

Evaluation of microbial contamination in frequently used Fomites in Kuwait

Abstract

The occurrence of potential pathogens and the levels of contamination at individual sites, particularly in fitness club, gas station, home, hospital, and supermarket in Kuwait is described in this paper. The samples were collected using sterile swab sticks. Results obtained showed the highest bacterial count of 2.8 log₁₀ CFU/ml in bathroom doorknobs at gas station, while the least was obtained from pantry in hospital with 1.3 log₁₀ CFU/ml. Prevalent isolated bacterial contaminants were *Staphylococcus epidermidis* (34%), followed by *Enterococcus faecalis* (26%), *Staphylococcus aureus* (14%), *Escherichia coli* (10%), *Streptococcus pyogenes* and *Pseudomonas alcaligenes* (4%), *Streptococcus agalactiae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (2%). Eighty percent of all swab samples cultured were gram positive and 20% were gram negative bacteria. The types and numbers of disease-causing microbes reported in previous epidemiological studies were also found in this study. Appropriate hygienic measures to suppress any potential microbial cross-contamination are therefore needed. It is also imperative to conduct regular testing to check for bacterial contamination and increase community awareness and education for hygienic standards.

Keywords: Environmental contamination, microorganisms, public health

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Introduction

In today's diverse human activities, hidden micro-organisms in indoor and outdoor environments are inescapable and impose dangerous health issues. In recent years, with the introduction of advanced technologies in houses, hospitals, industry, and other environments, the apprehension has increased. The interest in evaluating the level of microbial contamination in places at risk has increased and is considered to be an essential step toward infection prevention.^{1,2} Recent epidemiological studies have reported fomites in the transmission of human pathogens within high-exposure environments such as hospitals, child-care facilities, long-term care facilities, and sports facilities.^{3,4} Various types of microorganisms were identified, including rotavirus, rhinovirus, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Serratia marcescens*, to cause gastrointestinal diseases, the common cold, necrotizing fasciitis, and catheter associated bacteremia, respectively.⁴

Microbial contaminations are widely reported in different indoor and outdoor environments. Tunc & Olgun⁵ investigated the bacterial contaminations of 50 public telephones in Afyon city, Turkey.⁵ Twelve different types of bacteria including *Escherichia (E.) coli*, *Pseudomonas (P.) aeruginosa* and *Staphylococcus (S.) aureus* were found on the surface of telephones. Similar observations were also reported for hospital telephones and personal pagers.⁶ Rutala et al.⁷ studied the extent of microbial contamination, the efficiency of different disinfectants, and the cosmetic and functional impacts of the disinfectants on computer keyboards.⁷ The main findings were that microbial contamination of keyboards is ubiquitous and contamination can be cleansed with disinfectants. Narmeen et al.⁸ isolated and identified *S. aureus* pathogen in different locations in Azadi General Hospital including patients, health care staff, and hospital environment using both bacteriological as well as molecular markers.⁸ Of the 224 specimens collected from different sites, only 52 isolates were found to be *S. aureus*, which accounted for 23.21%

of the total isolates. *S. aureus* can cause infections habitually in newborns, surgical, burns, diabetic patients, and persons who are taking drugs suppressing immunodeficiency diseases. Harrison et al.⁹ addressed the risk of cross-contamination between hands, towels, and dispenser if either one is contaminated, using *Micrococcus (M.) luteus* and *Serratia (S.) marcescens*; both have a distinctive colonial morphology on the plate count agar used.⁹ Results demonstrated that zig-zag transfer of bacteria between paper-towel dispensers and hands can occur if any of these is contaminated.

Several factors have been identified to affect the transfer rate of bacteria from surface to another surface. These include bacteria type, source and destination surfaces, time post inoculation, and moisture level.^{10,11} Montville et al.¹² investigated bacterial transfer rates from food to hands and from hands to food with and without a glove barrier.¹² The authors found that a glove barrier can decelerate the transfer rate of microorganisms from food to hands. Additionally, it was reported that the majority of gloves are permeable to bacteria in a setting simulating actual use. It was also speculated that the glove barrier may be influenced by inoculum size. Tunc & Olgun⁵ found that the frequent usage of public telephones is a key factor increasing the cross-contamination.⁵

Several hygienic measures were reported to prevent cross-contamination from surface to another surface. Hand hygiene is one of the imperative tools to reduce and prevent surface-to-surface cross-contamination.¹³ Cogan et al.¹⁴ reported that no *Campylobacter jejuni* could be recovered from hand-washed cutlery. In contrast, some studies confirm the ineffectiveness of consumer-style hand-washing procedures,^{15,16} thus either the hand-washing procedure must be intensified or hand contact with the meat must be prevented.

