[1] Food-borne diseases are a serious public health issue, but little is known about their impact on worldwide social and economic growth. It is linked to high rates of illness and mortality over the world, making it a severe public health concern. In addition to the sheltering and transmission of infection, the kitchen is gradually becoming recognised as the most major area in the residence for cross-contamination of foodborne pathogens. The main concern in the home has been highlighted as cross contamination of pathogenic organisms, such as *Staphylococcus aureus, Salmonella* spp., *Listeria monocytogenes* and *Campylobacter* spp.,[2] which can be either direct or indirect. Direct cross contamination occurs when bacteria are transferred directly from raw food, whereas indirect cross contamination occurs when microorganisms are transferred via a vehicle such as kitchen towels and sponges, hands, utensils, and surfaces.

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A sponge is a cleansing tool that can be used for a variety of tasks, including scrubbing bathrooms and cleaning kitchen equipment. Kitchen sponges, in particular, are used on a daily basis during kitchen cleaning because of their capacity to remove food residues. As a result, a considerable percentage of the remains will invariably be absorbed by the sponge, creating an ideal environment for the growth of diverse bacteria.[3] Sponges are often used to clean surfaces such as cutting boards, pots and pans, dishes, counters, sinks, refrigerators, faucet handles, and stovetops in kitchens all over the world. Foodborne pathogen-infested kitchen sponges require special attention in the home since they can stay moist and function as a reservoir and carrier for germs to cause disease. Food borne pathogens such as *Cronobacter sakazakii,* which can cause necrotizing enterocolitis, bacteremia, and meningitis in children and infants with a 40–80% mortality rate, was isolated from kitchen sponges by Kilonzo-Nthenge *et al*.,[4] and *Listeria monocytogenes,* which can cause listeriosis, was isolated from kitchen sponges by Mattick *et al*.,[5]

Sponge disinfection can help to prevent the risk of germ emergence and spread in the kitchen. Simple, quick, and effective approaches for disinfecting kitchen sponges could help to limit the spread of spoilage and pathogenic germs in home kitchens, resulting in better food preservation and fewer incidents of foodborne disease. The main objective of the study was to determine the level of microbial contamination on used kitchen sponges. The following step was to isolate and identify the most common microbes, as well as study their antibiotic sensitivity patterns. The ultimate goal was to determine the efficacy of natural products (ginger extract, vinegar, and lemon juice) and chemical disinfectants (phenol, hydrogen peroxide, and alcohol) against potential human pathogens, including antibiotic-resistant bacteria. The current study attempted to examine the most efficient and effective method for disinfecting a heavily contaminated kitchen sponge in a short period of time.

MATERIALS AND METHODS

Sample collection

Fresh synthetic kitchen sponges of the same brands having polyester (soft yellow side) and polyurethane (abrasive side) used in the study were purchased from local markets and distributed to a total of 20 houses in the area of Ayyampilly, Vypin, Ernakulam, Kerala. We instructed them to use the kitchen sponges on a regular basis. After two weeks, the sponges were collected aseptically in germ-free polythene bags separately from the participants and taken back and transported to the Microbiology Laboratory, Department of Biosciences, MES College, Marampally, Aluva, Kerala. Samples were processed in an hour. In addition, a questionnaire designed by the investigators for this study was completed by the participants. The questionnaire consisted of two parts: The first part included questions on the history of food-borne illnesses, whereas the second part included questions on their kitchen sponge usage and disinfection procedures adopted in their daily life.

Sample preparation for analysis

Each sponge was coded and cut aseptically into pieces using a sterile knife. A small piece around 25 mm^3 diameter from each sample sponge is transferred in to 5ml peptone water (1%), and kept the tubes in incubator at 37°C for 24 hr. Nine mL sterilized water taken in 6 tubes for each sample sponge. One mL of sample is inoculated into these tubes and using serial dilution method the sample is serially diluted in to other tubes. Each sample (0.1mL) from 10^5 the dilution is transferred in to nutrient agar plate (for total bacterial count) and follow spread plating. Inoculated plates kept at 37°C for 24 hr. Suspensions and serial dilutions were plated on eosin methylene blue agar (EMB) to determine total coliform count and potato dextrose agar (PDA) to determine counts of yeasts and molds. EMB plates were incubated at 37°C for 24 hr before enumeration; the culture plates with no growth were further incubated for 48 hr. PDA plates were incubated at 25°C for 5 days before enumeration.

