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Assessment of door handles as potential reservoirs of drug-resistant enterococci

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Abstract

Background: Fomites have long been known to play a key role in the spread of disease causing agents. Hospital-associated fomites in particular have been linked with transmission of members of the *Enterococci* sp which are key human pathogens. Few studies have explored the role non-hospital door handles might play as potential sources of these isolates. This study therefore set out to explore this role.

Results: A total of hundred toilet and office door handles in a tertiary institution in Rivers State, Nigeria, were sampled using the swab and rinse method. The presence and drug susceptibility of *Enterococcus* was determined using the selective bile esculin agar (BEA) and standard microbiological methods. Growth on BEA was observed in 71% of cases, with more growth (38/50, 76%) observed from toilet door handles. Only 35% of samples produced the characteristic black pigmentation associated with *Enterococcus* sp. Six different bacterial groups were identified from this subset with *Enterococcus* sp. making up only 14% (5/35) of the isolates. All (100%) *Enterococci* were isolated from toilet door handles. Antibiotic susceptibility testing revealed very high levels of resistance (80–100%) against 75% of the test antibiotics. An analysis of the antibiotic resistance pattern of each isolate revealed 11 unique antibiogram patterns. Only 2 of these patterns were associated with the enterococci, with majority (4/5) exhibiting resistance to Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL) (antibiogram of AUG–CAZ–CRX–CTR–CXC–ERY). None of the enterococci, however, was resistant to vancomycin.

Conclusion: This study reports low level contamination of door handles by enterococci. Identical antibiogram patterns linked with majority of the enterococci could however point at the occurrence of a single clone perhaps indicating single source contamination. Reports of high levels of ampicillin resistance among these isolates are problematic as ampicillin–gentamicin combination is the treatment of choice for nosocomial enterococci pathogens.

Keywords: Drug resistance, Enterococci, Vancomycin, Fomites, Nigeria

Background

Though initially known for being commensals, presently, enterococci are notorious as key human pathogens. These organisms are predominantly associated with nosocomial infections and have been described as the third most commonly occurring nosocomial pathogen (Murray 1990; Selleck et al. 2019). A key problem associated with the enterococci is the issue of drug resistance.

Nosocomial strains of this organism are known for the rapid acquisition of drug resistance determinants and subsequent spread within an already susceptible population. The enterococci are a hardy species, able to survive harsh environmental conditions and exhibiting resistance to things such as desiccation, disinfection (García-Solache and Rice 2019). A critical step in the journey of the enterococci to act as pathogens is the colonisation of the patient (Selleck et al. 2019).

This colonisation has been thought to occur either prior to hospitalisation or following hospitalisation. A number of studies have focused on exploring the presence

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of these organisms in hospital environments in a bid to understand transmission and initiate control measures (Michael et al. 2017). Though some clones of enterococci appear adapted to hospital environments, a number of clones particularly of *Enterococcus faecalis* have been associated with both hospitals and other reservoirs (Guzman Prieto et al. 2016). Studies have therefore explored colonisation of enterococci, particularly vancomycin-resistant enterococci (VRE) in healthy individuals in a bid to fine tune control measures and explore effectiveness. Gurnee and colleagues reported lower carriage rates in healthy children as opposed to children with prior exposure to health care settings (Gurnee et al. 2014). One risk for VRE carriage has been found to be exposure to health care settings (Davis et al. 2020). Transmission occurs between colonised patients, healthcare workers and non-colonised patients often via fomites and hand colonisation (Rosenthal et al. 2014; Ferng et al. 2015; Kim et al. 2018). Few studies have however explored the role non-hospital surfaces might play as potential sources of these isolates. This study therefore set out to explore the role various non-hospital door handles play as potential reservoirs of drug-resistant enterococci.

Methods

Sample collection and processing

A total of 100 samples were aseptically collected from toilet and office door handles in a tertiary institution in Rivers State, using the swab and rinse method (Reynolds et al. 2005), using sterile swab sticks. Following sample collection and transport to the Medical Microbiology laboratory of the University of Port Harcourt, samples were immediately processed by inoculation to bile esculin agar.

