Isolation and Identification of Bacterial and Fungal Isolates from Door Handles in University of Benin Male and Female Post-Graduate Hostels

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Abstract: The hands are the chief organs for physical manipulation of the environment. They serve as a medium for the propagation of microorganisms from place to place and from person to person. Human hands usually harbour microorganisms both as part of the body's normal flora as well as transient microorganisms contracted from the environment. This study was aimed at isolating and identifying bacterial and fungal isolates from door handles in University of Benin male and female post-graduate hostels. Samples from door handles were subjected to standard microbiological analysis such as culturing, enumeration, Gram staining, biochemical test, lactophenol cotton blue assay and multiple antibiotic resistance tests. The bacterial count ranged from $6.94\pm2.52^{a}x10^{2}$ cfu/m^2 to $13.54\pm0.72^{a}x10^{2}$cfu/m^2 while the highest fungal count in this study was 10.35 ± 0.79^{a} x 10^{2}cfu/cm^2 the across the female and male hostels. The World Health Organization (WHO) acceptable level of fungal contamination is 10^{8}cfu/g or ml of which the amount present in the door handle surface was found to be in between the range. The results of this present study showed that *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa*, *Escherichia coli, Aspergillus niger, Mucor mucedo, Yeast* sp and *Penicillium notatum* were present on the door handle surfaces. This study also shows that there were more bacterial population in male hostels compared to the female hostels from both Ugbowo and Ekenwan campus indicating that individual door handles are not identical with respect to their design, usage patterns, surface materials, and environmental conditions (e.g. temperature, moisture, and ventilation rates) and any of these factors could influence the composition of bacterial communities found on door handle surfaces. *S. aureus* and *E. coli* demonstrated a very clear *β* hemolysis in the form of clearance zone along the streak on blood agar plate within 24 hours of incubation at room temperature. *E. coli* was also resistant to more than two (2) tested antibiotics. It is imperative that the culture of hand-washing be imbibed as this would reduce the chances of getting infections from the microorganisms a door user would be exposed to. Proper sanitation of the door surfaces should be constantly carried out and maintained.
Key word: Antibiotics resistance, door users, microorganisms, hemolysis, sanitation, WHO.

INTRODUCTION

The hands are the chief organs for physical manipulation of the environment. 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 microbes may lead to chronic or acute illness. Human hands usually harbour microorganisms both as part of the body's normal flora as well as transient microbes contracted from the environment (Burton *et al*., 2011). Door handles and surfaces are known to be a reservoir for the transmission of pathogens in the environment directly, by surface contact with the mouth or abraded skin, or indirectly by contamination of fingers and subsequent hand-to-mouth, hand-to-eye, or hand-to-nose contact (Appiah *et al*., 2025). The occurrence and spread of pathogens have also been studied to better understand the role of fomites in pathogen exposure and acquired infections (Medrano-Félix *et al*., 2011). Door handles host relatively diverse microbial communities dominated by human associated bacteria with clean linkages between environment and the communities at large. Human associated microbes are commonly found on door handles suggesting that bacteria, fungi and pathogens could readily be transmitted between individuals by the bacterial communities (Pastuszka *et al.,* 2015).

Currently, information concerning microbial contamination of door handles particularly in Nigeria, is still lacking (Appiah *et al.,* 2025). The increased availability of multiple door handles users in means that door handles are handled by numerous users on a daily basis. Given that door handles surfaces

are not routinely disinfected, the opportunity for the transmission of contaminating microorganisms is potentially great (Omoigberale *et al*., 2014). Previous studies have shown that door handles may be contaminated with different types of microorganisms including *Enterococcus feacalis*, Coagulase negative staphylococci, *Streptococcus* spp., *Klebsiella* spp., *Bacillus* spp., *Escherichia coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa* and *Staphylococcus aureus* (Nworie *et al*., 2012; Onwubiko and Chinyeaka, 2015).

