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Comparison of Microbial Contamination on Fomite Surfaces in Public Restrooms Based on Location and Gender Factors

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ABSTRACT

Objective: This study aims to investigate the microbial contamination on fomite surfaces such as door handles and faucets in student and staff toilets at Balıkesir University and to examine the relationship of contamination with surface location and gender. **Materials and Methods:** A total of 128 samples were collected using a sterile sponge swab method; 64 samples were obtained from fomite surfaces in female (32) and male (32) restrooms, while another 64 samples were taken from fomites on the right (32) and left (32) sides. The presence of total aerobic mesophilic bacteria (TAMB), *Staphylococcus/Micrococcus* spp., coliform bacteria, and *Escherichia coli* was assessed. **Results:** TAMB levels on faucet surfaces did not significantly differ between genders ($p>0.05$), whereas *Staphylococcus/Micrococcus* spp. counts were significantly higher in male restrooms ($p<0.05$). Exit door handles exhibited a higher bacterial load compared to entrance door handles, with statistically significant differences observed in TAMB and *Staphylococcus/Micrococcus* spp. counts ($p<0.05$). Contamination levels varied depending on fomite location, with the highest *Staphylococcus/Micrococcus* spp. counts detected on faucets on the right side and door handles on the left side. No coliform bacteria or *E. coli* were detected in any sample. **Conclusion:** A significant level of microbial contamination was detected on restroom fomite surfaces, with higher *Staphylococcus/Micrococcus* spp. levels in male restrooms and increased contamination on exit door handles. These findings indicate that microbial contamination on toilet fomite surfaces may pose a risk to public health and that hygiene measures should be improved, especially on surfaces with a high risk of microbial contamination.

Keywords: Fomite surfaces, Gender, Hand hygiene, Microbial contamination.

Genel Tuvaletlerdeki Fomit Yüzeylerde Mikrobiyal Kontaminasyonun Konum ve Cinsiyet Faktörlerine Göre Karşılaştırılması

ÖZ

Amaç: Bu çalışma, Balıkesir Üniversitesi'ndeki öğrenci ve personel tuvaletlerinde kapı kolları ve musluklar gibi fomit yüzeylerdeki mikrobiyal kontaminasyonu araştırarak, kontaminasyonun yüzey konumu ve cinsiyet ile ilişkisini incelemeyi amaçlamaktadır. **Gereç ve Yöntemler:** Toplam 128 örnek, steril sünger swab yöntemiyle toplanmıştır; bunların 64'ü kadın (32) ve erkek (32) tuvaletlerindeki fomit yüzeylerden, 64'ü ise sağ taraftaki (32) ve sol taraftaki (32) fomit yüzeylerden alınmıştır. Toplam aerobik mezofilik bakteri (TAMB), *Staphylococcus/Micrococcus* spp., koliform bakteriler ve *Escherichia coli* varlığı değerlendirilmiştir. **Bulgular:** Musluk yüzeylerindeki TAMB seviyeleri cinsiyetler arasında anlamlı bir fark göstermemiştir ($p>0.05$), ancak *Staphylococcus/Micrococcus* spp. sayıları erkek tuvaletlerinde daha yüksek bulunmuştur ($p<0.05$). Çıkış kapı kollarında giriş kapı kollarına kıyasla daha yüksek bakteriyel yük tespit edilmiştir ve TAMB ile *Staphylococcus/Micrococcus* spp. sayıları açısından istatistiksel olarak anlamlı farklılık gözlenmiştir ($p<0.05$). Kontaminasyon fomit konumuna bağlı olarak değişiklik göstermiştir; en yüksek *Staphylococcus/Micrococcus* spp. sayıları sağ taraftaki musluklarda ve sol taraftaki kapı kollarında bulunmuştur. Hiçbir örnekte koliform bakteri veya *E. coli* tespit edilmemiştir. **Sonuç:** Tuvalet fomit yüzeylerinde önemli düzeyde mikrobiyal kontaminasyon saptanmış olup, erkek tuvaletlerinde *Staphylococcus/Micrococcus* spp. seviyelerinin daha yüksek olduğu ve çıkış kapı kollarında artan kontaminasyonun olduğu belirlenmiştir. Bu bulgular, tuvalet fomit yüzeylerindeki mikrobiyal kontaminasyonun halk sağlığı açısından risk oluşturabileceğini ve özellikle mikrobiyal bulaşma riski yüksek yüzeylerde hijyen önlemlerinin iyileştirilmesi gerektiğini göstermektedir.

Anahtar Kelimeler: Fomit yüzeyler, Cinsiyet, El hijyeni, Mikrobiyal kontaminasyon.

