Detection of *mecA* gene and methicillin resistant *Staphylococcus aureus* isolated from Inanimate Surfaces and Healthcare Workers in Tertiary Hospitals in Abia State, Nigeria

Running title: Detection of *mecA* gene and MRSA isolated from Inanimate Surfaces and Healthcare Workers

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Abstract

Background Methicillin resistant *Staphylococcus aureus* (MRSA) has become a public health issue. The purpose of this study was to detect the MRSA amongst the *S. aureus* isolates and also to detect the presence of *mecA* gene amongst some selected MRSA isolates.

Method: A total of 206 *S. aureus* isolates identified after the necessary biochemical tests were used to confirm the isolates were tested for methicillin resistant using Cefoxitin disc diffusion method. The disc diffusion method by Kirby-Bauer was used to carry out the antibiotic susceptibility pattern of the isolates. Some of the MRSA were tested for the presence of *mecA* gene.

Results A total of 122 two *S. aureus* was recovered from inanimate surfaces with footwear showing the highest contamination rate (39.34%, n=48) while 84 *S. aureus* were isolated from the palm of hands and nostrils of healthcare workers with the nostrils, 83.33% (n=70) harboring the most. The Antibiotic susceptibility test was carried out on the *S. aureus* isolates. *S.aureus* isolates were more resistant to Ampicillin, Erythromycin, Cotrimoxazole and Gentamicin. A total of 206 *S. aureus* isolates were tested for MRSA using Cefoxitin disc diffusion, and 43.69% (n=90) were methicillin resistant. A total of 20 *S. aureus* isolates suspected to be MRSA were tested for *mecA* gene and only 4 isolates (20% of the total isolates tested by Polymerase Chain Reaction) were positive for *mecA* gene.

Conclusion MRSA presence in the two tertiary hospitals give rise to risk in healthcare and community environments by contaminating healthcare workers, patients, and visitors with contaminated surfaces.

Keywords: *Staphylococcus aureus*; Healthcare workers; MRSA; Inanimate surfaces.

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Introduction

Infections acquired in hospitals or other healthcare facilities are a significant public health issue (1). In developed nations, the prevalence of HAI is already high, affecting between 5% and 15% of patients admitted in standard wards and up to 50% of critically ill patients in intensive care units (ICUs) (2, 3).

It is obvious that keeping an eye on the hospital environment is crucial to preventing nosocomial infections. Even though hand-to-hand contact is likely the biggest danger, surface contamination may be suggested as a potential source of infection. Multidrug-resistant microorganisms (MDR) may contaminate high-contract communal, such as telephones and medical charts, as well as the surfaces of frequently used medical equipment that necessitate laborious, expensive, and complicated operations in order to improve patient safety (4, 5). The problem of environmental contamination presents an even bigger challenge in the intensive care unit (ICU), because patients who are critically ill develop multiple risk factors for nosocomial infections (6).

This issue becomes especially significant when multiresistant bacteria, such as non-fermenting rods, methicillin-resistant *Staphylococcus* and vancomycin-resistant enterococci, arise in a hospital setting (7-9).

MRSA-methicillin-resistant *S. aureus* is among the most prevalent bacterial strains that cause hard-to-control illnesses in hospital settings (10).

Coagulase-positive (like *S. aureus*) and coagulase-negative staphylococci (*S.epidermis* and *S. saprophyticus*) are germs that readily contaminate the hospital environment. People with venous catheters, diabetics, and healthcare workers are more susceptible to *S. aureus*. Methicillin-resistant *S. aureus* (MRSA) poses the most threat, though, as it causes nosocomial infections with exceptionally high rates of morbidity and mortality (11). *S. aureus* was one of the frequently isolated pathogens between 2006 and 2007 linked to nosocomial infections reported to the National Healthcare Safety Network. Out of every hospital supply and equipment that are *S. aureus*, 56% are MRSA. Colonized or infected individuals are the most significant source of MRSA in a hospital because they can readily contaminate nearby electronic and medical equipment. For several months, MRSA can last on arid surfaces (12, 13). While healthcare workers' infested hands are the main way that MRSA is spread to patients, there is evidence that patient infections can also result via exposure to MRSA-contaminated surfaces (14, 15).

