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THE PREVALENCE OF *Enterococcus spp* IN TOILET DOOR HANDLES OF MALE AND FEMALE HOSTEL IN DELTA STATE POLYTECHNIC OZORO

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ABSTRACT

The prevalence of *Enterococcus spp*. on fomites such as the toilet door handles in the male and female hostel of Delta State Polytechnic Ozoro was examined to determine the level of bacterial contamination on the toilet door handles which may pose a risk to the students through transmission of infection. A total of 15 samples were analyzed and coded as. A to H (Male toilet door handles) and I to O (Female toilet door handles). Seven Isolates were obtained; *Staphylococcus sp., E. coli, Streptococcus sp., Pseudomonas sp., Enterococcus sp., Bacillus spp.* The total heterotrophic plate count ranges from 5.3 X 10^3CFU/ML to 9.2 X 10^3CFU/ML. The present study showed that *Enterococcus sp.* was the most prevalent organism (25%) and the least prevalent organism was *Bacillus spp*. The spread of microorganism and prevention of infection from door handles can be minimized by thorough hand washing and use of hand sanitizer as well as daily washing and cleaning of restrooms with disinfectants.

INTRODUCTION

Restrooms are contaminated with microbes from human source such as saliva, skin, faeces and urine. Many infected infants shed high concentration of bacteria in their faeces and these readily transmit it through improperly washed hands (Dancer *et al.,* 2009). 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. (Reynolds *et al., 2005;* Ashgar *et al.,* 2012). 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 use of public toilets include diarrhea food borne illness, urinary tract infections (UTI), and severe acute respiratory syndrome (SARS) (Boone *et al.,* 2007).

The hands are the chief organs for physical manipulation of the environment. As a paired organ, the hand is controlled by the opposing brain hemisphere (Maria *et al.,* 2004) and enables one to do all manner of things. They serve as a medium for the propagation of microorganisms from place to place and from person to person. Although it is nearly impossible for the hand to be free of microorganisms, the presence of pathogenic bacteria may lead to acute illness. Human hands usually harbor microorganisms both a part of the body normal flora as transient microbes contacted from the environment (Dodril *et al.,* 2011). One common way by which organisms that are not resident in the hand are picked up by contact with surfaces such as table tops, door knobs or handles, banisters, toilet handles and taps in restrooms.

Hand washing is thought to be effective for the transmission of diarrhea Pathogens. However, it is not conclusive that hand washing with soap is more effective at reducing contamination with bacteria associated with diarrhea than using water only (Burton *et al.,* 2011). Many authors considered that hand washing is normal practice though to be effective for the prevention of disease, keeping hands clean and improving the hand hygiene is one of the most important steps taken to avoid getting sick and spreading germs to others (Chinakwe *et al.,* 2012).

The transmission of diseases through hand contact has been an area of major concern. Microbes in various environments live either freely or as parasites (Sleigh *et al.,* 1999) Human hands usually harbor microorganisms as part of body normal flora as well as transient microbes contacted from the environment (Lindberg *et al.,* 2004; Oranusi *et al.,* 2013). In some cases, they live as transient contaminants in fomites or hands where they constitute a major hazard as sources of community and hospital acquired infections (Pittet *et al.,* 1999).

In the polytechnic community, students have access to service offices regularly for different purposes. Given that the door handles are not routinely disinfected, the opportunity for the transmission of contaminating microorganisms is increasing day after day.

People are in the danger from the use of public toilets as public toilets have large interchange of users who deposit on the door handle their own microbial flora and other organisms that they have picked elsewhere. This study portrays the prevalence of both pathogenic and non-pathogenic organisms on fomites such as door handles of

toilets in the female and male hostels which may pose a risk to the community through transmission of infection.

MATERIALS AND METHODS

Study Area
This study was conducted in the male and female hostel of Delta State Polytechnic, Ozoro from which the sample was obtained.

Sample Collection
The samples were collected from door handles (8 toilet doors handle from male and 7 from female hostels) by means of sterile swab sticks. The swab was wiped firmly on the entire surface of the door knob. Each swab was placed in small tubes and normal saline was added to it properly covered at the place of collection and the samples were labeled. A to H (Male toilet door handles) and I to O (Female toilet door handles) and they were all transported to the laboratory, where analysis was carried out.