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INTRODUCTION

The level of civilization's development today is directly associated with the importance individuals place on cleanliness and hygiene (Tunç, 1999). Hygiene is not only concerned with physical cleanliness but also plays a crucial role in maintaining and sustaining health. Personal hygiene, in particular, is a fundamental requirement for individuals to preserve their overall health status and contributes to their well-being (Seçim, 1991). Developing personal hygiene habits enhances an individual's quality of life and plays a significant role in preventing infections and diseases. However, assessments of whether personal hygiene is adequate may vary among individuals. Various factors, including cultural values, economic conditions, education level, social environment, and the individual's perception of hygiene, influence these differences (Yalçın & Özkalp, 2005).

The skin is the organ most exposed to external environments and serves as the primary interface between the body and microorganisms. The skin surface of an adult's hands covers approximately 2 square meters and is in constant contact with various microorganisms. Notably, enteric bacteria can be transferred to the skin surface via fecal contamination (Madigan et al., 2010). Awareness of toilet use and post-toilet hygiene practices play a crucial role in maintaining personal hygiene. Restrooms provide favorable conditions for microbial proliferation due to their warm and humid environments. Insufficient hygiene in these settings significantly increases the risk of infection. Pathogens such as *Shigella* spp., *Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii, Staphylococcus aureus*, and norovirus can persist on environmental surfaces for extended periods while maintaining their infectious potential (Suen et al., 2019a).

In restrooms, fomite surfaces play a crucial role in the transmission of microorganisms from person to person. Fomite surfaces include inanimate objects or materials that act as intermediaries in contamination, such as door handles, faucet heads, light switches, kitchen utensils, pens, desks, towels, money, and books (Madigan et al., 2010; Maori et al., 2013; Tiku et al., 2019; Türkseven, 2018). The location of fomite surfaces within or outside the restroom, the frequency of cleaning, the properties of detergents and disinfectants used, and user-related factors all influence the level of contamination. In an experimental study, it was reported that 30–60% of bacteria present on these surfaces could be transferred to hands with a single touch (Rusin et al., 2002). Faucets in restrooms, in particular, pose a bidirectional contamination risk. Since hands are contaminated before washing, touching the faucet handles to turn them on or off may transfer microorganisms to these surfaces. If the same surface is touched again after handwashing, hands can become recontaminated (Trampuz & Widmer, 2004). Therefore, in our study, faucet handles and door handles were identified as critical surfaces for sampling. Additionally, this study aimed to compare contamination levels between fomite surfaces located on the right and left sides.

In this context, the study aims to determine the level of microbial contamination on entrance doors and faucets in staff and student restrooms located within the Tourism Faculty, Engineering Faculty, and Veterinary Faculty of Balıkesir University, considering surface location and gender factors.

MATERIALS AND METHODS

To determine the effect of gender on microbial contamination, samples were collected from bidet faucet heads and entrance-exit door handles in male and female restrooms. The sampling process was conducted over a three-week period during the mid-term break of the academic calendar, with samples collected twice a week.

Sampling surfaces were selected as bidet faucet heads and entrance-exit door handles. To compare contamination levels based on the location of fomite surfaces, a coding system was implemented during sampling, categorizing fomites according to whether they were positioned on the user’s right or left side. Specifically, for bidet faucet samples, the position of the faucet relative to the user's toilet posture (right or left) was considered. For door handles, the likelihood of being held with the right or left hand was determined based on the door's opening direction.

For the sampling process, sterile sponge swabs measuring 2 cm × 5 cm, pre-moistened with sterile physiological saline (SPS), were used. A total of 128 samples were collected. The distribution of the samples was as follows: 64 samples were obtained based on the gender factor, with 32 from female restrooms and 32 from male restrooms. The remaining 64 samples were collected based on fomite location, with 32 from the right side and 32 from the left side. The collected swab samples were homogenized in stomacher bags containing 25 mL of SPS. Serial dilutions were then prepared from the homogenized samples using glass tubes containing 9 mL of SPS.

Microbial analyses

For microbial analysis, serial dilutions were prepared, and the following culture media and methods were used: total aerobic mesophilic bacteria (TAMB) were enumerated using the pour plate method on Plate Count Agar (PCA; Merck, Germany). Coliform bacteria were counted using the double-layer pour plate method on Violet Red Bile Lactose Agar (VRBL; Merck, Germany). *E. coli* counts were determined using the spread plate method on Tryptone Bile X-Glucuronide Agar (TBX; Merck, Germany). *Staphylococcus*/*Micrococcus* spp. were enumerated using the spread plate method on Baird-Parker Agar (BPA; Merck, Germany). Following the incubation period, the resulting colonies were evaluated.

