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Isolation and Identification of Bacterial Contaminants from Door Handles of Public Toilets in Federal University Dutse, Jigawa State- Nigeria

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Abstract: *Public contact surfaces such as door handles frequently touched with hands can act as vehicles of diseases transmission. The aim of this study was to isolate, identify and evaluate the presence or absence of bacterial contaminants present on the door handles of public toilet in the Federal University, Dutse, Jigawa State, north-western Nigeria in order to take the necessary remedial measures. The study analyzed eighty-five (85) swab samples from public toilets door handles within the university campus. Sterile swabs were firmly pressed on the various door handles; up/down, left/right and diagonally. Samples collected were cultured and incubated at 37^0C for 24 hours. Isolation and identification of bacteria was done using standard microbiological procedures. A total of one hundred and fifteen (115) colonies were isolated in this study based on cultural, morphological and biochemical characteristics. Frequency distribution of the isolates showed that Staphylococcus aureus were 44(38.3%), Bacillus species 26(22.6%), Escherichia coli 16(13.9%), Micrococcus spp 13(11.3%), Salmonella spp 10(8.7%) and Klebsiella spp 6(5.2%). The level of contamination varies depending on the traffic exposure and the environment. This means that it is necessary to practice good personal hygiene through hand washing and use of hand sanitizer as well as daily washing and cleaning of toilets to reduce the incidence of microbial transmission.*

Keywords: *Public toilets, Door handles, Bacteria, Contamination, University.*

I. Introduction

Many factors have been shown to influence the bacterial transfers between surfaces, including the source and destination surfaces feature, bacterial species involved, moisture levels, pressure and friction between the contact surfaces and inoculums size on surfaces (Chen *et al.*, 2002; Rusin *et al.*, 2002). Studies have also shown that household surfaces can easily be contaminated with bacteria and that viruses can easily be transferred to hands and from hands to mouth (Rusin *et al.*, 2002). The risk of spreading of salmonella infection to other family members via the environment, including contaminated hands and surfaces in the toilet areas had been highlighted (Bloom field, 2000). As people come in contact with surfaces as desks, keyboards and office furniture, toilet lock handles, there is possibility of picking up microbes deposited on them. The toilet and offices lock handles are contact more frequently with their users and visitors, especially public toilets and offices. The hazards associated with toilet facilities office furniture and other formites had been established but less attention had been directed to toilets and offices lock handles as inanimate objects which could harbor and transmit infectious agents (Amala *et al.*, 2015).

Various bacteria have been isolated from public surfaces there by providing information on the relative hygiene of commonly encountered public surface, identifying the environments with contaminants and risk of exposures (Reynolds *et al*., 2005). The United State (US) center for disease control (CDC) and prevention indicate that contaminated publics surfaces most of which are of microorganisms are perhaps the most wide spread problems in contemporary world and is responsible for about one third of death world-wide through infections, with adverse effects which can reduce economic productivity (WHO, 2002). Human carriers are the main reservoir host of infections according to (Humphery, 1994). Both the feces and the urine may find their ways on the contact surfaces of the door handles via a hand touch since the hand is the major public serving as the vehicle of transmission of common human disease to a susceptible host. Thus, with respect to literature that points out dangers associated with disease transmission through formites, the present study aimed at isolating and identifying bacterial contaminants present on public toilets door handles surfaces in Federal University Dutse, Nigeria so as to determine its relative safety or otherwise to the general public constantly coming in contact with it.

II. Materials And Methods

Sampling Sites and Samples Collection

A bacteriological survey was conducted in eight (8) different sites within the university campus public toilets. Door handles of the university public toilets were swabbed from the University Clinic, University Library, University Mosque, Security Division, Physical Planning Unit, Faculty of General and Entrepreneurship Studies, Faculty of Agriculture and Male students' hostel. A total of eighty-five (85) public toilets door handles contact surfaces were swabbed. The samples were collected at noon when people made use of these toilets to maximize the chances of isolation (Amala *et al*., 2015). The swabs were moistened with 5ml of normal saline added to the swabs case and excesses were removed by pressing the swab stick against the inner side of the tube (Chesebrough, 2000). Individual moistened sterile cotton swabs were used to swab the door handles, which are hand-related public surfaces. This was accomplished in a tri-directional manner: up/down, left/right and diagonally, recapped and properly labeled. The samples were promptly transported to Microbiology laboratory of the Department of Microbiology and Biotechnology, Federal University, Dutse in an ice-cooled pack for analysis.

