

Research Paper

Determination of bacterial and fungal loads and antibiotic susceptibility testing of bacteria isolated from public toilet door handles in Vellore district, Tamilnadu, India

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Abstract

The main aim of this work is to portray the prevalence of both the pathogenic and non-pathogenic microorganisms on fomites such as public toilet door handles in Vellore district, India which may pose a risk to the community through transmission of infection. Swabs were collected from the door handles of different public toilets and it was found that the toilet door handles of hospital, bus station and railway station had a higher rate of contamination compared to schools and college. Majority of the bacterial isolates were coliforms and Gram negative bacteria. The following bacterial species were isolated such as *Staphylococcus aureus*, *Klebsiella sp*, *Escherichia coli*, *Salmonella sp*, *Shigella sp*, *Micrococcus sp*, *Bacillus sp*, *Pseudomonas sp* and *Proteus sp*. Fungus such as *Rhodotorula sp*, *Candida sp* and *Rhizopus sp* were found. Among Gram positive bacteria, *Micrococcus sp* (58%) showed highest resistance to antibiotics followed by *Staphylococcus sp* (50%) and *Bacillus sp* (42%). Among Gram negative bacteria, *Pseudomonas sp* (83%) showed highest resistance and *Proteus sp* (58%) showed the least resistance to antibiotics. To summarize the contamination of public toilet door handles largely go unnoticed but can cause serious infections and measures should be undertaken to control it.

Keywords: Microorganisms, toilet door handles, antibiotic susceptibility testing

Introduction

Microorganisms are ubiquitous and constitute a chief part of every ecosystem. The transmission of diseases through hand contact has been an area of major concern. Microbes in various environments live either freely or as parasites^[1]. Daily interaction of people contributes to spreading of disease but a major source and spread of community acquired infections are fomites^[2,3]. Such fomites include door handles, showers, toilet seats and faucets, sinks, lockers, chairs and tables, especially those found in schools, public offices, hospitals, hotels, restaurants and restrooms^[4,5]. Microbes live as transient contaminants in fomites or hands where they constitute a major health hazards as sources of community acquired infections. The increasing frequency of epidemic outbreaks of certain diseases and its rate of spread from one community to the other has become a major public health anxiety^[6,7]. Public toilets are the worst place to get infected as the total number of people using these places is more and variety of bacteria is deposited on the door handles.

Rest rooms are contaminated with microbes from human source such as saliva, skin, urine and feces. Many infected infants shed high concentration of bacteria in their feces and these readily transmit it through improperly washed hands^[8]. Public toilets have large interchange of users who deposit on the door handles their own microbial flora and the other organisms that they have picked elsewhere

[7,9]. People are in danger from the use of public toilets when the microbes enter the body through hand to mouth contact or hand to food contact. People cannot avoid the use of public restrooms to avert major health hazards. Some of the illnesses that result from the usage of public toilets include diarrhea, food borne illness, urinary tract infections (UTI), and severe acute respiratory syndrome (SARS)^[10].

The most concerned possible sources of infections are door handles of toilets and bathrooms^[7]. Even after multiple flushing and cleaning with antimicrobial fluids, bacteria seeded into toilets remain in the toilet for a long time^[11]. Bacteria such as *Staphylococcus aureus*, *E. coli*, *Klebsiella sp*, *Citrobacter sp* and *Salmonella sp* were found to be present on various contact surfaces such as chairs, tables, windows, door handles and many other common household fixtures^[12, 13]. The first line defense in preventing the spread of disease is by hand washing that is ignored and must be emphasized strongly by families, schools and health care professionals^[14]. On the other hand, many people wash their hands only with water without using detergents and some fail to wash their hands after using the public toilets. Studies have identified surfaces in kitchens and restrooms as being hot spots of bacterial contamination. These studies are of obvious importance in preventing the spread of human disease because several pathogenic bacteria are known to survive on surfaces for extended periods of time^[11,15]. The presence of pathogenic bacteria on environmental surfaces such as door handles poses a additional risk to vulnerable, immune-compromised individuals. It has been shown that hard, non-porous surfaces, such as door handles, have the highest bacterial transfer rates to hands^[5]. In recent years a lot of attempt has been invested in emphasizing hand hygiene through hand wipes and hand sanitizers in many public malls. Higher ethanol containing hand wipes are more effective in not only antimicrobial activity but also removal of endospores through the mechanical action^[16]. Even though people are commonly aware of such practices, the chance of inaccessibility or lack of use of these practices do occur. It has been reported that 60% of adults do not wash their hands when required^[17].

