ISOLATION AND IDENTIFICATION OF BACTERIAL CONTAMINANTS FROM DOOR HANDLES IN A TERTIARY INSTITUTION IN UMUAHIA, ABIA STATE, NIGERIA

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Abstract: Objects such as door handles, mobile phones, pens, often touched with hands can act as vectors of microbial pathogens. The aim of this study was to isolate, identify and evaluate the antibiotic sensitivity pattern of bacterial contaminants from door handles in Michael Okpara University of agriculture, Umudike.A total of one hundred door handles randomly scattered within the university campus of Michael Okpara University of Agriculture, Umudike (MOUAU) were swabbed and analyzed for bacterial contamination. Samples collected were cultured and incubated at 37°C for 24 hrs. Isolation and identification of bacterial pathogens was done by standard microbiological procedures. Antibiotic sensitivity testing was done by disc diffusion technique.A total of one hundred and thirty (130) bacteria were isolated in this study, they are; *Enterococcus feacalis* 6(4.8%), Coagulase negative *Staphylococcus(CoaNS)28(21.2%)*, *Streptococcus* spp 22(16.6%), *Klebsiella* spp 3(2.2%), *Bacillus* spp 22(16.6%), *E. coli* 4(3.0%), *Proteus mirabilis* 4(3.0%), *Proteus vulgaris* 6(4.6%), *Pseudomonasaeruginosa* 2(1.5%) and *Staphylococcus aureus* 33(25.0%). Ofloxacin, Peflacine, and Ceftriaxone were effective against the bacteria and exhibited encouraging results while Cotrimoxazole was resistant to most isolates. 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 and canteens with disinfectants.

Keywords: Door handles, Bacterial isolates, antibiotic susceptibility.

Introduction

Scientific research has shown that commonly used surfaces such as computers, headsets, telephone, desks and ATM machines are potential sources of infectious bacteria and viruses leading to the spread of colds, flu, sickness and diarrhea (Reynolds *et al.*, 2005).

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They are constantly in contact with the environment wherever they go. Germs can survive in the microscopic grooves and cracks on surfaces and will go unnoticed. Oils in the skin, dust, grime, moisture and warmth from central heating systems provide an ideal environment for these germs to accumulate. Cold and flu viruses can survive on dry surfaces for more than 48hours, while some bacteria, such as *Escherichia coli*, can survive for months. Soft, wet surfaces (particularly those with plenty of food) are perfect for

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bacteria. Cloth, sponges and carpets that have gotten wet are excellent living places for bacteria because it protects them from exposure to the environment, dry air or sunlight (Samy and Hamdy, 2002).

Bacteria that can cause severe gastroenteritis have been found on ATM machine keypads, and handles (Rusin, 2002) which demonstrate that germs that can be readily transferred from your hands to almost any frequently used surface. Other studies have implicated environmental surfaces in the transmission of bacteria (Manning *et al.*, 2001).

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 and Eliane, 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 chronic or acute illness. Human hands usually harbor microorganisms both as part of the body normal flora as well as transient microbes contracted from the environment (Dodrill *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.

The dominant resident microbes are *Staphylococcus epiderdimis* which is found on almost every hand (Larson*et al.*, 1992). It's been estimated that the population of *Staphylococcus epiderdimis* far out numbers *Staphylococcus aureus* on healthy hands. Others are members of *Corynebacteria* and *Micrococci* spp (Leyden *et al.*, 1991) and certain members of Enterobacteriaceae family. Pathogens that may be present on the hand as transient types include *Escherichia coli, Salmonella typhi, Shigella* spp *Clostridium perfringes, Giardia lamblia,* Norwalk virus and Hepatitis A virus; *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Citrobacter freundii, Enterobacter* spp; *Streptococcus* spp, *Klebsiella* spp.(Orskov *et al.*,1997).

The aim of this study was to isolate, identify and evaluate the antibiotic sensitivity pattern of bacterial contaminants from door handles in Michael Okpara University of agriculture, Umudike.

Materials and methods

A total of one hundred door handles randomly scattered within the university campus were obtained from Michael Okpara University of agriculture, Umudike in October 2014. These were processed by standard bacteriological procedures (Cheesebrough, 1993).