Recent global analyses have reinforced these concerns, highlighting the persistent role of door handles as fomites in both public and healthcare settings. A comprehensive systematic review and meta-analysis estimated an overall microbial contamination prevalence of 9.96% on door handles worldwide, with bacterial contamination at 6.20% and viral at a higher 17.73%, underscoring the need for enhanced hygiene protocols in high-traffic areas (Appiah *et al*., 2025). Predominant bacteria identified in such studies include Bacillus spp. (55.74%), Acinetobacter baumannii (25.00%), and Staphylococcus aureus (17.54%), while viruses like rotavirus (62.50%) and even SARS-CoV-2 were noted, particularly on toilet door handles which showed elevated contamination rates of 23.04% compared to other surfaces (Appiah *et al*., 2025). As many of these organisms are disease causing organisms, the researchers concluded that these door handles play an important role in spreading many diseases. The aim of this study was to carry out the isolation and identification of bacterial and fungal isolates from door handles in University of Benin male and female post-graduate hostels

MATERIALS AND METHODS

Study Area/Population: This study was carried out in University of Benin, Benin City, Edo State, Nigeria.

Collection of samples: Door handles surfaces of Male and Female Hostels in Ekenwan and Ugbowo were swabbed with sterile, cotton-tipped applicators (swab stick) moistened with sterile peptone water. It was then introduced into a MacCartney bottle containing 5mls of sterile peptone water, shaken, and loosely capped and were taken immediately to the laboratory for further analysis. This was done for a period of four (4) weeks).

Enumeration of Microorganisms: The method described by Holt *et al.* (2000) for estimating bacterial and fungal counts was used to enumerate the total viable counts of the isolates. The discrete colonies on the Nutrient agar and Potato dextrose agar (PDA) were selected and counted. The mean colony count on the nutrient agar and PDA plates of each given dilution was used to estimate the total viable count for the samples in colony forming units per centimeter square (cfu/cm^2).

Sub-Culturing of Bacterial Isolates: A single colony of the bacteria was streaked on nutrient agar. The nutrient agar plates were incubated at 37ºC for 24 h. The isolated and purified bacterial strains was stored in agar slant at 4ºC.

Enumeration of bacterial isolates: All nutrient agar plates were incubated at 37 ºC for 48 hr. The total number of bacterial colony forming units per cubic centimetre were counted and recorded. The colonial morphology of the colonies formed was noted and colonies was sub cultured into nutrient agar plates and incubated at 37ºC for 24 h and stored for further examination.

Gram staining reaction: The Gram staining technique was carried out on the basis of the component of the cell wall. Organisms which retained the colour of the initial stain are called Gram-positive while those that do not retain the primary stain when decolorized are Gram negative.

Biochemical Test: Biochemical test such as indole, oxidase, citrate, triple sugar iron urease and catalase test was carried out.

Microscopic Identification of Fungal Isolates: Potato dextrose agar plates were incubated at a room temperature of 28±2 ºC for 3 to 5 days. The fungal colonies were enumerated after which morphological and

colonial characteristics of each colony was identified according to the manual of Pitt *et al*. (1992). Microscopic examination of the fungal hyphal characteristics was carried out. This was done by taking a tiny portion of the fungal colony using an inoculating needle and macerating it on a clean slide. Lactophenol cotton blue was then added to the microscopic slide after which a cover slip was placed on the emulsion before observing microscopically.

Determination of Susceptibility to Conventional Antibiotics: The Clinical Laboratory Institute Standard (CLIS, 2005) disk diffusion method was adopted. Five (5) colonies of the test bacteria from culture medium were inoculated into Mueller Hinton broth and incubated at 37º C for 24 hr. Using sterile syringe and needle 0.5ml of the culture was transferred on to the surfaces of Mueller Hinton agar and spread evenly by gently rotating the plates. Using sterile forceps, the antibiotic discs were placed appropriately and evenly on the inoculated plates. The plates were incubated at 37 °C overnight. The following commercially prepared antibiotic discs were used for susceptibility testing: septrin (SXT) 30µg, penicillin (PN) 30µg, amoxicillin (AM) 30µg, chloranphenicol (CH) 30µg, gentamicin (CN) 10µg, ciprofloxacin (CPX), pefloxacin (PEF) 30µg, augmentin (AU) 30µg, tarivid (OFX), streptomycin (S) 30µg and sparfloxacin (SP) 10µg.