Statistical analysis

Statistical analyses were performed using SPSS 25 statistical software. Data were expressed as mean ± standard deviation. The normality of data distribution was assessed using the Kolmogorov-Smirnov test. Differences among groups were analyzed using the Kruskal-Wallis test, while differences between two groups were evaluated using the t-test.

Ethical Approval

This study does not require ethics committee approval as it does not involve data collection from human or animal subjects.

Table 1. Microbial load results on fomite surfaces and external door handles in male and female restrooms (log_{10} CFU/surface±SD)

Surface Type	Faucet (log_{10} CFU/faucet±SD)		External Door Handle (log_{10} CFU/door handle±SD)	
	Female	Male	Entrance	Exit
TAMB	3.41±0.42	3.72±0.58	3.35±0.56[b]	4.09±0.78[a]
***Staphylococcus*/*Micrococcus* spp.**	2.83±0.53[b]	3.17±0.62[a]	2.82±0.53[b]	3.47±0.49[a]
Coliform	<1.39	<1.39	<1.39	<1.39
Escherichia coli	<2.39	<2.39	<2.39	<2.39

[a-b]: Mean values in the same row with different superscript letters indicate statistically significant differences ($p<0.05$)

RESULTS

The bacterial loads detected on fomite surfaces sampled from male and female restrooms are presented in Table 1. No statistically significant difference was detected in TAMB counts on faucet surfaces based on gender factor ($p>0.05$). The highest mean bacterial load was recorded on fomite surfaces in male restrooms, with 3.72 log_{10} CFU/faucet. However, a statistically significant difference was found between male and female restroom fomite surfaces in terms of *Staphylococcus*/*Micrococcus* spp. counts ($p<0.05$). The *Staphylococcus*/*Micrococcus* spp. count was 3.17 log_{10} CFU/faucet in male restrooms. For entrance and exit door handles, a statistically significant difference was detected in both TAMB and *Staphylococcus*/*Micrococcus* spp. counts ($p<0.05$). Higher bacterial loads were detected on exit door handles in both analyses. No coliform bacteria or *E. coli* were detected at either sampling point.

The bacterial loads on fomite surfaces are presented in Table 2. No statistically significant difference was observed in TAMB counts between faucet locations ($p>0.05$). The highest TAMB count was recorded in left-side faucet samples, with an average of 3.37 log_{10} CFU/faucet. A statistically significant difference was detected in *Staphylococcus*/*Micrococcus* spp. counts based on faucet positions ($p<0.05$). The highest *Staphylococcus*/*Micrococcus* spp. count, 2.95 log_{10} CFU/faucet, was found in faucets located on the right-hand side. For door handle samples held with the right and left hands, a statistically significant difference was observed in TAMB and *Staphylococcus*/*Micrococcus* spp. counts ($p<0.05$). The highest mean bacterial load for TAMB was detected on right door handles at 3.64 log_{10} CFU/door handle, while for *Staphylococcus*/*Micrococcus* spp., it was detected on left door handles at 2.89 log_{10} CFU/door handle. No coliform bacteria or *E. coli* were detected in any of the examined faucet or door handle samples.

Table 2. Microbial load results based on the location of fomite surfaces (log_{10} CFU/surface±SD)

Surface Type	Faucet (log_{10} CFU/faucet)		Door Handle (log_{10} CFU/door handle)	
	Right	Left	Right	Left
TAMB	3.34±0.52	3.37±0.37	3.64±0.69[a]	3.52±0.52[b]
***Staphylococcus*/*Micrococcus* spp.**	2.95±0.22[a]	2.74±0.41[b]	2.72±0.59[b]	2.89±0.35[a]
Coliform	<1.39	<1.39	<1.39	<1.39
Escherichia coli	<2.39	<2.39	<2.39	<2.39

[a-b]: Mean values in the same row with different superscript letters indicate statistically significant differences ($p<0.05$).

DISCUSSION

Restrooms are a crucial component of social hygiene areas and hold significant importance for public

health. Individuals using the restroom come into contact with various parts of their bodies with their hands and subsequently touch faucet handles or door handles, leading to the contamination of these surfaces. Until an individual effectively washes their hands, they continue to transfer microorganisms to door handles during exit, to people they shake hands with, to money used in transactions, to handrails in public transportation, and to other surfaces they touch (Erol, 2010).