Sterile swab sticks (Sterilin UK) were made wet slightly with physiological saline and rubbed throughout the entire surface of the door handles. This was to ensure that microorganisms in the door handles adhere to the swab sticks appropriately. Samples collected were cultured using the streak plate method on MacConkey agar, Mannitol salt agar, and blood agar and incubated at 37°C for 24 hrs. Gram staining technique, carbohydrate fermentation tests in triple sugar iron agar and other biochemical tests such as

catalase and coagulase were used for gram positive cocci while oxidase, urease, citrate utilization, nitrate reduction, indole, methyl red and Voges-Proskauer were used for identification of gram negative bacilli.

The antibiotic susceptibility of the isolates was tested against the following antibiotics: Ofloxacin (OFL) 5µg, Pefloxacin (PEF) 5µg, Ciprofloxacin (CPL) 5µg, Amoxicillin/clavulanate (AMC) 30µg, Gentamicin (CN) 10µg, Streptomycin (STR) 10µg, Cefuroxime (CEF) 10µg, Cotrimoxazole (COT) 30µg and Ampicillin (AMP) 10µg. Antibiotic sensitivity pattern was determined by disc diffusion method (Bauer *et al.*, 1966). A colony of the test organism was picked with sterile wire loop and immersed in peptone water. The turbidity of the suspension was compared against a reference 0.5 Mcfarland tube. The suspension of the organism was streaked on the entire plate of nutrient agar and the antibiotic disc was placed on the plate using forceps. The plates were incubated at 37°C for 24hours.

Sensitivity pattern was determined by measuring the zones of inhibition with a calibrated ruler and interpreted according to standard guidelines for Clinical Laboratory standards (CLSI) criteria (CLSI, 2012).

Statistical analysis

Epi-Info software Version 6 was used for chi-squared analysis while simple percentages were used to compare rates. The level of significance for p-values was accepted at $p<0.05$.

Results

Out of a total of 100 samples collected from various door handles, 130 bacterial isolates were identified. The microorganism isolated were; Coagulase negative *Staphylococcus*(CoNS), *Streptococccus* spp, *Klebsiella* spp, *Bacillus* spp,*Staphylococcus aureus*, *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris*, *Enterococcus feacalis*, and *Pseudomonas aeruginosa*.

Table 1 shows the bacteria isolates and degree of growth from various door handles. The type of growth was indicated in this table as follows; + = scanty growth, ++ = moderate growth and +++ = profuse growth.

Table 2 shows the incidence of bacterial positive specimen from various door handles in MOUAU. Out of a total of one hundred samples which were examined, 86 showed positive results. The predominant contaminated site was the female toilets in 12/12 (100%), followed by canteens 5/5(100%), Male hostel 18/20(90%), Female hostel 17/20(85%), Library restroom 4/5(80%), Offices 16/20(80%), Classroom 3/4(75%), and Clinic 4/5 (60%).

Table 3 shows the differentiation of isolates from study by Gram staining reaction. The most frequently isolated bacteria was *Staphylococcus aureus* 33(25.0%) followed by Coagulase negative *Staphylococcus*(CoNS), 28(21.2%), others were *Streptococcus* spp 22(16.6%), *Bacillus* spp 22(16.6%), *Enterococcus feacalis* 6(4.8%), *Klebsiella* spp 3(2.2%), *E. coli* 4(3.0%), *Proteus mirabilis* 4(3.0%), *Proteus vulgaris* 6(4.6%) and *Pseudomonas aeruginosa* 2(1.5%).There were 111 Gram positive isolates and 19 Gram negative isolates. There was significant difference in observed values (P□0.01).

Table 4 shows the antibiotic susceptibility of the bacteria isolates from various door handles in MOUAU. Ofloxacin, Pefloxacin and Ceftriaxone

exhibited encouraging results. Bacterial pathogens showed the most resistance to Cotrimoxazole.