The present study showed the striking presence of pathogenic bacteria on the toilet door handles of trains, bus stand, schools, college, hostel and hospital in Vellore district, Tamil Nadu state, India. This will help evaluate the effect of unhygienic sanitation on public health and ensure the need for basic sanitation practices at public toilets.

Materials and Methods

Sample collection

Samples were collected from the public toilet door handles using sterile cotton swab moistened with sterile Trypticase soy broth (TSB) (Himedia, ILA). It was then introduced into a tube containing sterile TSB, cotton plugged and transported to laboratory and incubated overnight at 37°C.

Bacterial analysis

Each swab was aseptically placed into sterile tubes containing 10 ml of TSB. Samples were incubated over night to dislodge the microorganisms into the medium. The swabs were aseptically removed with forceps. The undiluted samples and the serial dilutions (tenfold from 1×10^1 to 1×10^7) in sterile TSB were prepared and 0.1 ml of the initial sample and each of the dilution was spread onto nutrient agar, blood agar, MacConkey plates, in duplicates. The plates were incubated for 24–48 hours at 37°C. The colonies that developed were counted and the total viable cells, referred to as colony forming units (CFU) per swabs were calculated. Bacteria were characterized on the basis of colony and cell morphology, Gram staining, motility and biochemical tests. Additionally antibiotic susceptibility tests were performed.

Sample Processing

Each collected swab was processed to identify the bacteria in the sample. The following processing techniques were employed;

- a) Culture
- b) Motility
- c) Gram staining
- d) Biochemical tests

Additionally fungal analysis was done by culturing on Sabouraud dextrose agar and identified by microscopic examination and germ tube test.

Culture

Each door handle sample was gently shaken, incubated overnight, serially diluted and aseptically inoculated into the three media namely; nutrient agar, MacConkey agar and blood agar and spread evenly over the entire surface of the media using a spreader (a sterile bent-glass rod). This was to allow for complete recovery of all organisms picked up in the swab. The plates were incubated overnight at 37°C and examined. Bacterial isolates were first differentiated by macroscopic examination of the colony. The colonies were differentiated based on size, color, pigmentation, elevation surface texture, and margin, hemolysis on blood agar and lactose fermentation on MacConkey agar.

Motility test

The hanging drop method was performed according to the method described by Barrow and Feltham^[18].

Gram staining

Gram staining was performed according to the method described in Cheesbrough^[19].

Biochemical tests

Several biochemical tests were carried out to further identify the various bacterial isolates.

Catalase test

Catalase test was carried out as described by Ochei and Kolhatkar^[20]. A colony of each Gram positive isolate was emulsified in distilled water on a clean grease-free slide. Two drops of hydrogen peroxide were added and observed for effervescence.

Oxidase test

Oxidase disc was placed on the clean glass slide and the overnight culture was placed on the disc and observed for deep purple color development within 10 seconds^[21].

Indole test

To a 24-hour culture of the bacterial isolate in peptone water, 0.5 ml of Kovac's reagent was added and shaken and examined after a minute for red color^[21].

Methyl red Test

To a 24-hour culture of the bacterial isolate in peptone water, 0.5 ml of methyl red reagent was added and shaken and examined after a minute for red colour.

Voges Proskauers Test

To a 24-hour culture of the bacterial isolate in peptone water, 0.5 ml of reagent Voges Proskauers A and Voges Proskauers B was added and shaken and examined after a minute for red colour.