Fecal matter is a major source of human pathogens and can lead to infectious diseases under inadequate hygiene conditions. Infections originating from feces are often associated with poor hygiene in shared environments. Surfaces such as door handles, faucets, soap dispensers, and light switches in restrooms are among the critical points for bacterial transmission. Even after washing their hands, individuals may come into contact with these surfaces, reintroducing pathogenic microorganisms and increasing the risk of transmission (Türkseven, 2018).

Societal gender norms play a significant role in shaping cleanliness and hygiene habits among men and women. In many cultures, women are expected to be more meticulous and hygienic, whereas similar expectations for men tend to be lower. A global study examining 56 countries found that hygiene-related norms are generally stricter for women compared to men (Eriksson et al., 2022). Additionally, cultural values and religious beliefs lead to significant differences in toilet hygiene practices across countries and societies. In many Asian, Middle Eastern, and African cultures, there is a cultural division of labor between the right and left hand: the left hand is considered "unclean" and is traditionally used for post-toilet cleaning, while the right hand is used for activities such as eating and greeting others (Bartram & Cairncross, 2010).

Studies evaluating post-toilet handwashing habits in terms of gender have identified significant differences between men and women. An observational study conducted in the United States with approximately 4,000 participants reported that women washed their hands more frequently and more correctly (using soap and for a longer duration) compared to men. In this study, 14.6% of men did not wash their hands at all after using the restroom, while 35.1% rinsed their hands with water only, without using soap. In contrast, these rates were 7.1% and 15.1% for women, respectively. Similarly, earlier studies conducted in the United Kingdom and the United States also found that men's post-toilet handwashing rates were lower than those of women. For instance, one study revealed that only 31% of men washed their hands, whereas this rate was 65% among women (Kelland, 2020). These differences suggest that men may be more prone to risky hygiene behaviors. All these findings indicate that gender disparities are clearly observed in hygiene practices, with men exhibiting more relaxed adherence to hygiene rules due to social and psychological factors. Consequently, numerous academic studies confirm that gender differences influence hygiene behaviors, attributing these disparities to sociocultural norms, risk perception, and knowledge levels (Suen et al., 2019b).

Studies on this subject have consistently reported that women wash their hands more frequently and for a longer duration compared to men, placing greater emphasis on hand hygiene before and after using the restroom (Üner et al., 2009; Deveci et al., 2010; Kaya et al., 2006; Balcı et al., 2005). Similarly, the study by Taşkıran et al. (2019) found that women had better hygiene habits than men, and that handwashing frequency was lower among men. Likewise, Oz et al. (2021) reported that handwashing frequency directly affects microbial load, with higher bacterial levels observed on the hands of male participants. The lower adherence to hand hygiene among men is a significant factor that can contribute to increased microbial contamination on restroom surfaces. Notably, one study found that men were more likely to skip handwashing when alone in the restroom or after urination only (Aunger et al., 2016). Moreover, women generally place greater importance on personal hygiene practices compared to men. It has been reported that women are more likely to engage in routines such as washing hands and face in the morning, washing hands after meals, cleaning food before consumption, practicing oral hygiene after breakfast, and changing underwear and socks daily (Erkal & Şahin, 2011). Women have also been shown to have better body hygiene practices than men, including more frequent bathing (Kaya et al., 2006; Şimşek et al., 2010). These behavioral differences may explain the higher levels of *Staphylococcus/Micrococcus* spp. detected in male restrooms in our study. There may be several reasons for these hygiene differences. For example, a large-scale survey conducted in the United States in 2025 identified the main reasons for individuals choosing to leave a public restroom without washing their hands. These included the absence of soap or paper towels, malfunctioning or dirty sinks, excessive crowding in the restroom, and unpleasant odors (Cleaning & Maintenance Management, 2023).

The literature review did not reveal any studies specifically examining the difference in microbial contamination between restroom entrance and exit door handles. However, the higher microbial load detected on exit door handles in our study suggests that post-toilet hand hygiene may be inadequate or that handwashing is being neglected. Indeed, a report indicated that the rate of handwashing with soap after using the restroom varies between 0% and 34% (World Bank, 2005). Similarly, according to CDC data, the global post-toilet handwashing rate is only around 19% (CDC, 2024). These findings support the high microbial contamination levels detected on restroom exit door handles in our study and highlight

behavioral deficiencies in hand hygiene as a potential explanation.

In our study, the TAMB count on fomite surfaces, based on their location, was detected at an average level of approximately 3.5 $\log_{10}$ CFU/faucet/door handle. A literature review did not reveal any studies specifically evaluating microbial contamination levels based on the location of restroom fomite surfaces. One relevant study in the literature, conducted by Suen et al. (2019a) in Hong Kong, reported that the TAMB count on fomite surfaces in public restrooms was approximately 2 $\log_{10}$ CFU, which is lower than the levels observed in our study. Additionally, our findings indicate that TAMB levels were higher on right-side door handle surfaces. This may be explained by the fact that over 90% of individuals worldwide are right-handed (McManus and Bryden, 1992), making the right hand more frequently exposed to surface contamination. Consistent with our findings, a study by De Alwis et al. (2012) also reported that dominant hand use (right hand) is a significant factor in door handle contamination.

