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Isolation of microbes from common household surfaces

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Summary:

Microorganisms are ubiquitous in nature. One might think that one is safe from these microorganisms at home, but they can be found there, too, especially in areas where food is prepared. It is common practice to clean food and food products prior to consumption. However, it is equally important to sanitize the places where food is stored and prepared, and also to sanitize the locations and items commonly used within a typical house. This work systematically investigated the occurrence of a variety of microorganisms on common household items and places. We hypothesized that a variety of microorganisms (e.g. bacteria and fungi) would be found from common household surfaces, even when those surfaces are regularly cleaned, and that a strong disinfecting agent, such as ethanol (70%) should eliminate the majority of these microorganisms. The appearance of microorganisms was observed on nutrient agar plates which were swabbed with samples from seven different sites in a house (kitchen counter, kitchen sink, bathroom floor, toilet seat, window, dishwasher, and bathtub) under non-sterile conditions, and the microbial load was observed to be significantly diminished after the surfaces were cleaned with 70% ethanol. We conclude that 70% ethanol based sanitation is of utmost importance to be safe at home.

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Introduction

Due to the ubiquitous nature of bacteria and fungi, the microbial-mediated contamination of food, air, water, and surfaces of common households may cause specific site-borne illnesses. Unless the cause of infection is determined to be due to microbial toxins (e.g. *Shigella, Salmonella, Campylobacter, Listeria, Clostridium*) (1, 2) the microbial contamination to humans is usually symptom-free (3). The bacteria *Salmonella*, *Shigella* and *Escherichia coli* are among the common microorganisms that cause fatal illnesses in humans (4, 5). However, molds also readily enter indoor environments by circulating through doorways, windows, ventilation systems, and air conditioning systems. The most common indoor, airborne molds' spores (e.g. *Penicillium, Aspergillus, Cladosporium, and Alternaria*) deposit on humans, food items, pets, and household items, causing allergic diseases while at the same time causing non-allergic symptoms (4, 5).

It is very easy to find locations in a house and to spot common household items that are inadequately cleaned, because these locations and items are used routinely. For example, kitchen counters, kitchen sinks, bathroom floors, toilet seats, and common household items such as dishwashers and bathtubs may not be adequately cleaned and hence can carry microorganisms that can cause life-threatening diseases (6-8). Microorganisms that can grow in these surroundings may remain undetectable, as they do not produce any bad odor or change the color or texture of their surroundings. If a kitchen has microbial presence due to inadequate cleaning, then even the safest food acquired from a grocery store could get contaminated and spread infection. While freezing of food items could inhibit microbial growth, these microbes can become active after thawing.

Many household locations are always vulnerable to microbial exposure, particularly in family dwellings where many facilities are shared. The bathroom is a good example of a place within a house that has a microbial presence. The microbial abundance found in toilet seats and bathtubs may not be sufficiently eliminated even if detergents are used to clean these places on a daily basis. These are some reasons as to why public health measures have promoted hand-hygiene as an effective tool against bacterial exposure (9). For example, The World Health Organization's (WHO) guidelines compared different hand hygiene methods (10). The alcohol-based sanitizer has been investigated and has been recommended as an effective tool to sanitize various surfaces, including hands (11-13).

The above-mentioned issues motivated us to examine the role of cleaning in the proliferation of microorganisms in common household locations and items. We hypothesized that a variety of microorganisms would be found from common household surfaces, even when those surfaces are regularly cleaned, and that a strong disinfecting agent, such as ethanol (70%) should eliminate the majority of the microorganisms. This work systematically investigates the occurrence of a variety of microorganisms in common household locations, and reports that the number of microorganisms significantly diminished, after the same locations were sterilized with 70% ethanol.

Results

It is interesting to demonstrate the ubiquity of microbes in a home environment, which we considered to be a safe place, devoid of microbes. We attempted to explore the microbial presence in a house built in 2005, adequately maintained for living conditions.