According to evidence from the literature review, *S. aureus* can survive surfaces that are dry for anywhere from one week to three years (16). Although there is evidence that *S. aureus* may survive in both home and clinical environments, the dissemination of these organisms, especially MRSA, across the population has received less attention (17). According to certain studies, *S. aureus* may survive on a wide range of inanimate surfaces, including polyethylene for 90 days, sterile packaging for 266 days, and screw-cap bottles for 318 days, and polypropylene for more than 1097 days (18). However, it appears that these results are the result of optimizing the experimental setup. They employed an inoculum of 107–109 CFU and staphylococcal strains that were highly resistant to desiccation. Although these findings were unambiguous and consistent in the lab, they might not be a true representation of *S. aureus* survival in the general population. Because of their limited survivability on surface that is dry for longer than 24 hours, it has been shown that dangerous and pathogenic *S. aureus* strains, such as MRSA, which cause illnesses linked to hospital, are rarely isolated from fomites in the environment (19).

The epidemiology of MRSA particularly on inanimate surfaces has remained understudied in Abia state, Nigeria and this might have contributed to its rise in hospital acquired infection. The main

aim of this study was to detect the MRSA amongst the *S. aureus* isolates and also to detect the presence of *mecA* gene amongst some selected MRSA isolates.
This study was necessary because majority of the isolates isolated from inanimate surfaces, nostrils and palm of hands of healthcare workers in the tertiary hospitals in Abia state were *S.aureus*.

Methods

This cross-sectional study was carried out in some selected hospital wards in Federal Medical Centre, Umuahia and Abia state University Teaching Hospital, Aba. The Ethical approval of the healthcare facilities was gotten before sampling and analysis. Informed consent was gotten from the participating healthcare workers with utmost confidentiality.
The present study focused on the 206 *S. aureus* isolates collected from inanimate surfaces, palm of hands and nostrils of healthcare workers after analysis in the laboratory. One hundred and twenty-two *S. aureus* isolates were collected from inanimate surfaces while 84 *S. aureus* isolates were collected from the palm of hands and nostrils of healthcare workers. A total of 14 *S. aureus* isolates were collected from the palm of hands while 70 *S. aureus* isolates were collected from nostrils. Swab sticks that are sterile dampened with sterile water was used to swab the surfaces of the mattress, tables, footwears, clinical coats, bed sheets, pillows. These inanimate surfaces were selected based on their frequent use and direct contact with patients, healthcare workers and visitors. Swab sticks that are sterile dampened with sterile water was used to swab the palm of hands of healthcare workers and only sterile swab sticks were used to swab the nostrils of healthcare workers. To guarantee each surface area was well covered, the swab sticks were oscillated.
Gram-stained isolates were identified using standard biochemical tests after the swab sticks were inoculated onto appropriate media (Blood agar, Mannitol salt agar) and incubated for 24 to 48 hours at 37°C. Pigment production, acid production, coagulase and catalase tests were the biochemical tests used to confirm *S. aureus* isolates from mannitol salt agar (20).
The disk diffusion method was used to test for antibiotic susceptibility, and the results were interpreted in accordance with the Clinical Laboratory Standards Institute (21) guidelines. Cotton wool swab that is sterile was dipped into a suspension of the organism's overnight growth that had been produced to the density of a McFarland no 0.5 opacity standard in order to inoculate Mueller Hinton culture plates. Any extra liquid from the swab was then expressed using the spread plate procedure prior to inoculation.
Antibiotic discs (Biomark, India) that were used have the following concentrations: Tetracycline 30 µg; Ampicillin 10µg; Meropenem 10 µg; Gentamicin 10 µg; Erythromycin 5 µg; Ciprofloxacin 5 µg; Cotrimoxazole 25µg; Cefuroxime 10 µg; Augmentin 30 µg; Cefalexin 10 µg; Vancomycin 30µg; Ceftazidime 10µg ; Chloramphenicol 10 µg; Ceftriaxone 30 µg; Cefotaxime 30µg; Amikacin 30µg.
To ensure the growth is confluent or near confluent, examination of the control and test plates was carried out after overnight incubation. The diameter of each zone of inhibition was measured in mm using a ruler on the underside of the plate. Growth starts at the endpoint of inhibition (22).
The Clinical and Laboratory Standards Institute's (23) criteria were followed when conducting the test. Each isolate was suspended to a turbidity of 0.5 McFarland Standard before being plated onto a Mueller Hinton agar plate (Hardy Diagnostics USA). On each plate, a 30 µg Cefoxitin disc (Oxoid) was placed. Zone sizes were assessed following a 24-hour incubation period at 35 ºC. Resistance was defined as isolates with an inhibitory zone size of less than or equal to 19 mm. ATCC 33591 (MRSA), ATCC 29213 (MSSA).

