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Bacterial isolates from toilet seats in a tertiary institution and their susceptibility to antibiotics and selected toilet cleaning agents marketed in Nigeria

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Abstract:

Background: Infections associated with the use of contaminated public toilets and the control of such infections are a major challenge especially with the high prevalence of substandard toilet cleaning agents in the Nigerian market. The study aimed to determine bacterial isolates contaminating public toilet seats and their susceptibility to commonly used antibiotics, and to assess *in vitro* antimicrobial activity of selected toilet cleaning agents commercially available in the Nigerian market.
Methodology: Swab samples were collected from 50 randomly selected toilet seats from three designated areas in Obafemi Awolowo University campus, Ile-Ife, Nigeria. Bacteria were isolated and identified using conventional culture and biochemical identification test scheme. Susceptibility of the isolates to conventional antibiotics was determined by the Kirby Bauer disk diffusion method and interpreted as resistant, intermediate or susceptible using the Clinical and Laboratory Standard Institute guidelines. The *in vitro* antimicrobial activity of selected toilet cleaning agents was evaluated using the agar well diffusion technique, with 1% cetrimide as the standard positive control.
Results: A total of 90 bacteria were isolated from the 50 toilet seats with a frequency of *Bacillus* spp (33.3%, n=30), *Escherichia coli* (23.3%, n=21), *Staphylococcus aureus* (15. 6%, n=14), *Klebsiella pneumoniae* (8. 9%, n=8), *Micrococcus luteus* (3.3%, n=3), *Enterococcus faecalis* (3.3%, n=3), *Salmonella typhi* (2.2%, n=2), *Proteus vulgaris* (2.2%, n=2), *Staphylococcus epidermidis* (2.2%, n=2), *Shigella sonnei* (1.1%, n=1), *Enterobacter aerogenes* (1.1%, n=1), *Staphylococcus intermedius* (1.1%, n=1), *Proteus mirabilis* (1.1%, n=1), and *Serratia marcescens* (1.1%, n=1). Majority of the isolates were resistant to multiple antibiotics. Susceptibility to ciprofloxacin antibiotic was the highest, while all the isolates were susceptible to all the toilet cleaning agents evaluated when compared to 1% cetrimide used as the standard positive control.
Conclusion: The study showed that toilet seats harbor pathogenic bacteria with varying susceptibilities to antibiotics and toilet cleaning agents.

Keywords: Toilet seats; Toilet cleaning agents; Bacteria; Antimicrobial activity; Susceptibility

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Isolats bactériens prélevés sur des sièges de toilettes dans un établissement d'enseignement supérieur et leur sensibilité aux antibiotiques et à certains produits d'entretien commercialisés au Nigéria

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Résumé:

Contexte: Les infections liées à l'utilisation de toilettes publiques contaminées et leur contrôle constituent un défi majeur, notamment en raison de la forte prévalence de produits d'entretien de qualité inférieure sur le marché nigérian. Cette étude visait à identifier les isolats bactériens contaminant les sièges de toilettes publiques et leur sensibilité aux antibiotiques couramment utilisés, ainsi qu'à évaluer l'activité antimicrobienne in vitro de certains produits d'entretien disponibles dans le commerce au Nigéria.
Méthodologie: Des prélèvements ont été effectués sur 50 sièges de toilettes sélectionnés aléatoirement dans

