Isolation and microbiological identification of bacterial contaminants in food and household surfaces: how to deal safely

Amal S. Othman

[a]Department of Microbiology, Faculty of Applied Medical Science, October 6th University, Cairo, Egypt

Correspondence to Amal S. Othman, PhD, Department of Microbiology, Faculty of Applied Medical Science, October 6th University, Central Axis, Part 1/1, 12588 Elsheikh Zayed, Governorate of 6 October, Egypt
Tel: +20 100 622 8919;
e-mail: amalothman_1976@yahoo.com

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Objective
This study investigates and reveals the relationship between pathogenic bacteria in some types of food and that present in different household sites (kitchens) and determines an effective disinfecting method to eliminate bacteria from common kitchen locations, some of which could be harmful or pathogenic.

Materials and methods
A total of 90 samples were collected; 85 samples were taken from different sites from five home kitchens and five samples were collected from different types of food. Samples were obtained (before and after disinfection) from kitchen towels, cooking gas stove knobs, refrigerator handles, water taps, and kitchen sponges used for washing utensils by using sterile cotton swabs. Bacteria were identified according to the conventional biochemical methods. DNA fragmentation was done to show the effect of disinfectants on the most common bacteria.

Results and conclusion
Escherichia coli, *Klebsiella* spp., and *Staphylococcus aureus* were the most abundant bacteria in the isolates. After disinfection using disinfectants containing sodium perborate and sodium silicate (detergent), sodium hypochlorite (Clorox), 5% amphoteric surfactant and chlorine (dishwashing powder), and Dettol, the samples were free of bacterial contamination. There was also a correlation between food contamination and bacteria isolated from the kitchens. As *E. coli* was the most highly abundant pathogen in the kitchen and was removed by the tested disinfectants, it was chosen for DNA fragmentation assay to examine the effect of the disinfectants on the bacterial DNA.
Kitchen towels, cooking gas stove knobs, refrigerator handles, water taps, and kitchen sponges are the most common sites in kitchens that transmit pathogenic bacteria. They must be disinfected routinely after preparing food.

Keywords:
cross-contamination, disinfection, DNA fragmentation, *Escherichia coli*, food contamination, food utensils
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Introduction

The kitchen is probably the most crucial area that harbors and transmits infection [1]. Germs are prevalent everywhere in the kitchen in sink sponges, countertops, cutting boards, kitchen utensils, refrigerators, sinks, towels, and even stove tops. Growth of undesirable contaminating bacteria not only causes deterioration in the sensory and organoleptic properties of food but can also cause illnesses. Most pathogenic microorganisms in food products are intestinal in origin; however, some are found in nasal passages, in the throat, on hair, and on skin [2]. Thus, food handlers are often a main source of contamination and cross-contamination. *Salmonella* spp. and *Campylobacter* spp. are easily transferred from chicken to a variety of kitchen surfaces, utensils, hands, and other food items [3]. The ability of bacteria to adhere to food contact surfaces compromises the hygiene of those surfaces. Surface physicochemical properties of the bacterial cell as well as of the materials, such as hydrophobicity and roughness, are determinants during the initial attachment phase [4–6]. It has also been demonstrated that, even after adhering to typical and specific hygienic procedures, pathogenic microorganisms can survive in kitchens, often for hours. The main sites in the kitchen responsible for cross-contamination are chopping boards, sinks, taps, dish cloths, knives, and other working surfaces [7].

The study was performed to ascertain whether there was a relation between pathogenic bacteria in some food samples and that present in common sites in the kitchen and determine an effective physical method to clean and eliminate these pathogenic bacteria.

Materials and methods

Food sample assessment

Samples collected

Five food samples were assessed from five different kitchens randomly selected for the study. Two of

them were assessed directly (raw milk and a pack of mango juice) and the other three samples (beef burger, chicken pane, and cheese samples) were homogenized by blending them separately (1 g/10 ml sterile distilled water). All samples were then serially diluted in nutrient broth media up to 10^3 [8].

