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High prevalence of multidrug-resistant bacteria in fomites in a tertiary institution in Southwestern Nigeria

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ABSTRACT

Background: The emergence and spread of multidrug-resistant (MDR) bacteria have become a major threat to public and environmental health because they limit treatment options against infections. Fomites have been implicated in the spread of pathogenic and resistant bacteria, making them an important source of disease. This study was carried out to determine the frequency and characteristics of resistant bacteria linked with environmental fomites in a tertiary institution in Nigeria as part of global efforts to provide information to contain the spread of antimicrobial resistance.

Methods: 300 swab samples, determined by factorial design, were aseptically collected from 10 fomites in 10 locations in three different facilities at Obafemi Awolowo University, Nigeria, and transported to the laboratory for processing. The isolates were identified by standard procedure and the Microbact™ identification kit. The susceptibility profile of the isolates was determined using Kirby-Bauer disc diffusion technique. MDR bacteria were examined for the presence of plasmids using the alkali lysis method, and plasmid-mediated quinolone resistance, extended-spectrum beta-lactamase, and methicillin resistance genes by polymerase chain reaction.

Results: 89 diverse bacteria were isolated from fomites in the study environment. *Staphylococcus aureus* (49.4%) predominated among the bacteria, followed by *Staphylococcus saprophyticus* (10.1%), *Providencia stuartti* (5.6%), *Proteus vulgaris* (4.5%), and *Acinetobacter baumannii* (3.4%). The isolates were commonly (84%-100%) resistant to ceftazidime, cefuroxime, and augmentin, while the least (8%-12.5%) resistance was to ciprofloxacin, amikacin, meropenem, and streptomycin. 58 (65.2%) isolates were MDR, and the majority (27.6%) were isolated from floors. 47 (81%) MDR isolates harbored plasmids, with their molecular weights between 2.697 kbp and 4.011 kbp. *mecA*, *TEM,* and *OqXAB* resistance genes were detected in *S. aureus*, *P. stuarttii*, *Pseudomonas* sp, and *K. oxytoca*.

Conclusions: This study has shown that fomites in the sampled locations are potential reservoirs for MDR bacteria, and may serve as sources of their transmission, thereby posing a threat to public health. Therefore, appropriate sanitary measures are required to prevent any potential cross-contamination. In addition, it is imperative to regularly survey environments for bacterial contamination to increase community awareness and education regarding hygiene standards.

Keywords: fomites, multidrug-resistance, bacteria, *TEM*, *OqxAB*, *Staphylococcus aureus*

INTRODUCTION

Antimicrobial-resistant infections have emerged as significant global public health concerns. Hence, treating these infections has become one of humanity's major challenges [1]. Antimicrobial resistance (AMR) threatens the foundations of modern medicine and the long-term viability of the global public health response to infectious diseases. Without concerted and urgent global action, the world is on the edge of entering a post-antibiotic age, where common diseases could kill again [2, 3].

AMR develops as bacterial populations are exposed to antimicrobial agents. These populations, which include human and animal microbiome interact with antibiotics used for therapeutic purposes [4]. Antibiotic-resistant bacteria are spread among individuals in public places like schools, hospitals, and other areas with a high concentration of people. A parallel genetic exchange takes place between the normal bacterial microflora and antibiotic-resistant microorganisms that have entered such environments through biological secretions from humans and animals [5]. Different types of bacteria, antimicrobial chemicals, and heavy metals mix in the environment, contributing to the development and dissemination of antibiotic resistance [6].

Available data indicate that fomites, which are generally considered as inanimate objects, can convey resistant pathogens to a new human host [7]. Fomites have been linked to the spread of resistant infections in settings with high-exposure levels, such as hospitals, daycare centers, nursing

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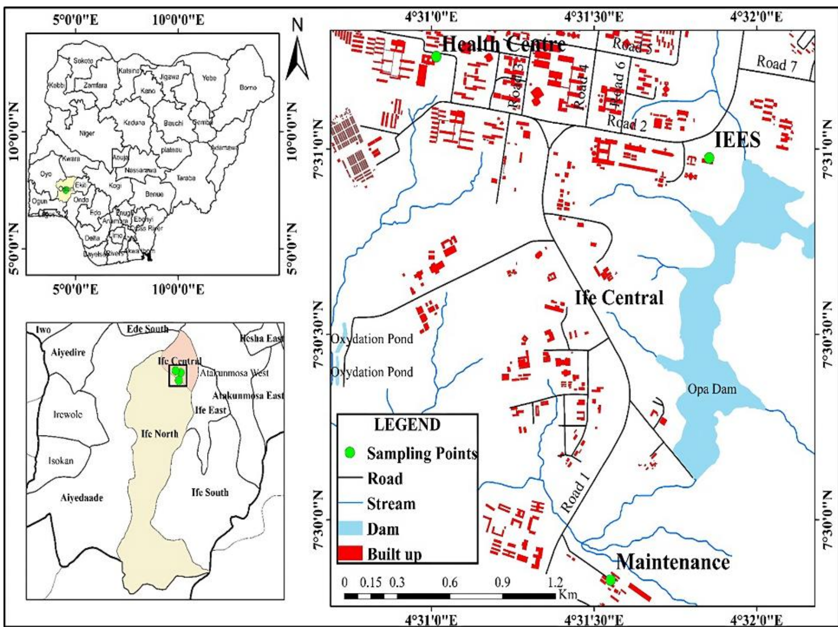

Figure 1. A map of Obafemi Awolowo University, Ile-Ife showing sampling points of selected facilities (Maintenance Unit, Health Center, & Institute of Ecology & Environmental Studies [IEES]) (Source: Authors' own elaboration)

homes, sports facilities. In such settings, various types of bacteria including extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* (MRSA), and *Salmonella enterica,* capable of causing catheter-associated bacteremia, necrotizing fasciitis, and gastroenteritis, have been identified. [8, 9] Similarly, a previous study by Kassem *et al*. in a university environment, found that computer keyboards from university students harbored MRSA, methicillin-resistant *S. epidermidis* (MRSE), and methicillin-resistant *S. hominis* (MRSH), which might contribute to spread of pathogens in community [10].