Isolation of microbial flora from kitchen surfaces

Table 1 indicates that the number of microorganisms was quantified as too many to count (TMTC) on a non-sterile kitchen counter, where food is prepared routinely. **Fig. 1 A-i** indicates the abundant growth of microorganisms from pre-cleaned surface conditions on nutrient agar (NA) medium plates. On the other hand, Fig. 1 A-ii, indicates a countable number of microorganisms (24) on the same surface after it was cleaned with 70% ethanol. The microbial colonies from ethanol-cleaned surfaces appeared to be white (6), yellow (13), and black (1). The colonies from the sterilized surfaces presented as both circular and circular filamentous. The black circular filamentous microbial colony appeared to be fungi and was the largest size found in this condition (4.5 mm) (**Fig. 1 A-ii**). No microbial growth was observed in the corresponding control condition (**Fig. 1 Ct.**).

Our results indicated a total of 90 microbial colonies from the swab of an un-cleaned dry kitchen sink surface streaked on an NA plate (**Table 1**). We distinguished the colonies of microorganisms based on their color, shape and average size (**Fig. 1 B-i**). A total of five colony types were differentiated based on the color, i.e. yellow (30), white (25), red (4), black (1), and brownish (30). Most colonies were circular and irregular, within the range of 1-2 mm in size. The cotton swab that was rubbed on the 70% ethanol cleaned surface and streaked on an NA plate revealed no appearance of microbial colonies (**Fig. 1 B-ii**), in comparison to the control set where a sterile cotton swab was streaked on NA plate (**Fig. 1 Ct.**).

The dishwasher, which uses strong detergents to clean dirty dishes, is also an important household item in the kitchen. The outer surface of an un-cleaned dishwasher yielded TMTC microorganisms on NA plates (**Fig. 1 C-i; Table 1**). Most of these microbial colonies were yellow and white in color, and circular with a small size (<1 mm). The orange microbial colonies were lesser in number than the yellow and white colonies; however, they were slightly bigger in size (**Fig. 1 C-i, Table 1**). The same surface when cleaned with 70% ethanol had diminished microbial presence and revealed the appearance of only two microbial colonies (**Fig. 1 C-ii**). A black colony was filamentous and larger in size (4 mm) than the white circular colonies (1 mm; **Fig. 1 C-ii; Table 2**). The corresponding control NA plate that was streaked with the control swab showed no microbial growth (**Fig. 1 Ct.**).

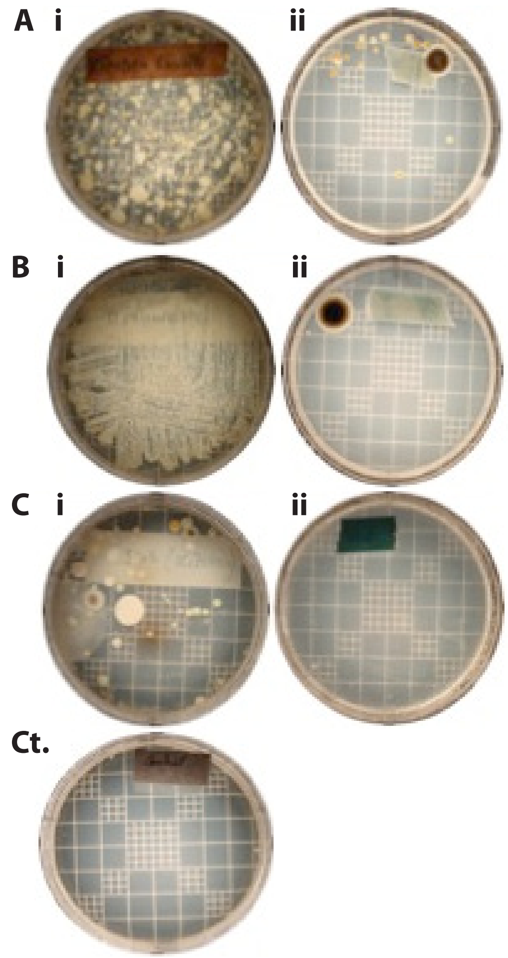

Figure 1. The microbial growth from non-cleaned (**i**) and 70% ethanol cleaned (**ii**) kitchen surfaces (**A**: Kitchen counter; **B**: Kitchen sink; **C**: Dishwasher) on nutrient agar (NA) medium plates. The control (**Ct.**) NA plate was streaked with a sterile cotton swab without swabbing on any surface.

