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BACTERIAL CONTAMINATION OF TOILET DOOR HANDLES ON BAZE UNIVERSITY CAMPUS ABUJA NIGERIA

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ABSTRACT

Aim: Contracting infectious diseases from microbial contaminated toilet door handles is a potential threat to public health and safety. Therefore we performed microbiological screening of toilet door handles in twelve public toilets on Baze University campus for bacterial contamination.

Methodology and Results: Biochemical analysis of bacterial isolates from entrance toilet door handles in six building blocks on Baze University campus, revealed a general contamination by mainly seven bacterial species associated with human gut and skin flora in order of decreasing frequency as follows: *Staphylococcus aureus* 42.9%; *Salmonella typhimurium* 21.4%; *Escherichia coli* 14.3%; *Pseudomonas aeruginosa* 9.5%; *Proteus mirabilis* 4.8%; *Klebsiella oxytoca* 4.8%; and *Klebsiella pneumoniae* with 2.3% prevalence.

Results of the total aerobic mesophilic count showed A-Block with the highest amount of contamination - 768*10⁵CFU/ml, while B-Block had the least amount of bacterial contamination - 473*10⁵CFU/ml. The levels of bacterial contamination in the other blocks were as follows: C-Block - 710*10⁵CFU/ml, D-Block - 695*10⁵CFU/ml, E-Block - 567*10⁵CFU/ml, and F-Block - 743*10⁵ CFU/ml.

Conclusion: Overall of the seven isolated bacterial species five were mainly gut-associated bacteria, suggesting fecal contamination. The others were skin-associated bacteria (*Staphylococcus aureus*), suggesting routine touch by hands, and soil-associated bacteria (*Pseudomonas aeruginosa*) suggesting contamination from settling dust particles. This study is relevant for public health and safety, as its findings reveal the presence of bacterial pathogens on toilet door handles, which is vital in preventing the spread of infectious disease.

Keywords: Toilet door handles, bacterial pathogens, fecal contamination, antibiotic resistance.

CONTAMINATION BACTÉRIENNE DE POIGNÉES DE PORTE DE TOILETTES SUR LE CAMPUS UNIVERSITAIRE DE BAZE AU NIGERIA D'ABUJA

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ABSTRAIT

Objectifs: Le fait de contracter une maladie infectieuse à partir de poignées de porte de toilettes contaminées par des microbes est une menace potentielle pour la santé et la sécurité publiques. Cette étude inédite à l'université Baze d'Abuja, au Nigéria, a pour but d'analyser des poignées de portes de toilettes sélectionnées provenant de six bâtiments du campus de l'Université pour détecter une contamination bactérienne.

Méthodologie et résultats: L'analyse biochimique des isolats bactériens provenant des échantillons de portes de toilettes sélectionnés a révélé une contamination générale des toilettes échantillonnées, principalement par sept espèces bactériennes associées à des maladies d'origine alimentaire et hydrique: *Staphylococcus aureus* 42,9%; *Salmonella typhimurium* 21,4%; *Escherichia coli* 14,3%; *Pseudomonas aeruginosa* 9,5%; *Proteus mirabilis* 4,8%; *Klebsiella oxytoca* 4,8%; et *Klebsiella pneumoniae* avec une prévalence de 2,3%. Les résultats de la numération mésophile aérobie totale sur des plaques de gélose nutritive ont montré la plus grande quantité de contamination dans le bloc A - 768 * 10⁵CFU / ml, alors que le B-Block présentait le moins de contamination bactérienne - 473 * 10⁵CFU / ml. Les niveaux de contamination bactérienne dans les autres blocs étaient les suivants: C-Block - 710 * 10⁵CFU / ml, D-Block - 695 * 10⁵CFU / ml, E-Block - 567 * 10⁵CFU / ml et F-Block - 743 * 10⁵ CFU / ml. **Conclusion, importance et impact de l'étude:** Les agents pathogènes bactériens isolés des poignées des portes de toilettes échantillonnées sont une source de préoccupation, car ils présentent un risque grave pour la santé et la sécurité publiques.