On a daily basis, people come in contact with many types of environmental fomites, some of which may harbor bacterial pathogens. Pathogen survival on fomites allows for possible spread to additional fomites and hands, raising the danger of person-to-person transmission and putting human health at risk if no preventive measures are taken [11]. Previous studies on bacteria on fomites in Nigeria have primarily focused on hospital settings, with a paucity of data from non-hospital settings, particularly in university environments with a large human presence and demand on existing facilities, which can contribute to contamination. This study, thus, was conducted to determine prevalence and characteristics of resistant bacteria from environmental fomites in a tertiary institution environment, to identify sources, where preventive interventions may be targeted, and to provide baseline data for AMR control.

METHODS

Study Area

The study was carried out at Obafemi Awolowo University, Ile-Ife, Osun State, Southwestern Nigeria from January 2018 to June 2018. Swab samples were collected from three selected facilities (Institute of Ecology and Environmental Studies, Maintenance Unit, and Health Center) within coordinates latitude 7° 31' 18'' N to 7° 29' 48'' N and longitude 4° 30' 50'' E to 4° 32' 0'' E (**Figure 1**).

Sample Collection and Processing

300 samples (using a factorial design [10×10×3]) of sterile swabs soaked in normal saline were used to swab ten environmental fomites, which included tables, chairs, shelves, floors, windows, door handles, drawer handles, computer keyboards, socket switches, and air-conditioner/fan in ten locations (staff offices, administration offices, lecture/patients waiting rooms, workshops, and laboratories) in three different facilities (Institute of Ecology and Environmental Studies, Maintenance Unit, and Health Center) within Obafemi Awolowo University, Ile-Ife (**Figure 1**).

All samples were collected and transported without delay to the laboratory for processing. In the laboratory, microbiological analyses such as culture, Gram staining, and biochemical tests were carried out to identify the bacterial isolates.

Isolation and Identification of Bacterial Isolates

The bacterial isolates were identified using morphology, Gram's staining reaction, and biochemical properties. Microbact™ Gram-negative bacilli (GNB) 24E identification kit was used to further identify Gram-negative bacteria (Oxoid Ltd., Basingstoke, United Kingdom). For Gram-positive isolates, *Staphylococcus aureus* was isolated from mannitol salt agar after an overnight incubation as a mannitol fermenting colony. The identification was confirmed microscopically by the characteristic appearance as Gram-positive cocci in clusters after Gram staining. Catalase test was used to distinguish between *Staphylococcus* (catalase-positive) and *Streptococcus* (catalase-negative). Coagulase test was used to distinguish *Staphylococcus aureus* (coagulase-positive) from *Staphylococcus* spp (coagulase-negative). *Bacillus* spp. were recovered from a blood agar medium that had been incubated for 24 hours at 37 °C. The identification was confirmed microscopically by the characteristic appearance as Gram-positive bacilli in chains after Gram staining.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of the isolates was performed using Kirby-Bauer disc diffusion method on Mueller-Hinton agar. 15 antibiotics were used, including ciprofloxacin (10 µg), augmentin (30 µg), gentamicin (10 µg), ofloxacin (10 µg), ceftazidime (30 µg), cefuroxime (30 µg), cloxacillin (5 µg), erythromycin (5 µg), cefoxitin (30 µg), ciprofloxacin (30 µg), meropenem (30 µg), tetracycline (30 µg), amikacin (30 µg), chloramphenicol (30 µg), and streptomycin (10 µg). The antibiotic discs were firmly placed on Mueller-Hinton agar plates that had previously been seeded with the standardized inocula and incubated for 24 hours at 37 °C. The diameter of the zones of inhibition was measured in millimeters and compared to the Clinical and Laboratory Standard Institute recommendations [12].

Determination of ESBL Production

All ceftazidime-resistant isolates were screened for ESBL production using the combined disk method. This involved the use of ceftazidime (30 µg) with and without 10 µg clavulanic acid placed on Mueller-Hinton agar previously inoculated with the test organisms [12]. A five-fold increase in the diameter of the inhibition zone when the cephalosporin disk was combined with clavulanic acid over cephalosporin alone indicated ESBL production. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

Plasmid Profiling

Plasmid deoxyribonucleic acid (DNA) was extracted using an alkaline lysis method [13]. Three colonies of a 24-hour-old bacterial culture (1.5 mL) were put in an Eppendorf tube and centrifuged at 14,000 rpm for five minutes in a micro-centrifuge machine. After centrifugation, the supernatant was discarded, leaving the bacterial pellet, which was resuspended in 300 µL of TENS solution and vortexed for five seconds to mix, which made the content of the tube slimy. Sodium acetate (150 µL) was added and vortexed for five seconds. The tube's contents were spun for two minutes in a micro-centrifuge, resulting in a white pellet containing bacterial debris at the bottom of the tube. The supernatant was transferred to a new Eppendorf tube, 0.9 ml of cold 100% ethanol was added, and the tube was spun for five minutes in a micro-centrifuge to generate white pellets containing plasmid DNA and bacterial RNA at the bottom. The supernatants were discarded, and 1 mL of 70% ethanol was added to wash the DNA. The procedure was repeated to remove as much liquid (ethanol) as possible before the pellet was air-dried at room temperature. The plasmids were then resuspended in 50 µL of TE buffer with 30 µL of RNase and visualized on a 1% agarose gel stained with 0.5 µg/mL ethidium bromide and ran at 100 V for 60 minutes.

DNA Extraction

The isolates' DNA was extracted using the boiling method [14]. Briefly, three colonies of each isolate were emulsified in 100 µL of sterile distilled water in an Eppendorf tube, heated for 15 minutes, then centrifuged in a micro-centrifuge at 10,000 rpm for five minutes. After centrifugation, the supernatant was transferred to a sterile Eppendorf tube and utilized as a DNA template for polymerase chain reaction (PCR).