Bacterial isolation

The food samples were cultured on blood agar, MacConkey's agar, and nutrient agar media. Plates were incubated at 37°C for 24 h. Bacterial identification was done using selective media and conventional biochemical methods as described by Weaver *et al*. [9].

Quantitative analysis of heterotrophic plate count

The pour plate method was used for determining the heterotrophic plate count (HPC) of bacteria in the five food samples after dilution. They were inoculated on nutrient agar media and incubated for 48 h at 37±1°C. The colony counts were determined using HPC standard protocol [8,10–12] and reported as colony-forming unit (CFU)/ml.

Kitchen assessment

Isolation of bacteria

A total of 25 samples were collected from the previously mentioned five home kitchens. Samples were collected by swabbing from five specific sites in the kitchen (kitchen towels, cooking gas stove knobs, refrigerator handles, water taps, and kitchen sponges used for washing utensils). Specimens were collected on brain–heart infusion broth and incubated at 37°C overnight. Subculture was done on blood agar, MacConkey's agar, and nutrient agar media. Plates were incubated at 37°C for 24 h. Bacterial identification was done using selective media and conventional biochemical methods.

Disinfection

The three most contaminated kitchens were chosen for reassessment after cleaning with four different types of disinfectants commonly used in homes. Soaking is an extremely economical and effective means of killing microbes. Each disinfectant was mixed with boiled water as per the manufacturer's instructions. The disinfectants contained sodium perborate and sodium silicate (detergent), sodium hypochlorite (Clorox, Egyptian co. for house detergent for Detto: Royal cosmetics co.), 5% amphoteric surfactant and chlorine (dishwashing powder), and (Dettol, Royal cosmetics co.).

After disinfecting the kitchen sponge it was washed in the dish washer, and after disinfecting the kitchen towel it was washed in the cloth washing machine. The other three sites were disinfected directly by the disinfectants using disposable tissue. Sixty samples were examined again, five sites in three kitchens using four types of disinfectants, using sterile cotton swabs. Specimens were collected on brain–heart infusion broth and incubated at 37°C for 24 h. Subculture was done on blood agar, MacConkey's, and nutrient agar media and was incubated at 37°C for 24 h.

DNA fragmentation

Sample preparation

The effect of each disinfectant on the selected common bacteria was examined to detect its effect on the bacterial DNA using the DNA fragmentation essay. Four tubes in duplicate contained 2 ml nutrient broth, 20 µl *Escherichia coli* suspension, and 50 µl disinfectant each and the fifth tube was a control tube without disinfectant.

DNA extraction

One milliliter was taken from each of the five tube cultures and was centrifuged at 10 000 rpm, after which the supernatant was discarded. The pellets were washed with 0.5 ml of deionized distilled water (ddH_2O) and centrifuged again. The pellets were then resuspended in 100 µl ddH_2O and subjected to heat block for 10 min at 98°C. Protein precipitation was carried out using 5.3 mol/l NaCl solution and centrifuged at 10 000 rpm for 10 min. The supernatant of each tube was then transferred to a new tube. DNA precipitation was carried out by adding a double volume of isopropanol and centrifuging at 14 000 rpm for 20 min. The DNA pellet was washed with 0.5 ml of 70% ethanol, and centrifuged at 14 000 rpm for 10 min. The supernatant was discarded and pellets were dried at room temperature and finally resuspended in 50 µl ddH_2O [13].

Agarose gel electrophoresis

Tris-acetate EDTA (TAE) electrophoresis buffer (50× TAE stock) was prepared and stored at room temperature and then diluted to 1× upon use in gel preparation or as running buffer. Gel loading dye (6×) was also prepared as bromophenol blue 0.25% (w/v), xylene cyanol FF 0.25% (w/v), and glycerol 30% (v/v).

Results

It is interesting to see the ubiquity of microbes in a home environment and in food samples that we consider safe and devoid of microbes. In this study five food samples were investigated for isolation of bacteria and for determination of HPC (Table 1 and Fig. 1).