Mots-clés: poignées de portes de toilettes, agents pathogènes bactériens, microorganismes indicateurs, résistance aux antibiotiques

INTRODUCTION

The transmission of infectious diseases from fomites in the surrounding environment is a potential threat to the public health and safety [1, 2, 3]. Remarkably, one of the sources of transmission of these infectious diseases is the previously unknown and seemingly harmless toilet door handles, which are often teeming with microorganisms due to the frequent and inevitable use [4, 1, 5]. Recent studies have shown the presence of bacterial pathogens on hard, non-porous surfaces such as kitchen surfaces, floor surfaces, toilet surfaces, door handles, etc., [6, 7, 8, 1, 9, 10, 5], from which pathogens are easily transmitted to unsuspecting members of the public posing a potential risk to vulnerable, immune-compromised individuals [11, 2, 5]. Currently, some of these bacterial pathogens have become antibiotic resistant, which is a major public health crisis facing the world today [12, 13, 14, 15]. Therefore there is a need to improve standards of toilet hygiene and toilet door handles in order to reduce the spread of infectious diseases [16, 11]. Hence the aim of this research was to examine twelve (six male and six female) public toilet door handles on Baze University campus for bacterial contamination. Our objectives for this research were to (i) ascertain the diversity and distribution of bacterial species on the toilet door handles on Baze University campus; (ii) determine the sources of bacterial contamination on the toilet door handles and, (iii) examine the susceptibility of the isolated bacterial species to antibiotics therapy. This study is expected to increase awareness of the university campus community on the potential threat posed by toilet door handles as a source of infectious disease transmission. The results of which will serve as a baseline data for future studies and reference, as well as improving the ways the public toilets are cleaned. Furthermore, identifying the sources of bacterial contamination on the door handles could be used to track the transmission of pathogens and help prevent the spread of infectious disease.

MATERIALS AND METHODS

Sample Collection and Study Area: A total of 24 microbial samples were collected aseptically from both the internal and external parts of the main toilet door entrances leading to the male and female toilets of each selected block using swab rinse method (Reynolds et al., 2005). The geographical coordinates of the six selected building blocks on Baze University, Abuja campus, are as follows: Block A (240° SW, 9°23'N, 7°24'17"E); Block B (145° SW, 9° 23'N, 7°24'17"E); Block C (328° NW, 9° 0'20"N, 7° 24'16"E); Block D (102° SE, 9° 0'20"N, 7° 24'16"E); Block E (242° SW, 9° 0'20"N, 7° 24'16"E); Block F (17° N 9° 0'20"N, 7° 24'16"E).

Microbiological Analyses: The cultural, morphological, biochemical and physiological characterization of the bacterial isolates were

performed using standard biochemical analysis [17]. Further antibiotic susceptibility test was performed by disc diffusion method [18]; and total bacterial count by aerobic plate counting [19]

The analyses were carried out in the microbiology laboratory of Baze University, Abuja from August to November, 2017. Statistical analysis of the experimental results was performed using OriginPro 8.5© 2010.

Methods of Biochemical Characterization of Isolates:

Door handles were swabbed, after which the swab stick were soaked in 0.9% of saline water for 10 minutes. A 1ml of 10³ serial dilution of the microbial solution was pipetted aseptically into the surface of sterile solid nutrient agar, Salmonella Shigella agar, MacConkey agar, Centrimide Agar, Eosin Methylene Blue agar and Mueller Hinton agar, plates and incubated in an inverted position at 37° C for 48 hours.

IDENTIFICATION OF BACTERIAL PATHOGENS IN THE SAMPLES

Detection of *Staphylococcus aureus*: A 1ml of 10³ dilutions of microbial solution was pipetted aseptically into the surface of a sterile mannitol salt agar plate, it was then spread and the plate incubated at 37°C for 24 hours. Yellow colonies were taken as *Staphylococcus aureus*.