trois zones désignées du campus de l'Université Obafemi Awolowo, à Ile-Ife, au Nigéria. Les bactéries ont été isolées et identifiées par culture conventionnelle et tests biochimiques. La sensibilité des isolats aux antibiotiques conventionnels a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer et interprétée comme résistante, intermédiaire ou sensible selon les recommandations du CLSI (Institut des Normes Cliniques et de Laboratoire). L'activité antimicrobienne *in vitro* de certains produits de nettoyage pour toilettes a été évaluée par la technique de diffusion en puits sur gélose, avec du cétrimide à 1 % comme témoin positif.
Résultats: Au total, 90 bactéries ont été isolées des 50 sièges de toilettes, avec les fréquences suivantes: *Bacillus* spp (33,3%, n=30), *Escherichia coli* (23,3%, n=21), *Staphylococcus aureus* (15,6%, n=14), *Klebsiella pneumoniae* (8,9%, n=8), *Micrococcus luteus* (3,3%, n=3), *Enterococcus faecalis* (3,3%, n=3), *Salmonella* Typhi (2,2 %, n=2), *Proteus vulgaris* (2,2%, n=2), *Staphylococcus epidermidis* (2,2%, n=2), *Shigella sonnei* (1,1%, n=1), *Enterobacter aerogenes* (1,1%, n=1), *Staphylococcus intermedius* (1,1%, n=1) et *Proteus mirabilis* (1,1%, n=1) et *Serratia marcescens* (1,1%, n=1). La majorité des isolats étaient résistants à plusieurs antibiotiques. La sensibilité à la ciprofloxacine était la plus élevée, tandis que tous les isolats étaient sensibles à tous les produits de nettoyage pour toilettes évalués, comparativement au cétrimide à 1% utilisé comme témoin positif. **Conclusion:** Cette étude a montré que les sièges de toilettes abritent des bactéries pathogènes présentant une sensibilité variable aux antibiotiques et aux produits de nettoyage pour toilettes.

Mots-clés: Sièges de toilettes; Produits de nettoyage pour toilettes; Bactéries; Activité antimicrobienne; Sensibilité

Introduction:

A toilet is a device that allows one to urinate or defecate. It usually has a flushing mechanism, a lid that can be removed, and a seat that can be pivoted (1). However, public toilets are facilities where individuals can address their hygienic needs. These can be found in all manner of places including markets, transport centers, schools, eateries, hostels, offices, factories, cinemas, bars, museum and more (1). Public restrooms, on the other hand, are any modest room or building with one or more toilets (2).

According to the World Health Organization (WHO), over 1.5 billion people of the global population still do not have basic sanitation services, such as private toilets or latrines of which 419 million still defecate in the open, for example in street gutters, behind bushes or into open bodies of water (3). Access to toilets has been recognized by the United Nations as a human right that provides privacy and ensures dignity. Public health depends on toilets and except that everyone in a community has a safe toilet, the health of everyone is threatened (4).

Unhygienic use of public toilets can lead to urine and faecal residues remaining on surfaces after use, potentially serving as significant reservoirs or sources of human pathogens (5). Public toilets exhibit microbial diversity due to the high activity levels of individuals with varying hygienic practices (6). Bacterial pathogens that have been reportedly associated with toilets include *Escherichia coli*, *Klebsiella*, *Pseudomonas, Salmonella, Proteus vulgaris, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Citrobacter* spp and *Bacillus* spp (7-9).

The presence of pathogenic organisms in toilets implies that toilets can serve as a source of infections aside their aiding the transmission of infections. Poor and unhygienic use of toilets has been linked to the transmission of diarrheal diseases such as cholera and dysentery, as well as typhoid, intestinal worm infections, and polio (10-12). Moreover, outbreaks of infectious diseases arising from toilets have been documented, largely from improper cleaning and disinfection of restroom facilities (13,14). However, despite that hazards associated with toilet seats have been reported by researchers, less attention has been given to toilet seats as inanimate objects which could harbor and transmit infectious agents (15).

One of the ways by which infections associated with toilet seats can be curtailed is through the use of toilet cleaning agents which are liquid solutions used in disinfecting the toilet bowl and even the seat. They are cleaning products specifically designed to sanitize and clean the toilet. They help remove germs, stains, and odors (16). They contain different active agents such as hydrochloric acid, sodium hypochlorite, sodium lauryl ether sulfate, sodium hydroxide, quaternary ammonium compounds and borax (17).

One of the factors militating against the increasing awareness on the need to maintain good toilet hygiene is the proliferation and preponderance of adulterated, substandard and fake toilet cleaning agents in Nigeria markets (18). This has become a national embarrassment and a public health concern. This study therefore aimed at evaluating some toilet cleaning agents in Nigeria markets for their antibacterial activity against bacterial isolates associated with toilet seats.

Materials and method:

Study area:

The study was carried out at the Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Obafemi Awolowo University (O.A.U), Ile-Ife, Nigeria.