Purification and identification

Colonies showing features characteristic of *Enterococcus* (black pigmentation), were then subcultured to nutrient agar, purified and characterised using an array of biochemical tests including: Gram stain, catalase, Voges Proskauer, starch hydrolysis, motility, fermentation of glucose, sucrose, lactose and mannitol and growth at 60 °C and 6.5% sodium chloride (Cowan and Steel 1985; Cheesbrough 2006).

Antibiotic susceptibility testing

Susceptibility testing was carried out on isolates using the disc diffusion method as previously described (Bauer et al. 1966). In brief an inoculum corresponding to 0.5 McFarland standard was inoculated to a Mueller Hinton agar plate using a sterile swab stick. After a 15 min preincubation period, commercially prepared antibiotic test discs were applied and incubated at 37 °C for 24 h.

Isolates were determined to be susceptible or resistant based on the diameter zone of inhibition using a standard interpretative chart (NCCLS 2000). Antibiotics tested include: ceftazidime, cefuroxime, gentamicin, ceftriaxone, erythromycin, cloxacillin, ofloxacin, augmentin and vancomycin.

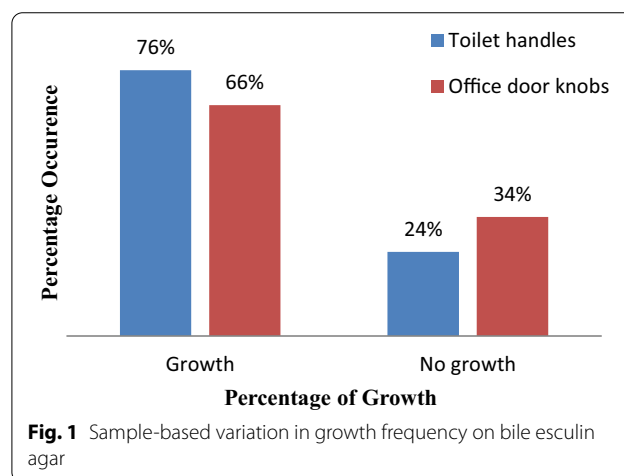
Results

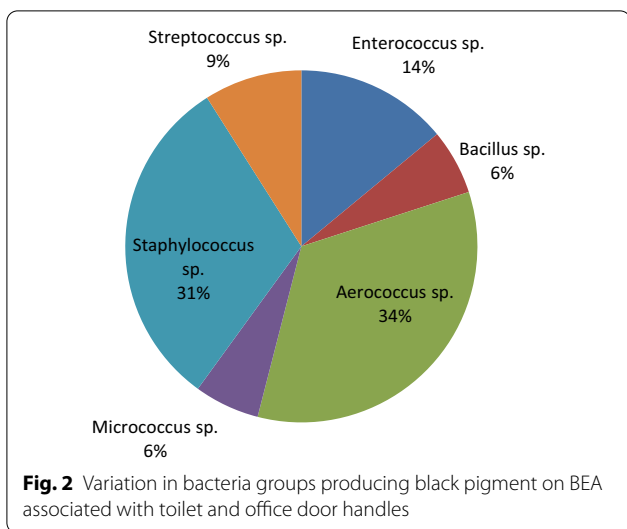
From the 100 samples collected (50 door handles; 50 toilet handles), growth on bile esculin agar (BEA) was observed in 71% of cases (Fig. 1). More growth was observed from toilet door handles (38/50; 76%) than office door handles (33/50; 66%). However, the characteristic black pigmentation was observed in only 35% of cases, majority of which (25/35) were from toilet door handles.

Biochemical characterisation of isolates with black pigmentation revealed a surprisingly wide array of bacterial strains (Fig. 2), considering the selective nature of the growth medium. Six different bacterial groups were represented among this subset with *Enterococcus* sp. making up only 14% of the isolates. All (100%) *Enterococci* were isolated from toilet door handles, with none (0%) identified from office door handles.