Citrate utilization test

A light suspension of the bacterial isolate was made on a normal saline. With a straight sterile wire, the suspension was stab inoculated into the Simmon's citrate agar and incubated overnight. This was examined for characteristic blue color indicating growth^[20].

Urease test

The entire surface of the Christensen's urease medium slant was inoculated with a suspension of the bacterial isolate and incubated overnight and then examined for reddish pink color^[20].

Growth of the bacterial isolates on selective media

The bacterial isolates from the toilet door handle (Table 1) was streaked on selective media based on the previous test results.

Fungal analysis

Evaluation of the presence of fungi was performed by plating 0.1 ml of overnight cultures from the toilet door handles on Sabouraud's Dextrose agar (SDA) (Himedia, ILA) and incubated at room temperature. The colonies were enumerated after 3 and 7 days of incubation. Mould fungi were identified on the basis of cultural and microscopic characteristics. Yeast fungi were identified on the basis of colony and cell morphology and germ tube test.

Germ tube test

0.5ml of human serum was pipetted into a small test tube; the colony from the culture plate was inoculated into the serum with a sterile inoculation loop and incubated at 35° C for 3 hours. A drop of this inoculated serum was placed onto a glass slide and covered with a cover slip and examined under 40X objective of the microscope for sprouting yeast cells^[20].

Antibiotic sensitivity test (Disk diffusion method)

The disk diffusion susceptibility method was performed by applying a bacterial inoculum of approximately 1–2×10⁸ CFU/ml to the surface of Mueller-Hinton agar (MHA) plate^[22,23]. Up to 12 commercially-prepared, fixed concentrations, paper antibiotic discs were placed on the MHA surface. Plates were incubated for 16–24 h at 37°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks were measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug were interpreted using the standard zone size interpretative chart (Himedia, ILA).

Results and Discussion

In this study, door handles of toilets in trains, railway stations, old bus stand, new bus stand, school, college, hostel, hospital and canteen toilets in Vellore district, Tamil Nadu, India were evaluated for the presence and frequency of occurrence of bacterial and fungal contaminants. Samples were collected from various sources of toilet door handles such as Trains (T), Railway stations (R), Old bus stand (O), New Bus stand (N), School (Sc), Boy's school (Sb), Girl's school (Sg), College (C), Canteen toilet (Ca), Hostel (Ha) and Hospital (H) in Vellore district, Tamil Nadu, India. It has been reported that fomites serve as carriers for transmission of infection and recontamination of washed hands^[5, 24]. Some of these organisms could be highly pathogenic and can be contagious to other people or may result in auto-inoculation.

Table 1: Bacteria isolated from different toilet door handles

Organism	Location										
	T	R	O	N	Sc	Sb	Sg	C	Ca	Ha	H
<i>Salmonella</i>	+	+	+	+	+	+	-	+	-	-	+
<i>Shigella</i>	+	+	+	+	+	-	-	+	+	-	+
<i>Staphylococcus</i>	+	-	+	+	+	+	+	-	-	+	+
<i>E. coli</i>	-	-	+	+	+	+	+	+	+	+	+
<i>Pseudomonas</i>	+	+	+	-	-	-	-	-	-	+	+
<i>Proteus</i>	+	-	+	-	-	-	-	-	-	+	+
<i>Bacillus</i>	+	-	+	-	-	-	-	-	-	-	-
<i>Micrococcus</i>	-	-	+	+	-	-	+	+	-	-	-
<i>Klebsiella</i>	+	+	-	-	+	+	+	+	+	+	+

The obtained results showed that all the swabs had microbial contamination (Table 1). Similar results have been reported where every surface tested was contaminated with microorganisms^[25]. Usage of public toilets by large number of people and the absence of the habit of washing their hands after using public toilets, or wash hands for short time with or without detergents could be a major reason for this result^[14,17,24,26]. Previous studies have shown that frequently used fomites were most likely contaminated and carried higher loads of heterotrophic bacteria^[5,7]. The present study demonstrates that majority of the bacteria, transmitted through door handles are Gram negative.