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To provide a detailed explanation, each isolate was cultured in 1ml of sterile water in different 1.5ml micro centrifuge tubes. For one minute, the samples were centrifuged at 10,000 rpm, and the supernatants were discarded.
After adding 200 liters of lysis buffer to each tube containing the pelleted samples, a vortex mixer was used to mix it thoroughly for a few seconds. The heating block machine (Biobase) was used to incubate the samples at 55ºC for 10 minutes. The samples were allowed to cool after incubation, and then centrifuged at 10,000 rpm for 30 seconds.
After this step, 200ul of absolute ethanol was added into each sample and the supernatants were carefully transferred into well labelled spin columns fitted into collection tubes without dislodging the pellets.
The spin columns were centrifuge at 10,000rpm for 1 min and the flow collected in the collection tubes were discarded.
500ul of wash buffer 1 and 2 respectively, the DNA which is now trapped on the silica membrane in the spin columns was washed.
At each stage of the washing, the spin columns were centrifuge at 10,000rpm for 30s each to allow the wash buffer pass into the collection tubes.
To remove any traces of ethanol, the spin columns were spin dried at 14,000rpm after this. DNA samples were eluted into well labelled and nuclease-free 1.5ml microcentrifuge tubes with 50ul of elution buffer. The DNA samples were stored at -20ºC until further analysis after the elution (24).
mecA gene was amplified by Polymerase Chain Reaction (PCR) using specific primer pairs (*mecA*-F:5'-AAAATCGATGGTAAAGGTTGGC-3;*mecA*-R:5'-AGTTCTGCAGTA CCGGATTTGC-3) (24).
For each isolate's DNA samples, a total of 25ul of reaction volume was prepared using 5X HOT FIREPol Blend Master mix with 7.5mM $MgCl_2$ (Solis Biodyne). This was brought down to 1X concentration using 1X Blend Master mix buffer buffer Buffer (Solis Biodyne), 1.5 mM $MgCl_2$, 200µM of each deoxynucleoside triphosphates (dNTP) (Solis Biodyne), 25pMol of each forward and reverse primer (BIOMERS, Germany), 2 units of Hot FIREPol DNA polymerase (Solis Biodyne), Proofreading Enzyme, 5µl of the extracted DNA, and sterile distilled water.
After five minutes of initial denaturation at 95°C, 30 amplification cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 45 seconds at 72°C were carried out using a PTC 200 gradient thermal cycler Eppendorf. A final extension phase of 10 minutes at 72°C came next. Electrophoresis was performed at 80V for 60 minutes after the resultant amplicons were separated on a 1.5% agarose gel. Following electrophoresis, ethidium bromide staining and a UV transilluminator were used to view the DNA bands. The benchmark for DNA molecular weight was a 100 bp DNA ladder (24).

Statistical analysis

Version 20.0 statistical software of the SPSS was used for data analysis. Categorical variables such as the frequency of the bacterial isolates were summarized using proportion expressed in percentages.

Results

The prevalence of *S. aureus* on inanimate surfaces is provided in **Table 1**. Out of the 122 *S. aureus* isolates collected from the inanimate surfaces, footwear, 39.34% (n=48) harbored the most followed by mattress, 38.52% (n=47) while clinical coat, 0.82% (n=1) had the least. At 95% confidence level, the calculated t - test (4.366) is greater than the critical t - test (2.015) which concludes that it is statistically significant and is unlikely to be due to random chance.

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Table 2 shows the prevalence of *S. aureus* amid healthcare workers. Eighty-four *S. aureus* were recovered from healthcare workers with the nostrils, 83.33% (n=70) having the highest. At 95% confidence level, the calculated t - test (4.320) is less than the critical t - test (6.314) which concludes that it is statistically not significant.

Comparing the prevalence of *S. aureus* on inanimate surfaces and among healthcare workers, the healthcare workers had a higher percentage (68.85%) as compared to inanimate surfaces (45.69%).

Antibiotic susceptibility profile of *S. aureus* isolates is provided in **Table 3**. The isolates were more susceptible to Meropenem, Augmentin, Tetracycline, Ceftaziime, Cefuroxime, and Cephalexin. Out of the 206 *S. aureus* isolates tested for MRSA using Cefoxitin disc diffusion, 43.69% (n=90) were resistant to methicillin (**Table 4**).

The agarose gel electrophoresis of *mecA* (532bp) gene shown in Figure 1. Most of the isolates tested were negative for *mecA* gene (Negative: 16; Positive: 4).

