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Original article

Biofilm formation of methicillin resistant *Staphylococcus aureus* on hospital environmental surfaces as potential sources of infection among hospitalized patients

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ABSTRACT

Background: Antibiotic resistance and biofilm production have become a significant and growing threat to public and environmental health. The occurrence of methicillin-resistant *Staphylococcus aureus* (MRSA) and Vancomycin Resistant *Staphylococcus aureus* (VRSA) in Nigeria is well documented in clinical samples. However, findings on MRSA and VRSA from hospital environmental surfaces (HES) are yet to be adequately reported. The current study determined the prevalence of MRSA and VRSA in some HES and their ability to form biofilms. **Methods:** A total of 60 samples obtained from some HES which included walls, door handles, toilet seats, floors, bed rails and bedside tables were assessed for Staphylococcus aureus using Blood agar and mannitol salt agar (MSA) by a standard culture-based approach. Methicillin and vancomycin susceptibility profile of the *Staphylococcus aureus* isolates were determined using the disc diffusion method. Biofilm production was determined using Congo red agar (CRA) assay. **Results:** Of the 60 samples tested, 42 (70.0%) Staphylococcus aureus were isolated, with a majority recovered from walls, bed rails and bedside tables. Among the *Staphylococcus aureus* isolates, 90.5% (38/42) were MRSA and all the MRSA isolates were resistant to vancomycin. Biofilm profile revealed 20 (52.6%) strong biofilm formers and 2 (5.3%) moderate biofilm formers. **Conclusion:** The high prevalence of MRSA, VRSA and strong biofilm forming capacity of isolates from HES is a threat to environmental health and risk of disease development in hospitalized patients.

Introduction

Environmental surfaces in hospital rooms represents a major public health problem, with their frequent contamination by pathogenic or potentially pathogenic bacteria. The potential transmission of bacteria during surgery operations and treatments of infected patients makes hospital environments easily contaminated with pathogens of treated patients [1]. Bacteria may also be deposited on hospital surfaces at close contact with patients such as doorknobs, bedrails, light switches, bedside table, and surfaces in and around toilets in patient rooms ("high-touched surfaces"), through the release of expectorate drops, fluids from infected wounds, excrements, urine, blood, other corporeal fluids, but also through clothes and blankets. Therefore, direct patient contact with the contaminated environment, person-to-person transmission, and hands of healthcare employers are risk factors [2].

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Methicillin Resistant Staphylococcus aureus (MRSA) is primarily a nosocomial infection in many hospitals which can persist from hours to months [2,3] and their dissemination is favoured by both patients and healthcare employers. MRSA infections continue to be a major concern globally and as such has been categorized as a high-priority pathogen by the World Health Organization (WHO) in 2017 [4, 5]. Hospital- associated MRSA is often acquired within the hospital setting and one of the infections exhibiting increased antimicrobial resistance. In multiple nosocomial and ongoing sporadic outbreaks, environmental contamination has been potentially associated with the transmission of the nosocomial pathogens [5, 6]. Epidemiologic studies have shown that organism acquisition from prior room occupants infected or colonized with MRSA are up to a three-fold higher risk of acquiring these organisms from contaminated environmental surfaces [5,7].

A large majority of MRSA are resistant to all available penicillin and other β-lactam antimicrobial drugs, leading to MDR and narrowing down the therapeutic options available to treat serious infections [8]. This contributes significantly to the rising treatment expenses, as well as increasing patients' morbidity and mortality. MRSA spreads more readily than other strains once introduced into hospitals, and are often difficult to eradicate once established, this in large part is due to the ability to develop surface attached polymicrobial communities known as biofilm [9]. Adaptation to surface attached growth within a biofilm is accompanied by significant changes in gene and protein expression, as well as metabolic activity [10] which confers a dramatic decrease in susceptibility to antimicrobial agents. This susceptibility may be intrinsic (as a natural outcome of growth in the biofilm) or acquired (due to transfer of mobile element). Many pathogenic and nosocomial bacteria predominantly exist as biofilms. Biofilm forming MRSA strains is considered an important virulence factor influencing its persistence in both natural environments and within infected tissues [11].

Compounding the problem even further is the emergence of MRSA resistance to the glycopeptide antibiotic vancomycin, an empiric drug considered to be most effective for the treatment of infection with MRSA [12; 13]. Unfortunately, decrease in sensitivity of S. aureus to vancomycin as an alternative for the treatment of MRSA infections has been detected in many countries all over the world [14,15]. However, most research on sources of S. aureus and MRSA spread had focused on patients and medical staff leaving the HES which contribute to within-facility transmission [9, 16, 17, 18]. Effective infection control measures must therefore include consideration of MRSA contamination in the environment. Limited data are available on MRSA colonization of environmental surfaces in hospital settings in Nigeria. Therefore, the objectives of this study was to determine the prevalence of MRSA and VRSA in HES and their ability to produce biofilm.