Detection of Extended Spectrum of Beta-Lactamase Genes

All ESBL-positive isolates were screened by PCR using SHV (SHV-F: CGCCTGTGTATTATCTCCCT and SHV-R: CGAGTAGTCCA CCAGATCCT), TEM (TEM-F: TTTCGTGTCGCCCTTATTCC and TEM-R: ATCGTTGTCAGAAGTAAGTTGG), and CTX-M (CTX-M-F: CGCTGTTGTTAGGAAGTGTG and CTX-M-R: GGCTGGGTGAAGTAA GTGAC) specific primers as previously described [15]. The targets were amplified using the following conditions: initial denaturation at 94 °C for five minutes, followed by 35 denaturation cycles at 94 °C for one minute, annealing at 52 °C for TEM, SHV, and CTX-M, extension at 72 °C for one minute, and final extension at 72 °C for three minutes. *Klebsiella pneumoniae* ATCC 700603 and *Escherichia coli* ATCC 25922 were used as positive and negative controls, respectively.

Detection of *mecA* Genes

All cefoxitin-resistant *Staphylococcus aureus* isolates were screened for the presence of *mecA* gene (533 bp) using the mecA forward primer CCAATTCCACATTGTTTCGGTCTA and mecA reverse primer GAAATGACTGAACGTCCGATAAA. The target was amplified using the following conditions: Initial denaturation for five minutes at 94 °C, followed by 35 denaturation cycles for one minute at 94 °C, annealing at 52 °C, extension for one minute at 72 °C, and final extension for three minutes at 72 °C [16].

Detection of PMQR Genes

For the ciprofloxacin-resistant isolates, the multiplex PCR protocol developed for the plasmid-mediated quinolone resistance (PMQR) genes was used [17]. The isolates were screened for the PMQR genes in two sets using specific primers for *qnrA* (forward: CAGCAAGAGGATTTCTCACG and reverse: AATCCGGCAGCACTATTACTC), *qnrB* (forward: GGCTGTCAGTT CTATGATCG and reverse: GAGCAACGATGCCTGGTAG), *qnrC* (forward: GCAGAATTCAGGGGTGTGAT and reverse: AACTGCT CCAAAAGCTGCTC), *aac(6')-lb-cr* (forward: TTGCGATGCTCTAT GAGTGGCTA and reverse: CTCGAATGCCTGGCGTGTTT) and *OqxAB* (forward: CCGCACCGATAAATTAGTCC and reverse: GGCG AGGTTTTGATAGTGGA). The amplification conditions for the first set of *qnrA*, *qnrC*, and *qnrB* were, as follows: an initial denaturation at 94 °C for four mins; 30 cycles of 94 °C for 30 seconds, optimized annealing temperature of 55 °C for 30 seconds and initial extension at 72 °C for one minute; followed by a final extension at 72 °C for five minutes. For the second set, *aac(6')-lb-cr* and *OqxAB*, the amplification conditions were initial denaturation at 94 °C for four minutes, followed by 30 cycles of denaturation at 94 °C for 45 seconds, annealing at 55 °C for 45 seconds, initial extension at 72 °C for one minute then a final extension at 72 °C for three minutes.

Visualization of the Genes

Each amplicon (10 µL) was electrophoresed on a 1.5% agarose gel, pre-stained with 0.5 µg/mL of ethidium bromide in 1X Tris-Borate-EDTA (TBE) buffer, and examined with a UVitec transilluminator (Avebury, Cambridge UK). The position of the 100 bp molecular weight marker was used to estimate the position of amplified products (Biolab, England).

Statistical Analysis

The data were analyzed using Excel software and Jamovi (1.6) statistical tool. Data were presented in the form of frequencies and percentages.

Table 1. Prevalence of bacteria isolated from the facilities

Bacterial isolates	IEES (n)	%	Health center (n)	%	Maintenance unit (n)	%	Total (n)	%
Gram-positive	**16**	18.0	**27**	30.3	21	23.6	**64**	71.9
Staphylococcus aureus	9	10.1	23	25.8	12	13.5	44	49.4
Streptococcus sp	0	0.0	0	0.0	2	2.2	2	2.2
Staphylococcus saprophyticus	4	4.5	1	1.1	4	4.5	9	10.1
Corynebacterium sp	3	3.4	3	3.4	3	3.4	9	10.1
Gram-negative	**9**	10.1	**4**	4.5	**12**	13.5	**25**	28.1
Escherichia coli	1	1.1	1	1.1	0	0.0	2	2.2
Klebsiella pneumoniae	1	1.1	0	0.0	1	1.1	2	2.2
Klebsiella oxytoca	0	0.0	0	0.0	1	1.1	1	1.1
Acinetobacter baumanii	1	1.1	1	1.1	1	1.1	3	3.4
Klebsiella ozaenae	1	1.1	0	0.0	0	0.0	1	1.1
Pseudomonas sp	1	1.1	0	0.0	2	2.2	3	3.4
Providencia stuartti	1	1.1	0	0.0	4	4.5	5	5.6
Proteus vulgaris	1	1.1	1	1.1	2	2.2	4	4.5
Yersinia enterocolytica	0	0.0	0	0.0	1	1.1	1	1.1
Morganella morganii	0	0.0	1	1.1	0	0.0	1	1.1
Acinetobacter iwoffii	2	2.2	0	0.0	0	0.0	2	2.2
Total	**25**	28.1	**31**	34.8	**33**	37.1	**89**	100.0