Collection of samples:

Samples were collected from 50 randomly selected toilet seats at 3 designated areas of the University; Faculty of Pharmacy (male and female toilets), a male hostel and a

female hostel using the swab-rinse technique. Briefly, sterile swab sticks pre-moistened with normal saline were used to swab the toilet seats, and then pre-incubated for 30 seconds in normal saline.

Bacteria isolation by conventional culture:

Samples were cultured on *Salmonella-Shigella*, MacConkey, Cetrimide, Mannitol salt and Blood agar plates. Following a 24-h incubation at 37^{o}C, distinct colonies were sub-cultured and purified for identification using conventional biochemical test schemes (19, 20).

Antibiotic susceptibility test of isolates:

Antimicrobial susceptibility testing of each bacterial isolate was determined using the Kirby Bauer disc diffusion technique (21). Briefly, colonies of each bacterial isolate were dissolved in 10ml sterile distilled water in McCartney bottle and standardized to 0.5 MacFarland standards using sterile distilled water. The resulting bacterial suspension was uniformly spread on the surface of Mueller-Hinton (MH) agar plate using a sterile swab stick.

Antibiotic discs (cefuroxime 30µg, ceftriaxone 30µg, cefotaxime 30µg, ceftazidime 30µg, gentamicin 10µg, amikacin 30µg, tetracycline 10µg, cotrimoxazole 25µg, chloramphenicol 10µg, ciprofloxacin 5µg, and meropenem 10µg) were placed on the agar plates. The plates were incubated at 37^{o}C for 24 hours after which the inhibition zone diameters were measured. The results were interpreted as susceptible, intermediate or resistant using the Clinical and Laboratory Standard Institute (CLSI) guidelines (22).

***In vitro* antibacterial activity of toilet cleaning agents:**

The toilet cleaning agents used were purchased from various supermarkets in Lagos and their containers disclosures are shown in Table 1. *In vitro* antibacterial activity of the cleaning agents was determined using the agar well diffusion method. Overnight grown culture of the bacterial isolates in Mueller-Hinton broth (MHB) was standardized to 0.5 McFarland standard (an equivalent of 1.5 x 10^8 CFU/ml) using sterile Mueller-Hinton broth. One in hundred (1 in 100) dilution of the standardized bacterial culture was made by adding 0.1ml to 10ml MHB to give 1.5 x 10^6 CFU/ml. A further 1 in 100 dilution was made by seeding 0.2ml in 20ml melted and cooled Mueller-Hinton agar in McCartney bottle, poured into Petri dish and allowed to set. This gave a final inoculum of 1.5 x 10^4 CFU/ml.

Wells were then bore into the MH agar using a sterile cork-borer. Exactly 4 drops of each of the toilet cleaning agents were put into each of the wells while 4 drops of 1% cetrimide solution was used as standard positive control. The plates were allowed to stand for one hour for diffusion of the toilet cleaning agent and then incubated at 37^{o}C for 24 hours. The antibacterial activity of each of the toilet cleaning agent was evaluated by measuring the diameter of the zones of inhibition. All the plates were made in duplicates.

Results:

The frequency distribution of 90 bacterial isolates from the 50 toilet seats is shown in Table 2, with 53 (58.9%) Gram-positive and 37 (41.1%) Gram-negative isolates. While *Bacillus* spp had the highest frequency of 33.3% among the Gram-positive isolates, *Escherichia coli* has the highest frequency of 23.3% among the Gram-negative isolates.

The detail susceptibility and resistance profiles of the bacterial isolates to selected conventional antibiotics are displayed on Table 3. Majority of the isolates were multiply-resistant to the antibiotics, with all *Bacillus* spp resistant to at least 4 antibiotics, and all *E. coli* and *S. aureus* resistant to at least 5 antibiotics. The percentage susceptibility of the isolates to the selected antibiotics is shown in Table 4, with majority of the isolates being most susceptible to ciprofloxacin but resistant to all the cephalosporins (cefotaxime, ceftazidime, ceftriaxone and cefuroxime) and meropenem.