Coagulase-negative and coagulase-positive *Staphylococcus* species, which are fundamental components of the normal human microbiota, have been increasingly recognized as causative agents of nosocomial infections in recent years (Schleifer & Bell, 2009). Consequently, the frequent presence of *Staphylococcus* spp. on restroom fomite surfaces where hands frequently come into contact has been reported in numerous studies. For example, in a study conducted by Nworie et al. (2012), 30.1% of total isolates obtained from public restroom fomite surfaces were identified as *S. aureus*. Similarly, Maori et al. (2013) reported that 43.3% of fomite surfaces in public restrooms were contaminated with *Staphylococcus* spp. Consistent with our study findings, a literature review revealed that Gram-positive microorganisms particularly *Staphylococcus* spp. are detected at higher rates on restroom fomite surfaces compared to *E. coli* and other Gram-negative microorganisms (Ngonda, 2017; Fakhoury & Nawas, 2018; Flores et al., 2011). This phenomenon may be explained by the greater adaptability of Gram-positive bacteria to dry environmental conditions, or by the inability of Gram-negative bacteria to effectively compete with Gram-positive microorganisms in such environments.

The *Enterobacteriaceae* family consists of Gram-negative, non-spore-forming, rod-shaped, oxidase-negative, facultative anaerobic, glucose-fermenting, and catalase-positive microorganisms. Some enterobacteria are potential pathogens for humans and, due to their ability to survive in various environments both inside and outside the body, they can cause opportunistic infections in immunocompromised individuals. The presence of fecal coliforms, a subgroup of enteric bacteria, is considered an indicator of fecal contamination or inadequate sanitary and hygiene conditions. The primary representative of this group is *E. coli*, which predominantly inhabits the intestines of humans and warm-blooded animals (Peixoto & Fontoura-da-Silva, 2007). In our study, no coliform bacteria or *E. coli* were detected on fomite surface samples. This situation may be attributed to several factors. First, considering the sampling method and analytical sensitivity, bacteria present on fomite surfaces must exceed a certain threshold level to be detected. This may be due to coliform bacteria being present on fomite surfaces (faucets and door handles) at levels below 25 CFU/faucet/door handle, and *E. coli* at levels below 250 CFU/faucet/door handle, making detection impossible. Additionally, due to sampling challenges, the study was conducted during the two-day weekend period when restrooms were not in use, which may have influenced the results. During this period, the lack of restroom use exposed bacteria on contaminated surfaces to environmental factors such as drying, temperature fluctuations, and ultraviolet (UV) radiation, which can significantly affect bacterial survival. Indeed, studies have shown that *E. coli* levels decline significantly within 24–48 hours on stainless steel and plastic surfaces under low humidity conditions (Visvalingam et al., 2017). Furthermore, at room temperature and low humidity (40–60% RH), *E. coli* has been reported to be largely eliminated within 24 hours (Shimoda et al., 2019). This suggests that *E. coli* is highly sensitive to desiccation and may not have survived on dried faucet and door handle surfaces, preventing its detection (Madigan et al., 2010; Gerhardts et al., 2012).

CONCLUSION

This study demonstrates that microbial contamination in restrooms is widespread and that hygiene habits are a key factor influencing contamination levels. Ensuring proper hand hygiene, along with the implementation of technological measures such as automatic door systems and sensor-operated faucets, are effective strategies to reduce the risk of transmission from fomite surfaces. Additionally, placing informational posters in public restrooms to raise hygiene awareness is considered another important measure that can encourage users to adopt proper hygiene practices.

Hygiene awareness campaigns conducted by health authorities and educational institutions are of great importance in reducing microbial contamination. The widespread promotion of hygiene education and the reinforcement of handwashing habits can play a critical role in infection prevention. In this context, it is essential to implement comprehensive educational programs and behavioral interventions aimed at increasing individual hygiene awareness.

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Conflict of Interest
The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions
Plan, design: TEG, HT; **Material, methods and data collection:** TEG, EBG, BM, SK, HT; **Data analysis and comments:** TEG, HT; **Writing and corrections:** TEG, EBG, BM, SK, HT.

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Ethical Approval
This study does not require ethics committee approval as it does not involve data collection from human or animal subjects.

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