Detection of *Salmonella* and *Shigella*: A 1ml of 10³ dilutions of microbial solution was pipetted aseptically into the surface of a sterile plate of salmonella shigella agar and incubated at 37°C for 24 hours. Colorless colonies with black spots at the center were taken as *Salmonella typhimurium*.

Detection of Coliforms: A 1ml of 10³ dilutions of microbial solution was pipetted aseptically into sterile eosine methylene blue agar plate and spread with a sterile spatula, after which the plates were incubated at 37°C for 24 hours. Colonies with small green-metallic sheen were presumptively taken as *Escherichia coli*.

Detection of *Pseudomonas aeruginosa*: A 1ml of 10³ dilutions of microbial solution was pipetted aseptically into the surface of sterile centrimide agar plates, it was then spread and plates were incubated at 37°C for 24 hours. Colonies that were blue-green in color were taken as *Pseudomonas aeruginosa*.

Antibiotic Sensitivity Test by Disc Diffusion Method: A 100 µl of standardized suspension of the total number of isolates of each identified bacteria isolate was dispensed into separate sterile petri dishes, and then 25ml of warm molten Mueller hinton agar (MHA) agar was poured into each, swirled, and allowed to solidify. Antibiotic disc (M&B Multi disc, Abtek biological Limited) containing Cepharox 30µg-Ceftriaxone; Oxavid 5µg-Ofloxacin; CIP-M & B CIPRO 5µg-

Ciprofloxacin; Aluclox 30µg- Ampicillin/Cloxacillin; Levotil 5µg-Levofloxacin; Loxaprim 30µg-Cotrimoxazole; Loxaclav 30µg-Amoxicillin/Clavulanate; Ceftin 30µg-Cefuroxime axetil; IPM - 5µg Imipenem and FOR - 5µg Cefoxitin; was placed in the plates and incubated at 37°C for 24hrs. Furthermore, the results were expressed in percentage of susceptibility/resistance according to the absence or presence / diameter of inhibition: - = No zone of inhibition (Resistant); + = 5mm zone of inhibition (low resistance), ++ =10-14mm (intermediate), +++ =15-17mm zone of inhibition (sensitive) [20, 21].

Aerobic Plate Count: A 1ml of 10⁻⁵ serial dilution of the microbial solution was pipetted aseptically into

the surface of a sterile solid blood agar plate and spread using a sterile spatula. The plates were incubated in an inverted position at 37°C for 48 hours after which colonies were counted using J-2 colony counter and results expressed using the formula: $CFU * 10^5/1ml$ [19].

RESULTS

Biochemical analysis of bacterial isolates from the selected toilet door samples revealed a general contamination of the sampled toilet door handles by mainly seven bacterial species (Table 1) associated with human gut and skin flora as follows: *Staphylococcus aureus*, *Salmonella typhimurium*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*.

TABLE 1: CHARACTERISTICS OF BACTERIAL ISOLATES FROM SELECTED TOILET DOOR HANDLES

Cell morphology	Rod	Cocci	Rod	Rod	Rod	Rod	Rod
Gram reaction	Negative	Positive	Negative	Negative	Negative	Negative	Negative
Catalase test	+	+	-	-	-	-	-
Coagulase test	-	+	-	-	-	-	-
Indole production	-	-	+	-	-	+	-
Citrate utilization	+	-	-	+	+	+	+
Urease test	-	-	-	-	+	-	+
Oxidase test	+	-	-	-	-	-	-
Motility test	+	+	+	+	-	-	+
Lactose test	+	+	+	-	+	+	-
Glucose test	+	+	+	+	+	+	+
Manitol	-	+	-	-	-	-	-
Kliger Iron Agar	Slope	R	-	Y	R	Y	R
	Butt	R	-	Y	Y	Y	Y
	H₂S	-	-	-	+	-	+
	Gas	-	-	+	+	+	+
Probable Identity	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>Salmonella typhimurium</i>	<i>Klebsiella pneumoniae</i>	<i>Klebsiella oxytoca</i>	<i>Proteus mirabilis</i>