Isolation of microbial flora from a bathroom

A white marble bathroom floor was swabbed and after five days of incubation, a total of 478 microbial colonies was observed (**Table 1**). The highest count (422) was for white circular colonies, with a total of six large colonies (3 mm). Among the yellow circular colonies, 22 microbial colonies were observed. A total of three black colonies were larger than the white and yellow microbial colonies that appeared on NA plates (**Fig. 2 A-i; Table 1**). The surface cleaned with 70% ethanol revealed eight microbial colonies on the NA plate. Among white (5) and yellow (2), one black colony was the largest (4.5 mm) (**Fig. 2 A-ii; Table 2**). The control streaking of a sterile cotton swab on the NA plate did not reveal any microbial

Sample	Total number of colonies*	Morphology of colony (Number of colonies)		
		Color (total number)	Shape (total number)	Average size (total number)
Kitchen counter	TMTC	Yellow (TMTC)	Circular (TMTC)	1-4 mm (TMTC)
		Brown (TMTC)	Round (TMTC)	1-3 mm (TMTC)
		Red (5)	Circular (2)	2 mm (2)
			Wrinkled (3)	3 mm (3)
Kitchen sink	90	Yellow (30)	Circular (30)	2 mm (30)
		White (25)	Circular (24)	2 mm (20)
				3 mm (3)
				5 mm (1)
			Irregular (1)	2 mm (1)
		Red (4)	Circular (2)	1 mm (2)
			Irregular (2)	2 mm (2)
		Black (1)	Circular (1)	4 mm (1)
		Brownish (30)	Circular (30)	1 mm (4)
				3 mm (26)
Dishwasher	TMTC	Yellowish (TMTC)	Circular (TMTC)	<1 mm (TMTC)
		Orange (TMTC)	Round (TMTC)	2 mm (TMTC)
		White (TMTC)	Circular (TMTC)	1 mm (TMTC)
Bathroom floor	478	White (422)	Circular (422)	1 mm (388)
				2 mm (28)
				3 mm (6)
		Yellow (48)	Circular (48)	1 mm (26)
				3 mm (22)
		Black (8)	Circular (8)	1 mm (5)
				4 mm (3)
Bathtub	TMTC	White (TMTC)	Circular (TMTC)	1 mm (TMTC)
		Yellow (2)	Circular (2)	2 mm (2)
Toilet seat	TMTC	Yellow (TMTC)	Circular (TMTC)	1 mm (TMTC)
			Smooth (TMTC)	2-4 mm (15)
			Wrinkled (TMTC)	3 mm (5)
			Flat (TMTC)	1 mm (TMTC)
			Opaque (TMTC)	<1 mm (TMTC)
		Red (TMTC)	Circular (TMTC)	2 mm (TMTC)
			Flat (TMTC)	<1 mm (TMTC)
		White (TMTC)	Circular (TMTC)	1 mm (TMTC)
			Smooth (TMTC)	3 mm (6)
			Flat (TMTC)	2 mm (5)
			Opaque (TMTC)	2 mm (6)
Window	72	Black (25)	Circular	3 mm (15)
			Filamentous (19)	4 mm (7)
			Irregular (6)	5 mm (3)
		White (10)	Circular (10)	1 mm (10)
		Yellow (35)	Opaque (31)	1 mm (28)
			Circular (4)	2 mm (7)

Table 1: The appearance of microorganisms on nutrient agar plates from common household items under non-sterilized (uncleaned) conditions. TMTC: too many to count. *Data represent the rounded average of three identical sets of experiments.

