COMPARATIVE STUDIES OF BACTERIA ASSOCIATED WITH SELECTED PUBLIC MALE AND FEMALE TOILETS DOOR HANDLES WITHIN FEDERAL POLYTECHNIC ILARO

Foluso Faparusi
Science Laboratory Technology Department, Federal Polytechnic, Ilaro, Ogun State
foluso.faparusi@federalpolyilaro.edu.ng

Abstract

Good toilet facilities are important and mandatory in every home and community; despite this, public toilet door handles serve as good route for transmitting enteric and respiratory pathogens, as a result of frequent use and poor personal hygiene. The study determined the level of hygiene of people using the public toilet in Federal Polytechnic Ilaro. A total of twelve (12) toilets (female and male) were randomly selected from East campus, Auditorium 1 and West campus. Swab door handle samples were collected for bacteriological analysis. Bacteriological profile of the selected door handles was determined using standard plate method, while the isolates were identified using conventional method. The results showed the presence of *Staphylococcus aureus* (25.0%), *Klebsiella* sp. (25.0%), *Pseudomonas aeruginosa* (22.2%), *Bacillus subtilis* (19.4%), *Escherichia coli* (2.7%), and *Enterobacter* sp. (2.70%) on the door handles. The isolates showed varied degrees of susceptibility to antibiotics. All the handles of the doors showed the presence of bacteria of public health concern. There was no difference between the species of bacteria isolated from the male and female toilets, thus, debunking the belief that female folks are more hygienic.

Keywords: Handles, hygiene, pathogens, public health, toilet

Introduction

Microorganisms are found everywhere and they constitute a major part of the ecosystem. Microorganisms play important roles in man's life and they can cause various infections if not properly managed. Contaminated hands play important role in the transmission of pathogens associated with fomites (Lopez *et al*., 2013). Diseases can be transmitted through hand shake or via surface to hand route. The frequency of interaction of humans with pathogens in the environment determines the rate of transmission of diseases (Li *et al.,* 2009). Microorganisms are present on all surfaces; fomites (inanimate surfaces) are potential reservoirs for direct or indirect transmission of pathogenic organisms (Lopez *et al*., 2013; Nicas & Sun, 2006). Since fomites harbour pathogenic organisms, the rate of human contact with them determines the rate of transmission of the organisms. The fomites include door handles, showers, toilet seat and faucets, sinks, lockers, and tables (Bright *et al.,* 2010). Fomites that are frequently touched serve as good route of transmitting infectious agents (Li *et al.,* 2009). Studies have revealed that inanimate surfaces in hospital and offices can be reservoirs of microorganisms of public health concern.

Toilet facility can serve as serious source of infection, most especially when utilized by different people. Unhygienic use of the toilet facilities and poor personal hygiene bring about contamination of the toilet. In developing countries, toilet doors are not automated, people access the facility with the aid of the door handles. Many toilets users wash their hands without the soap and some hardly wash at all after visiting toilets; same hands are used to open the doors. Several pathogenic bacteria can survive on toilet door handle surfaces for a long period of time (Baker *et al.,* 2004). Public toilet door handles can serve as good routes for transmitting enteric and respiratory pathogens due to frequent use and poor personal hygiene. Due to poor hygiene, the handles can become automatically reservoirs of infection when contaminated because the users must touch at entry and during exit from the toilet. Hence, users may end up picking the pathogen and he or she may get infected or transfer it to others (Maori *et al*., 2013). Door handles have been recognized as the most common possible source of infection in public toilet facilities (Reynolds & Hurst, 2005). Human hands harbour microorganisms that could be pathogenic, which

can be transferred to other persons that share same toilets (Aiello *et al.,* 2004). Man's hand, faecal matter and liquid secretion are sources of pathogens that can be transmitted through inanimate surfaces; these organisms are capable of surviving on fomite surfaces for a long period of time, although it depends on nature of fomites, type of microorganisms and environmental factors (Barker *et al*., 2004; Lopez *et al*., 2013).

Faecal matter is also main reservoir of enteric pathogens that contaminate the toilet door handles, if hands are not proper washed and disinfected when toilet one uses toilet. The contaminated door handles become vehicle for the transmission of infections and human health become in danger. When public toilet facilities are used and pathogens from door handles enter the body through hand to mouth contact or hand to food contact. Although it is nearly impossible for the hand to be free of microorganism; the presence of pathogenic bacteria may lead to chronic or acute illnesses (Oranusi *et al.,* 2013). Some of the illnesses that results from the use of public toilets includes food-borne illnesses, Urinary tract infection (UTI) and severe acute syndrome (SARS) (Bonne & Gerbe, 2010); these diseases have no sex boundary. There is a general belief that female folks are neater than their male counterparts, this study reports the use of toilet as a way of determining their level of neatness in terms of bacteria associated with door handles in public toilet facilities in Federal Polytechnic, Ilaro.