Table 1 and Fig. 1 show that chicken pane and beef burger were the most contaminated food samples with the highest HPC, followed by raw milk and white cheese. Mango juice had the least bacterial count. *Salmonella* spp., *Bacillus* spp., *Staphylococcus aureus*, *Shigella* spp., *and E. coli* were identified as the bacteria that contaminated the five food samples tested.

Table 2 shows that the five kitchens were contaminated with different types of bacteria, both Gram-positive and Gram-negative ones. *E. coli* was the most abundant bacteria in the kitchen samples. *E. coli*, *S. aureus*, *Staphylococcus epidermidis*, and *Shigella* spp., which were isolated from the five food samples (Table 1), were all found in the same kitchen. Kitchens 2, 3, and 5 were the most contaminated ones, whereas kitchen 4 was the least contaminated one.

The frequency of abundance of each isolated bacterial strain from the five sites in each kitchen is shown in Table 3. It is clear that *E. coli* and *Klebsiella* spp. were the most abundant bacteria in the five kitchens and *Shigella* spp. was the least contaminating organism.

Figure 2 shows that *E. coli* was the most abundant bacteria in the five kitchens, showing the highest prevalence in kitchens 1 and 5, followed by *Klebsiella* spp., which showed high abundance in kitchens 2 and 5 but was not present in kitchen 4, whereas *Salmonella* spp. and *Shigella* spp. showed low abundance in the five kitchens.

Figure 3 illustrates that *Micrococcus* spp. was the most abundant bacteria, whereas *S. aureus* was the least abundant Gram-positive bacteria in the kitchens. From Table 3 and Figs 2 and 3 we conclude that kitchens 2, 3, and 5 had the highest total frequency count of bacterial isolates and were chosen as the most contaminated kitchens for studying the effect of different disinfectants on the different contaminated sites.

After disinfection, isolation and identification of bacterial isolates was done as illustrated in Table 4. It is obvious that the different disinfectants had considerable effect in removing pathogenic bacteria from the kitchen sites tested, except that in kitchen 3 Dettol could not remove *S. aureus* from the sponge swab sample.

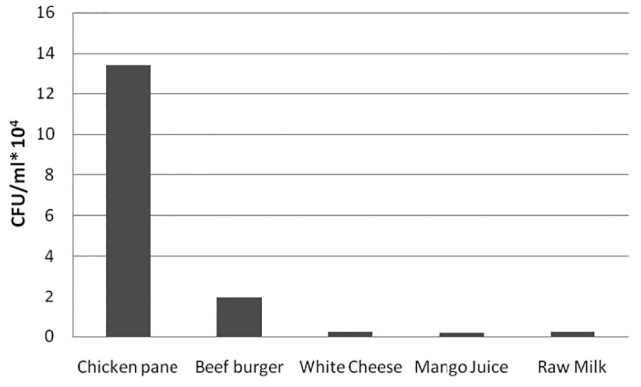
E. coli was chosen as the most highly prevalent bacteria in all examined sites and the effect of each of the four disinfectants was exposed to a DNA fragmentation test to see whether they could affect the bacterial DNA by fragmenting it (Fig. 4).

Figure 4 shows that the DNA of all samples was affected by the tested disinfectants, but with different fragmentation s (which was in the order of E3>E4>E2>E5). Detergent had the most powerful effect as there was no residual DNA in the sample to be fragmented. Clorox also showed nearly the same effect as detergent in E4, whereas dishwashing powder showed the least effect on fragmenting DNA.

Table 1 Diversity of bacteria in the five examined food samples

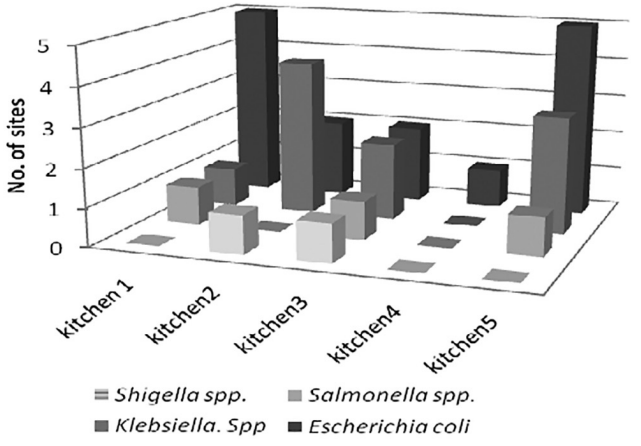
Kitchen nos	Type of food sample	Bacteria isolated
1	Chicken pane	*Salmonella* spp. and *Staphylococcus epidermidis*
2	Beef burger	*Salmonella* spp., *Bacillus* spp. and *Staphylococcus aureus*
3	White cheese	*Shigella* spp. and *Salmonella* spp.
4	Mango juice	*S. aureus*
5	Raw milk	*Escherichia coli* and *S. aureus*