In a recent study, Jong et al.¹⁷ examined the influence of hygienic measures on cross-contamination of *C. jejuni* and *L. casei* from chicken meat at home.¹⁷ Cross-contamination could be significantly reduced when cleaning cutting board and cutlery with hot water

(68°C). The author, however, conceded that this method alone is not sufficient to prevent cross-contamination. Instead, different cutting boards for raw meat and other ingredients should be used and meat-hand contact must be avoided or hands should be meticulously cleaned with soap. As a precautionary step to prevent computer keyboards cross-contamination, Rutala et al.⁷ suggested that keyboards must be disinfected daily or when visibly soiled or if they become contaminated with blood.⁷ Other researchers recommended that disinfection must be applied on computer keyboards that are in use in patient care areas.^{4,18,19} Tunc & Olgun⁵ recommended the use of hand-free telecommunication devices with magnetic card or voice activation to reduce bacterial contaminations from the use of public telephones.⁵ Other suggested techniques to decrease bacterial contaminations include use of antimicrobial additive materials and surface coatings to fabricate telephones, door knobs, fabrics and various building materials.^{20–22}

The current study was conducted to determine the type of microbial cross-contamination on different surfaces and locations in Kuwait. These include fitness club equipment, gas station, home, supermarket, and microbiology lab at hospital. Knowing the type of microbes in different localities and items would help in selecting the proper hygiene measures to suppress any potential microbial cross-contamination.

Methods

Specimens were collected from different surfaces and locations in Kuwait. These included fitness club equipment (exercise mats, weight training equipment, bathroom door knob, water cooler handle and stand, and cardio equipment), gas station (fuel handle, bathroom door knob), home (bathroom toilet seat, ipad, and TV remote control), supermarkets (e. g. , shopping carts and baskets) and microbiology lab at a hospital (lab coat, pantry, lab locker, chairs handle).

Isolation of different bacterial contaminants from numerous contaminated surfaces was performed through standard techniques. Single sterile swabs were wiped firmly over the entire contaminated surfaces. Subsequently, all collected swabs were placed in 2 ml of brain heart infusion broth in a sterile container, and vortexed for one minute. Samples were cultured on blood agar and MacConkey agar.

All samples were plated within 1-3 hours of specimen collection. Inoculated media were then incubated aerobically at 37°C for 24h. After that a bacterial suspension, made from the isolated bacteria, was cultured again on blood and MacConkey plates using a known loop size diameter so the colony forming unit (CFU) could be calculated. CFU count is the most crucial parameter, as it measures the live micro-organisms which can multiply.

To identify numerous types of microorganisms residing on the contaminated surface, the BIOMÉRIEUX VITEK®2 system was used. This system is an automated microbial identification system that gives very precise and reproducible results as proved in multiple independent studies. With its colorimetric reagent cards, and associated hardware and software advances, the VITEK®2 provides a state-of-the-art technology platform for phenotypic identification methods.²³ Twenty hours later, bacteria species along with the degree of contamination for each location can be obtained by VITEK®2 with percentage up to 99%.

Statistical analyses

Each measurement was a mean of at least three replicate determinations and reported in a concentration of colony forming unit (CFU) per ml. Data analyses were performed using SigmaPlot®12. A p-value of ≤ 0.05 was considered statistically significant.

Results and discussion

Microbial examination was carried out in five different locations. These locations are categorized as: (1) Fitness club; (2) Gas station; (3) Home; (4) Hospital; and (5) Supermarket. Table 1 displays 50 bacterial strains that were isolated from the five different localities, of which 7 strains were from the fitness club, 10 from the gas station, 10 from the private home, 13 from the hospital, and 10 from the supermarket. Among the isolated bacteria contaminants, *Staphylococcus epidermidis* had the highest prevalence (34%), followed by *Enterococcus faecalis* (26%), *Staphylococcus aureus* (14%), *Escherichia coli* (10%), *Streptococcus pyogenes* and *Pseudomonas alcaligenes* (4%), *Streptococcus agalactiae*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (2%). Of all tested items, 80% were gram positive bacteria and 20% were gram negative bacteria.