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Table 1. Prevalence of *Staphylococcus aureus* on inanimate surfaces**.**

ITEMS	NO EXAMINED	NO OF BACTERIAL ISOLATES	
		Staphylococcus aureus	Percentage
Mattress	256	47	38.52
Bedsheet	103	11	9.02
Clinical coat	68	1	0.82
Footwear	108	48	39.34
Pillow	69	7	5.74
Table	96	8	6.56
Total	700	122	
Percentage		45.69	

$P<0.05$ = Significant

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Table 2. Prevalence of *Staphylococcus aureus* amid healthcare workers

SITE OF COLLECTION	NO EXAMINED	NO OF BACTERIAL ISOLATES	
		Staphylococcus aureus	Percentage
Palm	125	14	16.67
Nostril	175	70	83.33
Total	300	84	
Percentage		68.85	

P>0.05 = not significant

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Table 3. Antibiotic susceptibility profile of *Staphylococcus aureus* isolates

Antibiotic	Isolates n (%)
Ampicillin	70 (33.98)
Meropenem	160 (77.67)
Erythromycin	60 (29.13)
Tetracycline	140 (67.96)
Cotrimoxazole	60 (29.13)
Cefuroxime	150 (72.81)
Gentamicin	70 (33.98)
Ciprofloxacin	75 (36.41)
Augmentin	140 (67.96)
Vancomycin	100 (48.54)
Ceftazidime	120 (58.25)
Cephalexin	130 (63.11)

Table 4. MRSA detection by Cefoxitin Disk Diffusion Method

ISOLATES	TOTAL NO TESTED	MRSA	PERCENTAGE
Staphylococcus aureus	206	90	43.69

Key: MRSA= Methicillin Resistant *Staphylococcus aureus*

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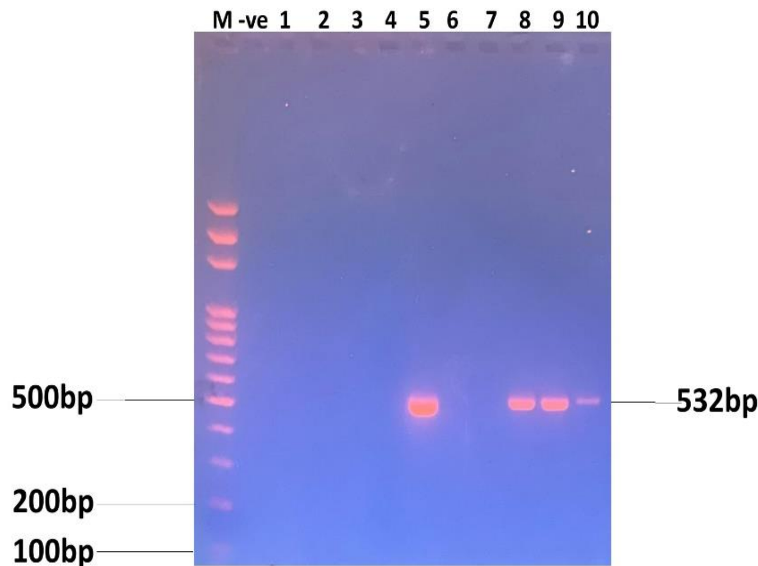

Figure 1. Agarose gel electrophoresis amplification of the *mecA* gene (532 bp) of *S. aureus* isolates. M; marker (100 bp), lines 1, 2, 3, 4, 6, 7 (Negative), Lines 5, 8, 9, 10 (Positive).

Discussion

S. aureus, 45.69% was the prevalent bacteria isolated from inanimate surfaces/fomites in this study. These results are comparable to those of the Brazillian study which found that *S. aureus* was the primary organism isolated from fomites 53.3 percent of the time (25). Munveshyaka et al., (26) findings on inanimate surfaces and equipment are also consistent with these findings. *S. aureus* can form biofilms on inanimate surfaces/fomites and these biofilms can be more resistant to disinfectants and cleaning efforts, making them a more persistent source of infection.

S.aureus was seen more from the nostrils as compared to the palm of hands in this study which corresponds to the findings of Pant and Sharma, (27); Junu et al., (28). This result is consistent with the notion that *S. aureus* most frequently colonizes the nostrils. Compared to nasal carriers, there are fewer reports of hand carriers. Compared to Mukhiya et al., (29) and Pant and Sharma (27), the prevalence of palm of hands carriers was lower, which may suggest that the healthcare workers who took part in this study practiced better hand cleanliness. In this investigation, every hand carrier was also a nasal carrier, and the isolates from both locations shared similar antibiogram, indicating that they were phenotypically identical. The notion that hands are the primary vector for spreading *S. aureus* from surfaces to the nostrils and from the nostrils to surfaces is further supported by the possibility that these carriers have a tendency of picking their noses.

