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Public Toilets in a tertiary institution in the Southern part of Nigeria as Potential Reservoirs of Drug Resistant Pathogens

***Otokunefor, K., Chijioke, D. C., Kalio, J. A. and Abu, G. O.**

Department of Microbiology, Faculty of Science, University of Port Harcourt. PMB 5323 Port Harcourt, Nigeria.

Abstract

Toilets have long been viewed as a significant potential contributor to human infectious diseases. Various studies worldwide have explored the bacterial communities associated with toilets but only few have focused on their possible role as reservoirs of drug resistant pathogens. To explore this role, four different surfaces from a pay-to-use toilet complex at a tertiary institution in the Southern part of Nigeria were sampled using the swab-rinse technique. Sample processing was done to determine bacterial load, identify bacterial types present in the samples and determine antibiotic susceptibility using standard techniques. Similar levels of bacterial contamination were observed at all the 14 sampling points ranging from 3.6×10^4 to 2.7×10^5 CFU. A higher level of contamination was generally noted on the door handles and floor surfaces. Of the ten different bacterial groups identified, *Shigella* sp. and *Salmonella* sp. were the predominant groups (20.6% each). The test isolates showed a wide rate of resistance to antibiotics, with the highest observed against ofloxacin (98.3%) and the least against ceftriaxone (44.4%). Forty-three different antibiogram patterns were detected among the test isolates. Most of the bacteria (63.2%) were associated with MAR index values greater than 0.8. This study shows that public toilets could play a role not just as a reservoir of potential pathogens but more specifically as a potential reservoir of drug resistant pathogenic microorganisms with high MAR indices.

Keywords: Toilet, Reservoir, MAR index, Nigeria
*Corresponding Author: kome.otokunefor@uniport.edu.ng +234-8051844470

Introduction

Toilets have long been viewed as a significant potential contributor to human infectious diseases, with the transmission of many diarrhoeal diseases thought to be associated with toilets (Mkrtchyan *et al.,* 2013; Kaewla and Wiwanikit, 2014; Johnson *et al.,* 2017). A large number of people worldwide rely on shared or public toilet facilities. These play a potentially crucial role in public health due to the higher number of users than private facilities, as well as often reduced levels of hygiene (Gerhardts *et al.,* 2012; McGinnis *et al.,* 2019). Transmission studies have clearly demonstrated the ability of specific bacteria to spread from toilet cisterns to surfaces in the toilets (Barker and Bloomfield 2000, Barker and Jones 2005). Additionally, various studies worldwide have explored the bacterial communities associated with toilets. These studies found a wide variety of bacterial types associated with different toilet surfaces, mostly potentially pathogenic species (Mkrtchyan *et al.,* 2013; Adewoyin *et al.,* 2013; Chengula *et al.,* 2014). A large scale study assaying 56 public restrooms for the presence of extra-intestinal pathogenic and drug resistant strains of *Escherichia coli* described sporadic contamination by these organisms (Mohamed *et al.,* 2015). Though the different studies often varied in design, bacterial load ranging from 10^3 to 10^7 CFU have been reported. A recent study (McGinnis *et al.,* 2019) noted a significant difference in the levels of bacterial load between community and household toilet facilities with higher levels observed in the community facilities. Majority of studies simply involved

isolation and identification of organisms, with extensive susceptibility studies carried out only in a few cases (Ogba and Obio, 2018). Furthermore, despite the currently emerging drug resistance pandemic, only a few studies have focused on the possible roles toilets play in this global scourge. This study therefore sets out to evaluate the possible role of public toilets as reservoirs for a wide variety of drug resistant pathogens.

Materials and Methods

Sample Collection

Samples were collected from a pay-to-use toilet complex at a tertiary institution in Southern Nigeria, using the swab-rinse technique. Briefly, sterile swab sticks pre-moistened with normal saline were used to swab several surfaces in the toilets, and then pre-incubated for 30 s in normal saline. A total of four surfaces per toilet were assayed, namely toilet seat, sink, floor and door handles.

Sample Processing

Following collection, sample processing was carried out to determine bacterial load and identify bacterial types present in the samples. To achieve this, 10-fold serial dilutions were first carried out and diluents cultured in duplicates on plate count agar (PCA). Additionally, samples were cultured onto Salmonella-Shigella agar, MacConkey agar, thiosulfate citrate bile salt agar and blood agar. Following a 24-h incubation at 37ºC, bacterial loads were determined and distinct colonies sub-cultured and purified for further identification using standard biochemical methodologies (Cowan and Steel, 1985; Cheesbrough 2006).