Table 1:Bacterial isolates and degree of growth from various door handles in MOUAU

SITES	Bacterial isolates/Degree of growth
Offices	Coagulase negative *Staphylococcus* (++), *Streptococcus* spp (++), *Klebsiella* spp, (+), *Bacillus* spp (++) *Staphylococcus aureus* (++).
Toilets/Bathroom	*Staphylococcusaureus* (+++), *Streptococcus* spp (+++), *Bacillus* spp (++), Coagulase negative *Staphylococcus* (++), E.*coli* (+++), *Proteus mirabilis* (+++), *Klebsiella* spp (+++).
Hostels	*Bacillus* spp (++), Coagulase negative *Staphylococcus* (++), *Escherichia* coli (+++), *Stapylococcus aureus* (+++), *Streptococcus* spp(+++), *Proteus* spp (++), *Klebsiella*spp (++) and *Pseudomonas aeruginosa* (+).
Classroom	*Streptococcus* spp(++), *Proteusvulgaris* (+).
Laboratory	E. *coli* (++), *Proteus vulgaris* (++), *Bacillus* spp (++), S. *aureus* (++), *Streptococcus* spp (++), Coagulase negative *Staphylococcus* (++)
Canteens	S. *aureus*(+++), *Streptococcus* spp(+),Coagulase negative *Staphylococcus* (+), *Proteus mirabilis*(++), *E. coli* (+++)
Clinic	*Staphylococcusaureus*(++), *Enterococcus feacalis*(++)
Banks	*Staphylococcusaureus*(+++),Coagulase negative *Staphylococcus* (+++), *Proteus mirabilis*(+++)

KEYS: + =Scanty Growth ++ = Moderate Growth +++ = Profuse Growth

Table 2: Incidence of positive specimens from various door handles

Sources	Total samples examined	No positive	% of positive samples
Offices	20	16	80.0
Female hostel	20	17	85.0
Male hostel	20	18	90.0
Female restroom	12	12	100
Classrooms	4	3	75.0
Library restroom	5	4	80.0
Laboratory	4	3	75.0
Banks	5	4	80.0
Canteens	5	5	100
Clinic	5	4	80.0
TOTAL	**100**	**86**	**86.0**

Table 3: Differentiation of isolates from study by Gram staining reaction

	Bacterial isolates	Number isolated	Percentage
Gram positive	*S. aureus*	33	25.0
	CoNS	28	21.2
	Streptococcus spp	22	16.6
	Bacillus spp	22	16.6
	Enterococcus feacalis	6	4.80
Gram negative	*Klebsiella* spp	3	2.20
	E. coli	4	3.00
	Proteus mirabilis	4	3.0
	Proteus vulgaris	6	4.6
	Pseudomonas aeruginosa	2	1.5
Total		130	100

χ^2 = 127.4 df = 1 p<0.0001 Odds Ratio (OR) = 34.13 (16.31 < OR < 72.58)

CoNS= Coagulase negative *Staphylococcus.*

Table 4: Antibiotic Susceptibility of the Bacterial isolates from various Door Handles in MOUAU

Isolates	No. tested	No.(%) of isolates sensitive to								
		OFX	PEF	CRO	AMC	CN	S	CEP	COT	AMP
Staphylococcusaureus	33	28(84.8)	26(78.7)	28(84.8)	20(60.6)	0(0)	9(27.2)	4(12.1)	0(0)	10(30.3)
CoNS	28	23(82.1)	24(85.7)	25(89.2)	18(64.2)	5(17.8)	2(7.1)	3(10.7)	6(21.4)	2(7.1)
Streptococcus spp	22	17(77.2)	12(54.5)	19(86.3)	9(40.9)	4(18.1)	6(27.2)	3(13.6)	0(0)	0(0)
Bacillus spp	22	15(68.1)	17(77.2)	20(90.9)	12(54.5)	13(59.0)	0(0.0)	4(18.1)	2(9.0)	8(36.3)
Enterobacter feacalis	6	4(66.6)	0(0)	0(0)	0(0)	2(33.3)	0(0)	0(0)	0(0)	0(0)
Klebsiella spp	3	3(100)	3(100)	3(100)	2(66.6)	3(100)	1(33.3)	3(100)	1(33.3)	2(66.6)
Escherichiacoli	4	4(100)	4(100)	4(100)	3(75.0)	2(50.0)	1(25.0)	3(75.0)	0(0)	1(25.0)
Proteus mirabilis	6	6(100)	6(100)	6(100)	2(33.3)	1(16.6)	3(50.0)	4(66.6)	0(0)	2(33.3)
Proteus vulgaris	4	4(100)	4(100)	4(100)	3(75)	1(25)	2(50.0)	3(75)	0(0)	2(50)
Pseudomonas aeruginosa	2	2(100)	2(100)	2(100)	1(50.0)	1(50.0)	1(50.0)	0(0)	0(0)	0(0)

KEY: OFX = Ofloxacin, PEF = Pefloxacin, CRO= Ceftriaxone, AMC = Amoxicillin/clavulanate, CN =Gentamicin, S= Streptomycin, CEP = Cefuroxime, COT=Cotrimoxazole, AMP = Ampicillin

CoNS= Coagulase negative *Staphylococcus*.