The bacterial isolates were resistant to the frequently used antibiotics. Erythromycin (29.13%), Cotrimoxazole (29.13%), Gentamicin (33.98%), Ampicillin (33.98%) and Ciprofloxacin (36.41%) had low susceptible rates to *S. aureus. S. aureus* was more resistant to these drugs. This could be owing to their availability, cost and misuse of these medications. This is in line with the findings of Sanusi et al., (30). Adam *et al*., (31), on the other hand, found that the highest rate of *S. aureus* resistance was found in trimethoprim-sulfamethoxazole. This could be due to development of resistance mechanisms like reduced antibiotic accumulation within the bacteria, and bypassing the antibiotic's inhibitory effects.

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Out of the 390 bacterial isolates obtained from the two tertiary hospitals in Abia state, 206(52.82%) were found to be positive for *S. aureus*. Reena et al., (32) and Atsedewoyn et al., (33) reported the presence of *S. aureus* on hospital surfaces in Nepal and Ethiopia respectively, despite the fact that there is a scarcity of data regarding the prevalence of MRSA in Nigeria. These results contradict with those of this investigation. The study's findings are in line with those of Anyadoh et al., (34), and Sanusi et al., (30), who found that more than 50% of hospital surfaces were infected with *S. aureus*.

The rise of MRSA, which is resistant to cephalosporin, a class of antibiotics commonly used to treat *Staphylococcus* infections, and monobactams as well as all beta lactam drugs has made the emergence of *S. aureus* infections worse (35). Identifying MRSA accurately requires prompt and early diagnosis because MRSA infections lead to treatment issues and spread (36).

In this study, 90(43.69%) *S. aureus* isolates were found to be resistant to Cefoxitin disks diffusion test for MRSA. This is comparable to Khairullah et al., (37) study, which revealed that 47.62% of the isolates tested positive for MRSA. In comparison, Adam et al., (31) and Muge et al., (38) reported 2.1% of MRSAs were positive, while Sanusi et al., (30) reported 5.4%. According to this study, a number of variables, such as improper hand hygiene and a failure to clean surfaces, might contribute to the development of MRSA contamination on fomites/inanimate surfaces and healthcare personnel. MRSA contamination raises the possibility of the spread of hard-to-treat Staphylococci, posing a major public health danger.

According to Miragaia (39), *mecA* genotyping by PCR remains the primary recommendation despite its inability to be carried out on a regular basis, and detecting MRSA phenotypically via disk diffusion has yet to yield reliable results. Nevertheless, due to its speed and affordability, disk diffusion identification of MRSA is still often utilized (40).

Oxacillin and cefoxitin diffusion disks exhibit the same 100% sensitivity and 74.07% and 92.59% specificities, respectively (41). But because there is still a high percentage of false positives with the oxacillin disk diffusion approach, a number of earlier investigations found that there was a higher sensitivity level with Cefoxitin disk diffusion method as compared to oxacillin in identifying MRSA (42). According to Vyas et al., (41), beta-lactamase hyper production may contribute to false positives by causing oxacillin resistance to manifest phenotypically without a genotypic resistance mechanism.

A total of 20 suspected methicillin resistant *S.aureus* isolates were tested for *mecA* gene and only 4 isolates (20% of all isolates that were tested by PCR) were *mecA* gene positive. This might be due to false positives in the phenotypic analysis. Suspected MRSA were found to have the *mecA* gene as shown by the result of the PCR. This is similar to Khirullah et al., (37) where 30% of the MRSA isolates tested were *mecA* gene positive. However, this was in contrast to Sanusi et al., (30) who had 83.33% of MRSA isolates positive to *mecA* gene. These findings are in line with Ramandinianto et al., (43) study. Because it can boost the expression of PBP2a, which the *mecA* gene encodes, the antibiotic cefoxitin is an effective inducer of *mecA* gene expression (44). This also agrees with Reichmann and Pinho, (45).

Conclusion

This study was conducted to detect the *mecA* gene and MRSA from the *S. aureus* isolated from inanimate surfaces, palm of hands and nostrils of healthcare workers in the selected hospital wards in the two tertiary hospitals in Abia state. This study had 43.69% MRSA prevalence and only 20% *mecA* gene positivity.

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The hospital wards evaluated in this study showed bacterial pathogens presence on inanimate surfaces and healthcare workers nostrils with *S. aureus* being the prevalent bacterial isolate in this study. There was higher nasal carriage than hand carriers in this study.