Materials
The materials used in this study includes laboratory coat, gloves, swab sticks, incubator, auto clave, measuring cylinder (50ml, 250ml, 500ml) and beakers (50ml, 250ml, 500ml), petri dishes, test tubes, conical flasks, Bunsen burner, nutrient agar, triple sugar iron agar, citrate agar, peptone water, wire loop.

Methods
Isolation of test organisms
The swab sticks containing the samples was used to streak the plates of prepared nutrient agar, each sample to each plate and was incubated at 37ºC per 24hours. Sub-culture was carried out on the growth and still incubated 24hours at 37ºC. Media prepared was according to the manufacturer instruction and then used for isolation of bacteria. Pure isolates were identified according to their morphological characteristics and reactions to biochemical test.

Morphological Characteristics Gram Staining
The procedure was carried out according to Cheesbrough (2006) as follows; smear was prepared from overnight culture in a clean dry slide. The slide was left to air dry. Fixation was done by rapidly passing the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30-60seconds, and then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. The back of the slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100x.

Biochemical Test
The biochemical analysis carried out was in accordance with procedures reported by (Cheesbrough, 2006).

Citrate Test
The bacteria isolate were tested for their ability to utilize citrate as the sole carbon source. Simmons citrate medium was used.

Bacteria isolates were inoculated into Simmons citrate medium in test tubes and incubated at 37ºC for 24-28hours. The culture media was observed for a colour change from green to blue. Positive showed no growth with intense blue colour, while negative test showed no growth and the colour of the medium remained green (Bello, 2002).

Triple Sugar Iron Agar Test (TSI)
Bacteria isolated were stabilized into TSI slant media and also streaked on the surface slant after while the medium was incubated at optimal temperature of 37ºC for 24hours. The TSI slant medium was used to check for the presence of the following:
GAS: if bubble is present in the media (Gas positive).
H_2S: if black is present in the media (H_2S positive).
LACTOSE: if the top of the media turn from pink to yellow (Lactose positive).
Glucose: if the bottom of the media turns from pink to yellow (Glucose Positive).

Catalase Test
This test detects the presence of catalase enzymes when present in a bacterium, it catalyze the breaking down of hydrogen peroxide with the release of oxygen as bubble.
$2H_2O_2 \xrightarrow{Catalase} 2H_2O + O_2$ With a wire loop, a colony was picked from the pure culture and was transferred to the centre of a glass slide 1-2drops of 3% hydrogen peroxide was added to the bacterial isolates immediate production of bubbles indicated positive result and if no bubble indicated negative.

Oxidase Test
The oxidase test is used to identify all organisms that produce oxidase enzyme. A piece of filter paper was soaked with oxidase reagent, a colony of test organism is then smeared on filter paper deep purple colour indicate that phenylene diamine in the reagent oxidize in the test organism (Cheesbrough, 2006).

Indole Test
This test demonstrates the ability of certain bacteria to decompose the amino acid tryptophan to indole production.

Bacteria isolates were inoculated into peptone water medium contained in sterile test tubes then incubated at 37ºC for 48hours. After the incubation period about 3drops of Kovac's indole reagent was added to the peptone water culture. The bottles were shaken thoroughly and allowed to stand and observed for colour development. A red colour ring at the interference of the medium denotes a positive result. And if the isolate is negative, the reagent layer will remain yellow or slightly cloudy (Bello, 2002)

Motility Test

The hanging drop method was performed according to the method described by (Barrow and Feltham, 1993).

RESULT AND DISCUSSION

Result

The organisms isolated from the samples are *Staphylococcus aureus, E. coli, Streptococcus sp., Pseudomonas sp., Enterococcus sp. Bacillus sp.* The bacteria isolated has the ability to utilize sugar as their substrate as shown in table 1. Shows the biochemical characteristics and identification of organisms. Table 2. Shows the total bacteria count of the isolates. Table 3 Shows the percentage occurrence of bacteria isolates.

Table 1: Shows biochemical characteristics and identification of organisms.