Key: + = positive; - = negative; KIA-Kliger Iron Agar: slope: Butt, H₂S, Gas R- Red-pink (alkaline reaction), Y - Yellow (acid reaction)

Unsurprisingly *Staphylococcus aureus* with the highest frequency of prevalence - 42.9%, was the sole bacterial specie isolated from all examined toilet door handles. While, *Klebsiella pneumoniae* had the lowest frequency of prevalence - 2.3%. The

prevalence rates of the other bacterial isolates were as follows: *Salmonella typhimurium* 21.4%; *Escherichia coli* 14.3%; *Pseudomonas aeruginosa* 9.5%; *Proteus mirabilis* 4.8%; and *Klebsiella oxytoca* 4.8% (Table 2).

TABLE 2: PREVALENCE OF BACTERIAL ISOLATES ON TOILET DOOR HANDLES IN THE SELECTED BLOCKS

Bacterial Isolate	Frequency of Isolates on Toilet Door Handle Samples by Blocks						Total	% Frequency
	Block A	Block B	Block C	Block D	Block E	Block F		
<i>Pseudomonas aeruginosa</i>	2	-	-	2	-	-	4	9.5
<i>Staphylococcus aureus</i>	3	4	2	3	3	3	18	42.9
<i>Escherichia coli</i>	-	-	-	3	1	2	6	9.5
<i>Salmonella typhimurium</i>	2	-	1	3	3	-	9	21.4
<i>Klebsiella pneumoniaea</i>	-	-	1	-	-	-	1	2.3
<i>Klebsiella oxytoca</i>	-	-	-	-	-	2	2	4.8
<i>Proteus mirabilis</i>	-	-	-	-	-	2	2	4.8
Total Number of Isolates per Block	7	4	4	11	7	9	42	100

Further antibiotic sensitivity testing on these bacterial isolates by disc diffusion method (Table 3) showed a general susceptibility to Ciprofloxacin, Ofloxacin, Levofloxacin, Imipenem, and Cefoxitin by all isolates. *Salmonella typhimurium* showed total resistance to Ampicillin/ Cloxacillin, Co-

trimoxazole, Amoxicillin/ Clavulanate, and Cefuroxime axetil. Ciprofloxacin was the most effective against all isolates: *Salmonella typhimurium* (77.8%), *Staphylococcus aureus* (100%), *Escherichia coli* (100%), *Klebsiella pneumoniaea* (100%), *Klebsiella oxytoca* (100%), and *Proteus mirabilis* (100%).

TABLE 3: ANTIBIOTICS SENSITIVITY TEST ON BACTERIAL ISOLATES BY DISC DIFFUSION METHOD

Antibiotic	S. aureus (n=18)		S. typhimurium (n=9)		E. coli (n=6)		P. aeruginosa (n=4)		P. mirabilis (n=2)		K. pneumoniaea (n=1)		K. oxytoca (n=2)	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
	Ceftriaxone	13	72.2	8	88.9	2	33.3	1	25.0	1	50.0	1	100.0	0
Ofloxacin	15	83.3	2	22.2	5	83.3	2	50.0	2	100.0	1	100.0	1	50.0
Ciprofloxacin	18	100.0	7	77.8	6	100.0	4	100.0	2	100.0	1	100.0	2	100.0
Ampicillin/ Cloxacillin	14	77.8	0	0.0	2	33.3	2	50.0	0	0.0	1	100.0	1	50.0
Levofloxacin	12	66.7	5	55.6	2	33.3	2	50.0	2	100.0	1	100.0	1	50.0
Co-trimoxazole	14	77.8	0	0.0	2	33.3	3	75.0	1	50.0	1	100.0	0	0.0
Amoxicillin/ Clavulanate	13	72.2	0	0.0	2	33.3	1	25.0	1	50.0	1	100.0	1	50.0
Cefuroxime axetil	10	55.6	0	0.0	2	33.3	0	0.0	0	0.0	0	0.0	0	0.0
Imipenem	18	100.0	4	44.4	5	83.3	3	75.0	2	100.0	1	100.0	2	100.0
Cefoxitin	18	100.0	7	77.8	5	83.3	2	50.0	1	50.0	1	100.0	2	100.0

