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ORIGINAL RESEARCH ARTICLE

Occurrence and Molecular Detection of Methicillin-Resistant Staphylococcus Species in Sewage at Federal Teaching Hospital Gombe, Gombe State, Nigeria

Waziri, J. S.[1,2], Adamu, M. T.[1], Tawfiq, U. A.[1], Garba, L.[1], Salawudeen, A.[1] and Manga, M. M.[2]*

[1]Department of Microbiology, Faculty of Sciences, Gombe State University, Nigeria
[2]Department of Medical Microbiology and Immunology, Faculty of Basic Clinical Sciences, Gombe State University, Nigeria

ABSTRACT

Methicillin-resistant Staphylococcus is one of the pathogenic microorganisms found in hospital sewage. It can cause problems for public health by causing diseases ranging from skin infections to severe adverse conditions. This study investigated the prevalence and characteristics of methicillin-resistant Staphylococcus species in sewage at Federal Teaching Hospital, Gombe. Phenotypic and molecular analyses of sewage samples were performed to determine the most prevalent serotype and detect resistance genes. Phenotypic analysis of 50 sewage samples revealed Staphylococcus aureus as the predominant species, with a prevalence of 52% in the sewage. Coagulase-negative staphylococci (CoNS) occurred in 7 samples (14%), while 17 samples (34%) showed no growth. Among S. aureus isolates, methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) had prevalent of 69.3% and 30.7% respectively. Similarly, among the CoNS, methicillin-susceptible CoNS (MS-CoNS) were more common, with a prevalence of 71.5%, compared with 28.5% for methicillin-resistant CoNS (MR-CoNS). In molecular detection of resistance genes, *mecC* was detected in 100% of methicillin-resistant isolates (n=10), while *mecA* was detected in only 75% of methicillin-resistant isolates. This study was the first Nigerian study to report *mecC* dominance in hospital sewage, contradicting the global *mecA* prevalence. These findings suggest a significant presence of Staphylococcus species, particularly MSSA, in hospital sewage, and highlight the prevalence of *mecC* as a key resistance determinant in this environment.

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INTRODUCTION

Staphylococcus species are Gram-positive, facultatively anaerobic organisms that usually inhabit the skin and nasal cavities of healthy individuals, which serve as part of the human microbiota (Cheatham *et al.*, 2019). Direct contact with these organisms was found to be harmless; however, colonization may lead to infections in some individuals with compromised skin and immune function (Ouidri, 2018). It causes a variety of infections, including seborrheic pimples, carbuncles, pneumonia, bloodstream infections, and surgical site infections (Silva *et al.*, 2021). This bacterium may cause numerous infections, ranging from skin conditions to complex systemic illnesses (Siddiqui & Koirala, 2023).

These organisms possess a diverse array of virulence factors that enable them to cause many infections (Pidwill *et al.*, 2021). These factors enable the bacterium to adhere to host tissues, evade the immune system, and damage host cells (Igbinosa *et al.*, 2023). The pathogenicity of Staphylococcus species includes the production of toxins that damage host tissue, leading to a severe immunologic response (Neelam *et al.*, 2022). Antimicrobial agent misuse can readily enable Staphylococcus to develop resistance to specific antibiotics, such as methicillin, resulting in Methicillin-Resistant Staphylococcus Strains (MRSSs) (Houkes *et al.*, 2023).

Methicillin-resistant Staphylococcus aureus (MRSA) is a strain of *Staphylococcus aureus* that has developed resistance to several antibiotics, including methicillin (Asnakew Abebe & Birhanu, 2023). This underscores the difficulties in managing cases caused by methicillin-resistant Staphylococcus (MRS), as many common antibiotics are ineffective against it (Silva *et al.*, 2021). MRS can cause human infections ranging from mild skin infections to severe, difficult-to-treat, and life-threatening systemic infections (Adamu *et al.*, 2023). Healthcare settings are environments usually associated with these pathogens, where they can spread through direct contact with infected individuals or contaminated surfaces (Adeiza et