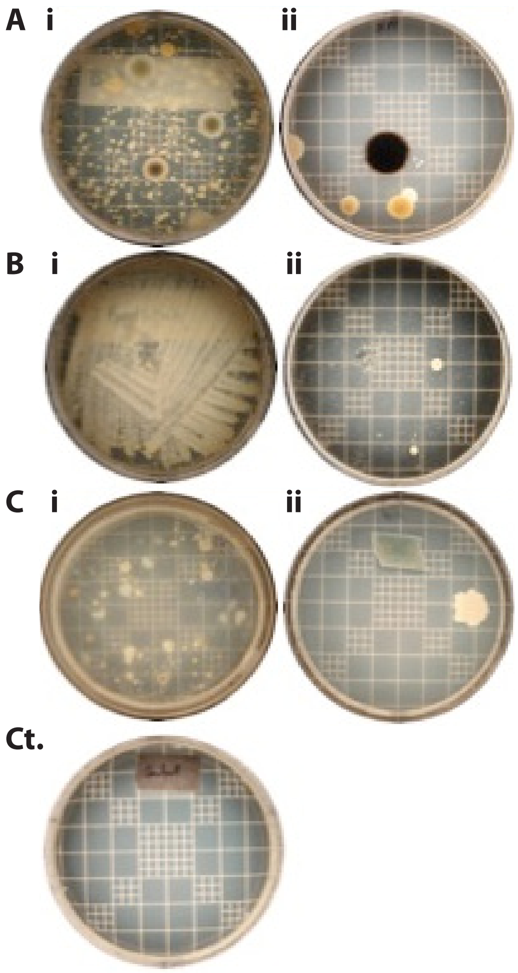

Figure 2. The microbial growth from non-cleaned (**i**) and 70% ethanol cleaned (**ii**) bathroom surfaces (**A**: Bathroom floor; **B**: Bathtub; **C**: Toilet seat) on nutrient agar (NA) medium plates. The control (**Ct.**) NA plate was streaked with a sterile cotton swab without swabbing on any surface.

growth (**Fig. 2 Ct.**).

Our studies showed TMTC microorganisms from an un-cleaned bathtub surface (**Fig. 2 B-i**). Most microbial colonies were white, circular, and small (1-2 mm) (**Table 1**). Two circular yellow microbial colonies that were observed on the NA plates were slightly bigger than the white colonies. Data from the same surface after cleaning with 70% ethanol indicated a significant decrease in microbial count on the NA plate. Only three microbial colonies appeared and they were white, circular, and varied in size (1-2 mm) (**Fig. 2 B-ii; Table 2**). The control streak on NA plate does not show a microbial presence (**Fig. 2 Ct.**).

The swab of the toilet revealed TMTC microorganisms in our studies (**Table 1**). Microbial colonies that appeared were mostly yellow and brown, and these colonies were noticeable on the NA plates from the un-cleaned surface of a toilet seat (**Fig. 2 C-i; Table 1**). Many small microbial colonies (1-2 mm) were observed in different shapes, colors, and textures. The number of microbial colonies diminished when the same area of toilet seat was cleaned with 70% ethanol and swabbed on NA plates (**Table 2; Fig. 2 C-ii**). Only one white colored irregular colony appeared from the same surface after the surface was cleaned using 70% ethanol (**Fig. 2 C-ii**). This microorganism appeared to be bigger than the microbial colonies from the same un-cleaned surface of the toilet seat. The corresponding control plate did not reveal any microbial growth (**Fig. 2 Ct.**).

Isolation of microbial flora from a window

A total of 72 microbial colonies appeared on the NA plates from the un-cleaned glass surface of the upper portion of a window, where human contact was minimal. Mostly yellow-colored microbial colonies appeared on the NA plate including black large (3-5 mm) filamentous colonies (**Table 1; Fig. 3-i**). The yellow color colonies were of two types: opaque and circular. Both colonies were very small (1-2 mm; **Table 1**). The microbial colonies were reduced in number when the same surface was cleaned with 70% ethanol. Only two small (1 mm), white colored colonies appeared on the NA plates (**Fig. 3-ii; Table 2**). The corresponding control plate revealed no growth of microorganism (**Fig. 3 Ct.**).

