

# A survey of public restrooms microbial contamination in Tehran city, capital of Iran, during 2019

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## ABSTRACT

**Introduction:** Daily use of public restrooms may have a significant impact on spreading infectious diseases. Human society could be affected by spreading of transitional infectious diseases through feces, urinary tract infection and poor personal hygiene. According to the World Health Organization reports, plenty of people's developed diseases caused by contaminated public restrooms that may result in severe health problems. **Methods:** This descriptive cross-sectional study was conducted on 7,482 samples that were collected randomly in 6 months (spring and summer 2019) in different regions of Tehran. The Data were obtained by analyzing 804 restroom's indoor and outdoor handles, 1062 toilet faucet, 826 washbasin taps, 1,062 toilet hoses, 804 flush tank levers, 643 soap dispenser bottoms, 643 liquid soaps, 99 bar soaps, 169 toilet papers and paper towels, and 50 hand dryer machines. Samples which were tested, based on bacteriology standard methods. **Result:** 7,482 samples were gathered of which 6,678 contaminated cases (89.25%) were observed and 804 cases (10.75%) were found non-contaminated. *Escherichia coli* with 28.48% and *Pseudomonas* with 0.39% were the most and the least common bacteria, respectively, in this study. **Conclusion:** The required tests to identify the bacteria that cause contamination through the use of public restrooms have been done. It is essential to inform the public of the mentioned items and teach how to prevent infectious diseases.

**Keywords:** Bacterial contamination, hand dryer soaps, public restrooms

## Introduction

Using public restrooms on a regular basis could have a significant effect on the transmission and diffusion of infectious diseases and other bacterial contamination. Due to many people using

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public toilets or washbasins and touching doorknobs several times a day, it can cause transmission of such contamination and pathogenic infectious disease. Therefore, the importance of toilets and washbasins as a source of transmission of bacterial contamination becomes more evident. It's obvious that if people's awareness of transitional contamination and related diseases enhances, it can be good for better social health and prevention of various infections. The purpose of this research is to boost health care in public services. In this study, by the

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survey of bacteriology and sampling of every facility that exist in restrooms and public services, we have measured the kind and amount of contamination that may be transferred by one to another people while they are using this places.

It seems that public services and home services must be more sanitized; absolutely in order to use public services and preventing problem for public health, more care of these services are recommended. Public services such as cinemas, hotels, hospitals, libraries, parks, etc., are more intended to be polluted because of severe public usage this should be done repeatedly to prevent transmission of disease and not to establish various illnesses such as skin disease, digestive disease, genital and venereal disease to the public. Such diseases are commonly transferable to public services because of sharing the same spaces of usage. All the points that are touchable for publics such as door handles, toilet paper, flush tanks, water hoses, valve handles, sink, liquid and solid soap, tissues, electrical driers, etc., subjects to be cleaned properly. The ventilation of public services areas is a very important factor to keep health care for public services.<sup>[1]</sup> Per annum, many cases of disease from public places occur to the people who are using these services, because of lack of sanitizing procedure; furthermore, by being infected with multidrug-resistant (MDR) bacteria or a harmful one, the process of recovery will become more complicated; this causes many economic and mental consequences to these people. Knowing the variety of contamination and checking infected facilities (such as an important one, toilet papers) that are existing in such services and have an important role in publishing the infection, McCusky *et al.*<sup>[2]</sup> and Robinton *et al.*<sup>[3]</sup> can help us to find means of preventing or diminishing infectious diffusion.

Recognizing the transmittal ways of "germs" and the means, help us to prevent establishing of contamination and to decrease the prevalence rate of disease that we expect to come after use of these facilities. Also, making people aware of the bad consequences of poor health services and encouraging them to keep their personal belongings clean will cause social behavior more confident in their health. This study examines whether electric driers, liquid or solid soaps, toilet paper and toilet valves, outdoor, and indoor handles etc., and whether they can play a positive or negative role in the transmission of diseases. In this article, we tried to answer this question by determining the type of microorganisms that we presume to exist. By the 7,482 samples we had taken from different points of many services, we get rich to achieve the trustful answer to the question. Definitely, by the numerous numbers of samples taken, its precision, accuracy, and reliability would be also higher.