The *in vitro* activity of selected toilet cleaning agents marketed and distributed in Nigerian markets on the bacterial isolates are presented in Table 5. Despite the fact that majority of the isolates were resistant to multiple antibiotics, all the toilet cleaning agents had high *in vitro* antibacterial activity against the bacterial isolates, higher than the 1% cetrimide used as the standard positive control agent.

Table 1: Detail of the contents of selected toilet cleaners marketed in Nigeria

S/N	Name of toilet washer	MFD	Expiry Date	Manufacturer's Address	Ingredients	Batch Number	NAFDAC Number
1.	Izal Germicide	03/24	02/26	GONGONI CO. LTD. 89A Sharada Industrial Estate, Phase III, Kano	Active Ingredient: Tar Acid Phenol 7%; Cresylic Creosote 2%	051	04-1130
2.	Jenic Power Plus Ceramic/toilet/floor Cleaner	2024	2027	Jenic Merchandise Industries Nig. LTD. H2 Plot 492 Signal Barracks Mile 2, Lagos. 08065378814	Active Ingredient: Hydrochloric acid	NA	05902
3.	Harpic Original	02/24	02/26	Reckitt Benckiser Nig LTD. Plot C2/3 Agbara Industrial Estate, Ogun State, Nigeria. 0902626262	Active Ingredient: 10%w/v Hydrochloric acid, 0.63%w/v Hexadecetrimethyl ammonium chloride	0352	02-2018
4.	Spark Toilet cleaner	2023	2025	Betholite Trust Int'l Venture, Km 42, Lagos- Ibadan Expressway, Pakoro, Ogun State. 08036740626	Deionised water, anionic surfactant, foam agent, Fragrance. Active Ingredient: Sodium Hydroxide, Hydrogen peroxide	NA	02-4971
5.	Squick Multiactive Ceramic/tiles/toilet cleaner	05/24	04/26	E-Summit multi-Global Technologies. 01 Orji Lane, Ojo, Lagos. +2347063584447	Aqua, surfactant, emulsifier, dye, fragrance, preservative Active Ingredients: Hydrochloric acid Disinfectant	0062	A2-1184
6.	Hypo Toilet Cleaner	09/23	09/25	Hypo Homecare Products LTD, Km 5, Itokin Rd, Ikorodu, Lagos. 07000004976	De-mineralised water, amine ethoxylate and color Active Ingredients: 10.5%w/v Hydrochloric acid	15 I	A2-1009
7.	Luvic Power Plus ceramic/toilet/ tiles cleaner	02/22	02/26	Luvic Nig ltd. 250, Old Ojo road, Agboju Amuwo odofin, Lagos State. 08100191319	Active Ingredient: Hydrochloric acid 85% Sulphuric acid 75%	NA	02-3982

Table 2: Frequency distribution of bacterial isolates from toilet seats

Bacterial isolates	Number	Percentage
Gram-positive bacteria		
Bacillus spp	30	33.3
Staphylococcus aureus	14	15.6
Micrococcus luteus	3	3.3
Enterococcus faecalis	3	3.3
Staphylococcus epidermidis	2	2.2
Staphylococcus intermedius	1	1.1
Gram-negative bacteria		
Escherichia coli	21	23.3
Klebsiella pneumoniae	8	8.9
Salmonella Typhi	2	2.2
Proteus vulgaris	2	2.2
Proteus mirabilis	1	1.1
Shigella sonnei	1	1.1
Enterobacter aerogenes	1	1.1
Serratia marcescens	1	1.1
Total	**90**	**100.0**

Table 3: Detailed antibiotic susceptibility and resistance profiles of bacterial isolates of selected toilet seats in Obafemi Awolowo University, Ile -Ife