Table 1 Prevalence of bacteria isolated from different items in fitness club, gas station, home, hospital, and supermarket

Bacteria	Fitness Club	Gas Station	Home	Hospital	Super Market	Total	Prevalence (%)
Gram positive							
<i>Enterococcus faecalis</i>	2	4	5	2	-	13	26
<i>Streptococcus agalactiae</i>	1	-	-	-	-	1	2
<i>Staphylococcus epidermidis</i>	1	6	4	2	4	17	34
<i>Staphylococcus aureus</i>	1	-	-	4	2	7	14
<i>Streptococcus Pyogenes</i>	-	-	1	1	-	2	4
Gram negative							
<i>Escherichia coli</i>	-	-	-	2	3	5	10
<i>Klebsiella oxytoca</i>	-	-	-	1	-	1	2
<i>Klebsiella pneumoniae</i>	-	-	-	-	1	1	2
<i>Pseudomonas aeruginosa</i>	1	-	-	-	-	1	2
<i>Pseudomonas alcaligenes</i>	1	-	-	1	-	2	4
Total	7	10	10	13	10	50	100

Figure 1 shows the total bacterial counts isolated from different items in the fitness club. Pathogenic microbes recovered from cultures of different items in fitness club swab specimens included *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, *Staphylococcus epidermidis*, and *Staphylococcus aureus*. Mean bacterial counts in the fitness club varied between 1.4 and 2.3 log₁₀ CFU/ml. The highest bacteria count (2.3 log₁₀ CFU/ml) was *Enterococcus faecalis*, found on bathroom doorknobs, while the lowest count was *Pseudomonas aeruginosa* (1.4 log₁₀ CFU/ml) found on exercise mats.

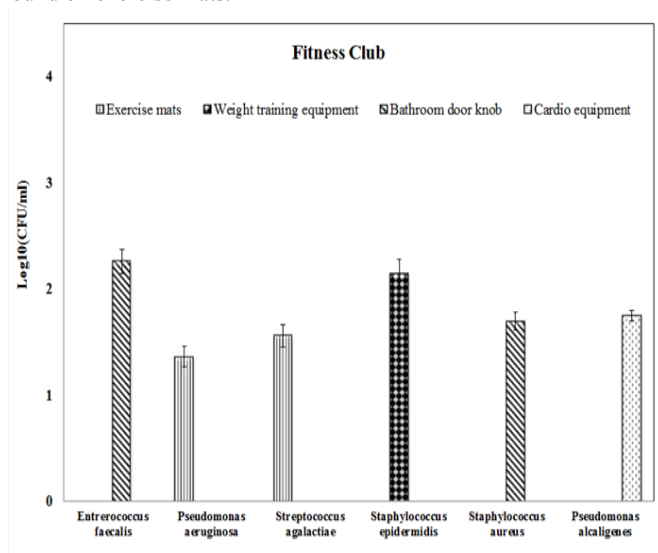


Figure 1 Mean total counts of bacteria \pm SD from different items in fitness club.

Door handles of toilets and bathrooms are one of the most implicated probable sources of infections. Nworie et al.²⁴ have investigated bacterial contamination in numerous public places in Abuja metropolis, Nigeria.²⁴ Among the 140 samples collected from toilet door handles/knobs, 122 (87.1%) showed bacterial contamination, whereas 34 (85%) of the 40 samples collected from bathroom door handles/knobs showed bacterial contamination. In Northern Ireland and UK, Moore et al.²⁵ found *P. aeruginosa* in 72% of hot tubs and 38% of swimming pools tested.²⁵ It was repeatedly reported that *P. aeruginosa* thrives in most environments. In this study, *P. aeruginosa* was also detected on exercise mats, which are meant to get wet due to perspiration fluids lost during exercise. Occurrence of various microorganisms in sport fitness in the current study is consistent with those previous studies discussed above.