## Materials and Methods

This descriptive cross-sectional study was conducted in different areas of Tehran during 2019. The subjects which were sampled are indoor and outdoor handles, taps, flush tank bottoms and levers, liquid and bar soaps. For bacterial type detecting, we have used of principals scientific sources and standard methods of bacteriology.

For a bacterial sampling of cases mentioned above, first provided wet sterile swabs which after sampling, transported on transport culture media and then as soon as possible it was transferred to the laboratory for passing them on culture medias such as nutrient agar (HIMEDIA, LOT45114591), blood agar (HIMEDIA, LOT45114591), MacConkey agar (HIMEDIA, LOTWe215), and EMB (HIMEDIA, LOT00000015320) which had been prepared before and were keeping on the refrigerator. Before passing the samples on culture media, the prepared media were brought out from the refrigerator in order to reach room temperature. After passage, in order to bacterial growth, the culture media which were passaged on were put into the incubator on 37 centigrade degrees (for 24–48 h). Finally, in order to assess the bacterial growth and colony-formation, the preserved culture media were examined precisely. When it manifested that the bacterial colonies had been grown on culture media, the Gram-staining method used to determine Gram-positive and Gram-negative bacteria.

Generally, all the species with their bacterial load of up to 100,000 CFU/g were designated detrimental even though those between 50,000 and 100,000 CFU/g were repeated for sampling and reassessed. Although, less than 50,000 colonies of bacteria/g are also considered normal and were excluded from the samples.

We realized that all the bacteria (whether Gram-positive and Gram-negative) could be able to grow on Nutrient agar media; also Gram-positive bacteria were grown on blood agar media and Gram-negative bacteria were grown on EMB and MacConkey agar media. For determining the specious of bacteria, these biochemical tests had been done; as mentioned below: For detecting the specious of Gram-negative bacilli such as *Shigella*, *Salmonella*, *Pseudomonas*, etc., Catalase, oxidase, urease test and triple sugar iron agar (TSI) (HIMEDIA, LOT00000015312) culture media were used. For detecting the specious of Gram-positive bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, etc., Catalase, coagulase, and MSA culture media (HIMEDIA, LOT0000287212) were used, too.

## Result

In the above study, we sampled 1,062 restrooms that in this survey: 2,124 restroom indoor and outdoor handles, 1,062 toilet faucet, 826 washbasin taps, 1,062 toilet hoses, 804 flush bottoms, 643 soap dispenser bottoms, 643 liquid soaps, 99 bar soaps, 169 toilet papers and paper towels, and 50 hand dryer machines. In total 7482 samples were tested from which 6,678 samples (89.25%) were contaminated and 804 samples (10.75%) uncontaminated.

## Discussion

In this study, 7,482 samples were taken from various cases. According to Table 1, there were 6,678 contaminated specimens, of which the highest rate was found in toilet hoses and taps with 99.72% (Out of 1,062 samples, 1,058 specimens were contaminated), followed by toilet outdoor handles with 99.62% (out of 1,062 samples, 1,058 specimens were

contaminated). Flush tank levers with 99.14% (out of 804 samples, 781 samples were contaminated), toilet indoor handles with 95.52%, soap dispenser bottoms with 97.82%, washbasin taps with 95.52%, bar soaps with 91.92%, hand dryer with 56% and towel papers with 20.12% were placed in terms of the amount of contamination.

According to results of Table 2. *E. coli* (28.5%) and *Klebsiella* (1.51%) were the most and least present bacteria in toilet indoor handles, respectively. On toilet outdoor handles, *E. coli* was the most (28.54%) and *Pseudomonas* was the least (1.32%). On flush tank levers, maximum bacteria was *E. coli* (35.08%) and *Pseudomonas* (0.26%) was the minimum. *E. coli* (30.6%) and *Salmonella* (1.52%) were the most and the least bacteria on