Code	Bacterial species	AMK	CHL	CIP	COT	CPZ	CRX	CTR	CTX	GEN	MEM	TET	MAR
	Gram-positive isolates												
	Bacillus												
F16b	*Bacillus* spp	S	S	S	R	S	R	S	S	R	S	R	4
F17a	*Bacillus* spp	S	S	S	I	R	R	R	R	S	R	S	5
F19b	*Bacillus* spp	S	S	S	S	R	R	R	R	S	R	S	5
F20	*Bacillus* spp	S	S	S	R	R	R	S	R	S	R	S	5
FOB2b	*Bacillus* spp	S	S	S	S	R	R	R	R	S	R	S	5
FOB5a	*Bacillus* spp	S	I	S	S	R	R	R	R	S	R	S	5
M14b	*Bacillus* spp	S	S	S	I	R	R	R	R	S	R	R	6
F6	*Bacillus* spp	S	S	S	R	R	R	R	R	S	R	S	6
F18	*Bacillus* spp	S	S	S	R	R	R	R	R	S	R	S	6
F21	*Bacillus* spp	S	S	S	R	R	R	R	R	S	R	I	6
FOB3a	*Bacillus* spp	R	S	S	S	R	R	R	R	S	R	S	6
FOB3b	*Bacillus* spp	S	S	S	S	R	R	R	R	S	R	R	6
S1	*Bacillus* spp	S	S	I	R	R	R	R	R	S	R	R	7
F2b	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	S	7
F3b	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	I	7
F5b	*Bacillus* spp	S	S	S	R	R	R	R	R	I	R	R	7
F8a	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	I	7
F16a	*Bacillus* spp	S	S	I	I	R	R	R	R	R	R	R	7
MOB3a	*Bacillus* spp	S	S	S	R	R	R	R	R	S	R	R	7
FOB4a	*Bacillus* spp	S	S	S	I	R	R	R	R	R	R	R	7
M15a	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	R	8
M15b	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	R	8
F7a	*Bacillus* spp	S	S	R	R	R	R	R	R	S	R	R	8
F10	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	R	8
FOB5b	*Bacillus* spp	S	S	S	R	R	R	R	R	R	R	R	8
M5b	*Bacillus* spp	R	S	S	R	R	R	R	R	R	R	R	9
M7bi	*Bacillus* spp	S	R	S	R	R	R	R	R	R	R	R	9
M9a	*Bacillus* spp	R	S	S	R	R	R	R	R	R	R	R	9
FOB4b	*Bacillus* spp	S	R	S	R	R	R	R	R	R	R	R	9
F14a	*Bacillus* spp	R	R	S	R	R	R	R	R	R	R	R	10
	Staphylococcus												
F17b	*Staphylococcus aureus*	S	S	S	S	R	R	R	R	S	R	S	5
M1c	*Staphylococcus aureus*	S	I	I	S	R	R	R	R	R	R	R	7
M1b	*Staphylococcus aureus*	R	S	R	S	R	R	R	R	R	R	S	8
M4b	*Staphylococcus aureus*	S	I	S	R	R	R	R	R	R	R	R	8
F1b	*Staphylococcus aureus*	S	S	S	R	R	R	R	R	R	R	R	8
F8c	*Staphylococcus aureus*	S	S	S	R	R	R	R	R	R	R	R	8
M3b	*Staphylococcus aureus*	S	S	R	R	R	R	R	R	R	R	R	9
M4a	*Staphylococcus aureus*	R	S	S	R	R	R	R	R	R	R	R	9
M6a	*Staphylococcus aureus*	R	S	I	R	R	R	R	R	R	R	R	9
F8b	*Staphylococcus aureus*	S	R	S	R	R	R	R	R	R	R	R	9
F12	*Staphylococcus aureus*	S	R	S	R	R	R	R	R	R	R	R	9
F13	*Staphylococcus aureus*	S	R	S	R	R	R	R	R	R	R	R	9
FNB3	*Staphylococcus aureus*	R	R	I	R	R	R	R	R	S	R	R	9
M3a	*Staphylococcus aureus*	S	R	R	R	R	R	R	R	R	R	R	10
MOB2a	*Staphylococcus epidermidis*	S	S	R	I	R	R	R	R	R	R	R	8
M12b	*Staphylococcus epidermidis*	S	R	S	R	R	R	R	R	R	R	R	9
F19a	*Staphylococcus intermedius*	R	S	S	S	R	R	R	R	I	R	S	6
	Micrococcus												
F4a	*Micrococcus luteus*	S	S	S	R	R	R	R	R	S	R	S	6

