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STUDIES ON BACTERIAL ISOLATES FROM DOOR HANDLES OF PUBLIC TOILETS IN SELECTED LOCATIONS IN GBOKO, BENUE STATE, NIGERIA

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ABSTRACT

The aim of this study was to isolate, identify and characterize the bacteria isolated from door handles of public toilets in some selected locations in Gboko, Benue State, Nigeria. A total of 100 samples of door handles were collected from 20 sampling points across 5 locations within 5 different landmarks: Main market toilets, Commercial banks, Nursery and Primary Schools (NPS), General Hospital Gboko and University of Mkar. Media preparation, microbial isolation, cultural practices and biochemical characterization followed standard procedures. Data were computed and described using counts and percentages. The chi-square test for homogeneity was employed in comparing the distribution of each bacteria type across the locations at 95% confidence limit ($P \leq 0.05$) on the SPSS version 25 software. A total of 150 bacterial isolates were identified in the overall results. Ten (10) species of bacteria were identified. They were: *Staphylococcus areus, S. pyogeny, Pseudomonas, S. epidemidis, Escherichia coli, Citrobacter, Proteus, Klebsiella, Bacillus* spp and *Enterobacter*. Toilet handles in main markets were the most contaminated (48 isolates), this was followed by General hospitals and banks (31 each) while University of Market toilet handle had the least isolates (18). The top three dominant bacteria were *E. coli* (21.3%), *S. areus* (16.7%) and *Bacillus* spp (13.3%). The distribution of bacteria across the five locations within each landmark was homogenous ($P>0.05$). Also, distribution was homogenous among the five landmarks ($P>0.05$). The information given in this report is of great importance in public health, disease control and epidemiology.

Keywords: Contaminants, Door handle, Public toilets, Public Health

1 INTRODUCTION

Many factors have been shown to influences the bacterial transfer between surface including the source and destination source of future. Beside the day-to-day interaction of people, which constitute one way of spreading disease, the major source of spread of these infections are formites (Prescott et al.,2005). Fomites when on constant contact with humans or natural habitats of pathogenic organism constitute a major source of spread of infectious diseases.The fomites include door handle of conveniences, showers, toilet, hand lockers especially those found in public offices, hospitals, hotels, restaurants and restrooms (Bright *et al.,* 2010). Microorganisms are found everywhere, bacteria and fungi contaminate our body, houses, work places, and whole environment. Fortunately among many billion of bacteria, only 1500 can be dangerous for our health, causing different disease such as pneumonia or skin infection (Eltablawy and Elhinfnawi, 2009). Human hands usually harbor microorganisms both as part of the body normal flora as well as trasiem microbes contracted from the environment (Dodnill *et al.,* 2011). One common way by which organisms that are not resident in the hand are picked up is by contact with surfaces such as table tops, door knob or handles, banisters, toilet handles and taps in restrooms (Lindberg *et al.,* 2004).

In the university environment, 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 great. Although it is accepted that the infection risk in general community is less than that associated with patients in hospital. The increasing incidence of Bacteria outbreaks of certain diseases and its rate of spread from one community to the other has become a major public health concern (Nworie *et al.,* 2012).People believe that microbes are only present in hospitals, clinics and research laboratories. Thus they have a misleading feeling of security in other places. This is due to lack of knowledge about microorganisms being ubiquitous and could be found on door handles of public toilet which when picked up could cause health problems. Researchers considered that 80% of infections are spread through hands contact with hands or other objects (Al-Ghamdi *et al.,* 2011). The main reasons are difficulties to prevent the transfer of microbes that are already present in human bodies (Lues and Tonder, 2007). It is established that unwashed hands can transmit pathogens, especially fecal pathogens, to food product after visit to the toilet.