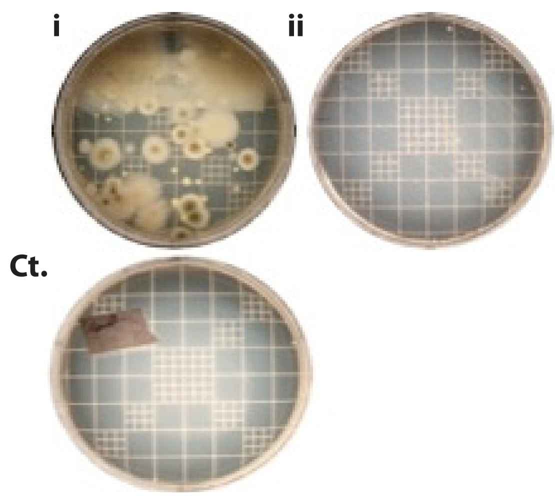

Figure 3. The microbial growth from a non-cleaned (**i**) and 70% ethanol cleaned (**ii**) window surfaces on nutrient agar (NA) medium plates. The control (**Ct.**) NA plate was streaked a with sterile cotton swab without swabbing on any surface.

Discussion

Home characteristics, particularly those associated with increased moisture levels, have been linked to respiratory symptoms due to the growth of microbial flora (i.e. bacteria and fungi) (14). Stark et al. reported that in the first year of a house becoming damp, the increased level of exposure to microorganisms, especially fungal, may increase the risk of allergic rhinitis (15). Our study revealed varied microbial loadings from routinely used

Sample	Total number of colonies*	Morphology of colony (Number of colonies)		
		Color (total number)	Shape (total number)	Average size (total number)
Kitchen counter	24	White (6)	Circular (6)	1 mm (1) 2 mm (5)
		Yellow (13)	Circular (13)	1 mm (5) 2 mm (8)
		Black (1)	Circular Filamentous (1)	4.5 mm (1)
Kitchen sink	0	ND	ND	ND
Bathroom floor	8	White (5)	Circular (4)	1 mm (3) 2.5 mm (1)
			Irregular (1)	3 mm (1)
		Black (1)	Circular (1)	4.5 mm (1)
		Yellow (2)	Circular (2)	2 mm (1) 3 mm (1)
Bathtub	3	White (3)	Circular (3)	1 mm (1) 2 mm (2)
Dishwasher	2	Black (1)	Circular Filamentous (1)	4 mm (1)
		White (1)	Circular (1)	1 mm (1)
Toilet seat	1	White (1)	Irregular (1)	4 mm (1)
Window	2	White (2)	Circular (2)	1 mm (2)

Table 2: The appearance of microorganisms on nutrient agar plates from common household items under non-sterilized (un-cleaned) conditions. ND: not detected. *Data represent the rounded average of three identical sets of experiments.

sites (i.e. kitchen counter, bathroom floor, toilet seat, and window) and common household items (i.e. kitchen sink, dishwasher, and bathtub).

A variety of microorganisms were observed from the studied locations. Even though these locations are routinely cleaned, the occurrence of microorganisms is assumed to be part of normal microbial flora. However, the unanticipated number of microorganisms at the various un-cleaned surfaces in the house is a major concern. The large number of microbial flora on NA plates from un-cleaned surfaces suggests that we should explore alternative and non-hazardous materials to sanitize our households routinely.

The microbial flora observed from the un-cleaned surfaces of various locations in the house can be considered as non-pathogenic microbial biota. However, we did not attempt to extend our study on microbial specificity towards pathogenic aspects. It is important to point out that the non-pathogenic microorganisms can mutate into a virulent phase, liberating toxins and causing illness (16). In general, the normal human microbial biota can defend the human body from several microbial types (17). The extensive use of antibiotics on the other hand has led to antibiotic resistance in many microbial types such as Methicillin-resistant *Staphylococcus aureus* (MRSA) (1) and *Enterococcus* (2). Consequently, microbial pathogenesis is currently a major concern within households, where most sources of microbial infection remain unnoticeable.