The results of the isolated Gram positive and Gram negative bacteria and their prevalence rate are shown in Table 2 and Figure 1. The isolated Gram positive bacteria constituted of *Staphylococcus sp*

(72.72%), *Micrococcus sp* (36.36%) and *Bacillus sp* (18.18%) (Table 2). The most frequently encountered Gram negative bacteria were *E. coli* (81.81%), *Klebsiella sp* (81.81%), *Salmonella sp* (72.72%), *Shigella sp* (72.72%), *Pseudomonas sp* (45.45%) and *Proteus sp* (36.36%) (Table 2). Therefore the present study has shown the frequency of occurrence of various bacterial contaminants. However, the frequency of *E. coli* and *Klebsiella sp* were higher compared to other bacterial isolates that may be source of UTI. *E. coli* has been reported to be the most common cause of UTI [27]. The levels of contamination vary depending on traffic, exposure and environment [10]. This observation could be due to poor sanitary conditions and lack of regular toilet maintenance like cleaning with disinfectants, not washing hands with disinfectants after using toilets. Compared to the toilet handles of other locations the Train, old bus stand and hostel toilet door handles showed greater diversity of bacteria (Table 1 and & Figure 2).

Table 2: List of bacterial isolates and their percentage of prevalence

Bacterial isolates	% of prevalence
<i>Staphylococcus sp</i>	72.72
<i>Micrococcus sp</i>	36.36
<i>Bacillus sp</i>	18.18
<i>Salmonella sp</i>	72.72
<i>Shigella sp</i>	72.72
<i>E.coli</i>	81.81
<i>Proteus sp</i>	36.36
<i>Pseudomonas sp</i>	45.45
<i>Klebsiella sp</i>	81.81

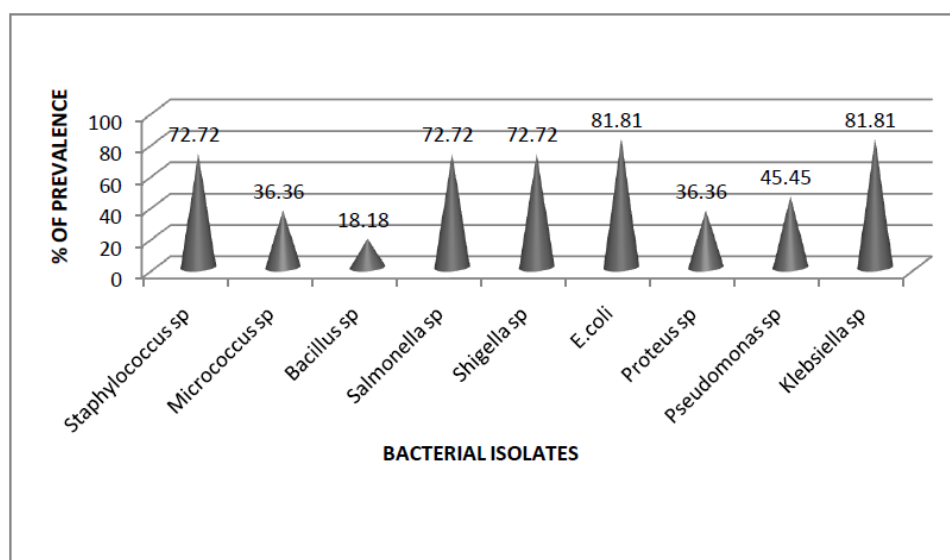


Figure 1: Bacterial isolates and their percentage of prevalence on public toilet door handles

