Research Article

Bacterial count and methicillin-resistant *Staphylococcus aureus* contamination of classroom and toilet door handles in a tertiary institution, Cameroon: Need for infection control in the non-hospital environment

Seraphine Nkie Esemu[1,2*], Mingo Victorine Ndiwago[1], Jerome Kfusi Achah[1,2], Lucy Mande Ndip[1,2]

[1]Department of Microbiology and Parasitology, Faculty of Science, University of Buea, P.O. Box 63, Buea, Cameroon.

[2]Laboratory for Emerging Infectious Diseases, University of Buea, P.O. Box 63, Buea, Cameroon

Corresponding author: esemu2003@yahoo.co.uk

Abstract

High bacterial load and the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) on high-touch surfaces in congregate settings like schools is a major public health problem. This study sought to determine the bacterial load, prevalence, and antimicrobial-resistant patterns of MRSA on classroom and toilet door handles at the University of Buea. A total of 306 swabs were collected from surfaces of classroom and toilet door handles in this cross-sectional study. Bacterial count, isolation, identification, and antibiotic susceptibility testing of MRSA were performed following standard microbiological methods. *S*taphylococcus *aureus* and MRSA were confirmed by PCR, using the *nuc* and *mecA* genes, respectively. Bacterial contamination of sampled surfaces was 100%, with bacterial counts ranging from 4.1 x 10^4 - 7.2 x 10^7cfu/swab for classrooms and 1.2 x 10^4 - 4.82 x 10^8 cfu/swab for toilets. There was a significant difference (p=0.004) between *Staphylococcus aureus* contamination of classroom door handles (77.6 %, 152/196) and toilets (90.9 %, 100/110). The difference between MRSA contamination for classroom (7.7%, 15/196) and toilet (21.8%, 24/110) surfaces was also significant (p=0.003). Similarly, MRSA contamination rates for staff toilets (10.4%, 5/48) and student toilets (30.6%, 19/62) showed a significant difference (p=0.003). The MRSA were resistant to penicillin (100%), ceftriaxone (100%), tetracycline (94.9%), clindamycin (87.2%) and gentamycin (76.9%). There was high susceptibility to amikacin (100%), kanamycin (97.4%) and vancomycin (94.9%). All 39 MRSA were multidrug resistant, and a total of 11 antibiotypes were identified. Results of this study expand our knowledge of the environmental reservoirs of MRSA and highlight the need to implement infection control measures in the study site.

Keywords: Methicillin-resistant *Staphylococcus aureus*, classroom door handles, toilet door handles, Cameroon.

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Résumé

La charge bactérienne élevée et la présence de *Staphylococcus aureus* résistant à la méthicilline (SARM) sur les surfaces à fort contact dans les lieux de rassemblement tels que les écoles constituent un problème majeur de santé publique. Cette étude visait à déterminer la charge bactérienne, la prévalence et les profils de résistance aux antimicrobiens du SARM sur les poignées de porte des salles de classe et des toilettes à l'université de Buea. Au total, 306 écouvillons ont été prélevés sur les surfaces des poignées de porte des salles de classe et des toilettes dans le cadre de cette étude transversale. La numération bactérienne, l'isolement, l'identification et l'antibiogramme des SARM ont été réalisés selon des méthodes microbiologiques standard. La presence *de S. aureus* et de SARM a été confirmée par PCR, en utilisant les gènes *nuc* et *mecA*, respectivement. La contamination bactérienne des surfaces échantillonnées était de 100 %, avec des numérations bactériennes allant de 4,1 x 10^4 - 7,2 x 107cfu/swab pour les salles de classe et de 1,2 x 10^4 - 4,82 x 10^8 cfu/swab pour les toilettes. Il existe une différence significative (p=0,004) entre la contamination par *Staphylococcus aureus* des poignées de porte des salles de classe (77,6 %, 152/196) et des toilettes (90,9 %, 100/110). La différence entre la contamination par le SARM des surfaces des salles de classe (7,7 %, 15/196) et des toilettes (21,8 %, 24/110) était également significative (p=0,003). De même, les taux de contamination par les SARM des toilettes du personnel (10,4 %, 5/48) et des toilettes des étudiants (30,6 %, 19/62) ont montré une différence significative (p=0,003). Les SARM étaient résistants à la pénicilline (100 %), à la ceftriaxone (100 %), à la tétracycline (94,9 %), à la clindamycine (87,2 %) et à la gentamycine (76,9 %). La sensibilité à l'amikacine (100 %), à la kanamycine (97,4 %) et à la vancomycine (94,9 %) était élevée. Les 39 SARM étaient multirésistants et 11 antibiotypes au total ont été identifiés. Les résultats de cette étude élargissent nos connaissances sur les réservoirs environnementaux de SARM et soulignent la nécessité de mettre en œuvre des mesures de contrôle des infections sur le site de l'étude.