The presence of MRSA in the two tertiary hospitals poses a risk to healthcare and community environments by contaminating healthcare workers, patients, and visitors with contaminated surfaces. This could ultimately lead to the community's exposure to resistant bacteria. Therefore, in order to stop the spread of *S. aureus* infections on fomites/inanimate surfaces as well as healthcare personnel at tertiary hospitals in Abia state, prevention and control measures are required.

However, the unavailability of molecular laboratories at close proximity to run the *mecA* gene analysis immediately limited this study.

Funding source

Not applicable

Ethical approval

The Ethics committee of the Federal Medical Centre, Umuahia (code: FMC/QEH/G.596/Vol.10/746) and Abia state University Teaching Hospital (code: ABSUTH/MAC/117/VOL1/60) approved the study.

Conflict of interest

The authors have no competing interests.

Authors contributions

EUO: Conceptualization; Methodology; Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Visualization; Writing-original draft; Writing-Review and editing

IO**:** Supervision; Methodology.

EON: Methodology; Investigation, Interpretation.

Consent for publication

Not applicable

Data availability statement

All data are available upon request from the corresponding author.

References

1. Amazian K, Rossello J, Castella A. Prevalence of nosocomial infections in 27 hospitals in the Mediterranean region, East Med Health J. 2010; 16(10):1070–1078, https://doi.org/10.26719/2010.16.10.1070.
2. WHO. *WHO guidelines on hand hygiene in health care: First global patient safety challenge clean care is safer care [homepage on the Internet]*. Geneva: World Health Organization; 2009 [updated 2018 May 23; Available from: https://www.ncbi.nlm.nih.gov/books/NBK144013.

Accepted Article

3. Vincent JL, Rello J, Marshall J. International study of the prevalence and outcomes of infection in intensive care units, J Am Med Ass. 2009;302(21):2323–2329, https://doi.org/10.1001/jama.2009.1754,
4. Lalami AEO, Touijer H, Ettayebi M, et al. Microbiological monitoring of environment surfaces in a hospital in Fez city, J Mat Env Sci. 2016; **7**(1):123–130.
5. Galvin S, Dolan A, Cahill O, et al. Microbial monitoring of the hospital environment: why and how? J Hosp Infect. 2012;82(3):143–151, https://doi.org/10.1016/j.jhin.2012.06.015
6. Huslage K, Rutala WA, Sickbert-Bennett E, et al. A quantitative approach to defining "high-touch" surfaces in hospitals, Infect Control hosp epidemiol. 2010; 31(8):850–853, https://doi.org/10.1086/655016.
7. Beggs C, Knibbs LD, Johnson GR, et al. Environmental contamination and hospital-acquired infection: Factors that are easily overlooked. Indoor Air: 2015;25: 462–74, https://doi.org/10.1111/ina.12170.
8. Martinez JA, Ruthazer R, Hansjosten K, et al. Role of environmental contamination as a risk factor for acquisition of vancomycin-resistant enterococci in patients treated in a medical intensive care unit of a cancer center. Arch Int Med. *2003;*163(16):1905–12, https:// doi.org/10.1001/archinte.163.16.1905.
9. Ray AJ, Hoyen CK, Taub TF, et al. Nosocomial transmission of vancomycin-resistant enterococci from surface. J Am Med ass. 2002; 287:1395–6, https://doi.org/10.1001/ jama.287.11.1395.
10. Jabłońska-Trypuć A, Makuła M, Włodarczyk-Makuła M, et al. Inanimate Surfaces as a Source of Hospital Infections Caused by Fungi, Bacteria and Viruses with Particular Emphasis on SARS-CoV-2. Int J env res pub health. 2022;19(13):8121. Doi: 10.3390/ijerph19138121.
11. Lin D, Ou Q, Lin J, Peng Y, Yao Z. A meta-analysis of the rates of Staphylococcus aureus and methicillin-resistant S aureus contamination on the surfaces of environmental objects that health care workers frequently touch. Am J Infect Control. 2017 Apr 1;45(4):421-429. doi: 10.1016/j.ajic.2016.11.004.
12. O'Hara LM, Calfee DP, Miller LG., et al. Optimizing Contact Precautions to Curb the Spread of Antibiotic-Resistant Bacteria in Hospitals: A Multicenter Cohort Study to Identify Patient Characteristics and Healthcare Personnel Interactions Associated with Transmission of Methicillin-Resistant *Staphylococcus aureus*. Clin infect dis. *2019;*69:S171–S177. Doi: 10.1093/cid/ciz621.
13. Cimolai N. MRSA and the Environment: Implications for Comprehensive Control Measures. Eur J clin microbiol infect dis. *2008;*27:481–493. Doi: 10.1007/s10096-008-0471-0.
14. Hardy KJ, Oppenheim BA, Gossain S, Gao F, Hawkey PM. A study of the relationship between environmental contamination with methicillin-resistant Staphylococcus aureus (MRSA) and patients' acquisition of MRSA. Infect Control Hosp Epidemiol. 2006 Feb;27(2):127-32. doi: 10.1086/500622.
15. Aldeyab MA, McElnay JC, Elshibly SM, et al. Evaluation of the Efficacy of a Conventional Cleaning Regimen in Removing Methicillin-Resistant *Staphylococcus aureus* from Contaminated Surfaces in an Intensive Care Unit. Infect control hosp epidemiol. *2009;*30:304–306. Doi: 10.1086/595964.