Enumeration of Micro-organisms: The isolated microbial colonies were counted by using following Colony Forming Unit (CFU) formula and average counts for triplicate (three plates per each treatment), were recorded as total viable microorganisms in the samples. Plates containing 25 to 250 colonies were chosen for enumeration.

Formula: CFU = Number of colonies × Dilution factor / Volume of sample.[6] The results were expressed in log CFU/mL.

Identification of Bacterial Isolates

All isolates were purified by repeated sub culturing, streaked on nutrient agar slant and preserved in refrigerator set at 4°C until further analysis. Among them two prominent isolates were selected for this experiments. The cultural, morphological, physiological and biochemical properties of two isolates KSBT18 and KSBT32 were studied as part of the identification. Identification was done according to the guidelines in

Bergey's Manual of Systematic Bacteriology.[7] The results were also confirmed by 16s r RNA gene sequence based molecular identification. For this isolation of genomic DNA and PCR were conducted as per methods described by Sambrook *et al*.,[8]

Antibiotic Sensitivity Test

Antibiotic sensitivity testing was performed on the selected isolates (KSBT18 and KSBT32) using the disc diffusion method.[9] Plates were examined after overnight incubation at 37°C, and zones of inhibition were quantified using a millimetre scale from the edge to the disc. The tops of four to five colonies of *A. baumannii* and *S. caprae*, were picked up with a sterile loop. The colonies were suspended in a sterile physiologic saline solution of 5 mL. The turbidity of the inoculum was standardised to the McFarland standard of 0.5. A sterile swab was used to inoculate the whole surface of a Mueller-Hinton agar plate. Using a sterile forceps, discs containing 30 µg of tetracyclin, 10 µg of ampicillin, and 15 µg of erythromycin were gently pressed onto the agar surface to ensure contact and the plates were incubated for 20 hr at 35°C. The diameter of the inhibitory zone around each disc was then measured. The National Committee for Clinical Laboratory Standards (NCCLS) documents M31-A2 and M2-A7 are followed in this technique.[10]

Testing the effectiveness of disinfectants on kitchen sponges

The effects of disinfectants were checked by using natural and chemical disinfectants. Natural disinfectants include ginger, vinegar, and lemon juice. Pure ginger extract was prepared by grinding the cleaned ginger on a mortar and pestle. Pure lemon juice extracted by squeezing the fresh lemon aseptically. Vinegar was purchased from market. Chemical disinfectants include hydrogen peroxide (3%), Phenol (0.1%), and alcohol (100%). A small piece around 25 mm^3 diameter from each sample sponge (KS 5 and KS 15) and 1ml disinfectant were added into a sterile Petri plate and mixed aseptically. The mix, spread plated into nutrient agar plate after each 5min, 10 min and 15 min. At 37°C for 24 hr, the plate was incubated. On each sample, the CFU count was calculated after it was treated with various disinfectants at regular intervals. The standard disinfectant for the investigation was both diluted and undiluted Lysol solution. The manufacturer's instructions were followed while diluting the Lysol (12 ml of Lysol in 4 litres of water). Each experiment was carried out three times in total. ANOVA was used to statistically analyse the disinfection procedures, with $p < 0.05$ considered significant.

RESULTS

Microbial load of sponges

The microbial counts of used kitchen sponges collected from various homes are shown in Table 1. KS5 had the highest count in bacterial (3.4 x 10^7 CFU/mL) and fungal count (3.9 x 10^6 CFU/mL) while KS12sponge had the lowest bacterial count (0.6 x 10^2 CFU/mL) and fungal count (4.2 x 10^2 CFU/mL). Overall, 80% of sponges tested in the study had heavy bacterial growth. 60% (12/20) the analyzed sponges, had contamination with coliform bacteria.

Identification of predominant isolates, KSBT 18 and KSBT32

All isolates were purified by repeated subculturing, streaked on nutrient agar slant and preserved in refrigerator set at 4°C until further analysis. Among them two prominent isolates were subjected to identification. Morphological and physiochemical properties of KSBT 18 and KSBT32 was shown in the Table 2. As per the *Bergey's Manual of Systematic Bacteriology* KSBT18 were

Table 1: Shows the microbial counts of used kitchen sponges.

