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Bacterial Contamination of Selected Public Toilet Door Handles within Adekunle Ajasin University Campus, Akungba-Akoko, Ondo State, Nigeria

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Abstract

The bacterial contamination of selected public Toilet door-handles within Adekunle Ajasin University campus, Akungba-Akoko, Ondo State, Nigeria were evaluated. A total of 200 toilet door handles were swabbed and cultured for bacteria isolation. The samples were cultured on MacConkey agar and Blood agar Figures, after which the isolates were identified with API-20E test kit. The following bacteria were isolated *Escherichia coli* (21.1%), Coagulase negative Staphylococcus (21.1%), *Pseudomonas aeruginosa* (15.8%), *Staphylococcus aureus* (10.5%), *Proteus mirabilis* (10.5%), *Salmonella sp.* (10.5%), *Klebsiella sp.* (5.3%), *Shigella dysentariae* (5.3%). All the bacteria isolated were susceptible to Gentamycin and Erythromycin. Only *Pseudomonas aureginosa* and *Staphylococcus aureus* were susceptible to Amoxicilin. The result of this study indicates that there are contaminations of bacterial origin on public toilets door-handles within Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria which requires urgent and proper intervention.

Key words: Toilet handles; Adekunle Ajasin University; *Escherichia coli*; environment contamination.

1. Introduction

Bacteria are microscopic organisms found everywhere in the Universe,that is, in the environment we stay and in the human body. They could either be pathogenic or non-pathogenic. They are found in the environment and within each one of us, there are trillions and trillions of them.

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Majority of them are harmless (non-pathogenic) to human and animals but those few that are harmful (pathogenic) can cause the death of affected individuals [1; 2]. Bacteria were among the first living organisms created and found everywhere on earth and probably constitute the largest of the earth's biomass as asserted by Prescott and his colleagues [3] Microbes can be found from the depth of earth's crust, on the polar ice and oceans and the bodies of plants and animals. Being mostly invisible, the actions of microorganisms are usually not as obvious or familiar as those of larger plants and animals [4].

Public toilets may contain a variety of pathogenic bacteria, mostly of the genus *Escherichia, Salmonella,* and *Staphylococcus* including Methicillin-resistant *Staphylococcus aureus* (MRSA) and *Streptococcus.* They get in the public toilets via human wastes which are mostly urine and faces [5; 6].

The hazards associated with public toilet facilities had been established but, less attention had been directed to door-handle/knobs of the public toilets as inanimate objects which could harbour and transmit infectious agents. As people come in contact with surfaces such as door-handles, there is possibility of picking up bacteria cells deposited on them. The door-handles of public toilets are made contact with more frequently by their users and visitors. Since human hands usually harbour microorganisms as normal flora and transient microbes acquired from the environment, it could be conceivable that the transfer of pathogens could occur among people who handle the same objects [7; 8]. The chance that another may acquire the organisms is dependent on how long the bacteria can survive in the environment. Although numerous studies have been carried out on the survival of bacteria on the surfaces of stainless steel subsequent to contamination [9; 8], most of these studies reported that they are relatively non-toxic to bacteria and they concluded by stressing the potential role of stainless steel as a fomite for human diseases [10; 8]. The chain of infection is completed when uninfected persons touch the mucus contaminated surfaces and contaminate their hands. The individual then contaminates self by touching his/ her nose, eye, edibles etc. [11; 8].

Faecal matter remains a major reservoir and source of human pathogens, which in adverse situation may bring about outbreaks of infection, example shigellosis. The occurrence of this may be attributed to the unhygienic use of the public toilet facilities, which can results in the gross contamination of the toilet door-handles, which individuals are less likely to see as contaminated [4]. Other possible organism laden-formites showers, toilet seats and faucets, sinks, lockers, chairs, tables, especially those found in public offices, hospitals, hotels, restaurants and restrooms [12]. However, the most implicated probable sources of infections is door handles of toilets [13].

The status of bacteria load in the public toilets of Adekunle Ajasin University, Akungba-Akoko has not been established. The number of public toilets in the University community is very few compared to the number of staff and students using the toilets. This may likely increase the frequency of the toilets usage.