This study is expected to highlight the problem of door handles of public toilets in some places in Gboko Local Government Area of Benue State. It is believed that microorganisms are ubiquitous comparatively. They can also be found on the door handles of public toilets. There are health risk associated with the exposure to these microorganisms on the door handles of these toilets. It is on this note that the researcher intends to carry out the study to verify the claim and to identify microorganisms associated with door handles of public toilets in some places in Gboko. The aim of this study was to isolate, identify and characterize the bacteria isolated from door handles of public toilets in some selected locations in Gboko, Benue State, Nigeria

2 MATERIALS AND METHODS

Study Area and Sample Collection

The research was conducted in Gboko town. It is 82km away from the State Capital Makurdi, with a population figure of approximately 4,253,641 inhabitants (2006 Census). A total of 100 samples of door handles were collected for this study.

Five (5) different landmarks were used, each with 5 locations and 20 sampling points (4 sampling points from each location) as distributed in Table 1. Sterilized swab sticks were made wet slightly with physiological saline and robbed throughout the entire surface of the door handle to ensure that micro-organisms adhere to the stick appropriately.

Table 1: Landmarks and Locations of Sample Collection in the Study Area

Landmarks of Sample Collection	Sample Locations	Total Samples
Main market toilets	Main park Mbayion park Kpamber park Adekaa park Anyiin park	20
Commercial banks	Unity Bank Eco Bank Union Bank Zenith Bank United Banks for Africa (UBA)	20
Nursery and Primary Schools (NPS)	Demonstration NPS Mkar Daystar NPS Mkar NKST Orphanage Mkar Ichigh International NPS Mkar Laurel NPS Mkar	20
General Hospital Gboko	Female ward Male ward General staffs toilets OPD DOT	20
University of Mkar	Staffs toilets Accounting lecture hall toilets Microbiology laboratory toilets Female hostel toilets Male hostel toilets	20
Total Samples		100

Media Preparations

The culture media used for the isolation, identification, and characterization of bacteria isolates were: Blood agar, MacConkey agar, Nutrient agar (NA), pepton water and Simon Citrate agar. Media were prepared in accordance with the manufacturer's specifications and sterilized using an autoclave at 121℃ for 15minutes (Cheesbrough, 2006).

Sample Preparation, Culture and Isolation of Pure Culture

Samples were processed by standard bacteriological procedures (Cheesbrough, 2006). Collected samples were pre-enriched with peptone water for 24hrs (overnight incubation) at 37^{o}C to dislodge adhered bacteria. Samples were also cultured using streak plate method on MacConkey agar, and blood agar and incubated at 37^{o}C for 24 hours. (Cheesbrough, 2006). The blood agar and MacConkey agar plates were examined for cultural characteristics. Different colonies on the MacConkey agar and Blood agar plates were picked carefully and inoculated on nutrients agar plate to obtain pure growth. Organisms that were identified to be the same from the blood and MacConkey agar were grouped as one isolate. Bacteria identification was done using the pure culture on the Nutrient Agar plates.

Biochemical Characterization of Isolates

Colonies were identified presumptively by cultural characteristics (colonial morphology) and gram staining characteristics. Isolates were further characterized biochemically using catalase, coagulase, oxidase, citrate utilization, indole and sugar fermentation tests. Triple sugar iron agar (TSIA) was used for the differentiation of the *Enterobactericeae* by Triple sugar fermentation and hydrogen sulphide production (Cheesbrough, 2006).

Statistical analysis

Data were computed and described using counts and percentages. The chi-square test for homogeneity was employed in comparing the distribution of each bacteria type across the locations at 95% confidence limit ($P \leq 0.05$) on the SPSS version 25 software.

3 RESULTS AND DISCUSSIONS

Ten (10) species of bacteria were isolated and identified. Reactions to different biochemical tests are explained in Table 2. They were: *Staphylococcus areus, S. pyogeny, Pseudomonas, S. epidemidis, E. coli, Citrobacter, Proteus, Klebsiella, Bacillus* spp and *Enterobacter*. Table 3 shows the distribution of bacteria isolates on door handles of public toilets from main market location. Out of 48 bacteria isolates in this location, *E. coli* was the most dominant (20.8%) followed by *S. areus* and *Bacillus* spp (16.7% each) while *Citrobacter* and *Proteus* were the least (4.2% each). Gboko main park and Kpamber park recorded the highest contamination of toilet door handles (12 isolates each out of 48). At the University of Mkar toilet handles (Table 4), a total of 18 bacterial isolates were obtained. *E. coli* and *Enterobacter* were 4 (22.2%) as the most dominant species followed by *Klebsiella* with 3 isolates (16.7%). The Female toilet handle was the most contaminated having recorded 7 out of 18 isolates.