Mots-clés : *Staphylococcus aureus* résistant à la méthicilline, poignées de porte des salles de classe, poignées de porte des toilettes, Cameroun.

Introduction

In congregate non-healthcare settings such as schools, the density and proximity of students and staff within the facilities provide a ready environment for infectious disease transmission (Blumberg *et al*., 2022). High-touch surfaces such as door handles in these settings play an important role in the chain of transmission (Cobrado *et al*., 2017). In healthcare settings, it is established that infections, called hospital-acquired infections (HAIs), are frequently caused by pathogens that contaminate and persist in the hospital environment especially high-touch surfaces such as bedrails, sinks, water dispensers and door handles (JabBoDska-Trypu et al., 2022). HAIs caused by methicillin-resistant *Staphylococcus aureus* (MRSA), a notorious antibiotic-resistant variant of *S. aureus*, are the most frequently reported (Ali Alghamdi *et al*., 2023). Unfortunately, MRSA, initially thought to be restricted to the hospital milieu, is presently a growing threat to the community.

In fact, the presence of pathogens on high-touch surfaces in non-healthcare environments is also increasingly being reported. For example, recent studies have reported potential pathogenic bacteria on the surfaces of door handles and computer keyboards (AL-Harmoosh *et al*., 2019), handles of public restrooms (Fakhoury and Nawas, 2018), vending machine buttons of ATM and touch screens (Dakroub and Nawas, 2017), books in libraries (Cave *et al*., 2021), mobile phones (Olsen *et al*., 2020), computer screens and

mice in public centers (Jaradat *et al.*, 2020), schools (Mbogori *et al.*, 2013), and public buses (Ly *et al.*, 2024).

While several studies focus on studying *Staphylococcus aureus* and its antibiotic-resistant variants (particularly MRSA) among patients and medical staff in hospitals, few studies have focused on the epidemiology of MRSA in public place environments, such as transportation, beaches and schools. Some studies have found that environmental surfaces of transportation buses showed particularly higher prevalence of MRSA (Lutz *et al.*, 2014). Previous studies that mainly focused on MRSA contamination in the environment of public transports, reported that environment surfaces in public transports are a hazardous reservoir for the transmission of *S. aureus* (including MRSA) to passengers (Jaradat *et al.*, 2020). A study of airport door handles revealed that the contamination rate was 5.5% for *S. aureus* and 0.3% for MRSA (Schaumburg *et al.*, 2016).

The presence of MRSA in schools, could be a source of infection to the community and possible entry of MRSA in the health facilities. Members of the school community are more likely to transfer the MRSA to their homes. The home is the meeting place for all the other family members who might spread the bacteria to other areas in the community (Mbogori *et al.*, 2013). School toilets, especially when not cleaned routinely, can act as a major source of microbial contamination (Jaradat *et al.*, 2020). Similarly, door handles, have been reported to have the highest bacterial transfer rates to hands and thereby constitute a public health hazard (Edi *et al.*, 2023).

The bacterial load on environmental surfaces is a general indicator of microbiological quality. However, there are no widely accepted quantitative thresholds for permissible levels of microbiological contaminants on surfaces in non-healthcare congregate environments (Giovinazzo *et al.*, 2017). In the hospitals, the bacterial load considered as microbio-logical standard for the surfaces, is generally indicated between $\leq$ 2.5 cfu/cm^2 and < 5 cfu/cm^2. The index organisms that must be absent or <1 cfu/ cm^2 are mostly *Staphylococcus aureus* (including MRSA and MSSA), *Aspergillus* spp., *Pseudomonas* spp. and Enterobacte-riaceae (Giovinazzo *et al.*, 2017).

Inadequate cleaning of environmental surfaces may lead to accumulation of dust and nonvisible ecological niches, which can promote biological contaminants including bacteria and viruses (Reynolds *et al.*, 2005). Therefore, enhanced cleaning in buildings should be promoted to encourage routine surface cleaning through education, policy and the provison of supplies and to routinely clean high-touch surfaces (Chen *et al.*, 2018; Shaughnessy *et al.*, 2013). Several studies have demonstrated that basic cleaning leads to MRSA elimination from environmental surfaces (Shaughnessy *et al.*, 2013; Rampling *et al.*, 2001).