Correspondence: Manga M. M. Department of Medical Microbiology and Immunology, Faculty of Basic Clinical Sciences, College of Medical Sciences, Gombe State University, Nigeria. ✉ drmmanga@gmail.com.
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al., 2020). The methicillin-resistant *Staphylococcus aureus* was first isolated in the 1960s, a highly resistant strain that causes significant healthcare-associated outbreaks and community-acquired infections (Hung *et al.*, 2022). The first cases of MRSA were reported in the United Kingdom in 1961, just a year after the introduction of the antibiotic methicillin (Enright *et al.*, 2002). MRSA continued to spread between the 1970s and 1980s, becoming a major problem in hospitals worldwide (Harkins *et al.*, 2017). Community-acquired MRSA (CA-MRSA) emerged between the 1990s and 2000s and could cause infections in healthy individuals who had not been hospitalised (Romero & de Souza da Cunha, 2021). Athletes and young people are the most vulnerable group to the acquisition of CA-MRSA (Moellering, 2012).

Staphylococcus aureus exhibits resistance to the methicillin group of antibiotics primarily due to the acquisition of the *mecA* and *mecC* gene. These genes encode a novel penicillin-binding protein (PBP2a) with low affinity for β-lactam antibiotics, including methicillin (Asnakew Abebe & Birhanu, 2023). The gene is typically located on a mobile genetic element called Staphylococcal Cassette Chromosome mec (SCCmec). This element is responsible for the dissemination of methicillin resistance among Staphylococcus strains (Marciniak *et al.*, 2024). The acquisition of resistant genes through horizontal gene transfer, particularly via conjugation, is the primary mechanism by which Staphylococcus becomes resistant to methicillin and other β-lactam antibiotics. This process highlights the importance of antibiotic and infection control measures to prevent the further spread of antibiotic resistance (Iregbu *et al.*, 2021). It's important to note that methicillin resistance is not exclusive to *S. aureus*. Other Staphylococcus species, such as *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, can also acquire methicillin resistance through similar mechanisms (Marciniak *et al., 2024)*. Proper hygiene practices, such as frequent handwashing and wound care, are essential to prevent the spread of staph infections (Asnakew Abebe & Birhanu, 2023).

MATERIALS AND METHODS

Study Area

The study area is the Federal Teaching Hospital, Gombe. The area was chosen because of its 95 underground sewage chambers and reservoirs. The hospital is one of the federal referral centres in northeastern Nigeria, where people with diverse norms, cultures, taboos, and beliefs receive health care services.

Sample size and collection

Fifty (50) samples were collected from each of the fifty exit chambers across the hospital units, and 10 ml of the samples were collected using sterile sewage sample containers. The containers were properly labelled with the assigned sample numbers. Safety measures were taken to avoid contact with the sewage and prevent sample contamination (Martín-Pozo *et al.*, 2019). The samples were aseptically transported to the Medical Microbiology and Immunology Teaching Laboratory, Gombe State University for further analysis.

Bacterial isolation

The samples were centrifuged to concentrate the pellet; the pellets were picked using a sterile wire loop, streaked onto the prepared mannitol salt agar (MSA) plate, and incubated at 37°C for 24 hours. After 24 hours of incubation, the plates were observed for colony growth and colour. *S. aureus* produces golden yellow colonies with yellow zones on MSA as a result of mannitol fermentation, where coagulase-negative staphylococci do not (Igbinosa *et al.*, 2023). A representation of the colonies was picked for identification (Tiwari, 2008).

Bacterial identification

Gram Staining and biochemical tests

A thin smear was prepared from golden-yellow and pinkish colonies and allowed to air-dry. The smears were heat-fixed, then covered with crystal violet (primary stain), and flooded in running water after 60 seconds. This was followed by covering the smears with Lugol's iodine (Mordant) for 60 seconds, then flooding with running water. Acetone (Decolorizer) was then added and washed immediately, then safranin (secondary stain) was added and allowed to stay for one minute and then washed with running water, the slides were allowed to air-dried and a drop of oil immersion was added to the stained slides and viewed under a microscope with X4 and then X100 objective lenses (Silva *et al.*, 2021). Biochemical tests such as catalase, coagulase, DNase, and mannitol fermentation were carried out on the isolates according to Adamu et al. (2023), Brown et al. (2021), and Chen et al. (2017).