This study aimed at investigating the bacteria contamination of door handle/knobs in selected public toilets within Adekunle Ajasin University campus, Akungba-Akoko, Ondo State, Nigeria, determining their percentage of occurrence and the susceptibility of the isolates to common antimicrobial agents.

2. Materials and Methods

Study Area

The study was carried out at selected public toilets in Adekunle Ajasin University, Akungba-Akoko. The university community has close to 10,000 students in population. The entire University community is divided into four, for the purpose of this study as indicated on the map.

Figure 1: Map of the Temporary site of Adekunle Ajasin University, Akungba Akoko Ondo State, Nigeria

Source: AAUA Physical Planning Unit.

KEY: Area A = Zenith hostel area

Area B = Education and Social & Management Science Lecture Theater area

Area C = E.T.F. 750 Lecture theatre area

Area D = Pioneer hostel area

Sample Collection

Samples were collected from the toilet handles using the swab-rinse method of the American Public Health Association as described by Reynolds and his colleagues [13]. Samples were collected at noon when reasonable number of people would have made use of these facilities to maximize the chances of isolation. Door handles 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.

The swab containing MacCartney bottles were transported to the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko and incubated at 37^0C for 24hrs. The rinsed fluids were subcultured on MacConkey agar and Blood agar and incubated for 24hours at 37^0C. Bacterial isolates were first differentiated by macroscopic examination of the colony based on size, colour, pigmentation, elevation surface texture, and margin, haemolysis on blood agar and lactose fermentation on MacConkey agar [14]. The API-20E test kit (from bioMerieux, Inc.) was used to identify the enteric gram negative rods

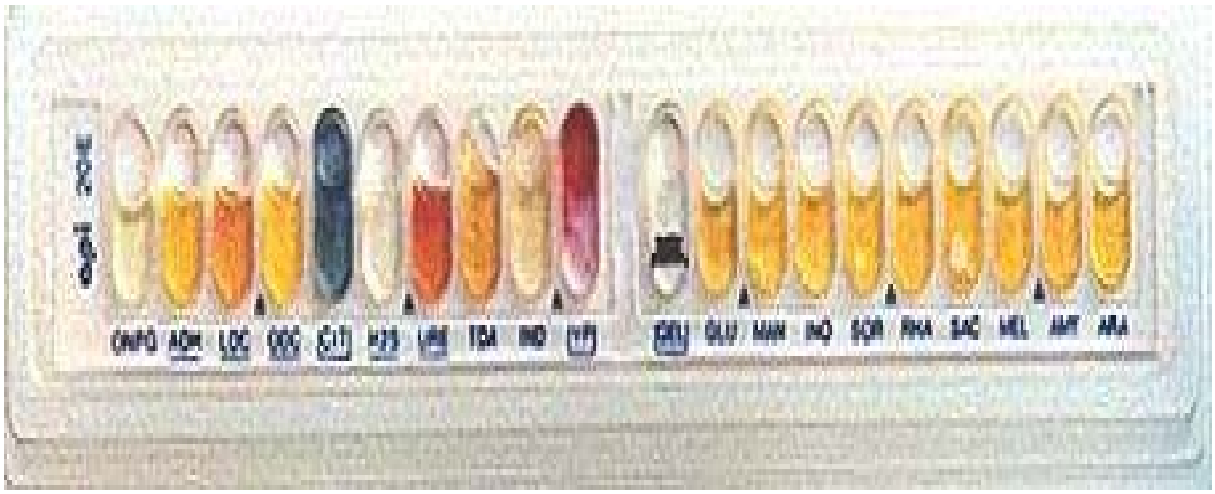

Figure 2: API-test strip for the identification of enteric pathogen.

Antibiotics susceptibility testing of the bacteria isolates

This test was performed to determine the phenotypic resistance traits of the bacteria isolated to the commonly used antibiotics. The test was carried out following the modified Kirby-Bauer-NCCLS disk diffusion method [15]. Inocula were prepared from the isolates and were standardized before use.