Materials and Methods

Study Area

The study was conducted on selected female and male public toilets in Federal Polytechnic Ilaro. The campus is traditionally divided into two sections; east campus and west campus. Both sections of the campus have a number of public toilets for the students and staff use.

Collection of Samples

A total of twelve (12) public toilet door handle swab samples were collected between 4pm and 5pm, in order to allow more people to use the facilities. The samples were obtained from the door handles using swab-rinse method of the American Public Health Association as described by Reynolds and Hurst (2005). Each swab sick was introduced into sterile peptone water in McCartney bottle. The bottles were labeled accordingly and transported to the Microbiology Laboratory of Science Laboratory Technology Department, The Federal polytechnic, Ilaro. The samples were analysed immediately.

Bacteriological Analysis

The McCartney bottles containing samples were shaken very well and loosely capped. The bottles were incubated at 37ºC for 24 h. The propagated cells were sub-cultured on Nutrient agar, MacConkey agar and Baird Parker agar plates using Streak method. The plates were incubated at 37^{o}C for 24 h. The pure cultures were maintained on Nutrient agar slants and kept at refrigerated temperature (4^{o}C) until further analysis. The pure isolates were identified using conventional method, on the bases of cultural characteristics, cellular characteristics, and biochemical tests as described by Cheesbrough (2006). Bergey's Manual of Determinative Bacteriology was used as reference for their identity (Holt *et al*., 1994).

Antibiotics Susceptibility Test

The antibiotic susceptibility of the isolated was determined using Kirby-Bauer-NCCLS disk diffusion method (CLSI, 2012). The bacteria were seeded on Mueller Hinton agar plates. The plates incubated 37ºC for 24 h. Diameter of the zone of inhibition was measured using transparent meter ruler. The diameter of inhibition zone was used as parameter to determine the sensitivity of the bacterial isolates to the antibiotics.

Results

The identities of isolates are shown in table 1. They showed different colonial and cellular characteristics. The response to biochemical tests varied according to isolates. The probable isolates were *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella* sp., *Enterobacter* sp. and *Escherichia coli.*

Bacterial isolates associated with various toilet door handle samples, collected at different locations within Federal Polytechnic, Ilaro are shown in table 2. *Bacillus subtilis* was present in samples A, B, C, D, F, J, K and L. Samples G, H, K and L showed the presence of *Pseudomonas aeruginosa*. *Staphylococcus aureus* was present in samples A, B, C, E, F, H and I. Samples A, B, C, D, E, F, H, J and K had *Klebsiella* sp. *Escherichia coli* was present in sample G. The presence of *Enterobacte*r sp. recorded in Sample I.

The list of bacterial isolates and their percentage of prevalence were shown in table 3. The organisms showed varied percentage of occurrence. *Staphylococcus aureus* and *Klebsiella* sp. recorded the highest percentage of occurrence (25.0%) while the least occurred organism was *Enterobacter* sp. (2.70%). Antibiotics susceptibility of the isolates was as shown in table 4. The diameter of zone of inhibition of *Bacillus subtilis* and *Staphylococcus aureus* ranged from 13-18 mm. *Bacillus subtilis* and *Staphylococcus aureus* were susceptible to Ampiclox (APX), Gentamycin (GEN), Amoxicillin (AM), and Streptomycin (STR*); Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Enterobacter* sp. were sensitive to Pefloxacin (PEF), Travid (OFX), and Amoxicillin (AM) in varied degrees. The diameter of zones of inhibition of *Escherichia coli*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *Enterobacter* sp. by the antibiotics ranged from 08-17 mm.