Phenotypic detection of methicillin-resistant Staphylococcus strains

The McFarland standard was prepared according to the method described by Hou et al. (2023). The susceptibility of the isolates was determined by the Kirby-Bauer disk diffusion method. The standardised inocula of the isolates were spread on Mueller-Hinton agar plates using sterile swab sticks, and a Cefoxitin disk (30 µg) and a Gentamycin (high-level resistant) disk (200 µg) were placed on the agar surfaces aseptically. The plates were incubated at 37 °C for 18–24 hours, and the zones of inhibition around the disks were measured in mm (Anwar *et al.,* 2020).

Molecular detection of resistant genes

DNA Extraction using *QIAamp* DNA Extraction Kit Protocol of the isolates

The protocol involves four main stages: lysis, precipitation, washing, and elution. Firstly, the bacterial cells were lysed using a lysis buffer to release the DNA. The cell debris was then precipitated with absolute ethanol and removed by centrifugation. The DNA was washed to remove impurities and finally eluted with a buffer to obtain a purified DNA solution (Elsayed Naeim *et al.*, 2023; Monteiro *et al.*, 2021).

Polymerase chain reaction (PCR)

A PCR convention was performed using the extracted DNA of the isolates with designed, synthesized primers for *mecA* (forward and reverse) and *mecC* (forward and reverse), as shown in Table 1.

Table 1: Primers for *mecA* (Forward and Reverse) and *mecC* (Forward and Reverse)

S/N	Gene	Forward primer	Reverse primer	Size (bp)
1	*mecA*	5'-AAAATCGATGGTAAAGGTTGGC-3'	5'-AGTTCTGGAGTACCGGATTTGC-3'	310
2	*mecC*	5'-TCACCAGGTTCAACTCAAAA-3'	5'-CCTGAATCAGCTAATAATATTTC-3'	533

The final volume of 20 *µl* was required for the PCR mixture preparation, which included 4µl of PCR master mix (Thermo Scientific), *1µl* each of both forward and reverse primers, then 2µl of template DNA and 12µl of nuclease-free water. The cycling conditions were:

- ✓ Initial denaturation at 95°C for 5 minutes.
- ✓ Denaturation at 95°C for 30 seconds.
- ✓ Annealing at 55°C for 30 seconds.
- ✓ Extension at 72°C for 1 minute.

35 Cycles

Final extension at 72°C for 10 minutes

Gel Electrophoresis

The agarose powder (1g) is dissolved in 100 mL of TAE buffer to prepare a 1% agarose gel. The mixture was heated to complete dissolution on a hot plate and cooled to 45-50 °C. Once cooled, 3 µL ethidium bromide was added to the mixture, enabling band visualisation. The solution is then poured into a casting tray with a comb to create wells. The gel solidified, and the comb was removed to form wells for sample loading (Lee *et al.,* 2012). The PCR products (Amplicon) were loaded into agarose gel wells and subjected to electrophoresis at 80V and 200 mA for 40 minutes. A 533 bp band indicated the presence of the *mecA* gene, while a 310 bp band indicated the presence of the *mecC* gene. These genes were associated with methicillin resistance in *Staphylococcus aureus* (Rafif Khairullah *et al*., 2022).

Statistical analysis

Fisher's Exact test was used to test if MRSA and MSSA are present in sewage samples obtained from the study area as well as if MR-CoNS and MS-CoNS are present in sewage.

Results and Discussion

Out of fifty (50) sewage samples that were analyzed. Twenty-six 26 (52%) samples were identified as *S. aureus*, while seven 7 (14%) samples were found to be coagulase-negative Staphylococcus, with seventeen 17 (34%) samples yielding no growth after 24 hours of incubation. As shown in Table 2 below, another study reported a prevalence of 23.4% for *S. aureus* in hospital wastewater (Mohammed *et al.,* 2025).

Table 2: Distribution of Staphylococcus species in sewage sample of FTH Gombe.

Sewage	Frequency	Percentage (%)
S. aureus	26	52
CoNS	7	14
No growth	17	34
Total	**50**	**100.0**

Table 3: Distribution of Methicillin-Resistant *Staphylococcus aureus* in the isolated *S. aureus* and Methicillin-Resistant Coagulase Negative Staphylococcus in Sewage samples of FTH Gombe.