A sterile swab stick was dipped into the standardized inoculum and pressed firmly against a wall of the test tube to remove excess inoculum and it was used to streak the entire dried surface of sterile Mueller Hinton agar asceptically. The streaking was repeated two times by rotating the Figures appropriately 60^o each time to ensure that every part of the Figure was inoculated.

The Figures were then allowed to stay for 15minutes so as to allow excess surface moisture to be absorbed before applying the antibiotics impregnated discs. Predetermined commercial Gram-negative and Gram-positive

discs were applied to the surface of the inoculated agar Figures asceptically using a pair of sterile forceps.

These discs were placed firmly by slightly pressing on the inoculated agar with the sterile forceps to ensure complete contact with the agar. The Figures were inverted and incubated for 18 hours at 37^{o}C 15 minutes after the disc were applied. After 18hours of incubation, each Figure was examined, the susceptibility of each isolate to each antibiotic was determined by clear zone (growth inhibition) around each disc.

The diameter of the zone of inhibition was measured using a calibrated ruler which was held on the back of the inverted Petri dishes and recorded accordingly. The zone of margin was taken as showing no obvious visible growth that can be detected with the unaided eye. The isolates were then recorded as either sensitive to the antibiotics or resistant depending on the zone of inhibition in comparison with manufacturer's standard.

3. Results

The following bacteria species were identified as contaminants on the selected public toilets door-handles evaluated. *Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Salmonella sp,* Coagulase negative Staphylococcus (CoNS), *Shigella dysenteriae, Proteus mirabilis, Klebsiella sp.* as shown in Figure 1.

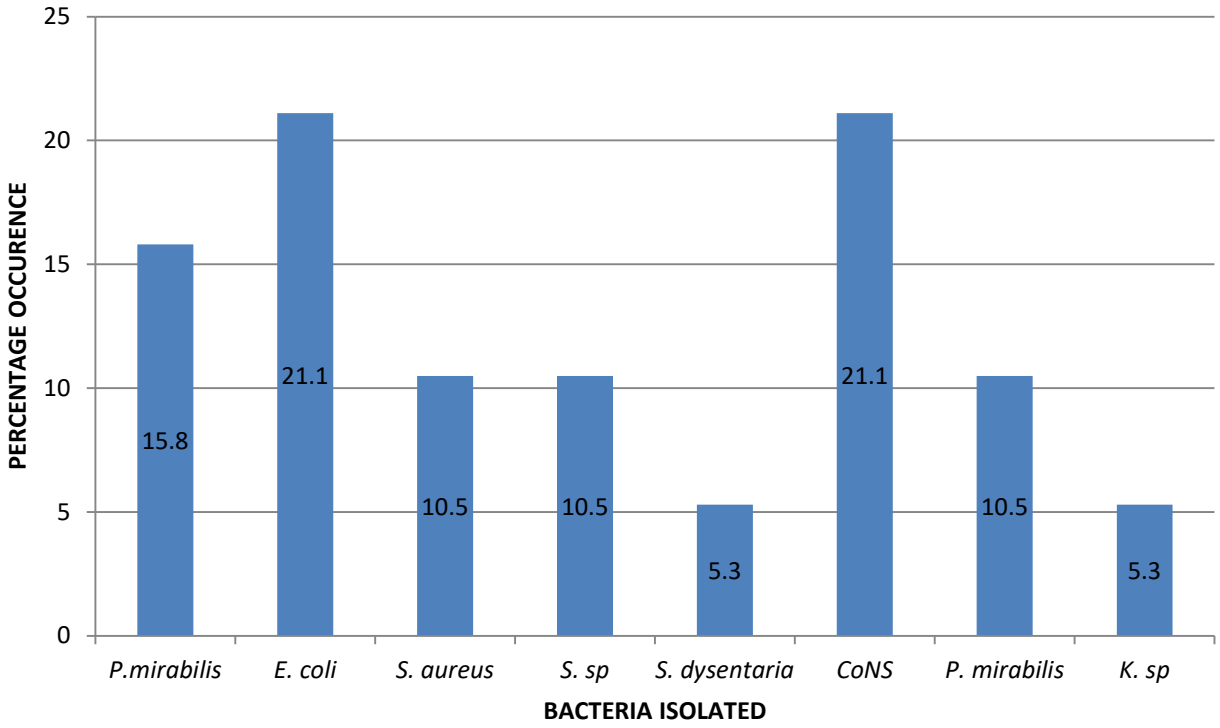

Figure 3: Number of bacteria isolated and percentage occurrence.