Methods

Ethical consideration: Prior to the commencement of the study, ethical clearance was obtained from the University ethics committee while permission was granted by the hospital management board of the hospital from which sampling was carried. All samples related to patients were anonymized and de-identified.

Sampling

This study was conducted within one month from March 1 to 31, 2022, in a government owned hospital in Delta State. We investigated the prevalence of MRSA contamination, vancomycin resistance and the association of MRSA with biofilm production in wards (walls, door handles, toilet seats, floors, bedrails and bedside tables) of inpatients of whom Staphylococcus aureus and MRSA was isolated via clinical samples.

S. aureus and MRSA was isolated from the following patient's specimens: 10 urine samples, 3 wound samples and 5 expectorated sputum samples. The clinical samples were collected aseptically with a sterile universal container and sterile cotton swab. samples were later inoculated on blood agar (Hi media, India) and subcultured on mannitol salt agar (Hi media, India) for pure isolates.

For the surface sampling, 10 samples each of the surfaces of walls, door handles, toilet seats, floors, bed rails and bedside tables were swabbed using sterile swabs moistened with physiological saline. An area of approximately 10 cm × 10 cm was examined. All collected samples were labelled and transferred into a sterile nutrient broth in a screw caped test tube and were delivered to the microbiology laboratory for culturing and antimicrobial susceptibility testing within 1 hour. Each sample was inoculated onto blood agar (Hi media, India) and pure culture of isolates was

obtained by subculturing onto mannitol salt agar (Hi media, India). All plates were incubated in aerobic atmosphere at 35–37°C for 24 h.

Identification

Presumptive S. aureus isolates were identified based on Gram-positive cocci in clusters, β-hemolytic colonies on blood agar, catalase and coagulase production, and yellow colony surrounded by yellow zone on mannitol salt agar. Other identification criteria included, methyl red positive, Voges–Proskauer positive, nitrate reduction positive, fermentative, urease positive, lactose, mannitol, maltose, mannose and sucrose fermenting.

Methicillin resistance profile

S. aureus isolates were characterized into Methicillin-resistant Staphylococcus aureus (MRSA) by using cefoxitin (30 μg) disc diffusion method ([19]). According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, MRSA was distinguished by a phenotype that was cefoxitin-resistant. Isolates with a diameter of zone of inhibition of ≤21 mm were identified as MRSA.

Vancomycin resistance

Following the CLSI guideline, vancomycin susceptibility testing and interpretation were carried out using the Kirby–Bauer disk diffusion method [19] The inhibition zone was measured and identified as susceptible (S) or resistant (R) following the norms of Clinical Laboratory Standards Institute (CSLI).

Biofilm production

Biofilm production was performed on Congo red agar (CRA) plates (Hi Media, India) as described by Freeman and coworkers [20]. A loop full of MRSA isolates were streaked on the CRA plate and incubated at 35°C under aerobic conditions for 24 hours. The Staphylococci biofilm producer strains formed black or very black colonies while the non-biofilm producer strains formed red or orange red colonies. Formation of very black colonies with a dry crystalline consistency indicated a strong biofilm formation. Black colonies with the absent of dry crystalline colonial morphology indicated a weak biofilm formation. The association between MRSA and biofilms formation was analysed with logistic regression.

Results

Among the total 18 urines, sputum and wound swab samples examined, S. aureus was isolated from 13 (72.2%) samples. Among the 13 (72.2%) S. aureus isolated, 10 (76.9%) were MRSA (Figure 1).

The prevalence of MRSA contamination of the HES (walls, door handles, toilet seats, floors, bedrails, and bedside tables) in the wards of 10 patients from whom S aureus and MRSA was detected is as shown in Table 1. The overall prevalence of S. aureus and MRSA was 42 (70.0%) and 38 (90.5%) respectively, with a majority (19.0%) of the MRSA coming from wall, bedrails, and side table (Table 2). All MRSA were resistant to vancomycin.

The result of biofilm production of MRSA by CRA method as shown in Table 2 demonstrates that MRSA either produced strong or moderate biofilm. Although majority of the 38 MRSA isolates [20 (52.6%)] formed strong biofilms, logistic regression analysis indicated that there was no significant association between MRSA and biofilms formation (OR, 1.013; 95% CL, 0.955-1.074; *P*=0.667). The fact that the OR is slightly greater than 1, is marginal and therefore suggests that there is some form of association that cannot be ignored.