Accepted Article

16. Chaibenjawong P, Foster SJ. Desiccation Tolerance in Staphylococcus aureus. Arch microbiol. *2011;*193:125–135. Doi: 10.1007/s00203-010-0653-x
17. Davis MF, Iverson SA, Baron P, Vasse A, Silbergeld EK, Lautenbach E, Morris DO. Household transmission of meticillin-resistant Staphylococcus aureus and other staphylococci. Lancet Infect Dis. 2012 Sep;12(9):703-16. doi: 10.1016/S1473-3099(12)70156-1.
18. Dietze B, Rath A, Wendt C, Martiny H. Survival of MRSA on sterile goods packaging. J Hosp Infect. 2001 Dec;49(4):255-61. doi: 10.1053/jhin.2001.1094. PMID: 11740873.

19. Domon H, Uehara Y, Oda M, et al. Poor Survival of Methicillin-Resistant Staphylococcus aureus on Inanimate Objects in the Public Spaces. Microbiol open. *2016;*5:39–46. Doi: 10.1002/mbo3.308.
20. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, 2nd edition, Cambridge University Press Publication, South Africa,, 2006. p.143-157.
21. CLSI. Performance Standards for Antimicrobial Susceptibility Testing; Clinical Laboratory Standard Institute, 2011. NCCLS document M100-S21. 940 West valley road. Wayne, PA 19687 USA.
22. Jorgensen JH, Turnidge JD. Susceptibility test methods dilution and disk diffusion methods. Manual of clinical microbiology, 9th Ed, ASM press 2007.p 1152-1172.
23. CLSI. Performance standards for Antimicrobial Susceptibility Testing. Clinical Laboratory Standard Institute, 2020. 30th Edition, M100-S. 940 West valley road. Wayne, PA 19687 USA.
24. Wielders CL, Fluit AC, Brisse S. MecA gene is widely disseminated in *Staphylococcus aureus* population. *J clin microbiol*. 2002;40(11):3970-5. Doi: 10.1128/JCM.40.11.3970-3975.2002.
25. Rodrigues DO, Peixoto LDP, Barros ETM, et al. Epidemiology of bacterial contamination of inert hospital surfaces and equipment in critical and non-critical care units: a Brazilian multicenter study, Microbiol res J Int. 2019;10:15, 793034.
26. Munveshyaka E, Cyuzuzo P, Yadufashije C, et al. Contribution of Medical Wards Contamination to Wound Infection among Patients Attending Ruhengeri Referral Hospital. Int J microbiol. 2021;11: 7838763 https://doi.org/10.1155/2021/7838763
27. Pant ND, Sharma M. Carriage of methicillin resistant Staphylococcus aureus and awareness of infection control among healthcare workers working in Intensive care unit of a hospital in Nepal. Braz J Infect Dis. 2016; 20: 218-9.
28. Junu RR, Rita A, Shiba RR. Hand and nasal carriage of Staphylococcus aureus and its rate of recolonization among healthcare workers of a tertiary care hospital in Nepal. JAC Antimicrob resist. 2022; 4:3 https://doi.org/10.1093/jacamr/dlac051
29. Mukhiya RK, Shrestha A, Rai SK. Prevalence of Methicillin resistant Staphylococcus aureus in hospitals of Kathmandu Valley, Nepal. J sci technol. 2012;13:185-90
30. Sanusi AA, Dangari MA, Salihu MK, et al. Molecular detection of mecAgene in Methicillin resistant Staphylococcus aureus isolated from surfaces of some public hospitals in Katsina state, Nigeria. UMYU J microbiol res. 2023;8(2):110-117. https://doi.org/10.47430/ujmr.2382.013.
31. Adam AS, Lisa M, Sarah KO, Ntulume I, Almustapha Aliero A. Antibiotic Susceptibility Pattern and Detection of mecA Gene in Methicillin resistant Staphylococcus epidermidis