Antibiotic susceptibility testing revealed very high levels of resistance (80–100%) against most of the antibiotics assayed (Fig. 3). These high levels were equally observed for isolates from toilet door handles as well as office door handles. Low levels of bacterial resistance (0–24%) were observed only against ofloxacin and gentamicin.

An analysis of the antibiotic resistance pattern of each isolate revealed 11 unique antibiogram patterns (Table 1). Office door isolates exhibited 4 different patterns, while toilet door isolates exhibited 10 different patterns. Only one of these patterns was unique to office door handles while 7 patterns were unique to toilet door handles.





Multidrug resistance of isolates

Based on the resistance pattern of each isolate, multidrug resistance (MDR, defined as resistance to 3 or more drug classes) was determined and revealed high levels of MDR in isolates from both toilet and office door handles (88% and 80%, respectively). Majority (58%) of these MDR isolates exhibited resistance to only 3 drug classes (Fig. 4).

Vancomycin resistance

An assessment of vancomycin resistance from all isolates producing characteristic black pigmentation of BEA showed a 17% (6/35) rate of vancomycin resistance. A higher level of vancomycin resistance was associated with toilet door rather than office door handles (Fig. 5).

From the six vancomycin-resistant isolates a 66.7% rate of MDR was noted (4/6), with 4 different antibiogram patterns represented (Table 2).

Multidrug resistance in enterococci

Special focus on the enterococci showed that despite the numerous antibiogram patterns identified in general, only 2 patterns were represented among the enterococci (Table 3), with most isolates (4/5) showing an identical pattern of resistance. In the predominant antibiogram pattern resistance to 3 drug classes was observed, thereby indicating an 80% rate of multidrug resistance among the enterococci. None of the enterococci, however, was resistant to vancomycin.

Discussion

One major route of transmission of enterococci in hospital settings is via inanimate objects called fomites. The role of such surfaces outside of hospital settings in transmission has not been widely explored. Bacteria may live as transient contaminants on inanimate objects, especially high touch surfaces where they could serve as sources of community acquired infections. The potential role of such surfaces called fomites (Xiao 2018) in transmission has been widely explored.

The total number of samples showing growth in this study (71%) is similar to previous reports both from within and outside Nigeria (Amala et al. 2015; Al-Har-moosh et al. 2019). Variations in number of samples showing growth could depend on a number of things such as hygiene, traffic and even temperature. This is further highlighted by the detection of more growth from toilet door handles (38/50; 76%) than office door