F4b	*Micrococcus luteus*	S	S	S	R	R	R	R	R	R	R	R	8
MOB1b	*Micrococcus luteus*	S	R	R	R	R	R	R	R	I	R	R	9
	Enterococcus												
F2a	*Enterococcus faecalis*	S	S	I	R	R	R	R	R	S	R	R	7
M11a	*Enterococcus faecalis*	S	R	S	S	R	R	R	R	I	R	R	7
M2bii	*Enterococcus faecalis*	S	S	R	R	R	R	R	R	R	R	R	9
	Gram-negative isolates												
	Escherichia												
MOB2b	*Escherichia coli*	S	S	S	S	R	R	R	R	S	R	S	5
M8ai	*Escherichia coli*	S	S	S	R	R	R	R	R	I	R	R	7
M12a	*Escherichia coli*	S	S	S	R	R	R	R	R	S	R	R	7
M14a	*Escherichia coli*	R	S	S	S	R	R	R	R	I	R	R	7
F5a	*Escherichia coli*	S	S	S	R	R	R	R	R	S	R	R	7
FOB1b	*Escherichia coli*	S	S	S	R	R	R	R	R	S	R	R	7
FOB2a	*Escherichia coli*	R	S	I	S	R	R	R	R	R	R	S	7
M8b	*Escherichia coli*	S	S	I	R	R	R	R	R	R	R	R	8
F3a	*Escherichia coli*	S	S	I	R	R	R	R	R	R	R	R	8
F9	*Escherichia coli*	S	S	S	R	R	R	R	R	R	R	R	8
FNB4	*Escherichia coli*	R	S	S	S	R	R	R	R	R	R	R	8
M6bii	*Escherichia coli*	S	S	R	R	R	R	R	R	R	R	R	9
M9b	*Escherichia coli*	R	R	S	R	R	R	R	R	S	R	R	9
F14b	*Escherichia coli*	S	R	S	R	R	R	R	R	R	R	R	9
FOB1a	*Escherichia coli*	S	R	S	R	R	R	R	R	R	R	R	9
M5a	*Escherichia coli*	S	R	R	R	R	R	R	R	R	R	R	10
M10a	*Escherichia coli*	R	R	S	R	R	R	R	R	R	R	R	10
M10b	*Escherichia coli*	R	R	S	R	R	R	R	R	R	R	R	10
F15	*Escherichia coli*	R	R	S	R	R	R	R	R	R	R	R	10
FNB1b	*Escherichia coli*	S	R	R	R	R	R	R	R	R	R	R	10
M6bi	*Escherichia coli*	R	R	R	R	R	R	R	R	R	R	R	11
	Klebsiella												
M13	*Klebsiella pneumoniae*	S	S	S	S	R	R	R	R	S	R	R	6
FNB2	*Klebsiella pneumoniae*	R	S	S	S	R	R	R	R	S	R	R	7
M7bii	*Klebsiella pneumoniae*	S	S	S	R	R	R	R	R	R	R	R	8
F11a	*Klebsiella pneumoniae*	R	S	S	R	R	R	R	R	R	R	I	8
F1a	*Klebsiella pneumoniae*	S	S	R	R	R	R	R	R	R	R	R	9
F7b	*Klebsiella pneumoniae*	S	S	R	R	R	R	R	R	R	R	R	9
F11b	*Klebsiella pneumoniae*	S	R	S	R	R	R	R	R	R	R	R	9
MOB3b	*Klebsiella pneumoniae*	S	R	S	R	R	R	R	R	R	R	R	9
	Salmonella												
M7a	*Salmonella* Typhi	S	R	S	R	R	R	R	R	R	R	R	9
M11b	Salmonella Typhi	S	R	S	R	R	R	R	R	R	R	R	10
	Proteus												
FNB5	*Proteus vulgaris*	S	S	S	R	R	R	R	R	R	R	R	8
M1a	*Proteus vulgaris*	S	R	R	R	R	R	R	R	R	R	R	10
MOB1a	*Proteus mirabilis*	S	R	I	R	R	R	R	R	R	R	R	9
	Shigella												
M2bi	*Shigella sonnei*	S	S	S	S	R	R	R	R	S	R	S	5
	Enterobacter												
M8aii	*Enterobacter aerogenes*	R	R	S	R	R	R	R	R	S	R	R	9
	Serratia												
FNB1a	*Serratia marcescens*	S	S	S	S	R	R	R	R	S	R	R	6