Note. IEES: Institute of Ecology and Environmental Studies

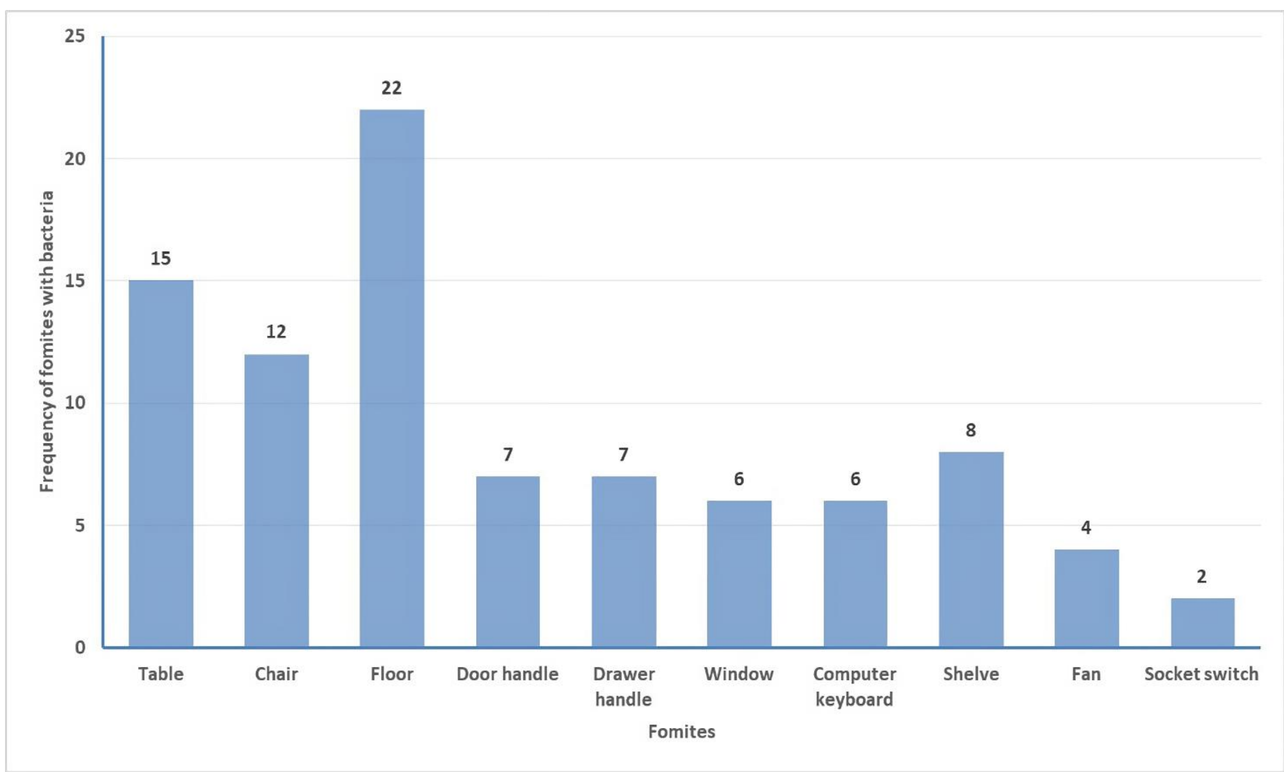

Figure 2. Frequency of bacteria in different fomites (n=89) (Source: Authors' own elaboration)

RESULTS

Percentage Distribution of Bacterial Isolates

In all, 89 bacteria were isolated from fomites in the three facilities. A total of 25 genera were identified with 71.9% as Gram-positive and 28.1% as Gram-negative bacteria. Among Gram-positive bacteria, *Staphylococcus aureus* (44; 49.4%) predominated, followed by *Staphylococcus saprophyticus* (nine; 10.1%), and *Corynebacterium* sp (nine; 10.1%). Among Gram-negative bacteria, *Providencia stuartti* (five; 5.6%) predominated followed by *Proteus vulgaris* (four; 4.5%), *Pseudomonas* sp (three; 3.4%), *Acinetobacter baumannii* (three; 3.4%), and *Klebsiella pneumoniae* (2; 2.2%) (**Table 1**).

The highest number of bacteria was isolated from floors (22; 24.7%) followed by tables (15; 16.3%), chairs (12; 13.5%), shelves (eight; 9%) and door handles (seven; 7.9%) (**Figure 2**).

Antibiotic Resistance Profile of the Isolates

As shown in **Table 2**, antibiotic resistance varied greatly among isolates. Among Gram-positive bacterial isolates, high rates of resistance to ceftazidime (100%), cefuroxime (100%), ceftriaxone (100%), cloxacillin (98.4%), and erythromycin (87.8%) were observed, while low rates of resistance to streptomycin (6.3%) and amikacin (6.3%) were observed. Among Gram-negative isolates, high rates of resistance to augmentin (84%), tetracycline (83.3%), and ceftazidime (83.3%) were observed, while low rates of resistance to ciprofloxacin (8%) and amikacin (12.5%) were observed.

Table 2. Antimicrobial resistance profile of Gram-positive isolates from selected facilities: n(%)

Bacterial isolates	CIP	AUG	OFLO	ERY	AMK	TET	AMP	MEM
Gram-positive (n=64)	**12(18.8)**	**60(93.6)**	**12(18.8)**	**56(87.8)**	**4(6.3)**	**ND**	**ND**	**ND**
Staphylococcus aureus (n=44)	6(13.6)	40(90.9)	7(15.9)	44(100)	0(0.0)	ND	ND	ND
Streptococcus spp (n=2)	0(0.0)	2(100)	0(0.0)	1(50.0)	0(0.0)	ND	ND	ND
Staphylococcus saprophyticus (n=9)	4(44.4)	9(100)	1(11.1)	9(100)	0(0.0)	ND	ND	ND
Corynebacterium spp (n=9)	2(22.2)	9(100)	4(44.4)	2(22.2)	4(44.4)	ND	ND	ND
Gram-negative (n=25)	**2(8)**	**21(84)**	**7(29.2)**	**15(62.5)**	**3(12.5)**	**20(83.3)**	**15(60)**	**15(60)**
Acinetobacter baumannii (n=3)	0(0.0)	2(66.7)	0(0)	1(33.3)	2(66.7)	2(66.7)	1(33.3)	1(33.3)
E. coli (n=2)	0(0.0)	1(50.0)	0(0)	1(50.0)	0(0.0)	2(100)	2(100)	1(50.0)
Morganella morganii (n=1)	0(0.0)	0(0.0)	0(0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)
Proteus vulgaris (n=4)	0(0.0)	4(100)	0(0)	3(75.0)	0(0.0)	4(100)	1(25.0)	3(75.0)
Klebsiella pneumoniae (n=2)	0(0.0)	2(100)	0(0)	2(100)	0(0.0)	2(100)	2(100)	2(100)
Klebsiella oxytoca (n=1)	1(100)	1(100)	0(0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)
Klebsiella ozanae (n=1)	0(0.0)	1(100)	0(0)	0(0.0)	0(0.0)	0(0.0)	1(100)	0(0.0)
Acinetobacter iwoffi (n=2)	0(0.0)	2(100)	0(0)	1(50.0)	0(0.0)	2(100)	2(100)	1(50.0)
Yersinia enterocolytica (n=1)	0(0.0)	1(50.0)	0(0)	1(100)	0(0.0)	1(100)	0(0.0)	1(100)
Providencia stuartii (n=5)	1(20.0)	5(100)	5(100)	3(60.0)	1(20.0)	4(80.0)	3(60.0)	3(60.0)
Pseudomonas spp (n=3)	0(0.0)	2(667)	2(66.7)	3(100)	0(0.0)	3(100)	1(33.3)	3(100)
Total (n=89)	**14(15.7)**	**81(91)**	**19(21.3)**	**71(79.8)**	**7(7.9)**	**20/25(80.0)**	**15/25(60.0)**	**15/25(60.0)**