Susceptibility Testing

Antibiotic susceptibility testing was carried out on the isolates using the Kirby Bauer disc diffusion technique (Bauer et al., 1966). In brief, a suspension of test isolate corresponding to 0.5 McFarland standard was inoculated onto a Mueller Hinton agar plate using a sterile swab stick. Following a 5 min pre-incubation, the appropriate commercial multi disc was applied to the plate and the set- up incubated at 37ºC for 24 h. Organisms were then classed as resistant or sensitive based on the diameters of the zones of inhibition using the CLSI standards (NCCLS, 2000).

Results

Bacterial Load

Similar levels of bacterial contamination were observed at all the 14 sampling points (Figure 1) ranging from 3.6×10^4 to 2.7×10^5 CFU/cm^3 (4.56 – 5.43 Log_{10} CFU). A higher level of contamination was generally noted on the door handles and floor surfaces, with the least level of contamination observed on the toilet seat of toilet B.

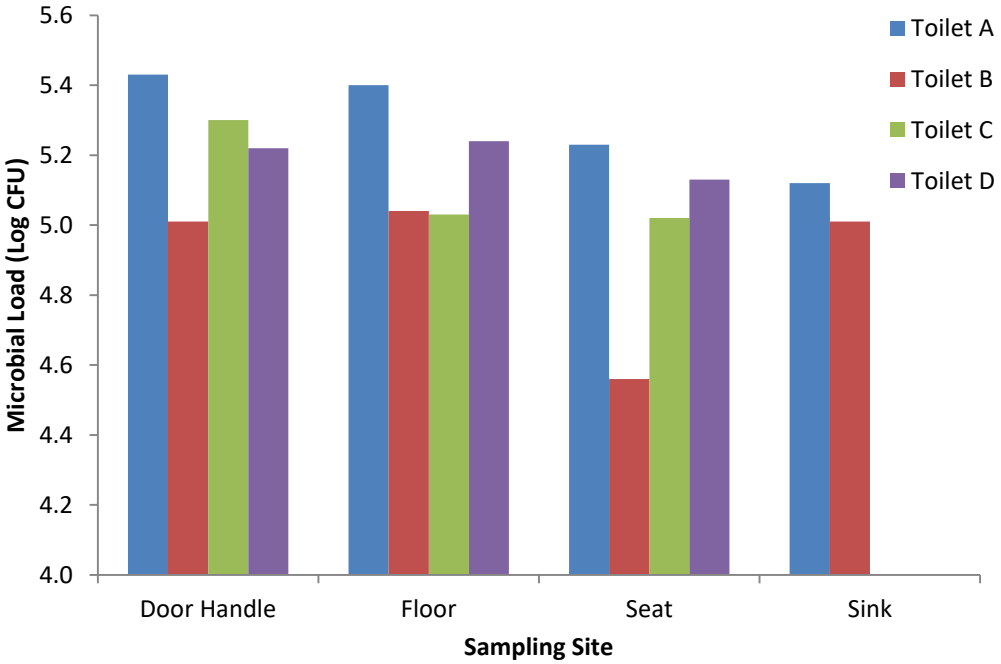

Figure 1: Variations in bacterial load of different sampling points in public toilets in a tertiary institution in the Southern part of Nigeria

Bacterial Identification and Distribution

Of the 68 non-repeat bacteria isolated from the various sampling points, a total of 10 different bacterial groups were identified (Figure 2). Members of the *Shigella* sp. and *Salmonella* sp. were the predominant groups isolated (20.6% each) while members of the *Enterobacter* sp. were the least predominant group (1.5%). Majority of the isolates (86.6%) were Gram negative, while only 1.3% were Gram positive.