A strategic disinfectant is very valuable in securing households from infections. While detergents, ammonia and bleach could have harmful effects on human health, antimicrobial hand soaps and sanitizers are safe, and are defined as drugs under 21CFR by The Food and Drug Administration (FDA) (18). Most hand-sanitizer products in the market generally contain anywhere from 60-90% ethyl alcohol, and are sold as a means to kill germs. Depending on the type of microorganism, ethanol, as a dehydrating agent, causes cell membrane damage, rapid denaturalization of proteins with subsequent metabolism interference, and cell lysis (19). In our study, we show the efficient elimination of microorganisms by cleaning the surfaces with 70% ethanol. However, alcohol may not be effective against a few non-lipid-enveloped microorganisms (e.g. noroviruses) or endospores of bacteria (e.g. *Clostridium difficile*) (20). Previous work suggests that barrier precautions may be an effective method to reduce *C. difficile* transmission (20). It may not be practical to use 70% ethanol to disinfect the house; however, it is a safe and effective disinfectant that could provide efficient elimination of microorganisms. Based on the data presented in this research, we recommend using hand-sanitizers containing at least 70% ethanol. Our research shows that when common household surfaces are cleaned with 70% ethanol, they do not support the same number of microorganisms, as compared to when the surfaces were not cleaned (**Fig. 1-3, Table 1, 2**). Seventy percent ethanol is one of the most efficient tools under the safety net of drugs approved by the FDA to destroy a wide group of microorganisms (18).

Our study of the complex microbial variety from routinely used locations within households was based on the color, size, and appearance of microorganisms (**Table 1 and 2**). However, the identification and measurement of commonly found microorganisms from other households and sites may help in understanding the longitudinal changes in home microbial levels. It will also enable us to determine the conditions under which most microbial outbreaks may occur within households. We assessed 70% ethanol as a cleaning material at different house locations, and demonstrated its efficiency and effectiveness on a wider group of microorganisms. These studies will further pave the way to reintroduce alcohol-based cleaning agents in the commercial market, avoiding the use of harmful chemicals in detergents, ammonia, and bleach in the environment.

Methods:

Preparation of nutrient agar plates

The dehydrated culture medium, nutrient agar (NA), obtained from Thermo Scientific was prepared according to manufacturer's recommendations. Twenty-three grams of NA medium was added to one liter distilled water and sterilized by autoclaving at 15 pounds per square inch (psi) and 121ºC for 45 minutes. The medium was cooled to 60-65ºC and poured into sterile disposable petri dishes (95 x 15 mm). The medium was solidified at room temperature and incubated at 37ºC overnight to check for possible contamination. The plates were stored at 4ºC in an airtight polyethylene bag until they were used for the isolation of microorganisms.

Isolation of microorganisms from house locations and household items

The microorganisms were examined from routinely used surfaces of different locations in a house (i.e. kitchen counter, kitchen sink, toilet seat, bathroom floor, and window) and common household items (i.e. bathtub, dishwasher). The cotton swabs, obtained from a local store, were sterilized by autoclaving at 15 psi and 121ºC for 15 minutes, and stored at room temperature until ready to be used. The cleaning agent, 70% (v/v) ethanol solution, was prepared by mixing 70 mL absolute ethyl alcohol in 30 mL distilled water.

The sterile cotton swabs were swabbed at one-inch sections of kitchen counter, kitchen sink, toilet seat, bathroom floor, window, bathtub, and dishwasher and streaked individually on separate sterile NA plates. The same surfaces were then rubbed with a wet 70% ethanol cotton swab. After drying the surface within two minutes, the same surfaces were swabbed with another sterile cotton swab and streaked on NA plates. Streaking sterile cotton swabs on NA plates without swabbing at any surfaces was performed as a control experiment.

After streaking, the plates were incubated at 28ºC for five days. The microbial growth was observed at regular time intervals for all three experimental types. The total number of colony forming units was manually counted using a Reichert Darkfield Quebec colony counter (Fisher Scientific) and documented. The microbial morphology was studied, and final imaging was performed using an Olympus Stylus digital camera. All the experiments were carried out in triplicate, and the experimental results represent the rounded average of three identical sets of experiments.

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