Bacterial isolates	CAZ	CHL	CRX	S	GEN	CXC	CX
Gram-positive (n=64)	**64 (100)**	**ND**	**64 (100)**	**4(6.3)**	**26(40.6)**	**63(98.4)**	**33(51.6)**
Staphylococcus aureus (n=44)	44(100)	ND	44(100)	0(0.0)	23(52.3)	43(97.7)	21(47.7)
Streptococcus spp (n=2)	2(100)	ND	2(100)	0 (0.0)	0(0.0)	2(100)	0(0.0)
Staphylococcus saprophyticus(n=9)	9(100)	ND	9(100)	0(0.0)	3(33.3)	9(100)	3(33.3)
Corynebacterium spp (n=9)	9(100)	ND	9(100)	4(44.4)	0(0.0)	9(100)	9(100)
Gram-negative (n=25)	**20(83.3)**	**17(68)**	**ND**	**ND**	**ND**	**ND**	**ND**
Acinetobacter baumannii (n=3)	1(33.3)	1(33.3)	ND	ND	ND	ND	ND
E. coli (n=2)	1(100)	2(100)	ND	ND	ND	ND	ND
Morganella morganii (n=1)	1(100)	0(0.0)	ND	ND	ND	ND	ND
Proteus vulgaris (n=4)	4(100)	3(75.0)	ND	ND	ND	ND	ND
Klebsiella pneumoniae (n=2)	2(100)	2(100)	ND	ND	ND	ND	ND
Klebsiella oxytoca (n=1)	1(100)	1(100)	ND	ND	ND	ND	ND
Klebsiella ozanae (n=1)	0(0.0)	0(0.0)	ND	ND	ND	ND	ND
Acinetobacter iwoffi (n=2)	1(50.0)	0(0.0)	ND	ND	ND	ND	ND
Yersinia enterocolytica (n=1)	1(100)	1(100)	ND	ND	ND	ND	ND
Providencia stuartii (n=5)	5(100)	4(80.0)	ND	ND	ND	ND	ND
Pseudomonas spp (n=3)	3(100)	3(100)	ND	ND	ND	ND	ND
Total (n=89)	**84(94.4)**	**17/25(68.0)**	**64/64 (100)**	**4/64(6.3)**	**26/64(40.6)**	**63/64(98.4)**	**33/64(51.6)**

Note. CIP: Ciprofloxacin; AUG: Augmentin; OFL: Ofloxacin; MEM: Meropenem; AMP: Ampicillin; CAZ: Ceftazidime; TET: Tetracycline; CHL: Chloramphenicol; GEN: Gentamicin; ERY: Erythromycin; CXC: Cloxacillin; AMK: Amikacin; CX: Cefoxitin; CRX: Cefuroxime; S: Streptomycin; & ND: Not determined

Table 3. Multiple antibiotic resistance profile of isolated bacteria (n=58): n(%)

Organisms	n(%)										
	Table	**Chair**	**Floor**	**DH**	**DRH**	**Window**	**CK**	**Shelf**	**Fan**	**SS**	**Total**
Staphylococcus aureus	6(10.3)	7(12.1)	10(17.2)	1(1.7)	3(5.2)	1(1.7)	1(1.7)	1(1.7)	3(5.2)	0(0)	33(56.9)
Staphylococcus saprophyticus	0(0.0)	0(0.0)	2(3.4)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	3(5.2)
Escherichia coli	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.4)
Klebsiella pneumoniae	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	1(1.7)
Klebsiella oxytoca	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)
Acinetobacter baumanii	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	2(3.4)
Klebsiella ozaenae	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)
Pseudomonas sp	2(3.4)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	3(5.2)
Providencia stuartti	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(3.4)	1(1.7)	0(0.0)	0(0.0)	1(1.7)	5(8.6)
Proteus vulgaris	0(0.0)	1(1.7)	1(1.7)	0(0.0)	1(1.7)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	4(6.8)
Yersinia enterocolytica	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)
Acinetobacter iwoffii	1(1.7)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(1.7)	0(0.0)	0(0.0)	2(3.4)
Total	**11(19.0)**	**9(15.5)**	**16(27.6)**	**2(3.4)**	**4(6.9)**	**5(8.6)**	**3(5.2)**	**3(5.2)**	**3(5.2)**	**2(3.4)**	**58(100)**

Note. DH: Door handle; DRH: Drawer handle; CKB: Computer keyboard; & SS: Socket switch

Prevalence of Multidrug-Resistant Isolates

As shown in **Table 3**, 65.2% (58) of 89 isolates were multidrug-resistant (MDR). The majority of MDR bacteria were isolated from floors (n=16; 27.6%); tables (n=11; 19%) and chairs (n=9; 15.5%). *Staphylococcus aureus* exhibited the highest frequency of multidrug-resistance (n=33; 56.9%) followed by *Providencia stuartii* (n=5; 8.6%) and *Proteus vulgaris* (n=4; 6.8%). The majority of the *Staphylococcus aureus* strains were isolated from floors (n=10; 17.2%).