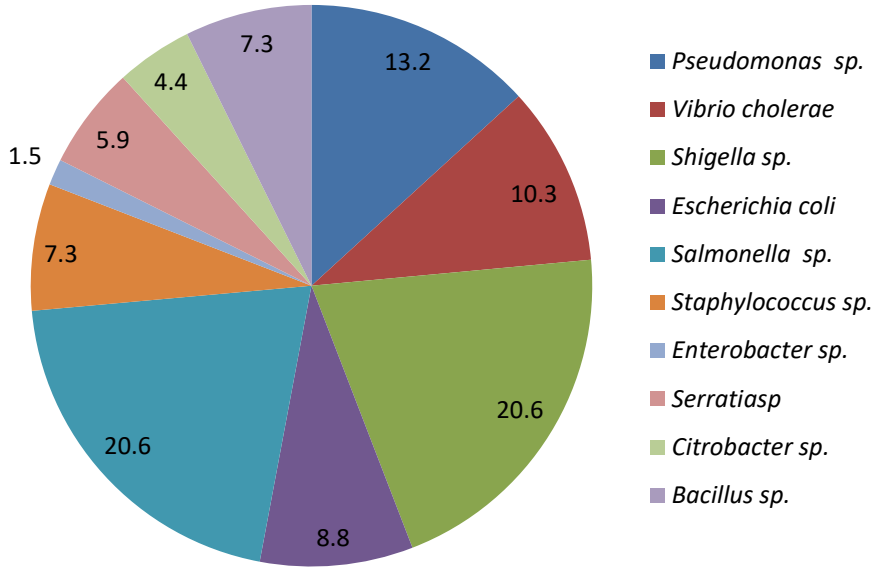

Figure 2: Percentage occurrence of the isolated bacteria from toilet surfaces in a tertiary institution in the Southern part of Nigeria

Antibiotic Susceptibility

Antibiotic susceptibility testing of the isolates revealed a wide rate of resistance ranging from 44.4% to 98.3%. The highest resistance was noted against ofloxacin while the lowest was noted against ceftriaxone. High rates of resistance by all bacteria (> 60%) were noted against 13 out of the 14 antibiotics tested. The Gram negative bacteria however contributed more to these high levels of resistance than the Gram positive bacteria (Figure 3).

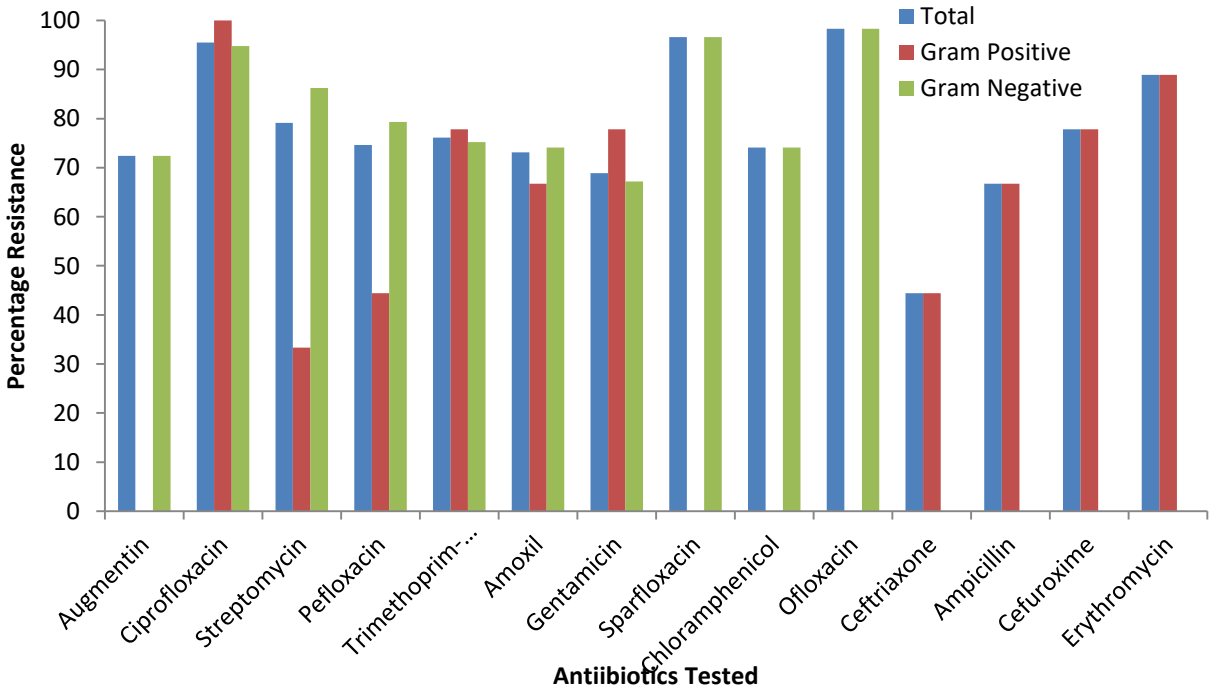

Figure 3: Frequency of antibiotic resistance of bacteria isolated from public toilets in a tertiary institution in Southern Nigeria