Escherichia coli (21.1%) and CoNS (21.1%) are more prevalent than the other isolates while *Klebsiella* sp and *Shigella dysentariae* were the least isolated as shown in Figure 3

Table 1: Susceptibility testing of bacterial isolates against antibiotics (percentage in bracket)

Isolates	**Number isolated (%)**	**GEN**	**SXT**	**E**	**CPX**	**OFL**	**AUG**	**AMX**	**COT**	**NIT**
Shigella dysenteriae	1(5.3)	1(100)	R	1(100)	1(100)	R	R	R	1(100)	R
Salmonella sp.	2(10.5)	1(50)	R	2(100)	1(50)	2(100)	1(50)	R	R	R
Pseudomonas aeruginosa	3(15.8)	2(66.7)	1(33.3)	3(100)	2(66.7)	3(100)	2(66.7)	2(66.7)	R	R
Proteus mirabilis	2(10.5)	2(100)	1(50)	1(50)	R	2(100)	1(50)	R	1(50)	2(100)
Escherichia coli	4(21.05)	4(100)	2(50)	2(50)	2(50)	3(75)	R	R	1(25)	3(75)
Staphylococcus aureus	2(10.5)	2(100)	1(50)	1(50)	1(50)	1(50)	2(100)	2(100)	2(100)	R
Klebsiella sp.	1(5.3)	1(100)	R	1(100)	1(100)	1(100)	1(100)	R	1(100)	1(100)
Coagulase – ve Staph.	4(21.05)	3(75)	2(50)	R	R	4(100)	R	R	R	1(25)
Total	19(100)	16(84.2)	7(36.8)	11(57.9)	8(42.1)	16(84.2)	7(36.8)	4(21.1)	6(31.6)	7(36.8)

KEY FOR TABLE 1:

GEN = Gentamycin, SXT = Septrin, E = Erythromycin, CPX = Ciprofloxacin, OFL = Ofloxacin, AUG = Augmentin, AMX = Amoxicillin, COT = Cotrimoxazole, NIT = Nitrofurantoin, R = Resistant.

Table 1 presented the sensitivity test of bacterial isolates against antibiotics and their percentages. Out of the 8 bacteria isolated, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were susceptible to Amoxicilin, all bacteria isolated are susceptible to Gentamycin and Ofloxacin as evident by their reported percentage

KEY FOR TABLE 2:

Area A = Zenith hostel area, Area B = Education and Social & Management Science Lecture Theater area

Area C = E.T.F. 750 Lecture theatre area, Area D = Pioneer hostel area

Table 2 contains the area distribution of the public toilets within the University. 129 (64.5%) out of the 200 of the public toilet door handles were contaminated. Eight (8) different bacteria species were isolated while the total organisms isolated was nineteen (19).

Table 2: Area distribution of the public toilets and Percentage number of contaminated handles

Area	No of handles	No of contaminated handles	Percentage no of contaminated handles (%)	No of different organism isolated
A	60	35	27.1	5
B	30	24	18.6	6
C	50	30	23.2	4
D	60	40	31.0	4
Total	200	129	100	19