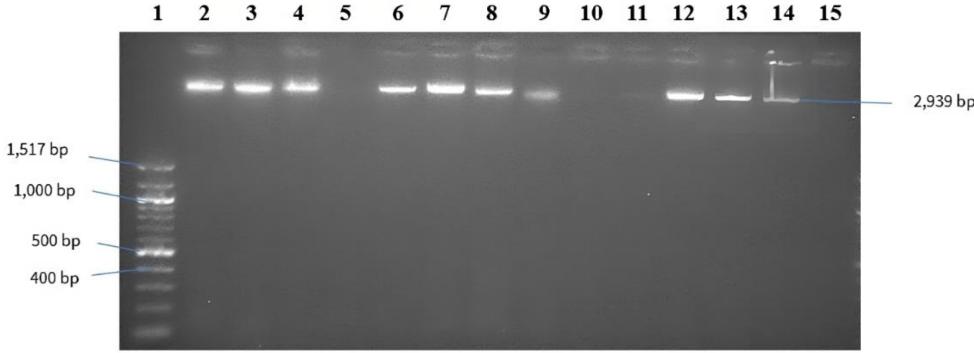

Figure 3. Plasmid picture of Gram-positive MDR isolates: lane 1: 100 bp DNA ladder; lane 2: *Pseudomonas* spp (3,109 bp); lane 3: *Klebsiella oxytoca* (3,109 bp); lane 4: *Acinetobacter iwoffi* (3,109 bp); lane 5: *Acinetobacter baumanii* (none); lane 10: *S. aureus* (none); lane 15: *Staphylococcus saprophyticus* (none); lanes 6-8: *Staphylococcus aureus* (3,109 bp); & lanes 9, 11-14: *Staphylococcus aureus* (2,939 bp) (Source: Authors' own elaboration)

Plasmid Profile of Multidrug-Resistant Bacteria and Identified Resistance Genes

58 MDR isolates were profiled for plasmid DNA analysis. 47 (81%) had at least one plasmid, while 12 (20.7%) had none. A significant proportion of the bacterial isolates (n=12; 25.5%) carried large plasmids with a high molecular weight (4011 bp), while 4 (8.5%) carried plasmids with the lowest molecular weight of 2697 bp (**Figure 3** and **Table 4**).

One cefoxitin-resistant *Staphylococcus aureus isolate,* two ceftazidime resistant (*Providencia stuarttii* and *Pseudomonas* sp) isolates, and one ciprofloxacin and ofloxacin resistant *Klebsiella oxytoca* isolate harbored *mecA* gene, *TEM*, and *OqxAB* genes, respectively. The bacteria (*Staphylococcus aureus*, *Providencia stuarttii, Pseudomonas sp,* & *Klebsiella oxytoca*) habored resistance genes were isolated from a door handle, computer keyboard, table, and socket switch, respectively.

DISCUSSION

This study reveals significant bacterial contamination of fomites in all the three facilities, with Gram-positive bacteria (71.9%) being isolated more frequently than Gram-negative bacteria (28.1%). The increased predominance of Gram-positive bacteria over Gram-negative bacteria is consistent with prior research and corroborates the assertion that Gram-positive bacteria outnumber Gram-negative bacteria as the primary group of bacteria recovered from fomites [18, 19]. This observation is due to the fact that Gram-positive bacteria are a natural part of both healthy and sick people's body flora and can be spread by hand, respiratory tract, or contact with animate or inanimate objects [20].

Staphylococcus aureus (49.4%) predominated among the isolates, followed by *Staphylococcus saprophyticus* (10.1%). The preponderance of *Staphylococcus aureus* may be due to its existence as normal flora of the skin and the upper respiratory tract, and its ability to be transmitted via various human activities such as sneezing, talking, and contact with moist skin [21]. Doorknobs are frequently handled, and *Staphylococcus* is part of the typical flora of hands, explaining its high prevalence on these surfaces in this study. This observation is consistent with what other researchers have reported [22, 23].

Our study revealed the presence of Gram-negative bacteria on a variety of inanimate surfaces. *Providencia stuartii* predominated among Gram-negative isolates followed by *Proteus vulgaris, Acinetobacter baumanii, Klebsiella spp., Acinetobacter iwoffi,* and *E. coli. Providencia stuartii* is a ubiquitous opportunistic pathogen belonging to the Enterobacteriaceae family. Its ubiquity may explain its isolation from fomites in this environment [24]. We observed a prevalence of 1.1% each for *Klebsiella oxytoca*, *Klebsiella pneumoniae*, and *Klebsiella ozanae*. Hospital-acquired infections, which primarily affect those with weakened immune systems or those that require critical care, are primarily caused by opportunistic microorganisms such as *Klebsiella oxytoca* [25]. *Klebsiella pneumoniae* are typically discovered living as commensals in the human gastrointestinal system due to their rampant nature in the environment, according to [26]. Once infected, the bacterium has a surprising proclivity to induce a wide spectrum of human ailments, from urinary tract infections to pneumonia [27]. The presence of faecal coliforms such as *E. coli* (2.2%) in this investigation suggests the presence of faecal contaminants on the doorknobs. This could be because most people after visiting the toilet, end up contaminating their hands with faecal and urinal material, and forget to wash their hands because they take cleanliness for granted and lack the concept of hand washing as a basic technique of preventing the transmission of infectious agents [28].

Antibiotic resistance varied widely among the isolates in this study. Gram-positive isolates had high rates of resistance to ceftazidime (100%), cefuroxime (100%), ceftriaxone (100%), cloxacillin (98.4%), and erythromycin (87.8%), while Gram-negative isolates were commonly resistant to augmentin (84%), tetracycline (83.3%), and ceftazidime (83.3%). These patterns confirm previous reports of substantial percentages of Gram-negative bacterial isolates that are resistant to various antibiotics, both in clinical and environmental settings [9, 29-31]. This indicates that these antibiotics cannot be used to treat infections caused by these bacteria. Antibiotics are reportedly consumed in vast quantities in clinical and environmental settings each year, which, among other causes, contributes considerably to the escalating prevalence of antibiotic-resistant bacteria [32]. Although low, the rate of resistance to ciprofloxacin in Gram-negative and Gram-positive isolates identified in this study may be related to the drug's increasing use in clinical settings. This rate of resistance to ciprofloxacin is worrying, as it used to be the drug of last resort in this part of the world for the treatment of infectious diseases [33, 34].