At the General hospitals (Table 5), 31 isolates were obtained out of which *S. areus* and *E. coli* were the most dominant (19.3% and 6 isolates each) followed by *Klebsiella* (16.1% and 5 isolates). The female and male wards toilet handles had the highest contaminants (9 isolates each) followed by OPD (7 isolates). Among the selected banks (Table 6), the Zenith bank toilet had the highest contaminants (11 out of 31 bacterial isolates) followed by UBA (7 out of 31 isolates). E. coli was the most dominant of the isolates (29%) while *Pseudomonas* was the least (3.2%). As given in Table 7, the toilet handle of the NKST Orphanage Nursery and Primary School (NPS) had the highest number of isolated contaminants (9 out of 22 isolates) while Laurel NPS had the least (2 out of 22). *Enterobacter* (27.2%) and *S. areus* (22.7%) were the highest among the contaminants.

A total of 150 bacterial isolates were identified in the overall results (Table 8). When all the landmarks of sampling were compared, toilet handles in main markets were the most contaminated (48 isolates), this was followed by General hospitals and banks (31 each) while University of Market toilet handle had the least isolates (18). The top three dominant bacteria were *E. coli* (21.3%), *S. areus* (16.7%) and *Bacillus* spp (13.3%). The distribution of bacteria across the five locations within each landmark was homogenous ($P>0.05$). Also, distribution was homogenous among the five landmarks ($P>0.05$).

The outcome of this study has shown that hands are the most important reservoir of microorganisms, which may act as source of bacteria transfer, particularly in the contamination of door handles of the public toilets. In the present study, the level of contamination of bacteria is less than what was reported (86.7%) in similar work (Nworie *et al.,* 2012). Moreover, majority of the Bacteria Isolated in this study are potential pathogens. Similar results were reported by Maryam *et al.* (2014) and Koing (2014) who stated that the occurrence bacteria on surfaces were coliform bacteria like *E. coli*. Differences in the level of contamination of toilet handles across the landmarks agree with the findings of Nworie *et al*. (2012) who reported that the levels of contamination vary depending on the traffic, exposure of the environment.

In this study, the most frequently isolated bacteria pathogens was *Escherichia coli* which may be due to the fact that it is a major component of the normal flora of the GIT, which probably explains its high prevalence as a contaminant, as it can easily be discharged by several human activities such as release of excretal. This observation is in agreement with the findings of other researchers (Nworie *et al*., 2012). The presence of species of *Staphylococcus* and *Streptococcus* could be attributed to the fact that they are resident flora of the human skin and they can easily contaminate surfaces on contact (Aiello *et al.,* 2004; Abdulla *et al*., 2008). Both species are known to be potential/ opportunistic human pathogens (Prescott *et al*., 2005). Enterotoxin producing strains of *staphylococci* have been implicated in food poisoning (Loir *et al*.2003; Linderberg *et al.,* 2004). *Bacillus* spp. are known to bear a resistant spore and common environmental contaminants; it has been shown to be a transient micro flora of the hand and surfaces due to the spore forming ability. Bacillus cereus has been implicated in food poisoning (Maori *et al.,* 2011; Schmitt *et al*., 1991). The spore forming ability of *Bacillus* and their wild distribution may be responsible for their spread on surfaces and on handle swabs (Itah and Ben, 2004).