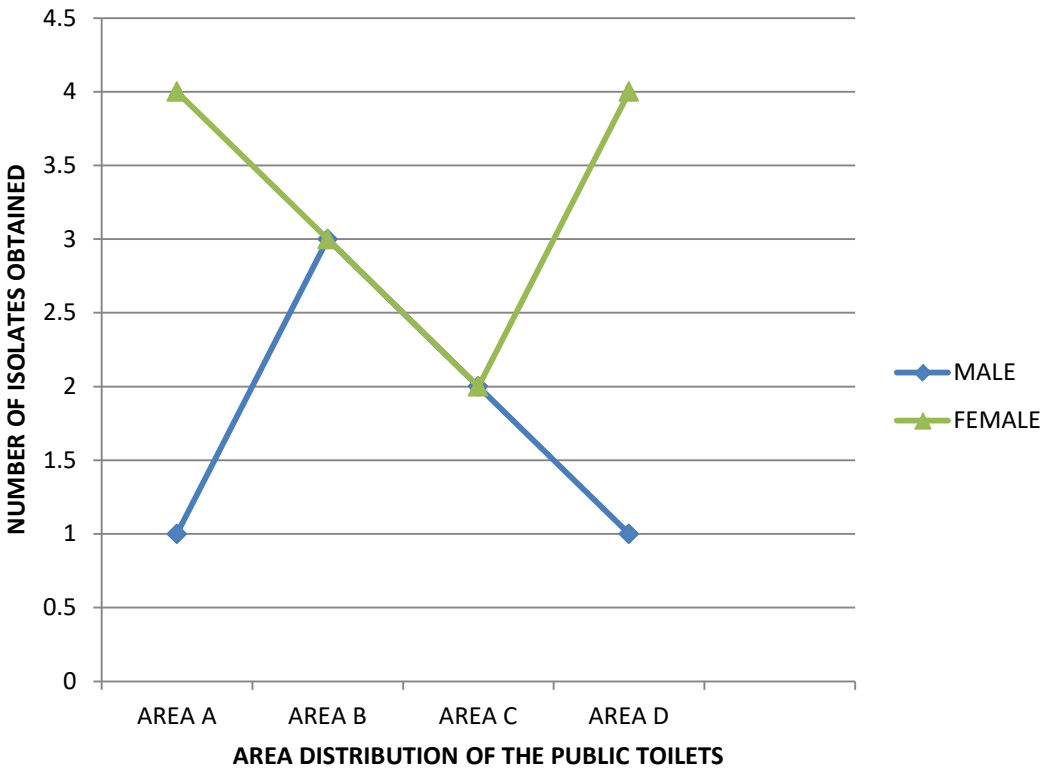

Figure 4: Gender distribution of the toilets in relation to the number of isolates.

Figure 4 shows the number of bacteria isolated from both male and female toilets in the various areas sampled. A total of 7 isolates were gotten from the male toilets while 12 isolates were gotten from the female toilets in the four areas sampled.

4. Discussion

The results presented in this study show a 64.5% prevalence of bacterial contamination of public toilets' door handles within Adekunle Ajasin University, Akungba-Akoko, Ondo State, Nigeria. Fresh sources of contamination could be possible with every toilet use, cleaners regularly trampling on and off the vehicles would themselves enable a steady microbial load of bacteria to be carried on the handles. This heavy load of bacteria on toilet handles might be associated with poor personal hygiene of some individuals, poor level of sanitation of the toilet facilities and low level of socio-economic status prevalent in the school society, which was equally noted by Wachukwu and his colleagues [16].

On a very good toilet door, there is a point at which the user opens it (i.e. door-handle) but some might have broken, thus leaving the user at the mercy of using some specific sites. These specific sites can become automatically a reservoir of infection when contaminated since the toilet user must have to touch the point both at entrance and exit from the toilet. Hence, one may pick the pathogen through which he may get him/herself infected or transfer it to another [4].

The microorganisms isolated from the toilet door-handles at the various selected public toilets door handles in their decreasing order of frequency are:- *Escherichia coli* (21.1%), CoNS (21.1%), *Pseudomonas aeruginosa* (15.8%), *Salmonella sp.* (10.5%), *Proteus mirabilis* (10.5%), *Staphylococcus aureus* (10.5%), *Klebsiella sp*.(5.3%) and *Shigella dysenteriae* (5.3%). The generally low recovery of bacteria in this study was expected and greatly in opposition to similar study done by Hammuel and his colleagues [17] in a hospital environment with high recovery of bacteria from toilet door handles. This result is in-line with the study carried out on the prevalence of bacterial organisms on toilet door handles in Secondary Schools in Bokkos L. G. A., Jos, Figureau Sate, Nigeria by Lynn and his colleagues [4], in which *Staphylococcus sp, Klebsiella species, Escherichia coli, Candida sp, Proteus sp, Salmonella sp.* were isolated and also the study carried out by Amala and his colleagues [8]. The difference in the result obtained by Amala and his colleagues [8] and this study is the isolation of *Enterococcus faecalis*, in their study but not in the current work. This might be due to the diversity in the source of the samples collected in their research which is absent in this study also the hygiene consciousness of the population using the toilet facilities may be a contributing factor. *E. faecalis* is an indicator organism for faecal contamination especially when isolated in water. These organisms, though are community acquired could become nosocomial, when carriers visit hospitals and drop them behind for patients to be infected by them [18; 19]