Table 4. Molecular weight of plasmid DNA isolated from MDR isolates

Sites	Sources	Organisms	Plasmids	Patterns
Health center	Table	*Staphylococcus aureus*	4,011 bp	AUG, CTR, CRX, ERY, CAZ, CXC, CX
		Staphylococcus aureus	4,011 bp	CTR, CAZ, CRX, ERY, CXC
	Chair	*Escherichia coli*	2,984 bp	AP, TET, CHL, CTX, CAZ
		Staphylococcus aureus	None	CRX, AUG, GEN, ERY, CTR, CAZ, CXC, CX
		Staphylococcus aureus	4,011 bp	CXC, OFL, CTR, CRX, ERY, CX, CAZ
		Staphylococcus aureus	None	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CTR, CAZ, CRX, CXC, ERY
	Floor	*Staphylococcus aureus*	None	CIP, AUG, AUG, CTR, CAZ, CRX, CXC, ERY
		Staphylococcus aureus	2,939 bp	AUG, GEN, CTR, CRX, CAZ, CXC, ERY
		Staphylococcus aureus	None	CIP, AUG, GEN, CTR, CRX, CAZ, CXC, ERY
		Staphylococcus aureus	3,009 bp	CAZ, AUG, CTR, CRX, CXC, ERY, CX
		Staphylococcus aureus	4,011 bp	AUG, GEN, OFL, CTR, CRX, CXC, CAZ, ERY, CX
		Staphylococcus aureus	4,011 bp	CIP, AUG, GEN, OFL, CTR, CRX, CAZ, CXC, ERY, CX
		Staphylococcus saprophyticus	4,011 bp	CIP, CAZ, AUG, CTR, CRX, CXC, ERY, CX
	Fan/AC	*Staphylococcus aureus*	2,939 bp	AUG, GEN, CTR, CRX, CXC, CAZ, ERY, CX
		Staphylococcus aureus	3,280 bp	CIP, AUG, CTR, CRX, CXC, CAZ, ERY, CX
		Staphylococcus aureus	4,011 bp	CIP, AUG, OFL, CTR, CRX, CXC, CAZ, ERY, CX
	Drawer handle	*Staphylococcus aureus*	3,109 bp	AUG, GEN, CTR, CRX, CXC, ERY, CAZ, CX
		Proteus vulgaris	2,838 bp	AP, TET, AUG, CTX, CAZ
	Shelf	*Staphylococcus aureus*	3,280 bp	AUG, CTR, CAZ, CRX, CXC, ERY
IEES	Floor	*Staphylococcus aureus*	3,109 bp	AUG, GEN, OFL, CTR, CAZ, CRX, CXC, ERY, CX
		Staphylococcus saprophyticus	3,109 bp	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
		Proteus vulgaris	2,984 bp	AP, TET, CHL, MEM, AUG, CTX, CAZ
		Klebsiella ozaenae	2,984 bp	OFL, CTR, CAZ, CRX, CXC, ERY, CX
		Staphylococcus aureus	2,838 bp	AP, AUG, CAZ
		Staphylococcus aureus	None	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
		Staphylococcus aureus	4,011 bp	AUG, CTR, CAZ, CRX, CXC, ERY
	Table	*Acinetobacter iwoffi*	3,109 bp	AP, TET, AUG, CTX, CAZ
		Providencia stuartii	2,838 bp	AP, TET, MEM, AUG, CTX, CAZ
	Chair	*Staphylococcus aureus*	3,109 bp	CIP, AUG, CTR, CRX, CXC, CAZ, ERY
		Staphylococcus aureus	3,575 bp	AUG, GEN, CTR, CRX, CXC, ERY, CAZ, CX
		Staphylococcus auerus	3,280 bp	CIP, AUG, CTR, CRX, CXC, CAZ, ERY
	Window	*Proteus vulgaris*	2,838 bp	TET, CHL, MEM, AUG, CTX, CAZ
		Providencia stuartii	2,838 bp	AP, TET, CHL, MEM, AK, AUG, CTX, CAZ, CIP
		Escherichia coli	2,697 bp	AP, TET, CHL, MEM, AUG, CAZ,
		Providencia stuartii	2,697 bp	AP, TET, CHL, AUG, CTX, CAZ, CIP
		Staphylococcus aureus	4,011 bp	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
	Door handle	*Pseudomonas spp*	2,838bp	AP, TET, CHL, MEM, AUG, CTX, CAZ
	Computer keyboard	*Staphylococcus saprophyticus*	None	CIP, AUG, CTR, CAZ, CRX, CXC, ERY, CX
	Shelve	*Acinetobacter iwoffi*	2,838 bp	AP, TET, CHL, MEM, AUG, CTX, CAZ
Maintenance unit	Door handle	*Staphylococcus aureus*	3,109 bp	CIP, AUG, GEN, OFL, CTR, CAZ, CRX, CXC, ERY, CX
	Chair	*Staphylococcus aureus*	None	AUG, CTR, CAZ,CRX, CXC, ERY
		Proteus vulgaris	None	TET, MEM, AUG, CTX, CAZ
		Staphylococcus aureus	4,011 bp	AUG, CTR, CAZ, CRX, CXC, ERY
	Table	*Pseudomonas spp*	3,109 bp	AP, TET, AUG, CAZ
		Klebsiella oxytoca	3,109 bp	AP, CHL, AUG, CTX, CAZ, CIP
		Staphylococcus aureus	3,109 bp	CIP, AUG, CTR, CAZ, CRX, CXC, ERY
		Staphylococcus aureus	3,575 bp	OFL, CTR, CAZ, CRX, CXC, ERY, CX
		Pseudomonas spp	4,011 bp	TET, MEM, AUG, CTX, CAZ, OFL
	Shelf	*Klebsiella pneumonae*	2,697 bp	AP, TET, CHL, MEM, AUG, CTX, CAZ
	Computer keyboard	*Provedencia stuartii*	2,838 bp	TET, CHL, MEM, AUG, CTX, CAZ
		Staphylococcus aureus	4,011 bp	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
	Drawer handle	*Staphylococcus aureus*	None	AUG, GEN, CTR, CAZ, CRX, CXC, ERY
		Staphylococcus aureus	None	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
	Floor	*Yersinia enterocolitica*	2,838 bp	TET, CHL, MEM, AUG, CTX, CAZ
		Acinetobacter baumanii	2,697 bp	CHL, AUG, CAZ
		Staphylococcus aureus	None	AUG, GEN, CTR, CAZ, CRX, CXC, ERY, CX
	Socket switch	*Acinectobacter baumanii*	None	TET, CHL, MEM, AUG, CTX, CAZ
		Providencia stuartii	2,838 bp	TET, CHL, MEM, AUG

Note. CIP: Ciprofloxacin; AUG: Augmentin; GEN: Gentamicin; ERY: Erythromycin; OFL: Ofloxacin; TET: Tetracycline; MEM: Meropenem; CX: Cefoxitin; CAZ: Ceftazidime; CRX: Cefuroxime; CTR: Ceftraxone; CXC: Cloxacillin; AMP: Ampicillin; & CHL: Chloramphenicol

In our study, we observed a high level of resistance to third-generation cephalosporins (ceftazidime and cefuroxime), erythromycin, and cloxacillin, which is consistent with previous reports of a high prevalence of resistance to these classes of antibiotics [9, 35, 36]. The low resistance percentage of the isolates to the aminoglycosides: amikacin (7.9%), and streptomycin (6.3%), is in line with a similar study that recorded 73.1% of the isolates' sensitivity to an aminoglycoside in public places [37].