In vitro-Pathogenicity Test

Hemolysin production: All isolates were cultured on sheep blood agar media as described by Pavlov *et al.*, 2004 and Ryan *et al*., 2014. Plates were incubated at 37 °C for 24 h and then checked for a zone of hemolysis around colonies. The results were recorded as follows: α-hemolysis (greenish zones), β-hemolysis (clear zone), or γ-hemolysis (no hemolysis).

Multiple antibiotic resistance (MAR) index: The MAR index is a good tool for health risk assessment which identifies if isolates are from a region of high or low antibiotic use. An MAR index ≥ 0.2 indicates a high-risk source of contamination.

MAR Index was calculated as follows: MAR $= \frac{a}{b}$

Where; a, number of antibiotics to which isolate is resistant; b, total number of antibiotics tested (Zhou *et al*., 1990). Values above 0.2 indicated that the source of contamination was introduced from a high-risk environment.

Statistical Analysis: The data generated were analyzed by one –way ANOVA (analysis of variance) using Genstat 12th edition analytical package as well as Non-Parametric T. test. Differences in mean were compared by Duncan's multiple range tests (Ogbeibu, 2015).

RESULTS

The total heterotrophic bacteria1 counts of door handles in Ekenwan hostel is shown in Table 1. Bacteria count from male hostel ranged from 8.03±0.24 x 10^2 - 10.53±1.57 x10^2 cfu/ml while bacterial counts in female hostel ranged from 4.31±0.90 x10^2 - 8.00±0.23 x10^2 cfu/ml. There was significant difference in bacterial count for all weeks ($p<0.05$) except for week 1 ($p>0.05$). Table 2 shows the total heterotrophic bacteria1 counts of door handles in Ugbowo hostel. Bacteria count in male hostel ranged from 6.76±0.76 x 10^2- 13.54±0.72 x10^2 cfu/ml while bacteria count in female hostel samples ranged from 6.94±2.52 x10^2 - 11.99±0.13 x10^2 cfu/ml. There was no significant difference in bacterial count for all weeks ($p>0.05$). Table 3 shows the total fungal counts of door handles in Ekenwan hostel. Fungal counts from male hostel ranged from 8.33±0.28 x 10^2 - 11.37±0.52 x10^2 sfu/ml while fungal count from female hostel samples ranged from 9.51±0.31 x 10^2 - 12.90±0.45 x10^2 sfu/ml. There was no significant difference in fungal count for all weeks ($p>0.05$). The total fungal counts of door handles in Ugbowo campus hostel is shown in Table 4. Fungal counts from male hostel ranged from 9.98±0.24 x 10^2- 12.61±0.20 x10^2 sfu/ml while fungal count from female hostel samples ranged from 9.07±0.60 x 10^2 - 14.01±0.28 x10^2 sfu/ml. There was no significant difference in fungal

count for all weeks ($p>0.05$). Table 5 represent the cultural, morphological and biochemical characterization of bacteria isolates. Bacterial isolated were two (2) Gram positive bacteria; *Bacillus subtilis* and *Staphylococcus aureus* and four (4) Gram negative bacteria; *Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli*.

In Table 6 *Staphylococcus aureus* and *Escherichia coli* showed β-hemolysin while *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Bacillus cereus* and *Proteus vulgaris* showed γ –hemolysin which means no zones of hemolysis.

Table 1: Total heterotrophic bacteria1 counts of door handles in Ekenwan hostel (x10^{2}cfu/ml)

Week	Male hostel	Female hostel	P-value
Week 1	8.03±0.24^a	5.26±0.62^a	0.07
Week 2	9.71±0.55^a	4.31±0.90^b	0.01
Week 3	10.53±1.57^a	8.00±0.23^b	0.02
Week 4	8.39±1.22^a	6.84±0.27^b	0.01

Key: Values are expressed as Mean ±Standard Error of duplicate experiments. Mean values with similar superscript within row are not significantly different from each other (p>0.05).