The high bacteria contamination recorded for door handle swab of public toilets could be a reflection of the level of exposure and this cross contamination. The hand is the main organ used for manipulating the environment and pick microorganism in these diverse environments (Chinakwe *et al.,* 2012; De Alwis *et al.,* 2012). Public toilets in places like market, hospitals, banks and schools receive large influx of people on daily basis are more likely to be contaminated. Improperly and unwashed hands contaminate door handles. The isolation of pathogenic bacteria from fomites in this study indicates that they can be vehicles for disease transmission. Poor hygiene is commonly linked to high incidence of salmonellosis and diarrhoea (Humphrey *et al.,* 1994; Rotter, 1999; Williams, 2000; Lambrechts *et al*., 2014). In the light of this, there is need for thorough hand washing, disinfection and conscientious contact control

procedures to minimize the spread of these pathogens. Proper hygiene and public enlightenment on the role of easy contact surface and the hands in disease dissemination is advocated.

Table 2: Biochemical Characterization of Bacteria Isolated from Door Handles of Public Toilets in Selected Locations in Gboko Metropolis

ISOLATES	SUGARS							TSIA					
	Lact.	Man.	Glu.	Suc.	Oxid.	Citr.	Indo.	Cata.	Coag.	Slop.	Butt.	H_2O	Ga.
S. areus	+	+	+	+	-	+	-	+	+	y	y	-	-
S. pyogeny	+	+	+	+	-	-	-	+	-	+	-	+	-
Pseudomonas	-	+	-	-	+	-	-	+	-	-	-	$+^2$	d
S. epidemidis	+	+	+	+	-	-	-	+	-	r	y	y	$+^2$
E. coli	+	+	+	+	-	-	$+^2$	-	-	y^2	y	-	+
Citrobacter	+	+	+	+	-	+	-	+	d	-	-	+	+
Proteus	-	-	+	+	-	d	+	-	-	y	y	+	d
Klebsiella	+	+	+	+	-	+	+	-	-	y	y	-	+
Bacillus spp	d	+	+	+	-	+	-	-	+	+	y	y^2	y
Enterobacter	-	+	+	+	-	+	-	+	-	y	y	-	-

KEY:
Lact. = Lactose, **Man.** = Manitol, **Gluc.** = Glucose **Ga.** = Gas **Suc.** = Sucrose, **+** = Positive, **-** = Negative, **H_2S** = Hydrogen sulphide, **Indo.** = Indol, **y** = yellow acid reaction ,**Coag.** =coagulase, **d** = different strains gave different results.

Table 3: Distribution of Bacteria Isolates on Door Handles of Public Toilets from Locations in Main Market.

Isolates	Motor Parks					
	Mbayion	**Gboko Main park**	**Kpamber**	**Adekaa**	**Anyii**	**Total**
S. areus	1 (12.5%)	2 (16.7%)	3 (25.0%)	1 (11.1%)	1 (14.3%)	8 (16.7%)
S. pyogeny	-	-	2 (16.7%)	1 (11.1%)	-	3 (6.2%)
Pseudomonas	1 (12.5%)	2 (16.7%)	-	-	-	3 (6.2%)
S. Epidemidis	1 (12.5%)	1 (8.3%)	2 (16.7%)	1 (11.1%)	-	5 (10.4%)
E. coli	2 (25.0%)	2 (16.7%)	1 (8.3%)	3 (33.3%)	2 (28.6%)	10 (20.8%)
Citrobacter	-	-	-	1 (11.1%)	1 (14.3%)	2 (4.2%)
Proteus	-	1 (8.3%)	1 (8.3%)	-	-	2 (4.2%)
Klebshiella	-	1 (8.3%)	2 (16.7%)	-	1 (14.3%)	4 (8.3%)
***Bacillus* spp**	2 (25.0%)	3 (25.0%)	1 (8.3%)	2 (22.2%)	-	8 (16.7%)
Enterobacter	1 (12.5%)	-	-	-	2 (28.6%)	3 (6.2%)
Total	**8 (100%)**	**12 100%)**	**12 (100%)**	**9 (100%)**	**7 (100%)**	**48 (100%)**

P=0.084 (P>0.05)