Resistance to three or more antibiotics was identified in 58 (65.2%) isolates, with *Staphylococcus aureus* demonstrating the highest rate of multidrug-resistance (56.9%). The majority

of MDR bacteria were isolated from floors (27.6%). Infections with antibiotic-resistant bacteria have a detrimental effect on public health, increasing the rate of treatment failure and disease severity [38]. Globally, disease treatment is complicated by the rise of bacterial resistance to several antibiotics [39]. Bacterial resistance to antibiotics may be caused by improper antibiotic use in humans and livestock, as well as incorrect and inferior prescriptions written by unqualified medical personnel and poor diagnosis [40, 41].

We also analyzed the plasmid DNA profiles of the 58 MDR isolates. 47 (81%) of the 58 isolates carried at least one plasmid. A considerable proportion (20.7%) of the bacterial isolates contained plasmids with a high molecular weight (4,011 bp), while 11 isolates (19%) carried low molecular weight plasmids (2,838 bp). Although some antibiotic resistance can be attributed to chromosomal alterations, it is most usually connected with extrachromosomal elements such as transposons, plasmids, and integrons that have been acquired from other bacteria in the environment [14, 42]. The presence of plasmids in several antibiotic-resistant isolates raises concerns about the possibility of antibiotic resistance genes being transferred between species, thereby increasing the diversity and quantity of antibiotic-resistant organisms on public interfaces [43]. Although plasmids have been implicated directly in the acquisition of resistance to antibiotics, several other mechanisms, such as antibiotic entry blockade, efflux mechanism, antibiotic enzymatic inactivation, and target site alteration, may also contribute to the antibiotic resistance phenomenon [44].

We identified *mecA* gene in a cefoxitin-resistant *Staphylococcus aureus* strain isolated from a door handle. Penicillin-binding protein 2a is encoded by the *mecA* gene identified in MRSA. This protein plays a role in methicillin resistance in *Staphylococcus* and results in a lower affinity for beta-lactam drugs [45]. MRSA has been detected in colleges and universities on a range of high-touch surfaces, including lockers, locker rooms, elevators, athletic training facilities, dorm floors, and public computers. Also, students' possessions, such as cell phones and door keys, have been found to be contaminated with MRSA [46]. The increased usage of antibiotics predisposed *S. aureus* to antibiotic resistance, especially methicillin resistance, leading to methicillin-resistant *S. aureus*. The presence of MRSA in public communal settings reflects a lack of basic hygiene standards among those who work or participate in activities related to these settings. This undoubtedly increases the likelihood of the spread of infectious diseases, which are notoriously difficult to regulate from a public health standpoint.

In this study, *TEM* genes were found in ceftazidime-resistant *Providencia stuarttii* and *Pseudomonas* spp isolated from computer keyboards and tables respectively. TEM enzymes are capable of hydrolyzing penicillin, older cephalosporins, and oxyimino-cephalosporins, such as cefotaxime and ceftazidime with relative ease. Other investigators have also detected this gene in Enterobacteriaceae and other Gram-negative bacteria in different settings globally [47, 48]. The detection of *TEM* gene could undermine the treatment of infections caused by third-generation cephalosporin-resistant isolates.

A quinolone-resistant isolate of *Klebsiella oxytoca* from a socket switch harbored *OqxAB* gene as observed in our study. According to [49], *OqxAB* gene encodes an efflux pump that confers resistance to quinoxaline-diNoxide olaquindox and raises the minimum inhibitory concentrations of flumequine, ciprofloxacin, and norfloxacin in bacterial cells. Our finding is noteworthy since it is the first time that an *OqxAB* gene carrying *Klebsiella oxytoca* has been isolated from fomites in this environment. *OqxAB* gene has been identified in human, animal, and environmental *E. coli* isolates [49-51]. It has previously been linked to IS26, which was discovered on the 43 kb to 115 kb IncF transferable plasmid [50]. The discovery of this PMQR gene in our investigation has potential to jeopardize the therapeutic use of fluoroquinolones in the treatment of infectious illnesses, posing a significant threat to public health.

CONCLUSIONS

A high diversity of bacteria was found on fomites in the study environments, with Gram-positive bacteria being isolated more frequently than Gram-negative bacteria. *S. aureus* was the most common bacteria found in the environmental fomites from the three selected facilities. The highest number of bacteria was isolated from floors and tables. Most of the bacteria, which included *Staphylococcus aureus, Staphylococcus saprophyticus, Providencia stuartti, Proteus vulgaris* and *Acinetobacter baumannii*, were clinically significant multiple antibiotic-resistant bacteria with 2.697 kbp to 4.011 kbp plasmids.

Recommendations

As part of infection control measures, to prevent indirect transmission through environmental fomites, appropriate hygienic measures to suppress any potential microbial cross-contamination are needed. Students and workers must practice personal hygiene and also carry hand-sanitizer to disinfect their hands regularly. There should also be proper regular cleaning of the environments with effective concentrations of disinfectant and decontamination solutions. Also, it is imperative to regularly survey the environments for bacterial contamination to increase community awareness and education regarding hygiene standards.

Limitations

The scale of the study was limited because it was not funded. Only the fomites in three selected facilities were investigated. Larger studies are needed to further investigate the magnitude of AMR in this environment. In spite of these limitations, the study has provided baseline data for further investigation of the problem of AMR in this environment. It has also identified critical areas where preventive interventions can be targeted in the environment to prevent outbreaks of resistant infections.

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