Antibiogram and Phenotypic diversity of the Isolates

An assessment of the antibiogram patterns of individual organisms revealed 43 different patterns associated with the 68 organisms. AMX-AUG-CHL-CIP-GEN-OFL-PEF-SPX-STR-SXT was the most commonly occurring antibiogram, exhibited by 22.1% of the isolates (Table 1). Thirty-five antibiogram patterns had only a single frequency of occurrence indicating a high diversity among the isolates. And most bacteria (63.2%) were associated with MAR index values greater than 0.8. No single isolate was fully susceptible to all the antibiotics.

Table 1: Phenotypic diversity of bacterial Isolates from toilet surfaces in a tertiary institution in Southern Nigeria

S/No	Antibiogram	Frequency	MAR Index*
	Gram Positive Organisms		
1.	CIP-GEN-STR	1	0.3
2.	AMP-CIP-STR	1	0.3
3.	CEF-CIP-CTX-ERY-GEN	1	0.5
4.	AMP-AMX-CIP-ERY-SXT	1	0.5
5.	AMX-CEF-CIP-CTX-ERY-STR-SXT	1	0.7
6.	AMP-AMX-CEF-CIP-ERY-GEN-PEF-SXT	1	0.8
7.	AMP-AMX-CEF-CIP-CTX-ERY-GEN-PEF-SXT	2	0.9
8.	AMP-AMX-CEF-CIP-ERY-GEN-PEF-STR-SXT	1	0.9
	Gram Negative Organisms		
9.	OFL-STR-SXT	1	0.3
10.	AMX-CIP-OFL-SPX	1	0.4
11.	AMX-CIP-OFL-SPX-STR	1	0.5
12.	CIP-OFL-PEF-SPX-STR	1	0.5
13.	AMX-AUG-CHL-CIP-OFL-SPX	1	0.6
14.	AUG-CIP-GEN-OFL-PEF-SPX	1	0.6
15.	CHL-CIP-OFL-PEF-SPX-SXT	1	0.6
16.	AMX-AUG-CIP-GEN-OFL-SPX-SXT	1	0.7
17.	AMX-AUG-CIP-OFL-PEF-SPX-STR	1	0.7
18.	AMX-CHL-CIP-GEN-OFL-SPX-STR	1	0.7
19.	AMX-CHL-CIP-OFL-PEF-SPX-SXT	1	0.7
20.	AMX-CHL-CIP-OFL-SPX-STR-SXT	1	0.7
21.	AMX-CIP-GEN-OFL-PEF-SPX-STR	1	0.7

22.	CHL-CIP-GEN-OFL-SPX-STR-SXT	1	0.7
23.	CHL-CIP-OFL-PEF-SPX-STR-SXT	1	0.7
24.	AMX-AUG-CHL-CIP-GEN-OFL-PEF-SPX	1	0.8
25.	AMX-AUG-CHL-CIP-OFL-PEF-SPX-SXT	1	0.8
26.	AMX-AUG-CHL-CIP-OFL-SPX-STR-SXT	2	0.8
27.	AMX-AUG-CIP-GEN-OFL-PEF-SPX-STR	1	0.8
28.	AMX-AUG-CIP-GEN-OFL-SPX-STR-SXT	1	0.8
29.	AMX-AUG-CIP-OFL-PEF-SPX-STR-SXT	1	0.8
30.	AMX-CIP-GEN-OFL-PEF-SPX-STR-SXT	2	0.8
31.	AUG-CHL-CIP-GEN-OFL-PEF-SPX-STR	1	0.8
32.	AUG-CHL-CIP-GEN-OFL-PEF-STR-SXT	1	0.8
33.	AUG-CHL-CIP-GEN-OFL-SPX-STR-SXT	1	0.8
34.	AUG-CHL-CIP-OFL-PEF-SPX-STR-SXT	1	0.8
35.	AUG-CIP-GEN-OFL-PEF-SPX-STR-SXT	1	0.8
36.	AMX-AUG-CHL-CIP-GEN-OFL-PEF-SPX-STR	1	0.9
37.	AMX-AUG-CHL-GEN-OFL-PEF-SPX-STR-SXT	1	0.9
38.	AMX-AUG-CHL-CIP-GEN-OFL-SPX-STR-SXT	1	0.9
39.	AMX-AUG-CHL-CIP-OFL-PEF-SPX-STR-SXT	3	0.9
40.	AMX-AUG-CIP-GEN-OFL-PEF-SPX-STR-SXT	2	0.9
41.	AMX-CHL-CIP-GEN-OFL-PEF-SPX-STR-SXT	3	0.9
42.	AUG-CHL-CIP-GEN-OFL-PEF-SPX-STR-SXT	3	0.9
43.	AMX-AUG-CHL-CIP-GEN-OFL-PEF-SPX-STR-SXT	15	1