TET: Tetracycline 10µg; COT: Cotrimoxazole 25µg; GEN: Gentamicin 10µg; CRX: Cefuroxime 30µg; CHL: Chloramphenicol 10µg; CTR: Ceftriaxone 30µg; CTX: Cefotaxime 30µg; CIP: Ciprofloxacin 5µg; AMK: Amikacin 30µg; VAN: Vancomycin 30µg; CPZ: Ceftazidime 30µg; MEM: Meropenem 10µg; R: Resistant; S: Sensitive; I: Intermediate; MAR: Multiple Antibiotic Resistance

Table 4: Susceptibility of bacterial isolates to selected antibiotics

Bacterial isolates	AMK (%)	CHL (%)	CIP (%)	COT (%)	CPZ (%)	CRX (%)	CTR (%)	CTX (%)	GEN (%)	MEM (%)	TET (%)
Gram-positives											
Staphylococcus aureus	11 (78.6)	9 (64.3)	12 (85.7)	4(28.6)	0	0	0	0	3 (21.4)	0	2 (14.3)
Staphylococcus epidermidis	2 (100)	1 (50)	1 (50)	1 (50)	0	0	0	0	0	0	0
Staphylococcus intermedius	0	1 (100)	1 (100)	1 (100)	0	0	0	0	1 (100)	0	1 (100)
Enterococcus faecalis	3 (100)	2 (66.7)	2 (66.7)	1 (33.3)	0	0	0	0	2 (66.7)	0	0
Micrococcus luteus	3 (100)	2 (66.7)	2 (66.7)	0	0	0	0	0	2 (66.7)	0	1 (33.4)
Bacillus spp	26 (86.7)	27 (90)	29 (96.7)	9 (30)	1 (3.3)	0	2 (6.7)	1 (3.3)	14 (46.7)	1 (3.3)	11 (36.7)
Gram-negatives											
Escherichia coli	13 (61.9)	12 (57.1)	19 (90.5)	3 (14.3)	0	0	0	0	7 (33.3)	0	2 (9.5)
Klebsiella pneumoniae	6 (75.0)	6 (75.0)	6 (75.0)	2 (25.0)	0	0	0	0	2 (25.0)	0	1 (12.5)
Salmonella Typhi	2 (100)	0	1 (50)	0	0	0	0	0	0	0	0
Proteus vulgaris	2 (100)	1 (50)	1 (50)	0	0	0	0	0	0	0	0
Shigella sonnei	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	1 (100)	0	1 (100)
Enterobacter aerogenes	0	0	1 (100)	0	0	0	0	0	1 (100)	0	0
Proteus mirabilis	1 (100)	0	1(100)	0	0	0	0	0	0	0	0
Serratia marcescens	1 (100)	1 (100)	1 (100)	1 (100)	0	0	0	0	1 (100)	0	0

TET: Tetracycline 10µg; COT: Cotrimoxazole 25µg; GEN: Gentamicin 10µg; CRX: Cefuroxime 30µg; CHL: Chloramphenicol 10µg; CTR: Ceftriaxone 30µg; CTX: Cefotaxime 30µg; CIP: Ciprofloxacin 5µg; AMK: Amikacin 30µg; VAN: Vancomycin 30µg; CPZ: Ceftazidime 30µg; MEM: Meropenem 10µg

Table 5: *In vitro* antimicrobial activity (average zone diameter of inhibition in mm) of bacterial species isolated from toilet seats to selected toilet cleaning agents