Foods commonly eaten by staff and students and commonly used to entertain visitors on campus are mostly "food on the run" (ready to eat foods) such as snacks. They are usually eaten with unwashed hands which might aid the transfer of enteric pathogens and thereby causing infection. It had been noted from other studies that infection outbreaks involving bacteria had surfaces as the source or means of transmission [20; 8]. Though, large infective doses are required of most pathogenic bacteria before a disease condition can be established, but for some bacteria, for example *Escherichia coli* 0517, it was estimated that at 100 to 500 colony forming units (cfu), can initiate a disease condition depending on the immunological status of the host [21]. Sanitary conditions in public places have always been a major problem, especially in the toilet since some organisms are

able to survive for weeks and months in dry areas, a very good example is the *Staphylococcus* species [20; 4]. The prevalence of *S. aureus* (10.5%) from the study was far less than 38% reported by Carvalho and his colleagues [22] and 53.5% by Nworie and his colleagues [23]. The hygiene of users and frequency of use due to population might be responsible for this sharp difference. Many students are weary of using public toilet and thus reduce the number of visits. They equally tend to be more conscious of their health and thus embrace good hygiene practices. *Pseudomonas aeruginosa* are known to be able to survive harsh environmental conditions and are found on environmental surfaces. A prevalence of 15.8% was recorded in this study as against 9.6% reported by Pal and his colleagues [24] in a similar study. *P. aeruginosa* has been isolated from wound infection and urinary tract infections. *Salmonella* sp and *Shigella dysentariae* though isolated at very low prevalence (10.5% and 5.3%) still call for serious concern for adequate hygiene measures to be put in place. These are enteric organisms that have been known to cause fatal illnesses such as Shigellosis and Typhoid fever. *Escherichia coli* is a major pathogen for Urinary tract infections [25]. It's isolation at a prevalence of 21.05% should be of health concern, though similar to the reported 20.6% by Hammuel and his colleagues [17] This becomes more serious among Undergraduates who with their age range are more sexually active, and an infection with one can easily and readily spread among them.

The general susceptibility of the isolates to Amoxicillin was poor (21.1%) except for *S. aureus* and *P. aeruginosa*, all other bacteria isolates were resistant. This is one of the commonly abused antibiotics which comes in capsular form and easy to use. The use and misuse of this antibiotic might be responsible for its poor performance against most of the bacteria. However, the issue of sub-standard products cannot be hurriedly overlooked, even when the drugs are genuinely prescribed by qualified clinician. Responses of the isolates to Ofloxacin (84.2%) and Gentamycin (84.2%) give a measure of hope in the treatment of infections caused by these pathogens in the locality. Gentamycin, being an injectable antibiotic may not readily be abused. *Shigella dysentariae* and CoNS were resistant to five different antibiotics from this study. This is a major concern with the global trend of increase in the discovery of multiple antibiotic resistant strains of both community and health care associated. Their isolation and multidrug resistant nature agrees with the study of Opere and his colleagues [26] and DubMandal, [27].

5. Conclusion

Bacterial isolated from the surface of the public toilets' door handles can be a threat to the user's health since they are known opportunistic pathogens. It is therefore recommended that public toilet door handles within the University environment should be cleansed and disinfected at frequent intervals. Regular water supply is germane to achieving proper usage and cleaning of the toilets so as to reduce soiling the door handles. Both the users and the cleaners should be enlightened about the presence and implication of been infected with these microscopic living cells.

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