Sample Analysis

Each sample was inoculated onto nutrient agar (oxoid) and Mac Conkey agar (oxoid) plates. Here, using the swab stick, a primary streak was made while secondary and tertiary streaks were made from the primary streak in parallel pattern with the aid of a sterilized wire loop to make a four-way streak plate-technique. All the plates were incubated invertedly for 24 hours at 37^0C. After the overnight incubation, the plates were removed from the incubator and presumptively observed for colony characteristics. Isolated colonies were then sub-cultured onto fresh nutrient agar (oxoid), Eosin-methylene blue (oxoid) and *Salmonella-Shigella* (oxoid) agar plates for proper preliminary identification (Chesebrough, 2000). Single isolated colonies from these plates were subjected to Gram's staining, spore staining and standard biochemical tests (catalase, coagulase, IMViC- indole, methyl red, voges-proskauer and citrate utilization tests).

III. Results

The results of this study showed that out of the 85 samples analyzed, 72 (84.7%) were found to be positive. Incidence of positive and negative samples from various door handles, Physical and biochemical characteristics of isolates obtained from the various sites in the study are shown in Table 1 and Table 2 respectively.

Table 1: Incidence of Positive and Negative Samples From Various Sample Sites

Sample Sites	Total Number of Sample Examined	Number of Positive Samples (%)
University Clinic	5	3(3.53)
University Library	10	8(9.41)
University Mosque	4	4(4.70)
Security Unit	2	2(2.35)
Physical Planning Unit	4	4(4.70)
Faculty of Gen. Studies	10	6(7.05)
Faculty of Agriculture	10	7(8.23)
Male Students' Hostel	40	38(44.70)
Total	**85**	**72(84.7)**

Table 2: Cultural, Morphological and Biochemical Characteristics of the Bacterial Isolates

Cultural Characteristics	Gram's Reaction	Biochemical Tests						Suspected Isolate
		Cat	Coa	Ind	MR	VP	Cit	
Large, small golden yellow colonies on nutrient agar (NA)	+	+	+					*Staphylococcus aureus*
Opaque, dried, white colonies on NA	+	+	-					*Bacillus* species
Red, orange color colonies on N.A	+	+	-					*Micrococcus* species
Small colonies with GMS on EMB	-			+	+	-	-	*Escherichia coli*
Large mucoid pink with LF colonies on Mac	-			-	-	+	+	*Klebsiella* species
Transparent colonies with black center on SSA.	-			-	+	-	+	*Salmonella* species

Key:

Cat = Catalase	Mac = MacConkey
Coa = Coagulase	EMB = Eosin Methylen Blue
Ind = Indole test	N.A = Nutrient Agar
MR=Methyl-red test	GMS = Green Metallic Sheen
VP = Voges proskeaur	LF = Lactose Fermentation
Cit = Citrate utilization	+ = Positive
SSA= Salmonella-Shigella Agar	- = Negative

Furthermore, the distribution of isolates on positive samples from various sites, the total number of isolates on positive samples from various door handles as well as the differentiation of isolates obtained from the study by Gram's staining are shown in Tables 3, 4 as well as 5 respectively.

Table 3: Frequency Distributions of Isolates According to Sample Sites

	Sites							
Bacterial isolates	UC	UL	UM	SU	PPU	GST	AGR	MSH
Staphylococcus aureus	3	6	4	3	2	3	6	17
Bacillus species	1	1	3	2	4	2	2	12
Micrococcus species	1	2	1	0	3	2	0	4
Escherichia coli	0	0	3	1	1	0	2	9
Klebsiella species	0	0	1	1	0	0	3	1
Salmonella species	1	0	1	0	1	0	0	7

Key:

UC=University clinic	MSH=Male student hostel
UM=University mosque	AGR=Faculty of Agriculture
UL=University library	GST= Faculty of General studies
SU=Security unit	PPU=Physical planning unit

Table 4: Incidence of Isolates on Positive Samples From Various Sample Sites

Bacterial isolates	Number of isolates	Percentage (%)
Staphylococcus aureus	44	38.3
Bacillus species	26	22.6
Micrococcus species	13	11.3
Escherichia coli	16	13.9
Klebsiella species	06	5.2
Salmonella species	10	8.7
Total	**115**	**100**

Table 5: Distribution of Isolates According to Gram's Reaction

Isolates	Number of isolates	Percentage (%)
Gram positive	83	72.2
Gram Negative	32	27.8

IV. Discussion

The results obtained from this study revealed high level of bacterial contaminants on door handles which were contaminated with considerable number of pathogenic bacteria most of which are Gram-positive bacteria 83(72.0%) as shown in Table 5. This is due to the fact that most skin flora bacteria are gram positive. The bacteria may had found their way onto the surfaces through cross contamination, poor personal hygiene of the users, diseases spreading vectors such as cockroaches and flies (Hood and Zottala, 1997). Of the total samples analyzed, 72 (84.7%) out of 85 shows growth (Table 1), which is in accordance with the reports of Amala *et al*., (2015) who observed (85%) bacterial contamination in offices and toilets door knobs. This variation in the number of positive samples from one place to another is likely to be as a result of the differences in hygiene and sanitary conditions in the environment