Bacterial isolates	Mean zone diameter of inhibition (mm)							
	IZAL	JENIC	HARPIC	SPARK	SQUICK	HYPO	LUVIC	1% CETRIMIDE
Gram-positives								
Staphylococcus aureus (n=17)	23	19	30	25	20	26	21	13
Micrococcus luteus (n=3)	22	22	32	26	22	28	20	14
Enterococcus faecalis (n=3)	25	22	30	29	23	29	25	17
Bacillus spp (n=30)	24	21	34	28	23	29	22	15
Gram-negatives								
Escherichia coli (n=21)	24	20	32	26	20	28	21	14
Klebsiella pneumoniae (n=8)	24	21	30	27	23	29	24	16
Salmonella Typhi (n=2)	23	22	35	28	22	30	23	13
Proteus mirabilis (n=3)	24	22	31	28	24	29	24	15
Shigella sonnei (n=1)	30	18	40	25	23	35	30	18
Enterobacter aerogenes (n=1)	25	16	35	25	25	25	20	10
Serratia marcescens (n=1)	21	18	45	35	21	25	22	12

Discussion:

Public toilets are required when there is a need to eliminate bodily waste in public settings. Safe, private and purposeful visits of public toilets are guided by factors as accessibility, availability and adaptability (23). Infections, which may be bacterial, fungal or viral in origin, are one of the hazards associated with public toilet use. All the pathogens responsible for toilet-associated infections may be harbored in any part of the toilet. However, less attention has been given to toilet seats as inanimate objects which could harbor and serve as a vehicle for transmission of infectious agents despite that hazards associated with toilet seats have been reported by researchers.

In this study, several species of bacteria were isolated and identified from the toilet seats. The finding in this study agrees with the report of Juliene et al., (24) on identification of bacteria from public toilets in Rwanda. Many of the isolates in our study have been reported by many researchers (1,7-9). The presence of these pathogens on toilet seats is an indication of unhygienic toilet use. For instance, the presence of *Bacillus* spp is an indication of a long-time settlement of dust on toilet seats without being cleaned, *Bacillus* spp being a spore-forming Gram-positive bacterium found in soil but also in the gastrointestinal tract of humans. The pathogenesis of the *Bacillus* species depends upon the survival of spores in the non-host environment, as dormant spores are the main infectious form of the bacterium (25). The presence of *E. coli* and *K. pneumoniae* indicates faecal contamination as well as human vectors involvement. These two species of bacteria are the leading causes of urinary tract infections. *Staphylococcus aureus* is a major component of the normal flora of the skin and nose, which can easily be discharged by several human activities.

Treatment of infections acquired from toilet seats often involves the use of antibiotics, the choice of which depends on the susceptibility of the organism responsible for the infection. In this study, the bacterial isolates displayed varying degrees of susceptibilities with majority of the isolates being multiple antibiotic-resistant. However, the presence of drug-resistant *Shigella* spp and *Salmonella*

Typhi in this study poses a threat to human health and requires a serious health control intervention. Although majority of the isolates were resistant to multiple antibiotics, they were however susceptible to ciprofloxacin, a fluoroquinolone antibiotic. This finding also corroborates the study of Lincy et al., (26) who reported high susceptibility of bacterial isolates from public toilets to ciprofloxacin.

Acquisition of infections as well as the potential for pathogen transmission through toilet seats can be curtailed through good toilet hygiene which can be achieved through the use of toilet cleaning agents (toilet sanitizers/cleaners). Toilet cleaners differ in their compositions and actions. They can be classified into cleaners containing acids for removing calcium or metal salts and cleaners containing a bleaching system (27). In this study, all the toilet cleaners demonstrated high *in vitro* antibacterial activity against all the bacterial isolates, compared to 1% cetrimide used as the standard positive control and irrespective of the resistance of the isolates to antibiotics.

Conclusion:

This study showed that toilet seats harbor pathogenic bacteria that are resistant to multiple antibiotics while all the brands of toilet cleaners marketed in Nigeria and tested in this study exhibited high *in vitro* antimicrobial activity against the isolates. However, toilets should be shared with caution among students of tertiary institutions since all the bacteria isolated in the study are known to be associated with various forms of infection in human.

Contributions of authors:

MOO designed the study and was involved in collation, analysis and interpretation of the data, and writing of the manuscript. OS-RO was involved in culture isolation, identification and antibiotic susceptibility testings of bacterial isolates. FOA was involved in culture isolation, identification, antibiotic susceptibility testings of bacterial isolates, collation, and analysis of data. DIO was involved in antibiotic susceptibility testings of bacterial isolates, collation and analysis of data.

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