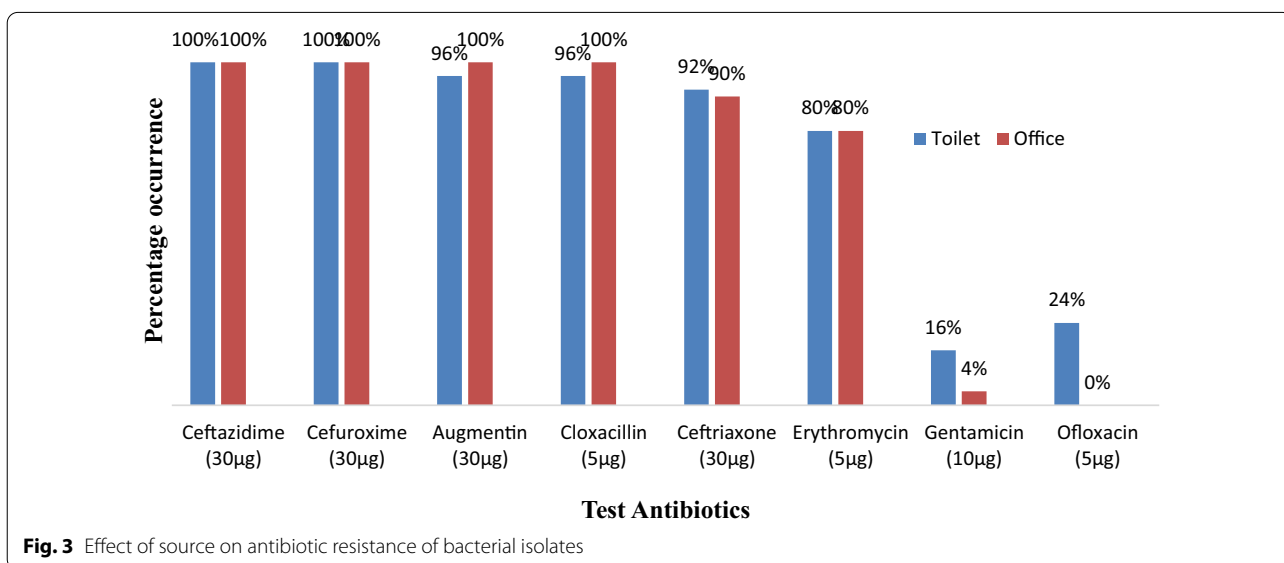


Table 1 Antibiotic resistance patterns and occurrence for toilet handles and office door knob isolates

Antibiogram	Number of toilet isolates	Number of door isolates
AUG-CAZ-CRX-CXC	1	-
AUG-CAZ-CRX-CTR-CXC	3	2
AUG-CAZ-CRX-CXC-ERY	-	1
AUG-CAZ-CRX-CTR-CXC-ERY	12	6
AUG-CAZ-CRX-CTR-CXC-GEN	1	-
AUG-CAZ-CRX-CXC-ERY-OFL	1	-
CAZ-CRX-CTR-CXC-ERY-GEN	1	-
AUG-CAZ-CRX-CTR-CXC-ERY-GEN	1	-
AUG-CAZ-CRX-CTR-CXC-ERY-OFL	2	-
AUG-CAZ-CRX-CTR-ERY-GEN-OFL	1	1
AUG-CAZ-CRX-CTR-CXC-ERY-GEN-OFL	2	-

Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL)

Table 2 Drug resistance pattern of vancomycin-resistant isolates

Antibiogram	Number of toilet isolates	Number of door isolates
AUG-CAZ-CRX-CTR-CXC	-	2
AUG-CAZ-CRX-CXC-ERY	-	1
AUG-CAZ-CRX-CTR-CXC-ERY	-	2
AUG-CAZ-CRX-CTR-CXC-ERY-GEN-OFL	1	-

Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL)

Table 3 Drug resistance among enterococci

Antibiogram	Isolate ID
AUG-CAZ-CRX-CTR-CXC	T27
AUG-CAZ-CRX-CTR-CXC-ERY	T19, T28, T37, T50

Augmentin (AUG), Ceftazidime (CAZ), Ceftriaxone (CTR), Cefuroxime (CRX), Cloxacillin (CXC), Erythromycin (ERY), Gentamicin (GEN), Ofloxacin (OFL)