The results of this study showed that *Staphylococcus aureus*, *Bacillus* spp, *Micrococcus* spp, *Escherichia coli*, *Salmonella* spp and *Klebisiella* spp are the main bacterial isolates frequently associated with the toilet door handles as shown in the (Table 2). These organisms may probably have their way to the door handles through the skin and hand-to-hand contacts. The most frequently isolated bacterium was *Staphylococcus*

aureus 44(38.3%) as shown in Table 4. This is because they are major components of the normal flora of the skin and nose, which probably explains its high prevalence as contaminant as it can easily be discharged by several human activities. This observation is in conformity with the finding of other researchers (Brooks *et al*., 2007; Nworie *et al.*, 2012). *Staphylococcus aureus* is the most important potential pathogen that cause boils, abscesses, wound infections toxic shock syndrome and pimples.

A high percentage of *Bacillus* spp isolated could be explained by their spore forming ability which makes them able to resist harsh environmental condition, withstand dry heat and certain chemical disinfectants for a considerable period. This is in accordance with the research carried out by Brooks *et al*., (2007) who reported that *Bacillus* spp was found to be among the predominant organism that was isolated from door handles, Samy *et al.*, (2012) also reported the isolation of *Bacillus* spp from environmental sites in Mecca city. The isolation of *Micrococcus* spp from this study was in conformity with the work of Opera *et al*., (2013) who reported the isolation of *Micrococcus* spp from public toilets.

Gram-negative rods isolated in this study indicate the possibility of the presence of fecal contamination on the door handles. This might be due to the fact that most people go to toilet and end up contaminating their hands with fecal and urinal material and fail to wash their hand because they take the issue of hygiene with levity, they also lack the concept of hand washing as a simple means of stopping this spread of infectious agents, this correspond with the work of Zhad *et al.* (1998), who reported that the high rate of isolation of these organisms is only achieved during epidemics in which human hands serve as the vehicle of transmission. Gram negative sepsis, urinary tract infections are most commonly caused by *E. coli* and *Klebsiella* spp. The significance of isolation of *Salmonella spp* from the door handles surfaces of the public toilets might be associated with the outbreak of diseases. *Salmonella* spp are the most important pathogens and one of the leading causes of gastroenteritis, typhoid fever in developing countries like ours where safe drinking water and basic sanitation is lacking with high morbidity and mortality rate. The occurrences of *Salmonella* spp on the toilets door handles was in accordance with the work of Amala *et al*. (2015) who reported the isolation of *Salmonella* spp from offices and toilets lock handles.

Male student hostel had the highest level of contamination (Table 3). This could be as a result of the number of samples examined 40(47.0%) as shown in Table 1, possible number of users, the surrounding that is unhygienic, the type of people using the toilets and also the lack of sanitary measures applied to the toilets, i.e., the number of cleaners as compared to other sites, for example the toilets located inside university clinic and university library which are in a close, healthy and clean environment. This is in accordance with the work of Boone and Gerba (2010), who also reported that the level of contamination varies depending on the traffic exposure and the environment.

V. Conclusion And Recommendations

Staphylococcus aureus, *Bacillus* spp, *Micrococcus* spp, *Escherichia coli*, *Salmonella* spp and *Klebsiella* spp were the main bacteria isolated in this research work so far. The results of the present study demonstrated that public contact surfaces such as door handles are often colonized by several bacteria and serve as a potential source of infections. Contaminated and improperly washed hands contaminate door handles, that is to say there is a high level of bacterial contamination which may lead to high level prevalence of the bacterial infectious disease due to contaminants. The isolation of pathogenic bacteria from the toilets door handles in this study indicates that they could be vehicle of disease transmission as microbial contamination of toilet door handles surfaces may be a common means of transfer of potentially pathogenic bacteria among users. On the basis of the above findings, it is therefore recommended that the university management should at least provide hand sanitizers to the users or spray disinfectants with regular cleaning of the toilets to ensure reduction in microbial load, more cleaners should be employed especially in male student hostel in considering the higher level of bacterial contamination in this site, Individuals both adult and young should adopt the habit of hand washing practice after using the toilets and routine surface disinfection of the toilets door handles, this can prevent cross contamination, Besides the encouraging for general good hygiene practice by all (staffs, students and visitors), there is a need for the university management to build more toilets so as to make it less busy, considering factors like the number of sites, the number of samples collected, conditions under which the research work was carried out, further research should be carried out particularly on the risk of fungal contamination.

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