Although contact with pathogens in the environment does not necessarily result in an infection, the environment acts both as a reservoir of pathogens and a means by which they can be broadly disseminated (Giovinazzo *et al.*, 2017). There are very few studies that have investigated inanimate surfaces for the presence of bacterial contaminants in Cameroon (Bissong and Moukou, 2022; Takemegni *et al.*, 2020; Akoachere *et al.*, 2014). Increasing attention paid to investigating bacterial contamination of environmental surfaces in crowded settings elsewhere stimulated our interest to determine the bacterial load, prevalence and antimicrobial resistant patterns of MRSA on classroom and toilet door handles in the University of Buea, one of the crowded tertiary institutions

in Cameroon, in order to provide baseline data to inform institutional infection control strategies.

Materials and methods

Study design

This was a cross-sectional study carried out from April - August 2019. The choice of door handles for investigation was purposive because they are highly touched surfaces while the selection of doors was by balloting from a list of classrooms and toilets in the institution. Doors that were permanently closed and not in use were excluded.

Study setting

This study was carried out in the University of Buea, a state-owned university chosen by balloting among other state-owned universities in the country. A common characteristic of these universities is the high student population. This university has a student population of over 25,000.

Sample size

The sample size for this study was calculated using a prevalence of 20.1% for *S. aureus* reported in a previous study in Cameroon (Akoachere *et al.*, 2014). This gave a minimum sample size of 247 at a confidence level of 95%.

Sample collection and transportation

A non-duplicate swab sample was collected from each door handle in the morning between 8:00 - 10:00 am. Each swab was collected using a sterile electrostatic swab cloth moistened with sterile buffered peptone water. The swab cloth was firmly rubbed on the door handle surface and rotated to 360 degrees. The swab was immersed in a plastic tube containing 5 mL sterile buffered peptone water and the tube placed in a cool box containing ice parks. Samples were transported to the laboratory for processing within 1 to 2 hours of collection. In the laboratory, each sample was mixed vigorously, using a vortex mixer (Thermo Scientific, USA) for 10 s, allowed to stand for 1 min and the swab cloth removed (Reynolds *et al.*, 2005). Each swab rinse fluid was used for microbiological processing. To confirm the sterility of the swab cloth, an unused swab cloth was processed similarly and served as negative control. Sampling was performed by the same individual to minimize variability of the sampling technique.

Determination of viable aerobic mesophilic bacterial count

The standard plate count method was used. Ten-fold serial dilution was done for each sample up to 10^6. Using the spread plate technique, aliquots of 100 µL from 10^{-5} and 10^{-6} dilutions were inoculated on nutrient agar (HiMedia laboratories, India) in duplicates. Plates were incubated aerobically at 37 °C for 24 h. For quality control, another negative control (uninoculated nutrient agar plate) was incubated to verify the sterility of the culture medium. At the end of incubation, mean counts from plates that had 30-300 colonies were used to calculate the viable aerobic mesophilic bacterial count according to the method of Public Health England (Public Health England, 2017) as follows:

Colony forming units/swab =

$$\frac{\textit{Number of colonies counted} * \textit{Dilution}}{\textit{Volume of sample plated}}$$

Sample enrichment, isolation and identification of *Staphylococcus aureus*

Sample enrichment is highly recommended for environmental samples to improve detection or isolation of pathogens (Rawlinson *et al.*, 2019). Hence, each sample (swab rinse fluid) was enriched by incubating at 37 °C for 18 h, before plating onto mannitol salt agar (MSA) (HiMedia laboratories, India) and incubating at 37 °C for 24 h. Growth on MSA was purified by streaking on nutrient agar and bacterial isolates presumptively identified using colony morphology, gram staining, catalase and coagulase tests. Only

one isolate was obtained from each sample. Using the QIAamp DNA Kit (Qiagen, Hilden, Germany), genomic DNA was extracted from the *S. aureus* isolates individually. The confirmation of *S. aureus* was based on the amplification of the *nuc* gene as previously described (Esemu *et al.*, 2021).

Phenotypic selection and genotypic confirmation of MRSA

Cefoxitin (30 µg) disc (HardyDisk™, USA) was used to detect methicillin resistance in the *S. aureus* following the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021). Genotypic confirmation of MRSA relied on the amplification of the *MecA* gene by polymerase chain reaction as previously described (Esemu *et al.*, 2021).