Table 2: Total heterotrophic bacteria1 counts of door handles in Ugbowo hostel (x10^{2}cfu/ml)

Week	**Male**	**Female**	**P value**
Week 1	13.54±0.72^a	11.99±0.13 ^{a}	0.38
Week 2	6.76±0.76 ^{a}	8.23±0.67 ^{a}	0.10
Week 3	9.72±1.20 ^{a}	10.91±0.30 ^{a}	0.93
Week 4	7.58±0.55 ^{a}	6.94±2.52 ^{a}	0.85

Key: Values are expressed as Mean ±Standard Error of duplicate experiments. Mean values with similar superscript within row are not significantly different from each other (p>0.05).

Table 3: Total fungal count of door handles in Ekenwan hostel (x10^{2}sfu/ml)

Week	**Male**	**Female**	**P value**
Week 1	11.26±0.34^a	12.21±0.11 ^{a}	0.46
Week 2	8.33±0.28 ^{a}	11.53±0.63 ^{a}	0.25
Week 3	11.37±0.52 ^{a}	9.51±0.31 ^{a}	0.14
Week 4	9.41±0.52 ^{a}	12.90±0.45 ^{a}	0.24

Key: Values are expressed as Mean ±Standard Error of duplicate experiments. Mean values with similar superscript within row are not significantly different from each other (p>0.05).

Table 4: Total fungal count of door handles in Ugbowo hostel (x10^2 sfu/ml)

Week	**Male**	**Female**	**P value**
Week 1	10.24±0.51^a	11.73±0.51 ^{a}	0.13
Week 2	12.61±0.20 ^{a}	14.01±0.28 ^{a}	0.29
Week 3	9.98±0.24 ^{a}	9.07±0.60 ^{a}	0.41
Week 4	10.67±0.66 ^{a}	12.95±0.25 ^{a}	0.16

Key: Values are expressed as Mean ±Standard Error of duplicate experiments. Mean values with similar superscript within row are not significantly different from each other (p>0.05).

Table 5: Cultural, morphological and biochemical characterization of bacterial isolates

Parameters	A	B	C	D	E	F
Cultural characteristics						
Shape	Rod	Irregular	Circular	Circular	Circular	Irregular
Colour	Milky	Milky	Milky	Yellow	Milky	Cream
Size	Large	Large	Large	Small	Entire	Medium
Elevation	Flat	Flat	Flat	Flat	Flat	Flat
Transparency	Opaque	Translucent	Opaque	Opaque	Opaque	Opaque
Morphology						
Gram stain	-	-	-	-	+	+
Cell type	Rod	Curve Rod	Cocci	Thick Rod	Short Rod	Cocci
Cell arrangement	Single	Single	Single	Pairs	In chains	Cluster
Biochemical test						
Citrate utilization	-	+	-	-	-	+
Spore forming	-	-	-	-	+	-
Catalase production	-	+	-	+	+	+
Indole	+	+	+	+	+	+
Motility	+	-	+	-	+	-
Coagulase	-	-	-	+	-	-
Oxidase	+	-	+	-	-	+
Urease	+	-	-	+	-	-
Fermentation test						
Lactose	+	+	+	+	-	-
Glucose	-	-	+	-	-	+
Galactose	+	+	-	-	+	-
Maltose	-	+	+	+	+	-
Manitol	+		+	+	+	+
Probable isolates	*Proteus vulgaris*	*Klebsiella pneumonaie*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Bacillus subtilis*	*Staphylococcus aureus*