Discussion

Contamination of toilet surfaces by potentially pathogenic bacteria and an association between these environments and diarrhoeal pathogens have long been clearly demonstrated. Information on the role these environments play as ~~a~~ reservoir of drug resistance is not as clearly understood. Similar to reports by previous studies, this study observed high levels of bacterial load ($3.6\times10^4 - 2.7\times10^5$ CFU/cm^3; 4.46 – 5.43 Log_{10} CFU) associated with the various toilet surfaces. The load in this study was slightly higher than that described by Odigie and colleagues (3.43 to 4.90 Log_{10} CFU), much lower than that described by Alonge and colleagues who reported figures above 1.0×10^7 CFU/ml and similar to reports by Sampson and colleagues (Odigie *et al.,* 2017; Alonge *et al.,* 2019; Sampson *et al.,* 2019). Comparison could not be made with a number of other studies which simply reported high levels of bacterial contamination without presenting information on specific levels of bacterial load (Bashir *et al.,* 2016; Abiose 2019).

Following isolation, this study identified similar types of bacterial contaminants as described in several other studies (Chengula *et al.,* 2014; Bashir *et al.,* 2016; Lincy *et al.,* 2016; Odigie *et al.,* 2017; Alonge *et al.,* 2018; Abiose 2019; Ogba and Obio, 2018). The exception to this was the lack of detection of *Streptococcus pneumoniae* which was reported by Odigie and colleagues. No Streptococcus was detected in the present study. One major difference observed between this study and others was in the predominant bacterial species identified. In most other studies, *Staphylococcus aureus* was a predominant group of bacteria identified. In this study, however, only 7.3% of the total isolates identified were *S. aureus*. Majority of these other studies, however, focused on toilet door handles rather than a variety of toilet surfaces. This difference therefore is possibly a reflection of this variation in sampling points. This hypothesis is supported by the results of Ogba and Obio who noted a 9.9% occurrence of *S. aureus* following the sampling of toilet seats only (Ogba and Obio 2018).

The results of susceptibility testing revealed that the public toilets sampled in this study could serve as potential reservoirs of drug resistant organisms. Majority of these organisms were associated with high MAR index values more commonly linked with areas of high antibiotic use promoting selective pressure selection (Adeleke and Omafuvbe, 2011). The values obtained in this study were in sharp contrast to MAR index values recently noted in environmental isolates where 67.7% of the isolates had a MAR index less than 0.21 (Abu *et al.,* 2020). This confirms that the source of isolates associated with toilet surfaces were not environmental but rather associated with humans. In general, the antibiogram generated for the various isolates revealed a high level of diversity of organisms on the toilet surfaces. This is expected from multi-source contamination.

This study shows that public toilets could play a role not just as a reservoir of potential pathogens but specifically, as a potential reservoir of drug resistant pathogenic microorganisms with high MAR indices.

References

Abiose, O. F. (2019). Bacterial Contamination of Selected Public Toilet Door Handles within Adekunle Ajasin University Campus, Akungba-Akoko, Ondo State, Nigeria. International J. Sciences: Basic Appl. Res. 43: 76 – 78.

Abu, G. O., Otokunefor, K. and Dappa, C. D. (2020). Bacteriological Analysis of Water Quality in a Recreational Park Pond in Rivers State, Nigeria. J. Appl. Sci. Environ. Manage. 24: 23 – 29.

Adeleke, E. O. and Omafuvbe B. O. (2011). Antibiotic Resistance of Aerobic Mesophilic Bacteria Isolated from Poultry Faeces. Research J. Microbiol. 6: 356 – 365.