Accepted Article

Isolated from Wards Surfaces of Kampala International University Teaching Hospital, Uganda. Romanian Arch microbiol Immunology. 2020; 79(1), 24-36.
32. Reena K, Mukhiya AS, Shiba K, et al. Prevalence of Methicillin-Resistant Staphylococcus aureus in Hospitals of Kathmandu Valley, Nepal J sci technol. 2012;13(2), 185-190. https://doi.org/10.3126/njst.v13i2.7734
33. Firesbhat A, Tigabu A, Tegene B, Gelaw B. Bacterial profile of high-touch surfaces, leftover drugs and antiseptics together with their antimicrobial susceptibility patterns at University of Gondar Comprehensive Specialized Hospital, Northwest Ethiopia. BMC Microbiol. 2021 Nov 8;21(1):309. doi: 10.1186/s12866-021-02378-w. https://doi.org/10.1186/s12866-021-02378-w
34. Anyadoh NO, Eri JC, Nwaokoro PO, Sylvia O. Anyadoh - Nwadike. Prevalence of *Staphylococcus aureus* within the Hospital Environment. Asian J med pharm res. 2011;1(1), 17-21
35. Bush K., Bradford PA. B-Lactams and β-Lactamase Inhibitors: An Overview. Cold Spring Harb Perspect Med. 2016;6(8). Doi: a025247. 10.1101/cshperspect.a025247
36. Mehta Y, Hegde A, Pande R. Methicillin-resistant Staphylococcus aureus in Intensive Care Unit Setting of India: A Review of Clinical Burden, Patterns of Prevalence, Preventive Measures, and Future Strategies. Indian j crit care med. 2020;24(1):55–62. 10.5005/jp-journals-10071-23337.
37. Rafif Khairullah A, Rehman S, Agus Sudjarwo S, Helmi Effendi M, Chasyer Ramandinianto S, Aega Gololodo M, et al. Detection of mecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from milk and risk factors from farms in Probolinggo, Indonesia. F1000Res. 2022 Jun 30;11:722. Doi: 10.12688/f1000res.122225.3.
38. Oguzkaya-Artan M, Baykan Z, Artan C, Avsarogullari L. Prevalence and risk factors for methicillin resistant Staphylococcus aureus carriage among emergency department workers and bacterial contamination on touch surfaces in Erciyes University Hospital, Kayseri, Turkey. Afr Health Sci. 2015 Dec; 15(4): 1289-94. https://doi.org/10.4314/ahs.v15i4.31
39. Miragaia M. Factors contributing to the Evolution of mecA-Mediated β-lactam Resistance in Staphylococci: Update and New Insights from Whole Genome Sequencing (WGS). Front microbiol. 2018; 9:2723. Doi: 10.3389/fmicb.2018.02723.
40. Bennett K, Sharp SE. Rapid differentiation of methicillin-resistant Staphylococcus aureus and methicillin-susceptible Staphylococcus aureus from blood cultures by use of a direct cefoxitin disk diffusion test. J clin microbiol. 2008; 46(11):3836–3838. Doi:10.1128/JCM.01276-08
41. Vyas A, Sharma M, Kumar S. A comparative study of Oxacillin screen agar, Oxacillin disk diffusion and Cefoxitin disk diffusion, Oxacillin E-test method for routine screening of methicillin-resistant Staphylococcus aureus. Int j curr res rev. 2015;7(10):55–60.
42. Broekema NM, Van TT, Monson TA. Comparison of Cefoxitin and Oxacillin Disk Diffusion Methods for Detection of mecA-Mediated Resistance in Staphylococcus aureus in a Large-Scale Study. J clin microbiol. 2009;47(1):217–219. Doi:10.1128/JCM.01506-08 40.

Accepted Article

43. Ramandinianto SC, Khairullah AR, Effendi MH MecA gene and methicillin-resistant Staphylococcus aureus (MRSA) isolated from dairy farms in East Java, Indonesia. Biodiversitas. 2020;21(8):3562–3568. Doi:10.13057/biodiv/d210819
44. Koupahi H, Jahromy SH, Rahbar M. Evaluation of Different Phenotypic and Genotypic Methods for Detection of Methicillin Resistant Staphylococcus aureus (MRSA). Iran J pathol. 2016;11(4):370–376
45. Reichmann NT, Pinho MG. Role of SCC mec type in resistance to the synergistic activity of oxacillin and cefoxitin in MRSA. Sci report. 2017;7:6154. Doi: 10.1038/s41598-017-06329-2

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