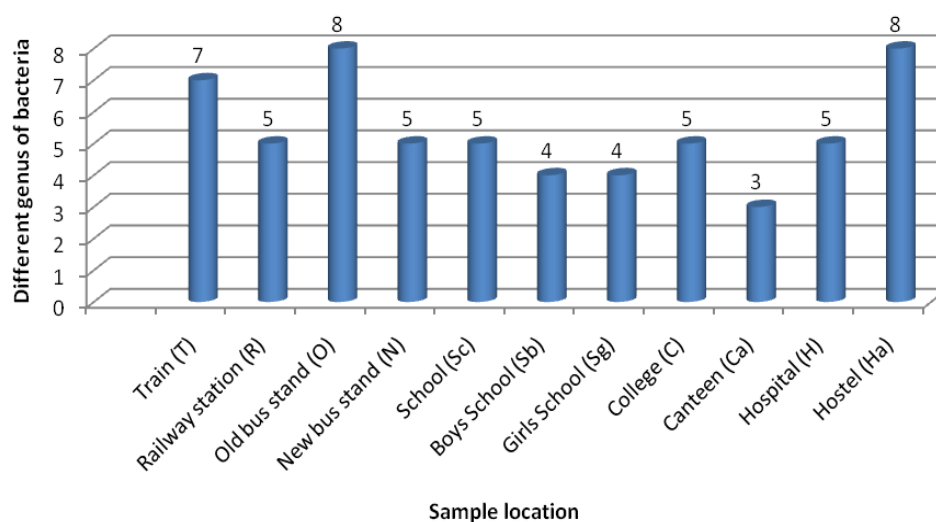


Figure 2: Diversity of bacteria isolated from various locations

Fungus such as *Rhodotorula sp*, *Candida sp* and *Rhizopus sp* were predominantly found to be present in the samples collected from the train toilet door handles that were not present in any other door handles of toilet (data not shown). In some of the previous research work, female toilets had higher bacterial contamination compared to male toilet door handles [28]. This may be due to certain habits of women which tend to increase contamination. For instance, women take a lot of beauty objects inside toilets like face creams, hand creams, and lotions. The consequences of using these things are that creams and lotions with contaminants may be smeared on the door handles which is rarely seen in male lavatories.

The present study highlights the importance of personal and especially hand hygiene while using public toilets. It has been reported that the bacterial loads has been reduced greatly by regular cleaning of the bathrooms, toilets, toilet seats, floor and door handles [29,30]. The fact that these contaminants at high levels in these environments is of great concern, especially for immunocompromised and transplantation patients. The results on the resistance pattern of the bacterial isolates to some antibiotics are presented in Table 3, 4 and Figure 3. Among Gram positive bacteria, *Micrococcus sp* (58%) showed highest resistance to antibiotics followed by *Staphylococcus sp* (50%) and *Bacillus sp* (42%) (Table 3). Among Gram negative bacteria, *Pseudomonas sp* (83%) showed highest resistance to antibiotics, whereas *Proteus sp* (58%) showed the least resistance to antibiotics (Table 3).

Table 3: Bacterial isolates and their percentage of resistance to various antibiotics

Bacterial isolates	% of resistance
<i>Staphylococcus sp</i>	50
<i>Micrococcus sp</i>	58
<i>Bacillus sp</i>	42
<i>Salmonella sp</i>	50
<i>Shigella sp</i>	75
<i>E.coli</i>	75
<i>Proteus sp</i>	58
<i>Pseudomonas sp</i>	83
<i>Klebsiella sp</i>	67