The levels of bacterial contaminations from different items in the gas stations are shown in Figure 2. The two bacteria species isolated were *Staphylococcus epidermidis*, which was detected on gas pump handles, and *Enterococcus faecalis* species, which were found on bathroom doorknobs. The average number of bacteria ranged from 2.1 to 2.8 log₁₀ CFU/ml. The gas pump handle is the most frequently touched surface at the gas station. In a recent study by Cezar-Vaz et al.²⁶ biological risk in gas stations was identified by 62.4% of workers.²⁶ The workers attributed the prevalence of microorganism contamination to their frequent contact with customers, insufficient measures for individual protection, and inappropriate hygienic conditions in the work environment.^{27,28} Jovic-Vranes et al.²⁹ identified the workers' perception of imminent infectious disease risk.²⁹ Results

demonstrated that risk perception depended on the frequency of the workers' exposure to contaminated fluids, knowledge of customers' diseases and history of previous accidents.

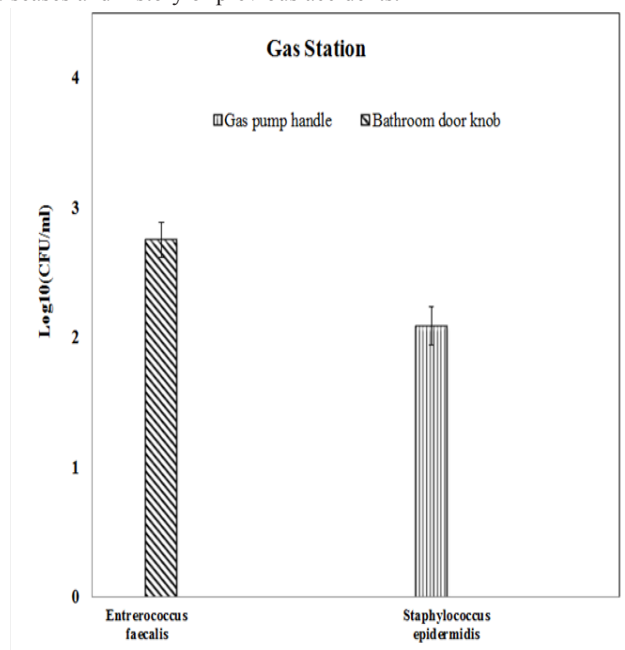


Figure 2 Mean total counts of bacteria from different items in gas station.

The bathroom door knob in gas stations, in this study, was found to be the second-most contaminated place, and *Enterococcus faecalis* species were identified with an average of 2.8 log₁₀ CFU/ml. Public toilets and bathrooms are of great threat due to high-volume traffic of users that contribute their own microbial flora and other organisms they have picked up elsewhere. Subsequently, these microorganisms will be deposited on door handles and knobs both on their way in and out.³⁰

Isolated bacteria counts from different items in homes (eg., bathroom toilet seats, TV remote controls, coffee table, and Ipads), are shown in Figure 3. Three bacteria strains were isolated; *Enterococcus faecalis*, *Streptococcus pyogenes*, and *Staphylococcus epidermidis*; the former was found only on bathroom toilet seats with a mean value of 1.5 log₁₀ CFU/ml. This result was lower than that observed by Kawo et al.³¹ where the toilet facility had the highest overall mean count of 7.52 log₁₀ CFU/ml.³¹ The authors attributed the high contamination count in toilets to high frequency of usage coincident with poor habits of sanitation and/or cleanliness. In one comparative study, the microbial contamination was quantified and compared between squat and sitting toilets.³² All tested toilets exposed to microbial contamination included *E. coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus*, though sitting toilets had fewer microorganisms. The risk of microbial contamination in toilet seats arises from the fact that large numbers of bacteria may remain in the bowl even after flushing, and even repeated flushing may not remove a persistent fraction. This is probably due to the adhesion of the organisms to the porcelain surfaces of the bowl, with gradual elution occurring after each flush. Thus, a possibility of infection from an aerosol created in a toilet is expected.

The second bacterial strain found in living room was *S. epidermidis*, which was isolated from TV remote controls and Ipads,

with a mean value ranging from 2.1 to 2.2 log₁₀ CFU/ml, as shown in Figure 3. Computer keyboards have become pools of pathogens, particularly in schools and hospitals.³³ Al-Ghamdi et al.³⁴ investigated the bacterial contamination of computers at home.³⁴ Results showed that the percent of contamination of tested computer keyboards and computer mice were 88 and 91%, respectively. One plausible reason for the increased contamination percentage of computers is the difficulty of cleaning and disinfection combined with the delusion that cleaning keyboards perhaps causes them to malfunction. Recent studies have confirmed that homes are important environments in the chain of infection transmission, and resulted in a renaissance of interest and concern about bacterial contamination as well as hygiene and sanitation in the home.^{35,36} Our study, along with previous studies, demonstrates the potential microbial risk in homes.