Figure 1



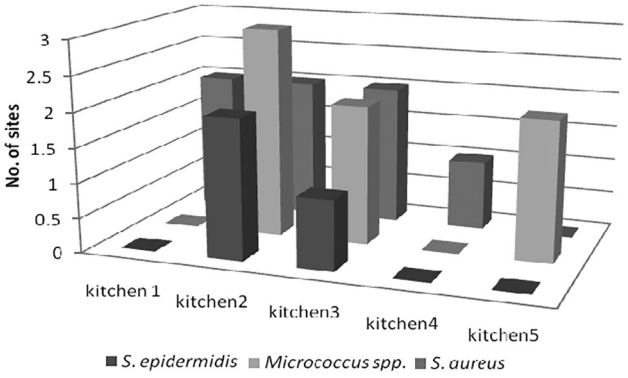
Heterotrophic plate count (CFU/ml) of bacteria in each food sample.

Figure 2



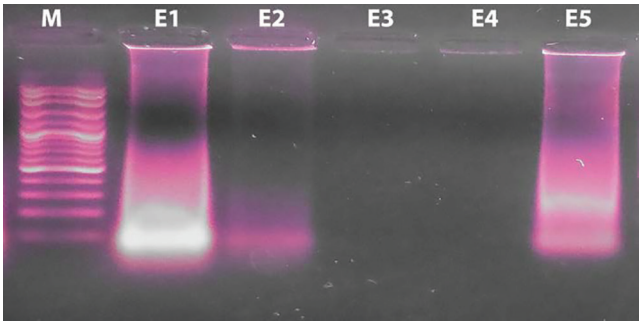
Prevalence of Gram-negative bacteria in the five kitchens.

Figure 3



Prevelance of Gram-positive bacteria in the five kitchens.

Figure 4



Agarose gel 1.5% showing DNA fragmentation in the different samples. M: 100 base pair molecular size marker; E1: control (*Escherichia coli* without treatment); E2: *E. coli* treated with Dettol; E3*: E. coli* treated with detergent; E4: *E. coli* treated with Clorox; and E5: *E. coli* treated with dishwashing powder.

Table 2 Diversity of bacterial isolates in the samples collected from the five tested sites in the kitchens

Kitchen nos	Site	Bacterial isolates
1	Kitchen stove knob	*Escherichia coli*
	Kitchen towel	*E. coli*, *S. aureus*
	Fridge handle	*E. coli*, *Klebsiella* spp.
	Water tap	*E. coli*
	Kitchen sponges	*Salmonella* spp., *E. coli*, *S. aureus*
2	Kitchen stove knob	*Micrococcus* spp., *E. coli*, *Klebsiella* spp.
	Kitchen towel	*Micrococcus* spp., *Shigella* spp.
	Fridge handle	*Klebsiella* spp., *E. coli*, *S. aureus*, *Staphylococcus epidermidis*
	Water tap	*Klebsiella* spp., *S. aureus*, *S. epidermidis*
	Kitchen sponges	*Klebsiella* spp., *Micrococcus* spp.
3	Kitchen stove knob	*Klebsiella* spp., *Shigella* spp.
	Kitchen towel	*E. coli*
	Fridge handle	*S. aureus*, *Micrococcus* spp.
	Water tap	*Klebsiella* spp., *E. coli*, *S. aureus* and *S. epidermidis*, *Micrococcus* spp.
	Kitchen sponges	*Salmonella* spp.
4	Kitchen stove knob	–
	Kitchen towel	–
	Fridge handle	–
	Water tap	*E. coli*
	Kitchen sponges	*S. aureus*
5	Kitchen stove knob	*Klebsiella* spp., *E. coli*, *Micrococcus* spp.
	Kitchen towel	*Klebsiella* spp., *E. coli*
	Fridge handle	*E. coli*
	Water tap	*E. coli*
	Kitchen sponges	*Klebsiella* spp., *S. aureus*, *Micrococcus* spp., *E. coli*