Table 6: Cultural and morphological characteristics of the fungal isolates

Characteristics	F1	F2	F3	F4
Cultural	Milk	Dark grey	Pale orange	Black fluffy colony with reverse side yellow
Morphological				
Hyphae type	Non-septate	Non septate	Non septate	Septate
Spore formed	Sporangiospores	Conidiophore	chlamydosphores	Conidiophores
Colour of spore	milk	Dark blue	cream coloured to yellowish	Green
Probable isolate	*Aspergillus niger*	*Mucor mucedo*	Yeast sp.	*Penicillium notatum*

Table 7: Hemolytic bacterial isolates from Door handles

Bacterial isolate	Hemolysin production
Staphylococcus aureus	β
Escherichia coli	β
Klebsiella pneumoniae	γ
Pseudomonas aeruginosa	γ
Bacillus subtilis	γ
Proteus vulgaris	γ

Keys: β-hemolysis (clear zone); γ-hemolysis (no hemolysis)

Table 8: Antibiotic susceptibility profile of bacterial isolates

Gram +ve	CAZ	CN	E	CXC	CTR	AU	OFX	CIP	MARI
Bacillus subtilis	18 (S)	20(S)	18 (S)	11(R)	19(S)	19(S)	18(S)	19(S)	0.1
Staphylococcus aureus	19(S)	11(R)	13(R)	10(R)	16(I)	12(R)	21(S)	11(R)	0.6
G-ve	CAZ	CN	CPX	CRO	AU	OFX	CIP	NIF	
Proteus vulgaris	14(I)	21(S)	16(I)	19(S)	16(I)	21(S)	23(S)	18(S)	0.1
Klebsiella pneumoniae	23(S)	20(S)	15(I)	17(I)	13(R)	19(S)	21(S)	18(S)	0.1
Pseudomonas aeruginosa	19(S)	7(R)	15(I)	19(S)	19(S)	18(S)	17(I)	14(I)	0.1
Escherichia coli	18(S)	12(R)	12(R)	23(S)	19(S)	18(S)	21(S)	11(R)	0.4

Keys: PEF: CAZ = Ceftazidime, CN: Gentamycin, CPX= Ciprofloxacin, E= Erythromycin, CXC = Cloxacillin, CTR= ceftriaxone, AU = Augumentin, OFX = Ofloxacin, CIP = cefuroxime (5µg), NIF = nitrofuratoin (5µg), CRO= cefixime, S = Sensitive, R = Resistance, I = Intermediate, R = 0-13, I = 14-17, S = 18 and above, MARI = Multiple Antibiotic Resistance index, MAR index ≥ 0.2 (public health significance)

Table 9: Antibiotic susceptibility profile of multi-drug resistant bacterial isolates before curing

S/N	Gram +ve	CAZ	CN	E	CXC	CTR	AU	OFX	CIP	MARI
19m	*Staphylococcus aureus*	R	I	I	R	R	R	R	R	0.8
37F	*Staphylococcus aureus*	R	R	R	R	S	R	S	R	0.8
63F	*Staphylococcus aureus*	R	R	I	R	R	R	R	R	0.9
79F	*Staphylococcus aureus*	R	R	R	R	R	I	R	R	0.9
	G-ve	**CAZ**	**CN**	**CPX**	**CRO**	**AU**	**OFX**	**CIP**	**NIF**	
40F	*Escherichia coli*	R	R	R	R	R	R	R	S	0.9

Keys: PEF: CAZ = Ceftazidime, CN: Gentamycin, CPX= Ciprofloxacin, E= Erythromycin, CXC = Cloxacillin, CTR= ceftriaxone, AU = Augumentin, OFX = Ofloxacin, CIP = cefuroxime (5µg), NIF = nitrofuratoin (5µg), CRO= cefixime, S = Sensitive, R = Resistance, I = Intermediate, R = 0-13, I = 14-17, S = 18 and above, MARI = Multiple Antibiotic Resistance index, MAR index ≥ 0.2 (public health significance)

DISCUSSION

The contamination level of door handle surfaces observed in this study indicates the level of hygienic practices in the hostels. The results of this present study showed that *Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *Escherichia coli* were present the door handle surfaces. This result is similar to the findings of Odigie *et al*. (2017) who isolated *Escherichia coli, Citrobacter* sp*, Enterobacter* sp., *Klebsiella* sp., *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus subtilis* from door handle surfaces. They concluded that door handle surfaces can be contaminated by microorganisms through body contacts, raw food items brought into the door handle, through aerosol and contaminated water. This finding corroborates earlier report of Boyce (2007) that surfaces can act as reservoirs of microbes which could in turn lead to the spread of infection upon being touched, by either healthcare workers, patients or visitors.