Sample	TVC (CFU/mL)	TCC (MPN/ml)	FC (CFU/mL
KS1	2.7×10^7 CFU/mL	0.4×10^2 CFU/mL	1.4×10^7 CFU/mL
KS2	3.2×10^7 CFU/mL	1 .3CFU/mL	5.2×10^3 CFU/mL
KS3	3.6×10^5 CFU/mL	2.5CFU/mL	2.7×10^6CFU/mL
KS4	3.7×10^4 CFU/mL	0.9×10^2 CFU/mL	1.6×10^3CFU/mL
KS5	3.4×10^7 CFU/mL	1.2×10^2CFU/mL	3.9×10^7 CFU/mL
KS6	1.6×10^7 CFU/mL	3.2CFU/mL	1.5×10^6CFU/mL
KS7	4.2×10^4 CFU/mL	6.2CFU/mL	2.2×10^2 CFU/mL
KS8	0.4×10^7 CFU/mL	0	2.4×10^6 CFU/mL
KS9	1.6×10^7CFU/mL	0	1.2×10^7 CFU/mL
KS10	1.5×10^7 CFU/mL	0	1.5×10^6CFU/mL
4KS11	1.2×10^7 CFU/mL	5.2CFU/mL	1.2×10^7 CFU/mL
KS12	0.6×10^7CFU/mL	0	4.2 CFU/mL
KS13	2.3×10^7 CFU/mL	1.3 CFU/mL	1.8×10^7 CFU/mL
KS14	1.6×10^7 CFU/mL	0	1.5×10^6CFU/mL
KS15	3.3×10^7 CFU/mL	0	3.7×10^7 CFU/mL
KS16	4.2×10^3 CFU/mL	5 CFU/mL	1.9×10^7 CFU/mL
KS17	2.2×10^7 CFU/mL	6 CFU/mL	1.1×10^7 CFU/mL
KS18	3.2×10^7CFU/mL	9 CFU/mL	1.5×10^6CFU/mL
KS19	1.2×10^7 CFU/mL	0	3.2×10^3 CFU/mL
KS20	1.3×10^7 CFU/mL	0	1.2×10^7 CFU/mL

TVC = Total Viable Count; TCC = Total Coliform Count; FC = Fungi Count; CFU = Colony forming unit; Ml = Millilitre;

Table 2: Physiochemical and morphological features of KSBT18 and KSBT32.

No	Test Name	KSBT 18	KSBT 32
1.	Colony Morphology	In nutrient agar media-Colonies are 1 to 2 mm, domed, mucoid, and non-pigmented	In Blood agar media- Smooth, shiny, round and convex colony with haemolysis.
2	Gram staining	short, almost round, rod-shaped (coccobacillus) Gram-negative bacterium	Gram-positive and appear in spherical shape. They are in clusters resembling bunch of grapes when observed under light microscope after Gram staining.
3	Mac Conkey	Non-fermenting	No growth
4.	Motility	Non-motile	Non-motile
4.	MR	-	+
5.	VP	-	-
6	Citrate Utilisation	+	+
7	Oxidase	-	-
8	catalase	+	+
9	H_2S production	-	+
10	Urease	-	-
11	Indole production	-	-
12	Sugar Fermentation Test Results		
	• Sucrose	-	-
	• Mannitol	-	+
	• Glucose	+	+
	• Lactose	-	-
13	Nitrate Reduction Test	-	+
14	Coagulase	-	+

Figure 3: MIC of *A. baumannii* KSBT18 and *S. caprae* KSBT32.

identified as *Acinetobacter baumannii* and bacterial isolate KSBT32 as *Staphylococus caprae*. 2. A BLAST search of the 16S rDNA sequence KSBT 18 and KSBT 32 against NCBI nucleotde database revealed 99% identity with *A. baumannii* and *S. caprae* respectively.

Minimal inhibitory concentration (MIC) test concentration (MIC) test

In our study the *A. baumannii* KSBT18 was found to be resistant to all antibiotics that we tested and the isolate *S. caprae* KSBT32 was resistant to ampicillin, while it was sensitive to tetracycline and erythromycin showing in Table 3 and the zone diameter was in Table 3. Data are presented as the mean ± standard error of three independent experiments.

Test Result of Disinfectant

Effect of disinfectants on selected kitchen sponges (KS5 and KS15) were shown in Table 4. Untreated sponge KS5, receiving no disinfecting treatment had total counts of 3.4×10^7 CFU/mL) and KS 15 has 3.3×10^7 CFU/mL bacteria. The sponges were disinfected, with both natural products (ginger extract, vinegar, and Lemon juice) and chemical disinfectants (phenol, hydrogen peroxide, and alcohol). Natural disinfection procedures with pure extracts for 15 min were unable to reduce bacterial counts, but 0.1 percent phenol disinfection showed a significant reduction ($P < 0.05$), i.e., the bacterial count was reduced from too numerous count (TNC) to 106 CFU/mL after 10 min of exposure and considerably lowered to 28 CFU/mL after 15 min of exposure. We proved that the treatment of phenol (0.1%) on heavily contaminated kitchen sponges was the most effective method to kill bacteria, than the method of disinfection by Lysol.