Antimicrobial susceptibility testing of MRSA

Using the Kirby-Bauer disc diffusion method on Mueller-Hinton agar (HI media, Mumbai, India), each MRSA strain was tested for antimicrobial susceptibility to nine antimicrobials (HardyDisk™, USA) representing seven antimicrobial classes as follows: aminoglycosides (amikacin; AK-30 µg, kanamycin: K-30 µg, gentamycin: CN-10 µg), penicillin (penicillin: P-10 IU), tetracycline (tetracycline: TE-30 µg), cephalosporin (ceftriaxone: CRO-30 µg), fluoroquinolone (ciprofloxacin: CIP-5 µg), lincosamide (clindamycin: DA-2 µg), and glycopeptide (vancomycin: VA-30 µg). In this study, MRSA strains which were resistant to e" 1 antimicrobial in e" 3 antimicrobial classes were reported as multi-drug resistant. The method of antimicrobial susceptibility testing and the interpretation of results followed the recent guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2021).

Data analysis

Data were analyzed using SPSS version 21 (Chicago Inc, USA). The differences in bacterial count and MRSA contamination between classroom and toilet doors, male and female toilet doors, staff and students' toilet doors were compared using the Chi-square test. Variables with $p<0.05$ were considered significant.

Quality control

To ensure the reliability and validity of our results, quality control measures were taken at the levels of sample collection and laboratory processing. Sample collection was done by the same person to minimize variability in the sampling technique. The sterility of each culture medium was tested by the incubation of uninoculated plates. Each laboratory procedure was carried out in accordance with an authorized standard operating procedure. Antimicrobial susceptibility testing and interpretation of results followed the CLSI guidelines.

Results

Bacterial counts on sampled surfaces

All the door handles investigated in this study had high bacterial counts, giving an overall bacterial contamination rate of 100 %. However, the bacterial counts on toilet door handles were higher than those of classrooms. Bacterial counts on classrooms ranged from $4.1 \times 10^4 – 7.2 \times 10^7$cfu/swab while for the toilets, it ranged from $1.2 \times 10^4 - 4.82 \times 10^8$ cfu/swab. Generally, the door handles of students' toilets had the highest bacterial counts which ranged from $1.19 \times 10^5 - 4.82 \times 10^8$ cfu/swab. Similarly, female toilets had higher bacterial counts than for the males (Table 1).

Staphylococcus aureus and MRSA contamination of sampled surfaces

The *S. aureus* contamination rates of the swabbed surfaces is shown in Table 1. Out of the 306 swab samples collected from the door handles, 252 (82.4 %) had evidence of *S. aureus* contamination. Of the 196 classroom door handles included in this study, 152 (77.6 %) were positive for *S. aureus*

and of the 110 swabs from toilets, 100 (90.9 %) were positive for *S. aureus*. The difference between S. aureus contamination on the classroom and toilet door handles was statistically significant (p = 0.003). The *S. aureus* contamination rates for staff toilets (85.4 %, 41/48) and student toilets (95.2 %, 59/62), did not show any statistically significant difference.

Table 1. Viable bacterial count, *S. aureus* and MRSA contamination on door handles

Door handles	Number of swabs	Viable bacterial count (CFU/swab)			Contamination with	
		Min	Max	Mean	*S. aureus* (%, 95% CI)	MRSA (%, 95% CI)
Overall	306				252 (82.4; 77.7-86.2)	39 (12.7; 9.5-17.0)
Classroom	196	4.10×10^4	7.20×10^7	5.60×10^6	152 (77.6; 71.2-82.8)	15 (7.7; 4.7-12.2)
Toilet	110	1.20×10^4	4.82×10^8	2.21×10^7	100 (90.9; 84.1-94.9)	24 (21.8; 15.1-30.4)
Staff toilet	48	1.20×10^4	9.81×10^5	4.94×10^5	41 (85.4; 72.8-92.8)	5 (10.4; 4.4-22.2)
Student toilet	62	1.19×10^5	4.82×10^8	3.89×10^7	59 (95.2; 86.7-98.3)	19 (30.6; 20.6-42.9)
Male toilet	55	1.20×10^4	1.23×10^7	1.11×10^6	41 (74.5; 61.7-84.2)	13 (23.6; 14.4-36.4)
Female toilet	55	1.23×10^5	4.82×10^8	4.31×10^7	49 (89.1; 78.2-94.9)	21 (38.2; 26.5-51.4)

Different MRSA contamination rates were recorded for the swabbed surfaces (Table 1). The MRSA contamination rate for the classroom surfaces was 7.7 % and 21.8 % for the toilets. This difference was statistically significant (p=0.003). Similarly, a statistically significant difference (p=0.004) was revealed between the MRSA contamination rates for staff toilets (10.4 %, 5/48) and student toilets (30.6 %, 19/62). The difference between the MRSA contamination rates of the male (23.6 %, 13/55) and female toilets (38.2 %, 21/55) was not significant.