**Table 1: Absolute and relative frequency table of contaminated and non-contaminated public restrooms**

Sample items	Contaminated items		Non-contaminated items		Total
	n.	Percentage	n	Percentage	
Restroom indoor handles	1042	98.12	20	1.88	1062
Restroom outdoor handles	1058	99.62	4	0.38	1062
Toilet faucet	1059	99.72	3	0.28	1062
Toilet hose	1059	99.72	3	0.28	1062
Flush bottoms and levers	781	99.14	23	2.86	804
Washbasin tap	789	95.52	37	4.48	826
Liquid Soap dispenser bottoms	629	97.82	14	2.18	643
Liquid soaps	108	16.80	535	83.2	643
Solid soaps and bar soaps	91	91.92	8	8.08	99
Toilet paper and paper towels	34	20.12	135	79.88	169
Hand dryer machines	28	56	22	44	50
Total	6678	89.25	804	10.75	7482

\* n.=number \*%=Percent

**Table 2: Absolute and relative frequency table of microorganisms isolated from public restrooms**

Microorganisms	S.epidermidis	S.aureus	salmonella	Entrococcus faecalis	Basillus subtilis	E.coli	Klebsiella	Citrobacter	Entrobacter	Pseudomonas	Proteus vulgaris	Shigella	Mix	Contaminating cases
	N. (%)	N. (%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)	N.(%)
Restroom indoor handles	138 (13.24%)	105 (10.08%)		16 (1.54%)	186 (17.85%)	297 (28.5%)	12 (1.15%)	18 (1.73%)	14 (1.34%)		128 (12.28%)	14 (1.44%)	114 (10.94%)	1042
Restroom outdoor handles	130 (12.29%)	98 (9.26%)	24 (2.27%)	38 (3.59%)	191 (18.05%)	302 (28.54%)	14 (1.32%)	21 (1.98%)	28 (2.65%)		80 (7.56%)	14 (1.44%)	112 (10.59%)	1058
Toilet faucet	61 (5.76%)	59 (5.57%)	26 (2.46%)	40 (3.78%)	189 (17.85%)	365 (34.47%)	16 (1.51%)	30 (2.83%)	35 (3.31%)		98 (9.25%)	20 (1.89%)	108 (10.2%)	1059
Toilet hose	107 (10.1%)	101 (9.54%)	18 (1.7%)	81 (7.65%)	197 (18.6%)	217 (20.49%)	22 (2.08%)	34 (3.21%)	38 (3.59%)	4 (0.38%)	109 (10.29%)	32 (3.02%)	110 (10.39%)	1059
Flush bottom and levers	90 (11.52%)	62 (7.94%)	24 (3.07%)	73 (9.35%)	60 (7.68%)	274 (35.08%)	67 (8.57%)	29 (3.71%)	22 (2.82%)	2 (0.26%)	45 (5.76%)	21 (1.98%)	8 (1.21%)	781
Whashbasin tap	76 (9.63%)	94 (11.91%)	12 (1.52%)	49 (6.21%)	128 (16.22%)	238 (30.6%)			46 (5.83%)		86 (10.9%)	25 (3.2%)	48 (6.08%)	789
Liquid soap dispenser bottoms	48 (7.63%)	38 (6.04%)	10 (1.59%)	92 (14.63%)	111 (17.65%)	187 (29.73%)		12 (1.91%)	9 (1.43%)	2 (0.32%)	63 (10.02%)	12 (1.52%)	40 (6.36%)	629
Liquid soaps				2 (1.85%)	22 (20.27%)			10 (9.26%)		18 (16.67%)	30 (27.78%)	17 (2.7%)	26 (24.07%)	108
Solid soaps and bar soaps	14 (15.83%)	10 (10.99%)	10 (10.99%)		10 (10.99%)	16 (17.58%)	1 (1.1%)	2 (2.2%)			14 (15.38%)		14 (15.38%)	91
Toilet paper and paper towels	10 (29.41%)				4 (11.76%)	6 (17.65%)					4 (11.76%)		10 (29.41%)	34
Hand dryer	10 (35.71%)	2 (7.14%)			2 (7.14%)						6 (21.43%)		8 (28.57%)	28
Total	684 (10.24%)	569 (8.52%)	124 (1.86%)	391 (5.86%)	1100 (16.47%)	1902 (28.84%)	1132 (1.98%)	156 (2.34%)	192 (2.86%)	26 (0.39%)	663 (9.93%)	141 (2.11%)	598 (8.95%)	6678