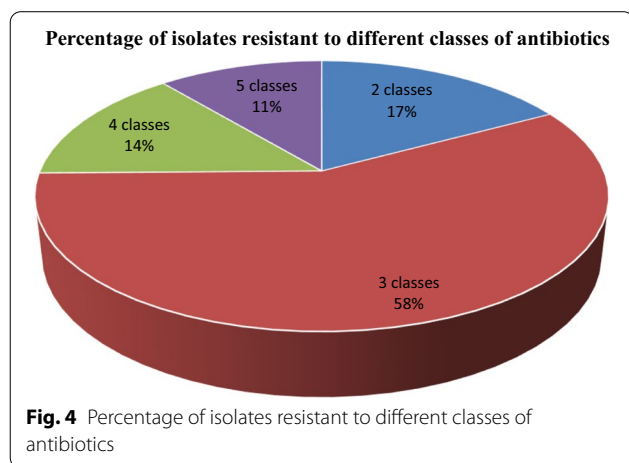


Fig. 4 Percentage of isolates resistant to different classes of antibiotics

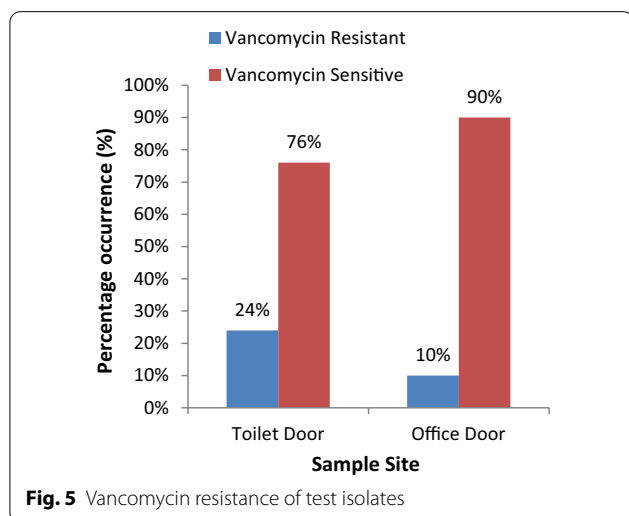


Fig. 5 Vancomycin resistance of test isolates

handles (33/50; 66%). This was similar to previous reports (Odigie et al. 2017; Al-Harbi et al. 2017) and could be an indication of a higher traffic with respect to toilet door handles than office door handles. Additionally, it could represent a breakdown in basic hygiene practices following the use of the toilet facilities. Or perhaps reflect the use of wet hands on toilet door handles as opposed to office door handles, considering the higher spread of microorganisms associated with a higher moisture and humidity levels (Lopez et al. 2013; Stephens et al. 2019; Varshney et al. 2020).

This study focused on detecting enterococcus using the selective bile esculin agar, from door handles found a variety of Gram positive bacteria presented. Gram positive bacteria had actually been reported as the predominant group of organisms by a number of previous studies on bacteria associated with door handles (Al-Harbi et al. 2017; Al-Harmoosh et al. 2018, 2019). The exception to this was a 2016 study in India by Lincy and colleagues which reported a predominance of Gram negative bacteria (Lincy et al. 2016). All bacterial types identified have previously been reported as associated with door handles. Furthermore, *Staphylococcus* sp, *Streptococcus* sp and *Bacillus* sp have often been found as one of the 5 most prominent genera represented (Lincy et al. 2016; Odigie et al. 2017; Al-Harmoosh et al. 2018; Alonge et al. 2019). For the Gram positive cocci, their presence has often been thought to be linked to their role as normal human flora, while the *Bacillus* sp are known hardy environmental strains due to their spore forming ability.

This study revealed only a 14% association of *Enterococcus* sp with the door handles. This is similar to a 2013 study by Sabra which reported a 13.4% detection rate but differs from some previous reports with higher detection rates (Sabra 2013; Ajayi and Ekozien 2014; Al-Harbi et al. 2017) of 21% and 26% and lower detection rates (Badger-Emeka et al. 2015; Nwankwo and Afurobi 2015) of 3.5% and 4.8%. Quite a number of studies though failed to report any association of the enterococci with toilet door handles (Nworie et al. 2012; Maori et al. 2013; Bashir et al. 2016; Fakhoury and Nawas 2018). These studies in general set out to explore the total bacterial population associated with the door handles, making use of general all-purpose media and some selective media, but not selective for enterococcus. This might explain the lack of detection of the *Enterococcus* sp. Interestingly though in this study, the enterococci were only associated with toilet door handles. The enterococci are widely known as normal flora of the human gastrointestinal tract, where they occur without causing any harm. Furthermore, infections due to this group of organisms are associated with prior colonisation. Results of this study show that toilet door handles especially might pose a risk in the transmission of the enterococci, especially to individuals with reduced immunity making use of these shared facilities. The relationship with toilet door handles specifically could highlight that this possible route of transmission could be eliminated by proper education on the importance of hand hygiene in infection control. A similar trend of the association of enterococci with toilet door knobs has been previously reported. Al-Harbi and colleagues observed that despite the fact enterococci was isolated from different buildings in their study; in most cases it was associated primarily with toilet door handles (Al-Harbi et al. 2017).