Antibiogram for MRSA identified in this study

All the MRSA were resistant to penicillin and ceftriaxone (Figure 1). High resistance rates of 94.9 %, 87.2 % and 76.9% were recorded for tetracycline, clindamycin and gentamycin respectively. The MRSA were highly susceptible to amikacin (100%), kanamycin (97.4%) and vancomycin (94.9%). No MRSA was susceptible to all the antimicrobials.

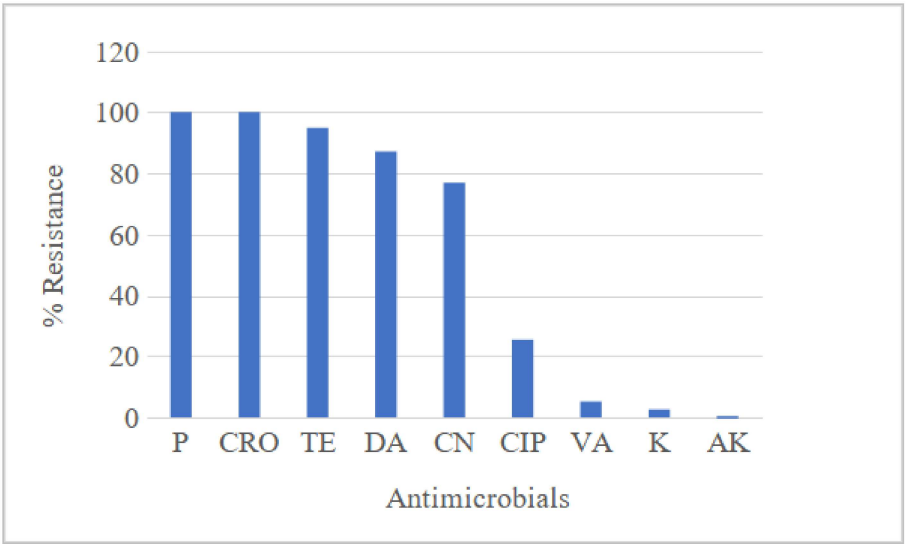

Figure 1. Methicillin-resistant *S. aureus* resistance to antimicrobials. P, penicillin; CRO, ceftriaxone; TE, tetracycline; DA, clindamycin; CN, gentamycin; CIP, ciprofloxacin; VA, vancomycin; K, kanamycin; AK, amikacin

Antibiotypes and multidrug resistance

All the 39 MRSA were multidrug resistant and a total of 11 antibiotypes were identified with CN-P-TE-CRO-DA having the highest frequency (51.3 %) (Table 2). Seven antibiotypes were recovered only from toilet door handles while two were present only on classroom door handles.

Table 2. Antibiotypes and the resistance pattern of the MRSA identified in this study

Profile	Antibiotype	No. of isolates (%)	Resistance pattern	Source
1	CN-P-TE-CRO-DA-VA	1 (2.5)	Multidrug	Toilet
2	CN-P-TE-CRO-CIP-DA	4 (10.3)	Multidrug	Toilet
3	K-CN-P-TE-CRO-DA	1 (2.5)	Multidrug	Classroom
4	P-TE-CRO-CIP-DA	4 (10.3)	Multidrug	Classroom, toilet
5	CN-P-TE-CRO-VA	1 (2.5)	Multidrug	Classroom
6	CN-P-TE-CRO-DA	20 (51.3)	Multidrug	Classroom, toilet
7	P-TE-CRO-CIP	2 (5.1)	Multidrug	Toilet
8	CN-P-CRO-DA	1 (2.5)	Multidrug	Toilet
9	P-TE-CRO-DA	3 (7.7)	Multidrug	Toilet
10	CN-P-TE-CRO	1 (2.5)	Multidrug	Toilet
11	CN-P-CRO	1 (2.5)	Multidrug	Toilet
	Total	**39 (100)**		