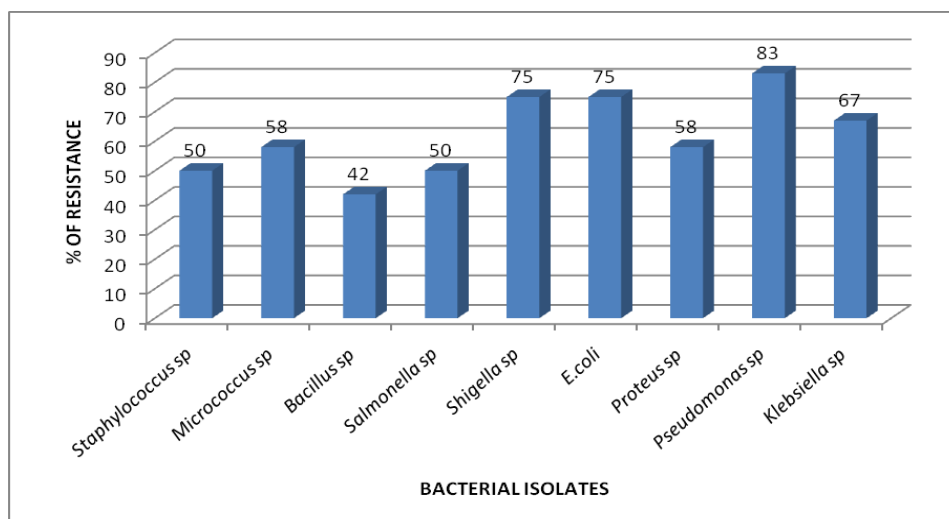


Figure 3: Bacterial isolates and their percentage of resistance to various antibiotics

Table 4: Antibiotic susceptibility test of the bacterial isolates

Organism	Zone of inhibition (in mm)											
	A	N	V	C	NE	G	T	PE	E	S	PI	AM
<i>Staphylococcus sp</i>	R	S	R	S	S	S	R	R	S	R	I	R
<i>Micrococcus sp</i>	R	R	I	S	I	S	R	R	R	S	R	R
<i>Bacillus sp</i>	S	S	R	S	I	S	R	R	I	I	R	R
<i>Salmonella sp</i>	R	S	S	S	R	S	R	R	S	R	R	S
<i>Shigella sp</i>	R	I	R	S	R	I	R	R	R	R	R	R
<i>E. coli sp</i>	R	S	R	S	R	S	R	R	R	R	R	R
<i>Proteus sp</i>	R	R	S	I	I	S	R	R	R	I	R	R
<i>Pseudomonas sp</i>	R	R	R	R	S	S	R	R	R	R	R	R
<i>Klebsiella sp</i>	R	I	R	S	R	S	R	R	R	R	R	S

Ampicillin (A) 10 µg, Norfloxacin (N) 100µg, Vancomycin (V) 5µg, Ciprofloxacin (C) 5µg, Neomycin (NE) 30µg, Gentamycin (G) 10µg, Ticarcillin (T) 75µg, Penicillin (PE) 10µg, Erythromycin (E) 15µg, Streptomycin (S) 10µg, Pipercillin (PI) 100µg, Amoxicillin (AM) 10µg,

Resistant (R), Sensitive (S), intermediately resistant (IR)

The determination of the antibiotic susceptibility patterns showed that all bacterial isolates tested were resistant or intermediately resistant to at least one antibiotic. The resistance of bacteria to commonly used antibiotics is an increasing problem worldwide and especially in developing countries^[31]. Also it has been reported that pathogens that cause UTI are developing resistance against commonly used antibiotics^[32]. Misuse of antibiotics is a major factor that leads to antibiotic resistance development by some strains of bacteria. In India, it is major concern as the antibiotics are available over the counter without prescription. People should be educated on the effects of indiscriminate use of antibiotics in humans and animals. Measures should be taken to reduce the antibiotic resistance of bacteria indirectly by preventing the availability of antibiotics over the counter without proper prescription. The data from our study may be useful to the medical doctors to avoid prescribing certain antibiotics to the bacterial sp that we have showed has already developed resistance.

Conclusion

Majority of public toilets found in bus stand, railway stations, parks, lack proper water system. Consequently, users can hardly wash their hands after usage, carrying the contaminants from such conveniences. Washing hands regularly is simply not enough to contain this form of germ breeding from occurring, although this should be a behavior that is instilled into people’s routines very early on

in life. Governmental organizations in developing countries including India should take measures to provide the basic amenities such as proper supply of water and detergents to the public toilets. A child should be educated from young age about the significance of cleaning hands before eating, cooking and after using the lavatories. Although this is something which we all think is done by everyone, many people fail to understand its importance.

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