Adewoyin, A. G., Majolagbe, O. N., Ogunmoyewa, T. and Abejide, M. A. (2013). Antibiotic Resistance Profile of Microbial Isolates of Toilet-Bowl of some Students Hostels in Ogbomoso, Nigeria. European J. Appl. Sci. 5: 76 – 78.

Alonge, O. O., Auwal, B. M. and Aboh, M. I. (2019). Bacterial contamination of toilet door handles on Baze University campus Abuja Nigeria. Afri. J. Clin Experimental Microbiol. 20: 35 - 41.

Barker, J. and Bloomfield, S. F. (2000). Survival of Salmonella in bathrooms and toilets in domestic homes following salmonellosis. J. Appl. Microbiol. 89:137 – 144.

Barker, J. and Jones, M. V. (2005). The potential spread of infection caused by aerosol contamination of surfaces after flushing a domestic toilet. J. Appl. Microbiol. 99: 339 – 347.

Bashir, S. F., Muhammad, H., Sani, N. M. and Kawo, A. H. (2016). Isolation and identification of bacterial contaminants from door handles of public toilets in Federal University Dutse, Jigawa State-Nigeria. IOSR J. Pharm. Bio. Sci. 11: 53 – 57.

Bauer, A. W., Kirby, W. M. M., Strerris, J. C. and Turk, M. (1966). Antibiotic Susceptibility Testing by a Standard Single Disk Method. American J. Clin. Pathol. 45: 493 – 496.

Cheesbrough, M. (2006). District laboratory practice in tropical countries Part II. Cambridge University Press, UK.

Chengula, A., Lushino, A., Mbise, J., Mzula, A., Mafie, E., Mwega, E., Makundi, I. and Peter, E. (2014). Determination of bacterial load and antibiotic susceptibility testing of bacteria isolated from students' toilets at Sokoine

University of Agriculture, Morogoro, Tanzania. Tanzania. J. Health Med. Nursing. 5: 1 – 5.

Cowan, S. T. and Steel, K. J. (1985) Manual for the identification of medical bacteria, 4th edition. London: Cambridge University Press, UK.

Johnson, D. L., Lynch, R. A., Villanella, S. M., Jones, J. F., Fang, H., Mead, K. R. and Hirst D. V. (2017). Persistence of Bowl Water Contamination during Sequential Flushes of Contaminated Toilets. J. Environ. Health. 80: 34 – 49.

Kaewla, W. and Wiwanitkit, V. (2014). Cleanness of the public restroom in a rural municipality, Thailand. J. Family Community Med. 21: 200.

Lincy, M., Vidhya, A., Ali, P. S. S. and John, J. (2016). Determination of bacterial and fungal loads and antibiotic susceptibility testing of bacteria isolated from public toilet door handles in Vellore district, Tamilnadu, India. International J. Res. Biosciences. 5: 69-78.

McGinnis, S., Marini, D., Amatya, P. and Murphy, H. M. (2019). Bacterial Contamination on Latrine Surfaces in Community and Household Latrines in Kathmandu, Nepal. International J. Environ. Res. Public Health. 16: 257.

Mkrtchyan, H. V., Russell, C. A., Wang, N. and Cutler, R. R. (2013). Could public restrooms be an environment for bacterial resistomes? PLoS One. 8: e54223.

Mohamed, M., Owens, K., Gajewski, A., Clabots, C., Johnston, B., Thuras, P., Kuskowski, M. A. and Johnson. J. R. (2015). Extraintestinal pathogenic and antimicrobial-resistant *Escherichia coli* Contamination of 56 public restrooms in the greater Minneapolis-St. Paul metropolitan area. Appl. Environ. Microbiol. 81: 4498 – 4506.

National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility tests; Approved standard. 7th ed. M2-A7. National Committee for Clinical Laboratory Standards, Wayne, PA; 2000.

Odigie, A. B., Ekhiase, F. O., Orjiakor, P. I. and Omozuwa, S., (2017). The role of door handles in the spread of microorganisms of public health consequences in University of Benin Teaching hospital (UBTH), Benin city, Edo state. Pharm. Sci. Tech. 2: 15 – 21.

Ogba, O. M. and Obio, O. M. (2018). Microbial Spectrum on Public Toilet Seats. Annals Microbiol. Infect. Dis. 1:58 – 62.

Sampson, T., Esheyigba, A. P. and Baridam, S. S. (2019). Bacteriological Assessment of Toilet Seats in a Nigerian University. J. Adv. Microbiol. 19: 1 – 11.