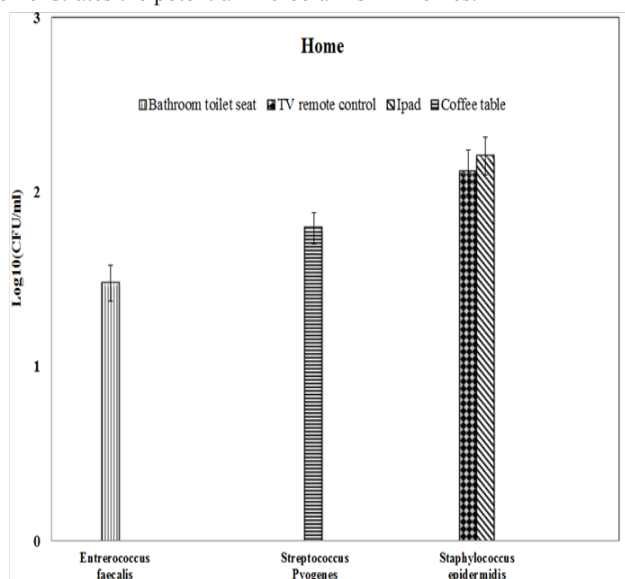


Figure 3 Mean total counts of bacteria ± SD from different items in homes

Microbial contamination counts in hospital microbiology labs are listed in Figure 4. The items selected were lab coat, pantry, lab locker, and chair handles. The isolated bacteria included *Pseudomonas alcaligenes* (n=1), *S. epidermidis* (n=2), *E. coli* (n=2), *S. aureus* (n=4), *Klebsiella oxytoca* (n=1), *S. pyogenes* (n=1) and *Enterococcus faecalis* (n=2). The highest bacterial count (2.6 log₁₀ CFU/ml) was *S. epidermidis* and was detected in a lab coat while the lowest bacteria count was *Klebsiella oxytoca* (1.32 log₁₀ CFU/ml); found in pantry. The average count of all isolated bacteria in all different items was 2.04 ± 0.4 log₁₀ CFU/ml. Carvalho et al.³⁷ investigated hospital ward surfaces in Brazil and about 40% of tested surfaces were found to be contaminated with *S. aureus*, which is consistent with this study, irrespective of whether they had infected patients or not. A similar observation was also reported by Narmeen & Jaladet,⁸ where about 23% of specimens were contaminated with *S. aureus*. Recently, Ulger et al.³⁸ showed that health care workers' hands and mobile phones were contaminated with numerous types of microorganisms.³⁸ These findings are consistent with our study, in which most of the tested surfaces were contaminated with different types of microorganisms, as shown in Table 1 and Figure 4.

Figure 5 shows the microbial contaminations from different items in supermarkets (e.g., shopping carts and baskets). Based upon the morphology, gram staining, and biochemical characterization, the

microorganisms found were *S. epidermidis*, detected on shopping carts and shopping baskets, and *Klebsiella pneumoniae*, *E. coli*, and *S. aureus* found only on shopping carts. The average number of *S. epidermidis* ranged from 1.5 to 2.1 log₁₀ CFU/ml, while the average numbers of *Klebsiella pneumoniae*, *E. coli*, and *S. aureus* were 2.5 log₁₀ CFU/ml, 2.14 log₁₀ CFU/ml, and 1.95 log₁₀ CFU/ml, respectively. Previous investigations found that 89-95% of tested shopping cart handles in four different locations in Saudi Arabia were contaminated by microorganisms including *Pseudomonas* species and *Staphylococcus*.³⁴