Table 1: Identification of the bacterial isolates

Sample Code	Colonial Morphology	Cellular Characteristics			Biochemical Test						Probable Organism
		Gram's	Reaction	Shape	Catalase	Coagulase	Indole	Glucose	Lactose	Sucrose	
A_1 (F)	Cream and spread all over the plates	+		Rod	+	-	-	+	+	+	*Bacillus subtilis*
A_2	Pinkish and mucoid	-		Rod	+	-	-	+	+	+	*Klebsiella* sp.
A_3	Golden yellow and smooth	+		Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
B_1	Golden yellow and smooth	+		Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
B_2	Cream and spread all over the plates	+		Rod	+	-	-	+	+	+	*Bacillus subtilis*
B_3	Pinkish and mucoid	-		Rod	+	-	-	+	+	+	*Klebsiella* sp.
C_1 (M)	Cream and spread all over the plates	+		Rod	+	-	-	+	+	+	*Bacillus subtilis*
C_2	Golden yellow and smooth	+		Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
C_3	Pinkish and mucoid	-		Rod	+	-	-	+	+	+	*Klebsiella* sp.
D_1 (M)	Cream and spread all over the plates	+		Rod	+	-	-	+	+	+	*Bacillus subtilis*
D_2	Pinkish and mucoid	-		Rod	+	-	-	+	+	+	*Klebsiella* sp.
D_3	Pinkish and mucoid	-		Rod	+	-	-	+	+	+	*Klebsiella* sp.
E_1 (M)	Cream and spread all over the plates	+		Rod	+	-	-	+	+	+	*Bacillus subtilis*
E_2	Greenish and flat	-		Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*

E_3	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
F_1 (F)	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
F_2	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
F_3	Pinkish and mucoid	-	Rod	+	-	-	+	+	+	*Klebsiella* sp.
G_1 (F)	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
G_2	Pinkish and smooth	-	Rod	+	-	+	+	+	+	*Escherichia coli*
G_3	Pinkish and smooth	-	Rod	+	-	+	+	+	+	*Escherichia coli*
H_1 (M)	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
H_2	Pinkish and mucoid	-	Rod	+	-	-	+	+	+	*Klebsiella* sp.
H_3	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
I_1 (F)	Pinkish and mucoid	-	Rod	+	-	-	+	-	+	*Enterobacter* sp.
I_2	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
I_3	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
J_1 (F)	Golden yellow and smooth	+	Cocci	+	+	-	+	+	+	*Staphylococcus aureus*
J_2	Pinkish and mucoid	-	Rod	+	-	-	+	+	+	*Klebsiella* sp.
J_3	Cream and spread all over the plates	+	Rod	+	-	-	+	+	+	*Bacillus subtilis*
K_1 (M)	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
K_2	Pinkish and mucoid	-	Rod	+	-	-	+	+	+	*Klebsiella* sp.

K_3	Cream and spread all over the plates	+	Rod	+	-	-	+	+	+	*Bacillus subtilis*
L_1 (M)	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
L_2	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*
L_3	Greenish and flat	-	Rod	+	-	-	+	-	-	*Pseudomonas aeruginosa*

Key: F = Female M = Male + = Positive - = Negative

Table 2: Isolates associated with the various toilet door handles

Sample code	Toilet Type	Isolates
A	Female	*Bacillus subtilis, Klebsiella* sp., *Staphylococcus aureus*
B	Female	*Staphylococcus aureus, Bacillus subtilis, Klebsiella* sp.
C	Male	*Bacillus subtilis, Klebsiella* sp., *Staphylococcus aureus*
D	Male	*Bacillus subtilis, Klebsiella* sp., *Klebsiella* sp.
E	Female	*Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus*
F	Female	*Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella* sp.
G	Male	*Pseudomonas aeruginosa, Escherichia coli, Escherichia coli*
H	Male	*Pseudomonas aeruginosa, Klebsiella* sp., *Staphylococcus aureus*
I	Female	*Enterobacter* sp., *Staphylococcus aureus, Staphylococcus aureus*
J	Female	*Staphylococcus aureus, Klebsiella* sp., *Bacillus subtilis*
K	Male	*Pseudomonas aeruginosa, Klebsiella* sp., *Bacillus subtilis*
L	Male	*Pseudomonas aeruginosa, Pseudomonas aeruginosa, Pseudomonas aeruginosa*

Table 3: Percentage of occurrence for the Isolates

Isolates	Percentage (%)
Pseudomonas aeruginosa	22.2%
Klebsiella sp.	25.0%
Enterobacter sp.	2.70%%
Staphylococcus aureus	25.0%
Escherichia coli	5.55%

Gram-Positive Bacteria	**Zone of Inhibition Diameter (mm)**								
	PEF	GEN	APX	AM	CPX	S	STX	R	Z
Bacillus subtilis	R	16	16	15	R	R	14	R	R
Staphylococcus aureus	R	14	18	15	R	R	13	R	R
Gram-Negative Bacteria	AUG	GEN	AM	OFL	SP	CPX	CH	STX	PEF
Escherichia coli	16	R	14	19	R	13	R	R	15
Klebsiella sp.	10	08	09	12	14	10	16	10	13
Pseudomonas aeruginosa	R	11	13	17	09	16	R	R	13
Enterobacter sp.	14	08	10	15	10	R	11	R	11