Discussion

Door handles are important reservoir of microorganisms. This study revealed high level of bacterial contaminants on door handles which were contaminated with considerable number of Gram positive bacteria and Gram negative bacteria. However, Gram positive bacteria were found to occur more than Gram negative bacteria. Most skin flora bacteria are Gram positive, which would account for their predominance on door handles. The study showed a statistically significant difference in this regard.

Out of 100 samples processed, 86(86%) showed bacterial contamination. This is in agreement with the reports of some researchers (Nworie *et al*., 2012) who observed 156(86.7%) bacterial contamination and slightly lower than the reports from London (Otter and French, 2009) who observed 95% positive cultures. This variation in the number of positive samples from one place to the other may not be unconnected with differences in hygiene and sanitary conditions in the environment.

In this study, the level of contamination was higher in canteens, Female restroom, Male hostel and Female hostels in that order as compared to Classrooms and Laboratory which were lower. The lower level of contamination in Laboratory and classrooms could be attributed to the fact that they are not being used as frequently as other places studied, this is in agreement with the findings of Boone and Gerba (2010) and Nworie *et al*., (2012) who reported that the levels of contamination vary depending on the traffic, exposure and environment. The high contamination of bacterial pathogens observed in female restrooms is in agreement with the reports by researchers from Abuja who attributed it to the fact that women carry a lot of artifact of beauty (hand creams, lotions, eye pencils, papers, mirrors, make ups and a lot more) in their bags and use it often each time they are in the restroom (Nworie *et al*., 2012).

In this study, the most frequently isolated bacteria pathogens was *Staphylococcus aureus* 33(25.0%) which may be due to the fact that it 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 be discharged by several human activities. This observation is in agreement with the findings of other researchers (Nworie *et al*., 2012; Ducel *et al*., 2002; Brooks *et al*., 2007).

The microorganisms isolated from toilet door handles in the stdudy were *S.aureus*, *Streptococcus* spp, *Bacillus* spp, Coagulase negative *Staphylococcus*(CoNS), *E. coli*, *Proteusmirabilis*and *Klebsiella* spp. However, the reports from Lynn *et al*., (2013) showed isolated microorganisms as; *Staphylococcus* spp, *Klebsiella* spp, *E. coli*, and *Proteus* spp but from toilet door handles at secondary schools in Bokkos L.G.A. Chris *et al*., (2002)reported the presence of the bacterial isolates such as *S. aureus*, and *E. coli* from the bathroom of students at the University of Miami USA. While Opere *et al*., (2013) also reported the isolation of *Bacillus* spp, *S. aureus, S.epidermidis, Micrococcus, Pseudomonas* and *Enterococcus feacalis* from public toilets. 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 feacal contamination of the hands as the origin.

A high percentage of *Bacillus* spp was isolated from this research and its predominance could be explained by the fact that *Bacillus* spp are ubiquitous in nature with their spores able to resist environmental changes, withstand dry heat and certain chemical disinfectants for moderate periods. This is also in agreement 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.

From the findings in this study, it was observed that most of the isolates obtained were resistant to most commonly used antibiotics such as Cotrimoxazole, Amoxicillin/clavulanate, Gentamicin, and Ampicillin which is in agreement with the research carried out by Adewoyin *et al.* (2013) who reported that antibiotic resistant microorganism contaminates environmental surfaces such as toilet and also reported that most of the isolates obtained in their study were resistant to commonly used antibiotics such as Cotrimoxazole, Amoxicillin /clavulanate and Ampicillin.

Conclusion

Individuals own hands are the lethal weapon. Contaminated and improperly washed hands contaminate door handles and it is important to note that there is a high level of bacterial contamination as well as high level of prevalence of the bacterial infectious disease due to contaminants. The isolation of pathogenic bacteria from fomites in this study indicates that they can be vehicles for disease transmission. In the light of this, there is need therefore for thorough hand washing, disinfection and conscientious contact control procedures to minimize the spread of these pathogens.

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