Table 4: Distribution of Bacteria Isolates on Door Handles of Public Toilets from Locations in University of Mkar, Mkar

Isolates	University of Mkar Public Toilets					
	Staff	**Accounting Lecture Hall**	**Microbiology Laboratory**	**Male Toilet**	**Female Toilet**	**Total**
S. areus	-	-	-	1 (20.0%)	1 (14.3%)	2 (11.1%)
Pseudomonas	-	-	-	1 (20.0%)	1 (14.3%)	2 (11.1%)
S. Epidemidis	-	-	-	-	1 (14.3%)	1 (5.6%)
E. coli	1 (50.0%)	1 (50.0%)	-	1 (20.0%)	1 (14.3%)	4 (22.2%)
Proteus	-	-	-	-	1 (14.3%)	1 (5.6%)
Klebsiella	-	-	1 (50.0%)	1 (20.0%)	1 (14.3%)	3 (16.7%)
Bacillus spp	-	-	-	-	1 (14.3%)	1 (5.6%)
Enterobacter	1 (50.0%)	1 (50.0%)	1 (50.0%)	1 (20.0%)	-	4 (22.2%)
Total	**2 (100%)**	**2 (100%)**	**2 (100%)**	**5 (100%)**	**7 (100%)**	**18(100%)**

P=0.726 (P>0.05)

Table 5: Distribution of Bacteria Isolates on Door Handles of Public Toilets from Locations in General Hospital, Gboko

Isolates	General Hospital, Gboko					
	Female Ward	**Male Ward**	**Staff**	**OPD**	**DOT**	**Total**
S. areus	1 (11.1%)	2 (22.2%)	-	2 (28.6%)	1 (20.0%)	6 (19.3%)
S. pyogeny	2 (22.2%)	2 (22.2%)	-	-	-	4 (12.9%
Pseudomonas	1 (11.1%)	1 (11.1%)	-	-	-	2 (6.5%)
S. Epidemidis	1 (11.1%)	-	-	-	1 (20.0%)	2 (6.5%)
E. coli	2 (22.2%)	1 (11.1%)	1 (50.0%)	1 (14.3%)	1 (20.0%)	6 (19.4%)
Citrobacter	-	-	1 (50.0%)	1 (14.3%)	-	2 (6.5%)
Proteus	-	-	-	2 (28.6%)	-	2 (6.5%)
Klebsiella	2 (22.2%)	1 (11.1%)	-	1 (14.3%)	1 (20.0%)	5 (16.1%)
Bacillus spp	-	2 (22.2%)	-	-	-	2 (6.5%)
Enterobacter	-	-	-	-	1 (20.0%)	1 (3.2%)
Total	**9 (100%)**	**9 (100%)**	**2 (100%)**	**7 (100%)**	**5 (100%)**	**31 (100%)**

P=0.332 (P<0.05)

Table 6: Distribution of Bacteria Isolates on Door Handles of Public Toilets in Some Selected Banks in Gboko Metropolis

Isolates	Bank Toilets					
	UBA	**Zenith**	**ECO**	**Unity**	**Union**	**Total**
S. areus	1 (14.3%)	2 (18.2%)	-	-	1 (16.7%)	4 (12.9%)
S. pyogeny	-	2 (18.2%)	-	-	1 (16.7%)	3 (9.7%)
Pseudomonas	-	1 (9.1%)	-	-	-	1 (3.2%)
S. Epidemidis	-	-	1 (25.0%)	-	1 (16.7%)	2 (6.5%)
E. coli	2 (28.6%)	3 (27.3%)	1 (25.0%)	1 (33.3%)	2 (33.3%)	9 (29.0%)
Citrobacter	2 (28.6%)	1 (9.1%)	-	-	-	3 (9.7%)
Klebshiella	1 (14.3%)	-	-	1 (33.3%)	-	2 (6.5%)
Bacillus spp	-	2 (18.2%)	1 (25.0%)	1 (33.3%)	1 (16.7%)	5 (16.1%)
Enterobacter	1 (14.3%)	-	1 (25.0%)	-	-	2 (6.5%)
Total	7 (100%)	11 (100%)	4 (100%)	3 (100%)	6 (100%)	31 (100%)