Table 3 Frequency of the isolated bacteria in the five kitchens

Bacterial isolates	Frequency of bacterial isolates	% of prevalence/ total
Escherichia coli	15	31.90
Klebsiella spp.	10	21.28
Staphylococcus aureus	7	14.90
Staphylococcus epidermidis	3	6.38
Micrococcus spp.	7	14.90
Salmonella spp.	3	6.38
Shigella spp.	2	4.26
Total	47	

Table 4 Effect of some disinfectants on survival of bacteria in the five sites in the three kitchens

Agent	Disinfecting area				
	Kitchen no.	Dishwashing powder	Dettol	Clorox	Detergent
Kitchen sponge	2	—	—	—	—
	3	—	*Staphylococcus aureus*	—	—
	5	—	—	—	—
Water tap	2	—	—	—	—
	3	—	—	—	—
	5	—	—	—	—
Kitchen towel	2	—	—	—	—
	3	—	—	—	—
	5	—	—	—	—
Fridge handle	2	—	—	—	—
	3	—	—	—	—
	5	—	—	—	—
Stove knob	2	—	—	—	—
	3	—	—	—	—
	5	—	—	—	—

Discussion

There are a number of methods that can be used to monitor the microbiological safety and quality of foods. HPC is used as an indicator of the level of contamination by bacteria in a food product [14]. According to another literature review [15] each year

an estimated 5.5–6.5 million cases of food poisoning are reported in the USA.

The results of this study show that the CFU/ml for the five tested food samples varied in their bacterial count. Beef burger and chicken pane were the most contaminated samples, followed by white cheese and raw milk, whereas the least contaminated was mango juice. *Salmonella* spp. was the most abundant pathogenic bacteria isolated from beef burger, chicken, and cheese samples. These results were in agreement with other studies, which found that among food samples the highest contamination was found in raw food, followed by cooked food and juices [8].

Exposure to pathogens may occur by either indirect contact with contaminated objects or indirectly through airborne particles. They also indicated that some bacteria, such as *E. coli*, *S. aureus*, *and Salmonella* spp., could survive on hands, sponges, and other objects for up to several days after contact [16].

In the study, 21 of 25 samples that were collected from the five (84%) kitchens were contaminated with pathogenic microorganisms such as *E. coli*, *Klebsiella* spp., *S. aureus*, *S. epidermidis*, *Salmonella* spp., *Shigella* spp., and *Micrococcus* spp.

A similar study [17] was conducted, which found that out of 50 samples collected – that is, five each from 10 kitchens – 32 samples (64% of sample collected) were found to harbor pathogenic microorganisms such as *Klebsiella pneumoniae*, *Proteus species*, *S. epidermidis*, *E. coli*, *S. aureus*, and *Enterobacter* spp. Among those 10 kitchens studied, only one showed contamination with *Enterobacter* organisms, which was found on the surface of the refrigerator handle. He also found that *K. pneumoniae* was the most abundantly found bacteria in the 10 kitchens and it was prevalent in all five sites in the kitchen. This result was in agreement with ours, as *Klebsiella* spp. was the most abundant bacteria following *E. coli* in the five sites tested in the five kitchens but it was not present in all five sites. The results also showed that the highly contaminated sites in the kitchens were water taps, fridge handles, stove knobs, kitchen sponges, and kitchen towels. This may be because water taps and stove knobs were often touched with unwashed hands during cleaning of raw food. This result was in agreement with a previously mentioned study by Adiga *et al*. [17], who revealed that among the different places in the kitchens tested water taps were the most contaminated, followed by stove knobs, towels, and refrigerator handles, whereas kitchen sponges were the least contaminated. The authors concluded that the high incidence of bacteria on kitchen towels was certainly due to the high frequency of using towels to wipe raw food or to dry hands.