Discussion

Bacterial contamination of high-touch surfaces like door handles is known to be a major risk factor for hospital-acquired infections. In the hospital environment, routine microbiological examination of high-touch surfaces for viable bacterial counts and the presence of specific pathogens is recommended to guide infection control measures (Bhatta *et al.*, 2022). Similarly, in the non-hospital environment, bacterial contamination of high-touch surfaces such as door handles has been reported as a potential threat to public health and safety. Such contaminated high-touch surfaces play a role in the transmission of human pathogens through several routes including hand-to-mouth contact or surface-to-mouth contact (Bhatta *et al.*, 2022; Reynolds *et al.*, 2005). High-touch surfaces and fomites in general, are thought to play a crucial role in the spread of *Staphylococcus aureus* and MRSA, particulary because these pathogens are known to survive long periods on environmental surfaces (JabBoDska-Trypu et al., 2022; Fakhoury and Nawas, 2018, Suleyman *et al.*, 2018). Therefore, tracking *S. aureus* and MRSA in the non-hospital environment is very important towards identifying environmental reservoirs and controlling the spread of MRSA infections in the community

In this study, bacterial contamination was recorded for all the swabbed surfaces. The bacterial counts on toilet door handles (range: 1.2 x 10^4 - 4.82 x 10^8 cfu/swab) were higher than those of classrooms (range: 4.1 x 10^4 - 7.2 x 10^7cfu/swab). The high bacterial count on toilet door handles could be due to the high traffic of students, staff, and visitors using these toilets (Odigie *et al.*, 2017). This study further corroborates the results of Ayuba et al. (Ayuba *et al.*, 2019) who investigated convenience places in Gombe State University in Nigeria and Nworie et al. (Nworie *et al.*, 2012) who reported that toilet environments usually contain higher microbial loads than other facilities within any public centre. In this study, the door handles of students' toilets had the highest bacterial counts which ranged from 1.19 x 10^5 - 4.82 x 10^8 cfu/swab compared to staff toilets. This may be due to a relatively higher level of compliance to hand hygiene practices which encompasses hand washing and sanitizing, by the staff and the relatively fewer users of the toilets.

Female toilets had higher bacterial counts than the males and similar findings have been reported in previous studies (Nworie *et al.*, 2012; Flores *et al.*, 2011). Generally, high bacterial counts on toilet doors handles have been reported to mirror inadequate cleaning and sanitation of the surfaces and also possible poor hand hygiene practices by the toilet users (Odigie *et al.*, 2017).

MRSA contamination of environmental surfaces has been significantly associated with nosocomial infections in developing countries (Manyahi *et al.*, 2014) and has been shown to vary from hospital to hospital depending on factors such as hand hygiene, crowding, infection control practices and MRSA carriage rate (Borg *et al.*, 2007). In this study, *S. aureus* and MRSA prevalence on classroom door handles was 77.6% and 7.7% respectively. Onwubiko and Chinyeaka (2015) reported *S. aureus* (25.0%) as the most frequently isolated bacteria from door handles of a tertiary Institution at Umuahia, Abia State, Nigeria. Similarly, *Staphylococcus* species (43.3%) were reported as the most prevalent bacterial contaminants of door handles of secondary schools in Bokkos Local Government of Jos Plateau State, Nigeria (Maori *et al.*, 2013). In addition, *Staphylococcus* species also have been reported to colonize environmental surfaces in several other places including Ghana (Donkor *et al.*, 2020), Nigeria (Alonge *et al.*, 2018) and Saudi Arabia (Ahmed and Sirag, 2016). The spread of antimicrobial-resistant pathogens like MRSA is a serious global health problem. In this study, all the MRSA were multidrug resistant, corroborating the results of Ababneh et al. (2022) who reported that all the 30 MRSA (2.85, n = 1078) isolated from high-touch surfaces in a university campus were multidrug-resistant.

Conclusions

This study was carried out in a single institution and the results may not be generalized. Although the virulence potential of the MRSA was not investigated, infection prevention measures in the study site such as regular cleaning and disinfection of door handles and constant provision of water in the institution are recommended. The availability of water can foster hand hygiene practices within the institution. The potential role of high-touch surfaces as reservoirs and vehicles for the transmission of other pathogenic bacterial species should be investigated. High bacterial counts and the presence of multidrug-resistant MRSA on door handles used by a high traffic of persons on daily basis is worrisome. These findings highlight the public health relevance of this study.

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