P=0.103 (P<0.05)

Table 7: Distribution of bacteria isolates on door handles of public toilets from selected Nursery/Primary Schools in Gboko metropolis

Isolates	Nursery/ Primary School Public Toilets					
	NKST Orphange Nur./ Pri. Sch., Mkar	Demonstration Nur./ Pri. Sch., Mkar	DayStar International Nur./ Pri. Sch., Mkar	Ichigh International Nur./ Pri. Sch., Mkar	Laurel Nur./ Pri. Sch., Mkar	Total
S. areus	2 (22.2%)	-	1 (33.3%)	1 (25.0%)	1 (50.0%)	5 (22.7%)
S. pyogeny	-	-	1 (33.3%)	-	-	1 (4.5%)
E. coli	2 (22.2%)	1 (25.0%)	-	-	-	3 (13.6%)
Citrobacter	1 (11.1%)	-	-	2 (50.0%)	-	3 (13.6%)
Klebshiella	1 (11.1%)	-	-	-	-	1 (4.5%)
Bacillus spp	2 (22.2%)	1 (25.0%)	-	-	-	3 (13.6%)
Enterobacter	1 (11.1%)	2 (50.0%)	1 (33.3%)	1 (25.0%)	1 (50.0%)	6 (27.2%)
Total	**9 (100%)**	**4 (100%)**	**3 (100%)**	**4 (100%)**	**2 (100%)**	**22 (100%)**

P=0.538 (P<0.05)

Table 8: Comparison of Distribution of Bacteria Isolates on Door Handles of Public Toilet Across Locations in Gboko Metropolis

ISOLATES	LOCATIONS					
	Main Market	**University**	**General Hospitals**	**Banks**	**Nursery/ Primary School**	**Total**
S. areus	8 (16.7%)	2 (11.1%)	6 (19.4%)	4 (12.9%)	5 (22.7%)	25 (16.7%)
E. coli	10 (20.7%)	4 (22.2%)	6 (19.4%)	9 (29.0%)	3 (13.63%)	32 (21.3%)
S. epidemidis	3 (6.3%)	0 (0%)	3 (9.7%)	3 (9.7%)	1 (4.6%)	10 (6.7%)
pseudomunas	3 (6.3%)	2 (11.1%)	2 (6.5%)	1 (3.2%)	0 (0%)	8 (5.3%)
S. pyogeny	5 (10.3%)	1 (5.6%)	2 (6.5%)	2 (6.5%)	0 (0%)	10 (6.7%)
Citrobacter	2 (4.2%)	0 (0%)	2 (6.5%)	3 (9.6%)	3 (13.6%)	10 (6.7%)
Proteus	2 (4.2%)	1 (5.6%)	2 (6.5%)	0 (0%)	0 (0%)	5 (3.3%)
Klebsiella	4 (8.3%)	3 (16.6%)	5 (16.1%)	2 (6.5%)	1 (4.6%)	15 (10.0%)
***Bacillus* spp**	8 (16.7%)	1 (5.6%)	2 (6.5%)	5 (16.2%)	4 (18.18%)	20 (13.3%)
Enterobacter	3 (6.3%)	4 (22.2%)	1 (3.2%)	2 (6.5%)	5 (22.0%)	15 (10.0%)
Total	**48 (100%)**	**18 (100%)**	**31 (100%)**	**31 (100%)**	**22 (100%)**	**150(100%)**

P>0.05

4 CONCLUSION

A total of 150 bacteria isolates were identified in the overall results. Toilet handles in markets were the most contaminated followed by General hospitals and banks The top three dominant bacterial isolates were *E. coli S. areus* and *Bacillus*. The distribution of bacteria within and between landmarks was homogenous. This information given in this report is of great importance in public health, disease control and epidemiology,

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