The presence of *Escherichia coli* in this study may be due to closeness of some of these hostels to wastewater disposal channels, refuse dump sites and toilet. This agrees with the report of Russell and Jarvis (2001), stating that *E. coli* is expelled into the faecal matter. The bacterium grows massively in fresh faecal matter under aerobic conditions for 3 days, but its numbers decline slowly afterwards (Russell and Jarvis, 2001). *E. coli* has also been reported to be harmless and part of normal flora but can cause serious food poisoning in their hosts, and is occasionally responsible for product recalls due to food contamination (Vogt and Dippold, 2005).

Faecal–oral transmission is the major route through which pathogenic strains of the bacterium cause disease. Cells are able to survive outside the body for a limited amount of time, which makes them potential indicator organisms to test environmental samples for fecal contamination (Uraih, 2004).

The presence of *Pseudomonas aeruginosa* in this study is in agreement with the studies of Bashir *et al*. (2016) who reported 46.1% cases of *Pseudomonas aeruginosa* from home door handle equipment. Ekhaise *et al*. (2010) also in their work have reported the role played by hospital surfaces in the transmission of emerging healthcare-associated pathogens. Crowded conditions within the door handle, frequent transfer of door handle materials from one unit to another may contribute to proliferation of microorganisms in door handle surfaces. Microbial flora may contaminate surfaces of objects, devices and materials which subsequently contact susceptible body sites (Kramer *et al*., 2006).

Escherichia coli was also resistant to more than two (2) tested antibiotics. The resistance of the bacterial isolates to the tested antibiotics in this study is of public health significance. This result is in agreement with the studies of Mwajuma (2010) who reported that *Escherichia coli, K. pneumoniae* and *Bacillus subtilis* were resistant to cefixime, augumentin, ampiclox, erythromycin and zinnacef. This finding was supported by Mbim *et al*. (2016), Jaran (2015) and Tagoe *et al*. (2011) who reported the existence of multidrug resistant *E. coli, S. aureus* and *B. subtilis*. This corroborates the findings of Igbinosa *et al*. (2014) who also noted that multidrug resistant phenotypes have been spread widely among some Gram-negative and positive bacteria.

In general, the development of drug resistant bacterial isolates can be linked to various aspects including the practice of indiscriminate use of antibiotics and due to the selective pressure to extensive use of antibiotics in the food industry (Ayandiran *et al*., 2014; Mohammed *et al*., 2014).

Upon curing, *Escherichia coli* isolates became sensitive while *Staphylococcus aureus* was resistant to some antibiotics revealing that their resistance could be plasmid borne. Plasmids are extrachromosomal DNA molecules which could exist independently of the host. They may carry genes responsible for resistance to antibiotics. The gel electrophoresis shows that *Staphylococcus aureus* and *Escherichia coli* had single plasmid bands (Opera and Ojo, 2013).

CONCLUSION

The result of this study indicated that door handle surfaces are contaminated by microorganisms. This suggests that contaminated environmental surfaces are reservoirs of potential pathogens. The chances of contracting infections from door handle surfaces due to touch of surfaces remains high, if necessary, hygiene steps are not taken. It is imperative that the culture of hand-washing be imbibed as this would reduce the chances of getting infections from the microorganisms a door user would be exposed to. Proper sanitation of the door surfaces should be constantly carried out and maintained. Proper disinfection of the environment from time to time will reduce the carriage rate of pathogens of doors. To reduce the incidence of drug resistance, the indiscriminate use of antibiotics by individuals whose health are impaired should be discouraged.

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