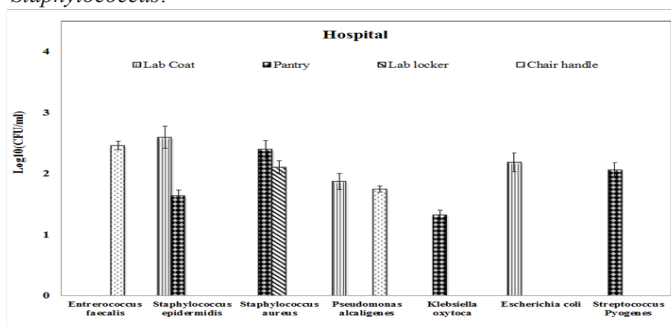


Figure 4 Mean total counts of bacteria ± SD from different items in hospital

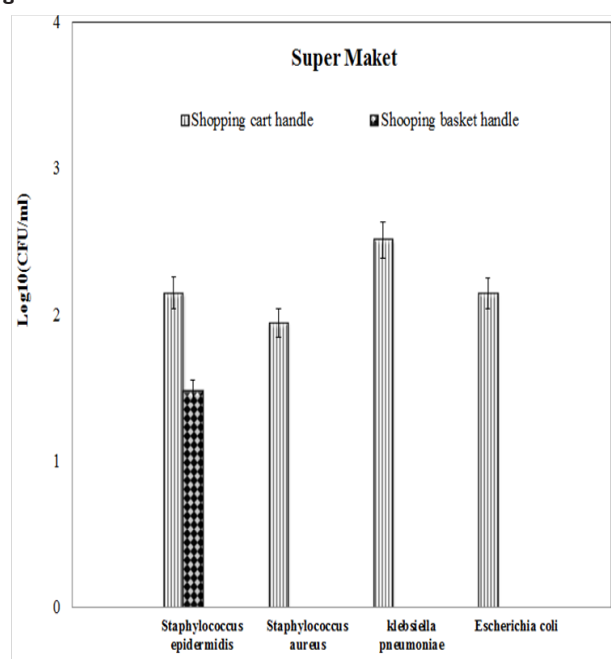


Figure 5 Mean total counts of bacteria ± SD from different items in supermarket.

Reynolds et al.³⁹ evaluated the occurrence of biological contamination on several environmental surfaces by quantifying protein and biochemical markers for human body fluids such as saliva, blood, sweat and urine, as well as fecal and total coliform bacteria.³⁹ Their results showed that shopping cart handles ranked third among the surfaces sampled for biological contamination. Previous epidemiological studies identified riding in shopping carts near meat or poultry products as an associated risk factor in infant Salmonellosis and Campylobacteriosis.^{40,41} Contamination of shopping carts and baskets handles could be ascribed to their frequent use by shoppers.

Shoppers differ in their hygienic status and when they hold shopping carts and baskets handles, transmission of microorganisms from hand to handles occurs, and vice versa. Items that shoppers move from shelves to carts and baskets could also be potential fomites of microorganisms. Subsequently, transferred microorganisms from these items to hands will transfer to shopping carts and baskets handles. The combined results of previous studies and this study suggest that shopping carts may indeed play a substantial role in the transmission of enteric pathogens.

Quantitative and qualitative bacterial analysis of the isolates in five different localities in this study, show moderate-to-high level and prevalence of bacterial contamination in frequently or heavily used fomites. Bacteria isolates in this study include *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Streptococcus agalactiae*, and *Pseudomonas alcaligenes*; *Enterococcus faecalis*, *Klebsiella oxytoca*, *Streptococcus pyogenes*, and *Klebsiella pneumoniae*.

S. epidermidis is one of the most common nosocomial pathogens together with *S. aureus*, *E. coli*, and *Pseudomonas aeruginosa*.^{42,43} *S. epidermidis* can cause an infection that can increase the risk of adverse outcome and substantially prolong hospital stay. *Staphylococcus* species are found in all individuals and usually expelled from the respiratory tract through the nose and mouth.⁴⁴ Previous studies^{45,46} have reported that the existence of *S. aureus* in food is a symptom of environmental and human contamination. *P. aeruginosa*, an opportunistic pathogen, causes bacteremia and gastrointestinal infections.⁴⁷ *S. agalactiae* is a main human and bovine pathogen. It was repeatedly reported as the most common agent of invasive infections in neonates, causing pneumonia, septicemia and meningitis, and is an increasingly common pathogen in adults.⁴⁸ *Enterococcus faecalis* is a commensal bacterium that colonizes the gastrointestinal tract. It has emerged as an important nosocomial pathogen with high-level resistance to antibiotics causing clinical infections including urinary tract infections, bacteremia and bacterial endocarditis in elderly and immune compromised patients.⁴⁹ *Klebsiella oxytoca* is an opportunistic pathogen that causes primarily hospital-acquired infections, most often involving immune compromised patients or those requiring intensive care.⁵⁰ *Streptococcus pyogenes* is one of the most frequent human pathogens capable of producing a wide variety of diseases ranging from pharyngitis to more severe and life-threatening infections like acute rheumatic fever.⁵¹ *Klebsiella pneumoniae* is prevalent in the environment and is frequently found as a commensal resident of the human gastrointestinal tract.⁵² Once inoculated, it has a significant ability to cause a wide range of human diseases, from urinary tract infections to pneumonia.⁵³ All previous studies highlight the severe health impacts originating from microorganisms like the ones found in this study; and subsequently, extensive efforts to minimize their occurrences are required.