Gram reaction	Cultural morpholy	Indole	Catalas	Oxidase	Motility	Gas	H^2S	Citrate	Name of organism
Gram +ve cocci		-	-	+	-	+	-	+	*Pseudomonas sp.*
Gram +ve cocci		-	-	+	-	+	-	-	*Enterococcus sp.*
Gram +ve cocci		-	-	+	+	+	-	-	*Streptococcus pyogen*
Gram +ve cocci		-	-	+	+	+	-	-	*Staphylococcus aureus*
Gram +ve rod		-	-	+	+	+	-	-	*Bacillus sp.*
Gram -ve rod		+	+	+	+	-	+	-	*E.coli*
Gram +ve cocci		+	+	+	+	+	+	-	*Campylobacter jejuni*

Table 2: shows the total bacteria count.

Samples	Numbers isolated
A	7.2×10^3
B	9.2×10^3
C	6.2×10^3
D	8.4×10^3
E	8.5×10^3
F	7.4×10^3
G	7.2×10^3
H	8.8×10^3
I	7.7×10^3
J	6.3×10^3
K	8.2×10^3
L	6.7×10^3
M	5.3×10^3
N	6.9×10^3
O	7.3×10^3

Table 3: Percentage occurrence of bacteria isolates.

Isolates	% Occurrence
Staphylococcus Sp.	20.83
E.coli	10.51
Streptococcus Sp.	20.83
Pseudomonas sp.	12.5
Enterococcus sp.	25
Bacillus Sp.	10.33
TOTAL	**100**

DISCUSSION

Bacterial contamination of door handles is well documented. These fomites in turn serve as vehicles for cross-infections (Monarca *et al*., 2000). Some of the contaminants can be highly pathogenic and can be transferred from one person to another or may result in auto-inoculation (Kennedy *et al.,* 2005).

In this study a total number of 15 door handles swabbed from both the male and female hostels investigated 15 of them yielded bacterial growth.

The present study showed that all the swabs had microbial contamination (Table 1). Similar results have been reported where every surface tested was contaminated with micro-organisms (Otter *et al.,* 2009). Usage of the public toilet 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. Previous studies have shown that frequently used fomites were most likely contaminated and carried highly loads of heterotrophic bacteria (Lopez *et al.,* 2013). The presents study demonstrates that majority of the bacterial transmitted through door handles are Gram positive.

The microorganisms isolated from toilet door handles in the study were *Staphylococcus sp., E. coli, Campylobacter jejuni, Streptococcus spp., Pseudomonas spp., Enterococcus spp., and Bacillus spp.* Each of these organisms has been implicated either as a major contaminant or as the most pathogenic bacteria recovered. The fact that bacteria of the enterobacteriaceae were regularly found on different door handles may indicate faecal contamination of the hands as the origin.

The present study showed that *Enterococcus sp.* was the most prevalent organized (25%), followed by *Staphylococcus sp.* (20.83%) and *Streptococcus sp.* (20.83%). These contaminants were of a great concern. It must be borne in mind that even small numbers of organisms such as *Staphylococcus sp.* may proliferate and become hazardous if transferred to food (Elizabeth *et al*., 1982). *Staphylococcus sp.* and *Bacillus spp.* recovered during this study constitute a major part of normal skin flora; they may be passed from person by direct contact or via surfaces, including door handles. The two organisms are potentially pathogenic and may cause disease due to their high resistance such as food poisoning, abscesses; if they enter the body can lead to bacteremia and sepsis, pneumonia, meningitis osteomyelitis.

Several studies have indicated that bacteria such as *Staphylococcus sp.* Survive on hands and surfaces like door handles for hours or even days (Jiang & Doyle., 1999; Scott *et al.,*1990). The present study confirmed their findings.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The isolation of pathogenic bacteria from toilet door handles in this study indicates that they can be vehicles for disease transmission. Contaminated and improperly washed hands contaminate door handles and it is important to note there is high level of prevalence of the bacterial infectious disease due to contaminants.

Recommendations

It is hereby recommended that

1. The door handles are instrumental in the spread of many infections thus the use of self-disinfecting door handles (e.g. Copper) is highly recommended particularly important in service offices.
2. There is need therefore for thorough hand washing, disinfection and conscientious contact control procedures to minimize the spread of these pathogens.
3. Regular surface cleaning and disinfection is also highly recommended to reduce chances of transmission of these potential pathogens.

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