Results of the total aerobic mesophilic plate count, showed a general trend of a considerably higher amount of bacterial contamination on the internal part of the entrance toilet door handles than the external parts of both male and female toilets in all the selected blocks as follows: Block A: male toilet - 10×10^5 CFU/ml, female - 6×10^5 CFU/ml; Block B:

male - 10×10^5 CFU/ml, female - 5×10^5 CFU/ml; Block C: male - 2×10^5 CFU/ml, female - 10×10^5 CFU/ml; Block D: male - 18×10^5 CFU/ml, female - 50×10^5 CFU/ml; Block E: male - 18×10^5 CFU/ml, female - 39×10^5 CFU/ml; Block F: male - 17×10^5 CFU/ml, female - 10×10^5 CFU/ml (Figure 1: A & B).

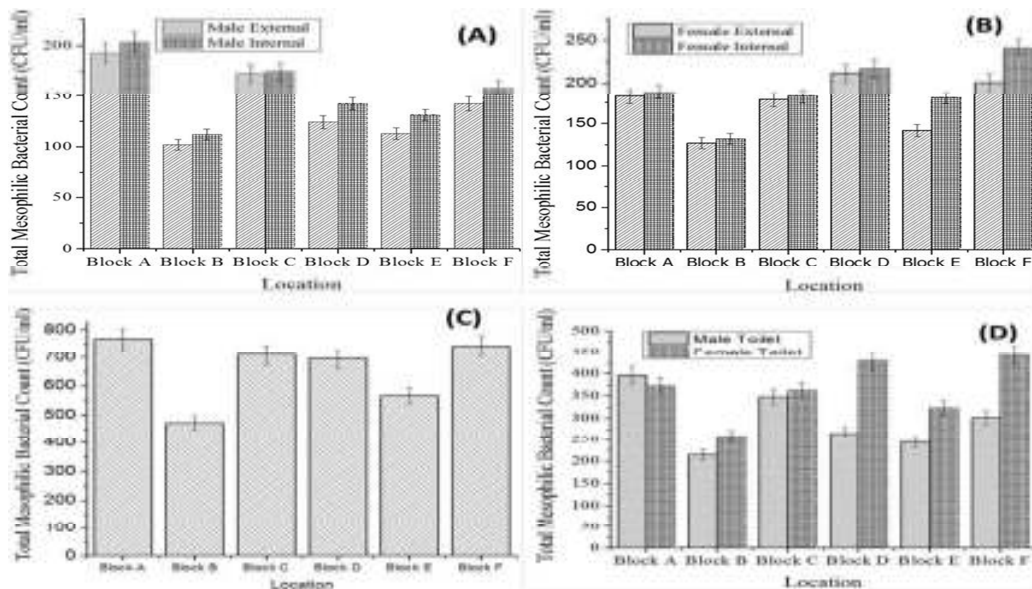


Figure 1: Comparative analysis on the level of bacterial contamination on toilet door handles: A- between the external and internal parts of male door handles; B - between the external and internal parts of female door handles; C - total amount of contamination by Blocks; B - between male and female toilets in all the Blocks

Comparative analysis of the combined total of bacterial contamination on door handles (internal and external) of both male and female toilets by blocks, showed Block A with the highest amount of contamination - $768 \times 10^5 \pm 38.4$ CFU/ml, while Block B had the least amount of bacterial contamination - $473 \times 10^5 \pm 23.7$ CFU/ml. The levels of bacterial contamination in other blocks were as follows: Block C - $710 \times 10^5 \pm 35.5$ CFU/ml, Block D - $695 \times 10^5 \pm 34.8$ CFU/ml, Block E - $567 \times 10^5 \pm 28.4$ CFU/ml, and Block F - $743 \times 10^5 \pm 37.2$ CFU/ml (figure 1C).