Sewage	Frequency	Percentage (%)
MRSA	8	30.7
MSSA	18	69.3
MR-CoNS	2	28.5
MS-CoNS	5	71.5
Total	**33**	**200.0**

The distribution of methicillin-resistant *Staphylococcus aureus* revealed that MSSA was the most common, with 18 (69.3%), followed by MRSA with 8 (30.7%) (Table 3). The distribution of Methicillin-resistant coagulase-negative strains (MR-CoNS) among isolated coagulase-negative staphylococci from sewage samples showed that MR-CoNS accounted for 5 (71.5%) occurrences, while MSCoNS accounted for 2 (28.5%) (Table 3). Another study recorded the prevalence of MR-CoNS. The results revealed that MSSA (69.3%) and MS-CoNS (71.3%) were more predominant in the sewage than MRSA (30.7%) and MS-CoNS (28.5%). Another study reported a prevalence of MRSA among isolated S. aureus of 42% (Hoseini Alfatemi *et al*., 2014). A study carried out by Xu et al. (2018) found an 11% occurrence of methicillin-resistant coagulase-negative staphylococcus. This could be due to environmental factors, such as disinfectants and toxic substances present in the environment (e.g., sewage).

Phenotypically confirmed MRS strain isolates were subjected to molecular analysis to detect resistance genes in *S. aureus* and coagulase-negative staphylococci. Following molecular screening, all samples were found to carry the *mecC* gene, while 75% carried the *mecA* gene (Figure 1), indicating that *mecC* was the most prevalent gene identified in this study (Figure 2). Rana et al. (2018) reported a 43.7% occurrence rate of *mecC* in S. aureus isolates, which appears lower than the result obtained in this study. A similar survey of Idrees et al. (2023) revealed

the prevalence of the *mecA* and *mecC* genes of 88.8% and 65%, respectively. A lower prevalence of the *mecA* gene (20%) was reported by Moges et al. (2023). In addition, Jayanthi et al. (2019) reported 33.3% *mecA*-positive S. aureus and 12.5% *mecC*-positive coagulase-negative Staphylococcus species.