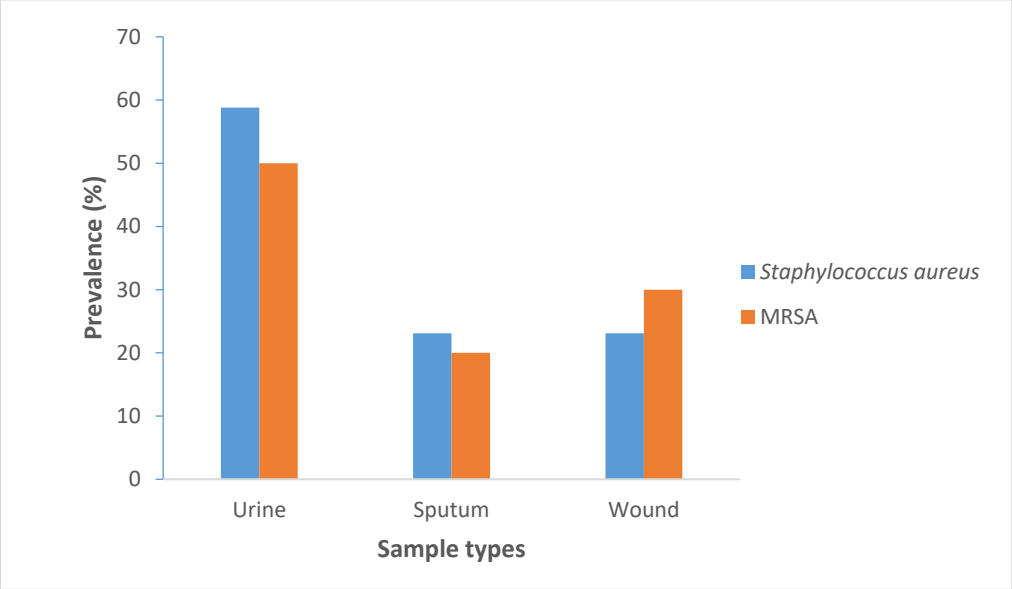
Table 1. Prevalence of MRSA and VRSA in HES

Environmental surfaces examined	Number of S. aureus isolates	Prevalence (*N*=38)	
		MRSA [% (*n*)]	Vancomycin resistance in MRSA (%)
Walls	19.0 (8)	21.1 (8)	21.1 (8)
Door handles	19.0 (8)	15.8 (6)	15.8 (6)
Toilet seats	14.3(6)	15.8 (6)	15.8 (6)
Floors	4 (9.5)	5.3 (2)	5.3 (2)
Bedrails	19.0 (8)	21.1(8)	21.1(8)
Side tables	19.0 (8)	21.1(8)	21.1(8)
Total	42 (70.0)	90.5(38)	90.5 (38)

Table 2. Biofilm formation in MRSA.

Strength of Biofilm	MRSA (%)
Strong(+++)	20 (52.6)
Moderate (++)	2 (5.3)
Weak(+/-)	0 (0.0)
None	16 (42.1)
Total	38

Figure 1. Prevalence of Staphylococcus aureus and MRSA in clinical samples.



Discussion

The hospital environment is an important reservoir for MRSA. When a prior occupant is infected by MRSA, hands of the hospital staff, and visitors tend to be contaminated, resulting in cross-infections and epidemics. This study investigated the prevalence of *S. aureus* and MRSA in wards previously occupied by patients from whom *S. aureus* and MRSA was isolated via clinical specimens. A total of 60 environmental surface swabs (walls, door handles, toilet seats, floors, bed rails, bedside tables) were collected. The overall recovery of *S. aureus* and MRSA from HES in this study was 70.0% and 90.5% respectively. The result of this study is in line with previous reports by **Piechota** et al. [21] who observed that hospitalised patients with MRSA colonized diarrheal stools impacted significantly on environmental contamination. [22], also observed that prolong use of bedspread contributeded to the cross contamination of wounds as the organism isolated from the wound was demonstrated to be present on bedspreads. These reports and that of this study buttresses the importance of colonised surfaces for nosocomial transmission and a risk factor for disease development and transmission. Furthermore, hospital surfaces with which individuals have prolonged, close contact or are highly accessible to patients are more likely to be frequently contaminated with MRSA. Our result indicated that the highest source (21.1%) of the MRSA isolates were obtained from bedside tables and bed rail surfaces. These are high torch surfaces with a possible spread of pathogens among the hospital staff and patients as well as the larger community. Thus, in order to control the spread of MRSA, contact precautions, thorough disinfection of patients' immediate surfaces, care equipment and the hospital environment, plus adequate antimicrobial stewardship are quite essential [11, 23]. There are several other important risk factors that influences MRSA contamination in hospitals such as the condition of the patient, the ward setting and over crowding [24, 25]. Activities of Healthcare workers including doctors and nurses are known to be the major vehicle of the environmental dispersal of micro-organisms [23]. The nursing station, ward rounds, and clinical activities (resuscitation, sampling, etc.) are all potential sources of MRSA