In the present study there were similarities between the types of bacteria detected in the food samples and that in the sites tested in the kitchens, except for *Bacillus* spp., which was present in the beef burger sample but never present in kitchen samples. This result may reveal the incidence of cross-contamination between contaminated food and kitchen sites. Another study [18] found that a high incidence of cross-contamination in 25 domestic kitchens by potential pathogens (*Salmonella* spp., *Campylobacter* spp., *E. coli and S. aureus*) was also detected during the preparation of a chicken lunch.

Sponges are commonly used in kitchens around the world to clean surfaces such as cutting boards, pots and pans, dishes, countertops, sinks, refrigerators, faucet handles, and stove tops [15]. Using sponges to clean surfaces, which may be covered with harmful bacteria, and then using them to clean items such as dishes and faucet handles, may allow the bacteria to spread to places where we can come in direct contact with them. Sponges, which may contain a large amount of pathogens, are a common way for bacteria and other food-borne pathogens, such as *Salmonella* spp. and *E. coli*, to spread throughout the kitchen. If the sponges are adequately cleaned, the spread of pathogens from kitchen surfaces and sponges to humans may be greatly reduced.

It is important to clean and sanitize any surface that comes into contact with food. When you clean a surface you are removing all signs of food and dirt. Cleaning only helps to remove some of the bacteria and germs. You can use warm water and dish detergent for general cleaning.

In the present study the most common disinfectants used in each home were assessed to determine their effect on the bacteria that contaminate the tested sites in the kitchen by using boiled water mixed with detergent, Clorox, dishwashing powder, and Dettol each. They showed excellent effect in removing the pathogenic bacteria except *S. aureus*, which was present in the kitchen sponge after using Dettol only. These results were in agreement with another study by Kusumaningrum *et al*. [16], who examined the use of dishwashing detergents as an aid to kill pathogens commonly found in the kitchen, such as *E. coli*, *Salmonella* spp., *S. aureus*, and *Bacillus cereus*. Their study tested detergents with and without food residue present.

Agarose gel electrophoresis is the easiest and most common way of separating, identifying, and analyzing DNA fragments using an agarose concentration appropriate for the size of the DNA fragments to be separated [13]. As the results of this study showed that *E. coli* was the most prevalent bacteria in the five kitchen sites across the five kitchens, it was chosen to investigate the effect of the four disinfectants on its DNA by means of a DNA fragmentation assay.

Exposure of strains of *E. coli*, *Pseudomonas* spp., and *Staphylococcus* spp. to lethal doses of hypochloric acid causes a decrease in ATP production. Chlorine dioxide acts on the permeability of the external membrane of *E. coli* through a primary lethal phenomenon that consists in a substantial leakage of K^+ ions; such leakage does not occur for macromolecules. Sublethal doses inhibit cellular respiration due to a nonspecific oxidizing effect [19]. This may explain why E3 and E4 lanes [sodium perborate and sodium silicate (detergent) and sodium hypochlorite (Clorox)] in the DNA fragmentation test showed no fragmentation as the disinfectant affected the cell membrane and destroyed the bacteria.

Conclusion

There were similarities in the type of bacteria present in the food samples and in some sites in the kitchens. *E. coli* and *Klebsiella* spp. were the most abundant bacteria in the kitchen, which reveals the poor hygiene in these kitchens. To prevent cross-contamination, surfaces and utensils that are used to make and prepare raw food, particularly poultry and meat, should be thoroughly cleaned with an antibacterial cleanser or disinfectant after each use. When using sponges and dish towels, it is imperative that they be disinfected. Sponges should be placed in a dish washer while dish cloths and towels should be soaked in the washing machine. When cleaning your kitchen, do not forget to clean kitchen handles, kitchen sinks, and refrigerator handles with a suitable detergent.

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Conflicts of interest
None declared.

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