DISCUSSION

The detection of coliforms in kitchen sponges shows the existence of faeces and could imply the existence of food pathogens. This could be due to poor hygienic and

Table 3: Inhibitory zone of *A.baumannii* KSBT18 and *S. caprae* KSBT32.

Antibiotic Name	Strength of disc	Zone of inhibition around the discs in mm		Degree of sensitivity	
		A. baumannii KSBT18	*S. caprae* KSBT32	*A. baumannii* KSBT18	*S. caprae* KSBT32
Ampicillin	10 µg	Nil	7±1.5mm	Resistant	Resistant
Tetracycline	30 µg	5 ±1.2mm	20±1.8mm	Resistant	Susceptible
Erythromycin	15 µg	5±2,3mm	20±1.3mm	Resistant	Intermediate

Table 4: Effect of disinfectant on selected kitchen sponges.

Sample	Type of Disinfectant		0 min	5 min	10 min	15 min
KS5	Positive control Lysol	Diluted	TNC	TNC	TNC	TNC
		Undiluted	TNC	TNC	568±11CFU/mL	365±3 CFU/mL
	Natural	Ginger extract	TNC	TNC	TNC	TNC
		Vinegar	TNC	TNC	TNC	TNC
		Lemon juice	TNC	TNC	TNC	TNC
	Chemical	Hydrogen peroxide	TNC	TNC	160 ±4 CFU/mL	48 ±7CFU/mL
		Phenol	TNC	TNC	88±3 CFU/mL	19±3 CFU/mL
		Alcohol	TNC	TNC	TNC	220 CFU/mL
KS15	Positive control Lysol	Diluted	TNC	TNC	TNC	TNC
		Undiluted	TNC	TNC	623±5 CFU/mL	354±6 CFU/mL
	Natural	Ginger extract	TNC	TNC	TNC	TNC
		Vinegar	TNC	TNC	TNC	TNC
		Lemon juice	TNC	TNC	TNC	TNC
	Chemical	Hydrogen peroxide	TNC	TNC	224 ±3CFU/mL	104 ±7CFU/mL
		Phenol	TNC	TNC	106 ±4CFU/mL	28±2CFU/mL
		Alcohol	TNC	TNC	TNC	TNC

sanitary practises during food preparation, raw product contamination, a lack of disinfection procedures, cross contamination, and the keeping sponges in places with high moisture content and an appropriate temperature to boost microbial growth. In numerous investigations, kitchen sponges have been found to be possible sources of cross contamination, along with the transfer of disease germs.[11,12] Sponge cleaning is quite common in household kitchens and commercial food services for cleaning equipment, utensils, and sinks. Cleaning techniques aim to remove food residues from surfaces, but as a normal part of that kind of process, particulate matter accumulates. These food remains, together with the moisture maintained in the sponges, provide an ideal habitat for bacterial growth,[11] increasing the risk of microbiological contamination.

In this study, 80% of sponges tested had heavy bacterial growth. 60% the sample, had contamination with coliform bacteria. Similar studies found that *Salmonella spp*., *Proteus* spp., *Campylobacter* spp., *Staphylococcus* spp., and *Moraxella* were on kitchen sponges sampled from different households,[12-16] As a result of a lack of sanitization measures, high concentrations of Enterobacteriaceae, aerobic mesophilic bacteria, coliforms, moulds, and yeasts were also reported in kitchen sponges.[17,18]

The isolated strains were identified and designated as *A. baumannii* KS18 and the bacterial isolate KSBT32 as *S. caprae*. *Acinetobacter*, has previously been isolated from used kitchen sponges.[19] *A. baumannii* is aerobic gram-negative coccobacillus responsible for opportunistic infections of the blood, skin, urogenital, and other tendon infections.[20] The prevalence of *A. baumannii* infections acquired in the community has been steadily increasing.[21] *S. caprae* is a commensal bacteria that colonises people' noses, nails, and skin, causing community-acquired illnesses such as acute otitis externa, peritonitis, urinary tract infections, pneumonia, endocarditis, meningitis, and many cases of bacteremia.[22] All antibiotics tested showed *A. baumannii* KSBT18 to be resistant. Many studies have found that *A. baumannii* rapidly acquires antibiotic resistance, and multidrug-resistant strains have been identified.[23] World Health Organization declared that, *A. baumannii* is one of the most dangerous ESKAPE pathogens that successfully evade the effects of antibacterial treatments.[24] Few

medications are effective in treating infections caused by multidrug resistant *A. baumannii*, owing to the high prevalence of illnesses and outbreaks produced by this organism.