Furthermore, with the exception of A-Block, the frequency of bacterial contamination on female toilet door handles was about 1.04 - 1.61 times higher than the frequency of prevalence on door handles of the male toilets (figure 1D).

DISCUSSION

The presence of *Escherichia coli*, *Proteus mirabilis*, *Salmonella typhimurium*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, infer contamination by fecal matter, suggesting poor hygienic practices and the settling of suspended microorganisms in the air after flushing of water closet systems without covering the lid [4, 1]. The presence of *Pseudomonas aeruginosa* a predominant soil bacterium suggest transmission from toilet floor surfaces brought in from foot wears, and settling dust suspensions [1, 3, 22].

The isolated bacterial pathogens from the toilet door handles in this study are consistent with the findings of other researchers [4, 9, 10, 5], with *Staphylococcus aureus* being the most prevalent [9, 10, 23]. Contamination by *Staphylococcus aureus*, a bacterium of the skin flora, suggests direct contact

of the toilet door by individual handlers [24, 1, 3]. Bacterial pathogens such as *Salmonella typhimurium* isolated from virtually all the blocks is a major cause for concern, causing typhoid fever which is a leading cause of disease and death in Nigeria today [25]. Opportunistic pathogens of the coliform group, *Staphylococcus* and *Pseudomonas* have been linked to urinary tract infections, bacterial diarrhea, bacterial meningitis and bacterial pneumonia (*K. pneumoniae*) [26].

Indications from the antibiotic sensitivity test, showed that the Quinolones (Ciprofloxacin, Ofloxacin, Levofloxacin), whose mode of action involves the inhibition of nucleic acid synthesis of Bacteria, were most effective against both the gram positive (*Staphylococcus aureus*) and gram negative (*Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Escherichia coli*, *Proteus mirabilis*, *Klebsiella oxytoca*, and *Klebsiella pneumoniae*) bacterial isolates. In contrast the Cephalosporins (Ampicillin/ Cloxacillin, Amoxicillin/ Clavulanate, and Cefuroxime axetil) with the exception of Ceftriaxone, Imipenem and Cefoxitin, were not as effective, especially against the gram negative isolates, these results were consistent with the findings of other researchers [14, 15]. This could be as a result of a growing resistance to the β -lactams component of these antibiotics, by β -lactamase producing bacteria such as *Staphylococcus aureus* [27], suggesting the development of *Methicillin Resistant Staphylococcus aureus* (MRSA); and especially extended-spectrum β -lactamases (ESBLs) producers such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli* and *Proteus mirabilis*, which possess grave consequences to public health [28, 12, 29].

The high prevalence of bacterial contamination on the internal parts of the entrance toilet door handles than the external parts of both male and female toilets in all the selected blocks can be attributed due to the close proximity of the internal parts of the toilet handles to the water closet system and basin sink. Hence contamination is more likely as a result of being exposed to settling air borne microbes from coughing, sneezing, flushing, vector borne spread (flies) and contact with unwashed human hands [1, 2].

The high frequency of bacterial contamination on toilet door handles in A-Block can be attributed to the high traffic of staff, students, and visitors using these toilets. A-Block houses both the administrative department and the largest Faculty in Baze University, the faculty of Management & Social Sciences. B-Block which houses the smallest faculty by departments had the least amount of contamination.

Finally, the higher abundance of bacterial contamination on female toilet door handles was also reported by other researchers in their work [9, 1]. This occurrence suggests additional source of contamination from the high concentration of vaginal-associated bacterial species contained in female discharges as reported by Flores et al., (2011).

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