There are several important factors that influence the survival of bacterial cells on surfaces; these include characteristics of an organism and its surrounding environment. The existence of specific surface structures such as pili, flagella, and extracellular polysaccharides have been suggested to affect the adhesion and survival of bacteria.⁵⁴ Numerous studies have reported that various bacteria can adhere and survive on hands, utensils, sponges, cloths, and currency for hours, days, or weeks after initial contact with the microorganisms.⁵⁵⁻⁵⁷

It is also important to note that potential hazard depends not only on type but also number of pathogenic organisms present. It was reported

that large doses (10^5 - 10^7 CFU) of enteropathogenic organisms and 10^6 CFU of *S. aureus* are required to cause an infection.³ In our study, isolated bacteria counts were between 21-563 CFU, in the range of previously reported results above; and therefore, the risk of infection is unavoidable.

Transmission of pathogens takes place by either direct or indirect pathways. Direct pathways include human-to-human transmissions, such as hand-shaking.³⁸ As shown in Figures 1-5, different microorganisms were detected on different fomites. These microorganisms could transfer from contaminated surfaces to hands via surface-surface cross contamination or any sort of direct pathways. Subsequently, these microorganisms could be ingested or inhaled if adequate hygiene practices are not applied. Indirect transmission occurs when a non-living agent is involved in the transfer of the pathogen to a susceptible individual such as through air, food, water and inanimate objects known as fomites. It was previously reported that bacteria can adhere to particles of grain dust, which are considered an effective infectious aerosol because its organic materials offer necessary nutrients for airborne microorganisms adherent to their surfaces.⁴⁶ Microorganisms found in contaminated surfaces in this study (Figures 1-5), if transferred via any media suggested above and ingested or inhaled by humans, could cause severe health problems like those reported in the above discussion. Overall, results in this study highlight the threat posed by contaminated surfaces if cross-contamination occurs followed by ingestion and/or inhalation.

Based on assessment of the prevalence of contamination and risks of transfer, it is imperative to adopt what action, if any, should be taken. Decontamination is a process that eliminates, inactivates, or reduces the level of microbes that have accumulated on personnel and equipment. Subsequently, this eliminates or reduces the possibility of microbial transmission to a susceptible site, causing infection or colonization. In the decontamination process, chemical or physical agents are used to prevent microbial growth, transmission, and infection. Several types of disinfectants have been previously and recently recognized as more effective antimicrobial tools over boiling, soap and water washing, which are often either ineffective or not feasible.³ A thorough discussion of disinfection and hand washing, as antimicrobial tools, is made in introduction section. In parallel with decontamination tools, community awareness and education for hygienic standards, respiratory etiquette and hand-washing should be taken seriously into consideration.

Conclusion

This study investigated the occurrence of potential pathogens and the levels of contamination at individual sites, particularly in fitness clubs, gas stations, homes, hospitals, and supermarkets. Various disease-causing microbes reported in previous epidemiological studies were isolated in this study. These include *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas alcaligenes*, *Streptococcus pyogenes*, *Klebsiella oxytoca*, *Streptococcus agalactiae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. This study shows that frequently or heavily used fomites harbor highly pathogenic bacteria, which have the potential of causing epidemics in the near future. Therefore, appropriate hygienic measures to suppress any potential microbial cross-contamination are needed. Besides, it is imperative to conduct regular testing to check for bacterial contamination and increase community awareness and education for hygienic standards.

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Conflict of interest

The authors declare that there is no conflict of interests to declare regarding the publication of this paper.

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