Because of the rise in antimicrobial resistance and the emergence of strains that are resistant to almost all current drugs, *A. baumannii* is attracting more and more attention.[25] Aminopenicillins, first- and second-generation cephalosporins, and chloramphenicol are among the antibiotics that this bacterium is innately resistant to Vila J, Seifert HA.[26,27] It also has a greater potential for acquiring multidrug resistance to broad-spectrum-lactams, aminoglycosides, fluoroquinolones, and tetracyclines.[28] Antibiotic resistance among bacteria is a serious public health concern, as it will result in a rise in foodborne infections and a reduction in treatment options.[29]

As a coagulase-negative *Staphylococcus* species, *S. caprae* is not regarded a clinically important member, but some *S. caprae* strains have gained resistance and their ability to persist in the environment for a long period (i.e., 28 months or more) has been documented.[30] Slime production and biofilm development are other *S. caprae* characteristics that may play a role in conferring pathogenicity.[31]

All of these characteristics of *A. baumannii* KSBT18 and *S. caprae* KSBT32 should make healthcare practitioners worried and vigilant. As a result, early detection and proper identification of this bacteria will aid in preventing major issues that may arise as a result of this microorganism's colonization of kitchen sponges and invasion.

There is a need to start public health surveillance for kitchen sponge sanitization and foodborne infections. From the farm to the table, food safety is paramount. Foodborne illness can arise at any time in the food chain. Basic hygiene, a germ-free preparation environment, and microbe-free dishes are all part of food sanitation. Food contamination can result in disease outbreaks but also foodborne illness and intoxication. Untreated kitchen sponges were heavily contaminated with bacteria. The use of appropriate antimicrobial agents is therefore a crucial step in sanitizing kitchen sponges because ineffective disinfection may increase the risk of transmission and horizontal spread of pathogens. According to our findings, immersing contaminated kitchen sponges in 0.1 percent phenol once a week kills foodborne germs in home kitchen sponges. It is a quick and effective way to improve household kitchen cleanliness and minimize the risk of foodborne illness in the home. Phenol has also been shown to induce severe leakage of critical metabolites as well as the release of K^+,[32] resulting in cellular damage and cell lysis, as well as acting as a protoplasmic toxin, causing cytoplasmic coagulation.[33]

Several studies have found that focused disinfection is the most effective way to lower the risk of infectious diseases in homes. A targeted and regular cleaning and disinfection strategy successfully reduced coliforms, and heterotrophic bacterial populations.[34] Regarding kitchen sponge sanitation and the frequency of foodborne illness, we conducted a survey among the people whose houses we collected used kitchen sponges from. Some of them lack (72%) adequate information about kitchen sponge sanitation, while others (28%) have adequate information but do not practice it on a regular basis, proving the old adage, "Knowledge is useless until it is put into practice." Among them, 25 % suffer foodborne illness once a month. Continuous food safety education in rural areas and motivation are needed to increase awareness about the sanitation of kitchen sponges once a week.

CONCLUSION

On the basis of the findings, it is feasible to assume that kitchen sponges are highly contaminated, but proper cleaning measures are available. Outbreaks infections of residential kitchen sponge can indeed be eradicated by soaking them in 0.1 percent phenol once a week, according to our research. In case of home hygiene, more exploration and awareness are recommended.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

TNC: Too numerous to count; **TVC:** Total Viable Count; **TCC:** Total Coliform Count; **FC:** Fungi Count; **CFU:** Colony forming unit; **Ml:** Millilitre.

SUMMARY

Used kitchen sponges were collected from houses in the Ayyampilly area near Vypin in the Ernakulum district of Kerala, India. The total viable bacteria (TVC), faecal coliforms (TCC), and fungus (FC) were then quantified Two prominent isolates, designated KSBT18 and KSBT32, were identified as *Acinetobacter baumannii* and *Staphylococcus caprae*, respectively. *Acinetobacter baumannii*

KSBT18 was resistant to all antibiotics tested, and *Staphylococus caprae* KSBT32 was found to be resistant to ampicillin, while sensitive to tetracycline and erythromycin. We found that treating badly polluted kitchen sponges with phenol (0.1 percent) was the most effective way to eliminate bacteria.

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