contamination. Another important group is the cleaning staff. The literacy level, attitudes and beliefs of the cleaning staff are considered as an additional factor as some may carry out the cleaning more effectively. On observation the cleaners were more interested in the mobbing of the floor, leaving surfaces with which patients have close contact or the highly accessible surfaces unattended to. Not surprising that the floor had the lowest prevalence of Staphylococcus and MRSA. The cleaning staff should consider including and increasing the frequency of cleaning the bedrails, side table and other high torch surfaces, including some high bacteria reservoir areas such as walls. **Abubakar and Sulaiman** [26] observed that most MRSA strains in Nigeria were hospital acquired-MRSA. This underlines the importance of infection control interventions and effective hand hygiene [11,23].

The acquisition of high-level vancomycin resistance by MRSA has been adjudged a major clinical and epidemiologic threat. A meta-analysis conducted by **Palmer & Onifade** [27] has shown that there is an emerging and increasing rate of resistance to vancomycin globally. Remarkably observed in this study is a 100% vancomycin resistance by MRSA. Being that vancomycin is a last resort drug for treatment of severe MRSA infections, the finding of *S. aureus* completely resistant to vancomycin is disconcerting. A local outbreak situation and possibly increasing rate of resistant plasmid as a cause for this observation is underlined. Mobile genetic elements (MGEs) are known to play integral part in the ability of *S. aureus* to adapt to environmental stresses [11] which include exposure to antibiotics. Staphylococcal plasmids have played a central role in conferring resistance to vancomycin [13]. However, **Zhang** et al, [28] obtaining similar resistance profile opined that *S. aureus* isolates with decreased sensitivity to vancomycin usually arise from previously existing MRSA infection. Many studies have suggested the existence of plasmid-mediated vancomycin resistance genes (*van*A, *van*B, *van*D, *van*E, *van*F, and *van*G) as one of the mechanisms for vancomycin resistance in MRSA [13,29]. Also suggested as factors involved in the higher number of VRSA in developing countries is lower public hygiene standards, different attitudes towards antimicrobial treatments and patients' population density which can enhance microbial transmission. Long-term vancomycin use and overuse which is consequent on inadequate monitoring and insufficient surveillance for vancomycin resistance.

Another troubling observation in this study was the high rate of strong biofilm formers (52.6%) among the MRSA. Although, the logistic regression indicated that the association was not significant. However, the OR (1.013) was marginal which could be attributed to the limited number of samples in this research therefore the association should not be ignored. Methicillin-resistant *S. aureus* is one of the main pathogens causing chronic infections, mainly due to its capacity to form biofilms. The association of MRSA with biofilm production favours their persistence in the environment and improves their survival for prolonged period in the environment. Furthermore, it is extremely difficult to determine the appropriate treatment option when biofilm-related infections are coexisting with methicillin resistance [30,31]. The importance of biofilm-producing MRSA in HES is not adequately documented in Nigeria and other developing countries. Epstein et al. [32] reported 62.5% MRSA biofilm formers from different hospital surfaces. Mohammed et al. [34] inferred that environmental conditions have a strong influence on bacterial biofilm formation. In concordance, **Neopane** et al. [35] revealed that strains obtained from hospital environments in their study significantly produced more biofilm than those isolated from community environments. More so, the biofilm producing staphylococci isolated from food [36] and nasal samples [33] are low in prevalence compared to the result of this study.

This research had some limitations. The molecular characterization of *S. aureus* and resistance genes was not performed. The genes responsible for biofilm production was not studied. Also, the study was carried out in one government owned hospital of Delta State, Nigeria, making the results of this study limited.

Conclusion

This study showed the high prevalence of MRSA as well as the alarming rising rate of VRSA infections in hospital environmental surfaces. This necessitates the increased disinfection of the immediate surroundings of all hospitalized patients particularly from whom MRSA has been isolated. The strong biofilm-forming capacity of MRSA indicates great ability to persist in hospital environment and increase the risk of disease development in hospitalized patients.

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Conflict of interest

The authors declare that there is no conflict of interest.

Data availability

All data generated or analyzed during this study are included in this puplished article.

Authors' contribution

All authors made significant contributions to the work presented, in the study design, implementation, data collection, analysis, and interpretation. They also contributed to the article's writing, revising, or critical evaluation, gave final approval for the version to be published.

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