Table 4: Antibiotic susceptibility of the Isolates

Keys: AU = Augmentin, GEN = Gentamycin, AM = Amoxicillin, OFX = Tarivid , SP = Sparfloxacilin, CPX = Ciprofloxacin, CH = Chloramphenicol, STX = Septrin, S = Streptomycin, R= Rocephin, Z= Zinnacef, PEF = Pefloxacin, APX = Ampiclox

Discussion

Door handles are important reservoirs of microorganisms; this study revealed the level of bacterial contaminants on the surfaces of door handles of male and female public toilets in the Federal Polytechnic, Ilaro. Gram-negative bacteria were found to occur more than Gram positive bacteria. The high level of contamination of the door handles in this study is in agreement with the reports of Nworie *et al.*(2012), who observed 86.7% bacterial contamination of door handles in selected public convinences in Abuja metropolis. High number contaminants could be due to similar poor hygiene practices of the users and other factors that encourage contamination of the doors.

The microorganisms isolated from the door handles in this study were: *Bacillus subtilis, Pseudomonas aeruginosa, Klebsiella* sp., *Enterobacter* sp., *Staphylococcus aureus* and *Escherichia coli*. In this study the most frequent isolated bacteria pathogens were *Staphylococcus aureus* and *Klebsiella pneumonia*, the frequent occurrence of *Staphylococcus aureus* in this study may be due to the fact that the organism is a major component of the normal flora of the skin and nostrils, which probably explains its high prevalence as a contaminant, as it can easily discharged by several activities of human. This observation is in agreement with the findings of Nworie *et al.*(2012) on bacterial contamination of door handles in selected public convinences in Abuja metropolis. Isolation of *Staphylococcus aureus, Klebsiella* sp. and Escherichia *coli* from the toilet door handles is in line with previous study of Lynn *et. al.* (2013), which reported the presence of coliforms, *Salmonella* spp., *Staphylococcu*s spp., and *Candida* spp. on toilet door handles in secondary schools in Bokkos local government area of Jos, Plateau State.

The occurrence of *Bacillus subtilis* and *Pseudomonas aeruginosa* on the surfaces of the toilet door handles is in agreement with previous work of Opere *et al.* (2013), who reported the isolation of *Bacillus subtilis, Staphylococcus aureus* and *Pseudomonas aeruginosa* from public toilets; these organisms have been implicated as the most pathogenic bacteria recovered. A high occurrence of *Bacillus subtilis* in this study can be explained by the fact that B*acillus* species are ubiquitous in nature and their spores are able to resist environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods. This is also in agreement with the study carried out by Brooks *et al.* (2007) who reported that *Bacillus* spp. as the predominant organisms on door handles.

The presence of *Escherichia coli* in the handles could be due to poor hygiene of the users of the toilets or in availability of hand washing soap and regular water supply in the toilets. *Escherichia coli* is one of the enteric bacteria that are associated with faecal matter. Presence of this organism can be due to long nails keeping by the users of the facilities and it can bring about foodborne infection or intoxication.

Some of the commercial antibiotics tested showed high level of inhibition against the isolates, they would serve as good means of combating in the infection emanation from the use of the toilets. More so, many of the isolates showed multiple antibiotic resistance, this could be a great threat to health seeker since bacteria are known to exchange gene mediated resistance between organisms of same and different genera. The presence of multiple antibiotic resistant could be due to drug abuse and poor hygienic condition. This study agrees with the findings of Olajubu (2019) that reported the presence of antibiotic resistant bacteria strains in doors of public toilet doors in Adekunle Ajasin University campus, Akungba-Akoko.

Conclusion

The isolation of pathogenic bacteria from fomites in this study indicates that they can be vehicles for diseases transmission. There was presence of similar bacteria genera across the various categories of public toilets samples. These bacteria can cause health related issues if not properly managed. The presence of the bacteria could be due to poor hygiene practices by both female and male users. Therefore the general belief that females are neater than their male counterparts does not hold. Neatness cannot be attached to any particular sex.

Although due to coronavirus infection pandemic, there is great awareness across the globe on the need for regular hand washing in controlling the scourge. Much effort should be geared towards improving the level of hygiene in

the toilet facilities across the campus, by providing hand washing soaps and regular water supply. Regular awareness should be created on the campus about the need for good personal hygiene and its implications.

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