In general, this study reports high levels of drug resistance. High levels of drug resistance are generally associated with human rather than environmental isolates (Abu et al. 2020), with purely environmental strains often associated with lower levels of drug resistance in the absence of antimicrobial selective pressure (Li et al. 2010; Chitanand et al. 2010; Hatha et al. 2015; Odonkor and Addo 2018). These results therefore indicate strongly that indeed the organisms isolated from these high touch surfaces are of human origin.

The rates of resistance described in this study were however much higher than those described in other studies. Alumbugu et al. described rates of resistance ranging from 13 to 68%, with the resistance levels of above 50% observed against 60% of antibiotics (Alumbugu et al. 2019). While a study similar to this from Nigeria (Nwankwo and Afurobi 2015), also reported high rates ranging from 14.6 to 93.1%, with resistance rates of above

70% against 55.6% of antibiotics. A 2017 study by Sultana (2017), reported much lower rates ranging from 5.41 to 100% but with resistance to majority of antibiotics (5/9, 55.6%) less than 11%.

Despite the limited number isolated, the resistance rates of the enterococci in this study were generally much higher than previously reported (Ferede et al. 2018; Ezeah et al. 2019; Kateete et al. 2019; Schell et al. 2020). Gentamicin and ofloxacin were the two antibiotics posing an exception, as all five enterococci were susceptible to these two. Gentamicin which belongs to the aminoglycoside family is one of the drugs of choice for the treatment of enterococcal infections (Arias et al. 2010; Ngbede et al. 2017). Some studies have reported increasing levels of gentamicin resistance (Olawale et al. 2011; Adesida et al. 2017; Ferede et al. 2018; Ezeah et al. 2019). This differs from the findings of this study which reports that despite the high level of resistance associated with the organisms, gentamicin sensitivity is still prevalent. A similar finding was recently reported (Shettima and Iregbu 2019). Another striking difference also in susceptibility pattern of the enterococcal isolates obtained from this study and others reported worldwide is the results of the ampicillin susceptibility. Ampicillin is also touted as another drug of choice for enterococcal therapy acting in synergy with the aminoglycosides. A high level of sensitivity has been noted by various other studies both within Africa and outside (Chakraborty et al. 2015; Farman et al. 2019; Kateete et al. 2019). This was not so in this present study which reported 100% resistance rates to ampicillin among the enterococci. These high level ampicillin resistance are however similar to reports from Nigeria (Olawale et al. 2011; Ezeah et al. 2019) and a 2018 Ethiopia study (Ferede et al. 2018). It could therefore point at a regional variation possibly reflective of ampicillin use in the area. Additionally, considering that *E. faecalis* appears to be the species specifically associated with ampicillin sensitivity, whereas *E. faecium* is associated with ampicillin resistance (Coombs et al. 2020), these variations could also be a reflection of variations in species within the Enterococci.

The limited number of Enterococcal isolates obtained in this study provide a major limitation for extrapolation of findings and at most could only serve as baseline data for future studies which would therefore need to employ much larger sample size to study possible roles of fomites in the spread of drug-resistant Enterococci.

Conclusion

This study exploring the potential role of door handles in the spread of drug-resistant pathogens, reports low level contamination of door handles by Enterococci. Identical antibiogram patterns linked with majority of

the Enterococci could however point at the occurrence of a single clone perhaps indicating single source contamination. Reports of high levels of ampicillin resistance among these isolates are problematic as ampicillin–gentamicin combination is the treatment of choice for nosocomial enterococci pathogens.

Abbreviations

BEA: Bile esculin agar; MDR: Multidrug resistance; VRE: Vancomycin-resistant enterococci.

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Authors' contributions

KO conceived and designed the study. BOF and DOD carried out most of the laboratory work. KO did most of the writing. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study are included in this published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that there are no financial or non-financial competing interests.

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