washbasin taps, respectively. In soap dispenser bottoms, *E. coli* was the most (29.73%) and *Pseudomonas* was the least (0.32%). The most and the least bacteria that were found in liquid soaps were *Proteus vulgaris* (27.78%) and *Enterococcus* (1.85%). The most bacteria in bar soaps was *E. coli* (17.58%) and the least was *Klebsiella* (1.1%). On toilet papers, *Staphylococcus epidermidis* and mix bacteria were the most with (29.41%) and *Proteus spp.* and *Bacillus spp.* with (11.76%) were the least and finally in hand dryer machines *S. epidermidis* (35.71%) was the most and *S. aureus* and *Bacillus spp.* (7.14%) were the least.

In general, *E. coli* is the highest rate of contamination related to flush tank levers or bottoms and *Pseudomonas* is the lowest rate of contamination. It seems that after using the bathroom, flush tank levers can be effective in transmitting bacterial infectious diseases due to non-adherence in health care. It seems that *E. coli*, which is an intestinal bacteria, causes various parts of restroom contamination during the use of toilets, which is a sign of non-adherence in health care. Also, *E. coli* bacteria are very sensitive to drying on the contaminated hands; so the high potential of this bacteria for cross-contamination is expected due to sloppy hands.<sup>[4]</sup> *Pseudomonas* in liquid soap and the other parts of restrooms, which were contaminated by these bacteria, is a sign that subjects and materials are not used correctly. People are infected, and they transmit diseases.

A study performed by Buffet-Bataillon et al.,<sup>[5]</sup> has questioned the outbreak of *Serratia marcescens* and its investigation and control in the neonatal intensive care unit (NICU). In this study, during 3 months period, five infants were colonized by a single strain of *Serratia marcescens*. The researchers of this study achieved that a bottle soap dispenser can be a reservoir of this nosocomial pathogenic bacteria. So, these microorganisms can be easily transferred to newborns by healthcare workers. Conversely, *P. vulgaris* (27.78%) were the most bacteria sampled from liquid soaps as well as *E. coli* (29.73%), *Bacillus subtilis* (17.65%), and *Enterococcus faecalis* (14.63%) liquid soap dispenser bottoms. Although some researchers have proved that washing hands with non-antibacterial soaps and water are more effective than with water alone, Burton et al.,<sup>[6]</sup> basically by regarding the contamination of soap dispensers, we suggest using of alcoholic hand antiseptic instead of liquid or solid soaps.

In the study of microbial biogeography of public restroom surfaces which have been done by Flores et al.,<sup>[7]</sup> the communities were clustered into three general categories: those found on surfaces associated with toilets, those on the restroom floor, and those found on the surface routinely touched with hands. However, by comparison to our study, the sample items and bacterial diversity were almost alike, also vagina-associated *Lactobacillaceae* were widely distributed in female restrooms.

In the study of Kanayama et al.,<sup>[8]</sup> 252 samples were contaminated from 292 specimens, taken from toilets and warm water taps. *S. aureus*, *Streptococcus spp.*, *Enterococcus spp.*, *Enterobacteriaceae* and other negative bacteria had been found. From the above items, *Enterobacteriaceae* were isolated as 84 (%28.8) bidets and *E. coli*,

*Enterobacter spp.*, *Klebsiella*, *Citrobacter spp.*, and *Enterobacteriaceae* by 38 (13.0%), 22 (7.5%), 13 (4.5%), 5 (1.7%) and 6 (2.1%) were isolated in toilet bidets warm water, respectively.

In the study of McCusky et al., *Bacillus licheniformis* was the most isolated bacteria with 20.2% that shows a remarkable difference to compare with our study.<sup>[2]</sup> Also, in the study of Harrison et al., *Micrococcus luteus* and some strains of *Serratia marcescens* were the two species of bacteria found in paper towel specimens.<sup>[9]</sup> In addition, Robinton et al.,<sup>[3]</sup> showed that paper towels have substantially fewer viable bacteria on them than cloth towels, although in the opposite of cloth towels, the number of bacteria found on paper towels does not seem to be a variable appreciably influenced by geographic and/or climatic differences. In the above study, *Bacillus spp.* were the most bacterial species found in both kinds of towels. This is in contrast to our results in which *S. epidermidis* was common. There is no difference in the type of infectious bacteria in the above study compared with our study, but there is a significant difference in the percentage of contamination.<sup>[10]</sup> In a study by Sabra in Egypt in 2011,<sup>[11]</sup> the contamination of the women's public toilets were examined. 71.9% of the samples being positively infected. Toilets door handles (91.3%), toilet doors (73.8%), toilet sinks (63.3%), and flush tank levers (50%) were contaminated. *S. aureus* (40.6%) and *E. coli* (22.5%) were the most isolated bacteria from positive samples and *P. vulgaris* was the least one. There is no significant difference to compare with our study.

In the study of Alharbi et al.,<sup>[12]</sup> five different bacterial isolations were sampled from the airflow of 15 warm air dryers used in washrooms; including *Staphylococcus haemolyticus*, *Micrococcus luteus*, *Pseudomonas alcaligenes*, *Bacillus cereus*, and *Brevandimonad diminuta (vascularis)*. In this survey, the most bacterial isolates were due to *S. haemolyticus* with 95% pathogenicity; however, in our study, hand dryer machines were highly contaminated by *S. epidermidis* (35.71%). It is obvious that hot air dryers can deposit the pathogenic bacteria onto the hands and body of users as well as distributing them into the general environment whenever dryers are running. Also, some microorganisms could be inhaled by users and nonusers alike. So, it is imperative to recommend the sanitization of this machines several times a day. It is notable that in some studies it's manifested by which using warm air dryers or some jet dryers, we actually have augmented the aerosolization of bacteria and facilitating the microbial cross-contamination via airborne dissemination to the environment.<sup>[1]</sup> Best et al. reported that higher levels of contamination were due to washrooms using a jet air dryers compared with those using paper towels.<sup>[13]</sup> The hand-drying method can affect the risk of (airborne) dissemination of bacteria in real-world settings. JADs may not be suitable for settings where microbial cross-contamination risks are high, including hospitals.

The study that was conducted by Zapka et al., in 2011,<sup>[14]</sup> the *K. pneumoniae* was isolated from samples after the bacteria were recovered and transferred by hand after washing with liquid soap which had been spontaneously infused and liquid soaps which were contaminated without control.



A study of bacteriological assessment of door handles/knobs of toilets and washrooms was conducted by Frank Ngonda in a hospital setting in 2017,<sup>[15]</sup> which revealed some bacteriological similar results. Among the total of 442 samples, 184 cases (41.6%) were contaminated and also *S. aureus* was the most bacteria had isolated. The male toilet handles were most contaminated than the females (35.5% beside 19.4%), followed by general sets (9.7%). Whilst the washroom was less contaminated in general, the highest contamination being observed in the male washroom 19.4% as compared to the female washroom at 9.7%.

In the study of Ogba *et al.*,<sup>[16]</sup> the researchers have checked on 151 samples of public toilet seats. Out of the 151 samples examined, *E. coli* 70 (46.4%) was the most prevalent isolate followed by *Salmonella spp.* 45 (29.8%) while *Staphylococcus aureus* 15 (9.9%) was the least encountered isolate. Nevertheless, most of the samples and isolates were from hostels 41 (44.0%). This study demonstrates that public toilet seats that have been washed still harbor a high number of bacterial organisms and may serve as a potential source of infections.

## Conclusion

The results of this study and other similar related studies, that have been presented, demonstrate that illnesses such as genitourinary tract infections as well as gastrointestinal diseases can be found in children and adults by using contaminated services. In females, some genitourinary tract disorders such as vulvovaginitis, acute and chronic pregnancy, premature rupture of membrane (PROM), and acute pyelonephritis would arise mostly due to *E. coli*. In males, acute and chronic urethritis, cystitis, and prostatitis are most likely. Also, *E. coli* contamination is principally qualified to lead on Infertility in males and females. Acute cystitis, urethritis, and vaginal discharges are the main problems that occur in children are affected by this bacteria, therefore, enhancing personal hygiene, sanitizing public restrooms regularly and correctly, and using public toilets safely can prevent the transmission, diffusion, and spread of bacterial infections.

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## Conflicts of interest

There are no conflicts of interest.

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