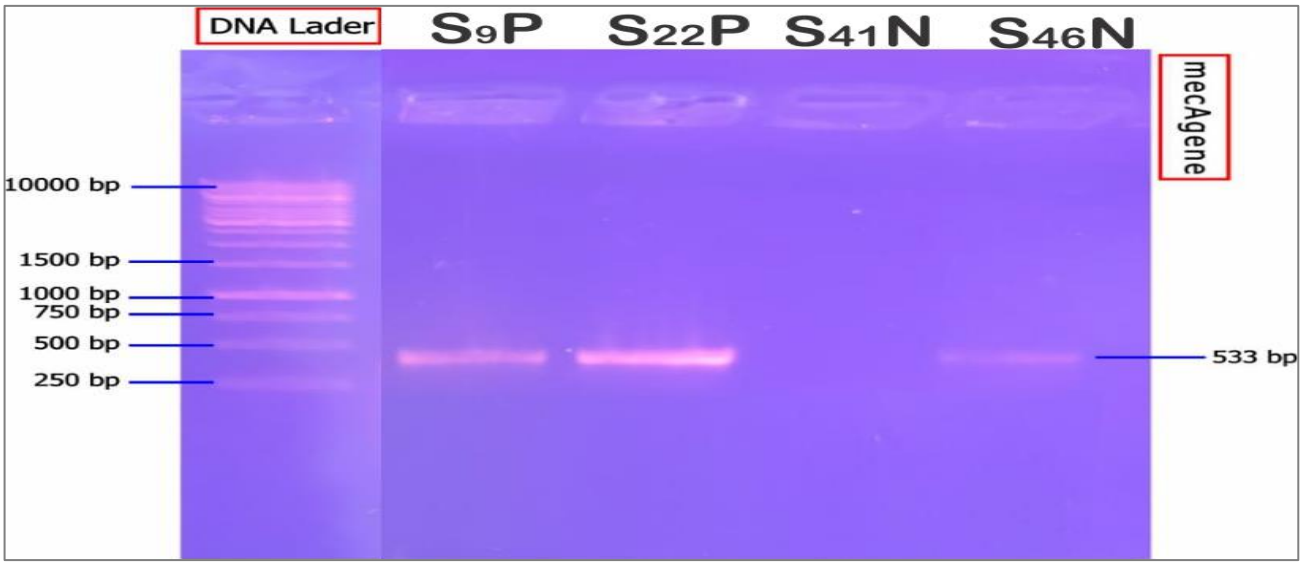

Figure 1: Result for molecular detection of the *mecA* gene from methicillin-resistant coagulase-positive and negative staphylococci

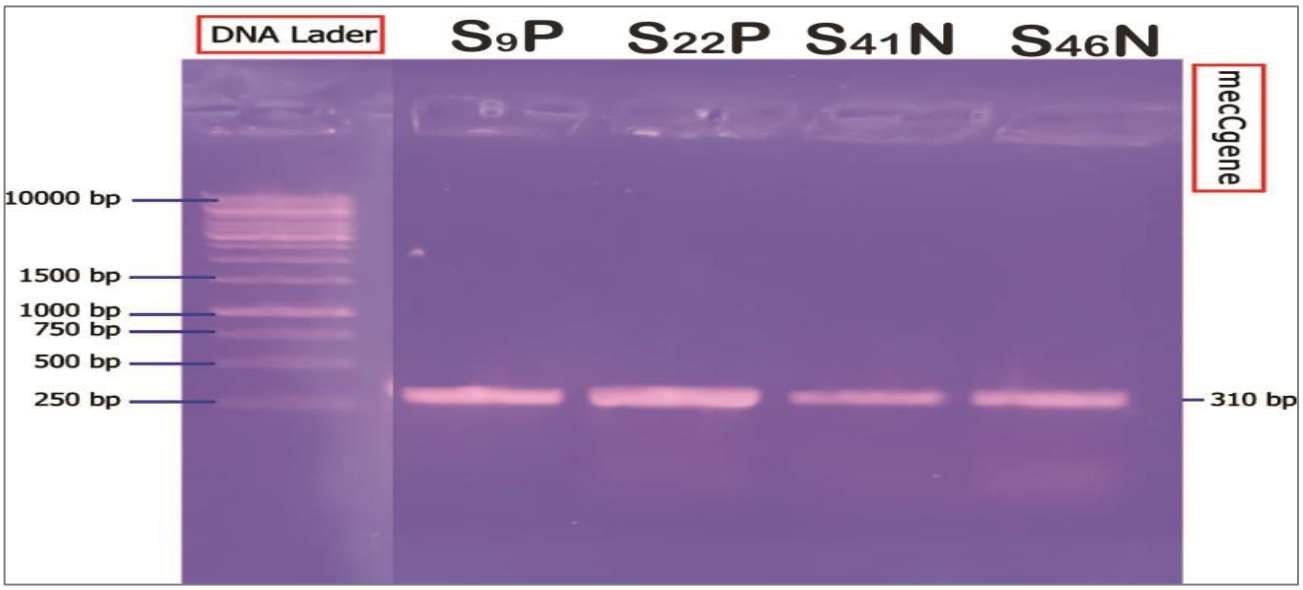

Figure 2: Result for molecular detection of *mecC* gene from methicillin-resistant coagulase-positive and negative staphylococci

Furthermore, the dominance of *mecC* genes over *mecA* observed in this study might be attributed to horizontal gene transfer or to mobile genetic elements, as reported by Marciniak et al. (2024). This finding stands out in this study (novelty) as the first study in Northeast Nigeria to report *mecC* dominance over the *mecA* gene. In the context of a clinical versus environmental comparison, the findings from this study contrast with the 88.8% *mecA* gene prevalence in Pakistani clinical isolates (Idrees et al., 2023). This, in turn, suggests an environmental section for *mecC*. The results of this study have clearly established that untreated sewage indeed serves as an AMR dissemination route, and when mistakenly discharged into the body of a local water source, it has serious detrimental consequences for public health, which could result in disease outbreaks in the community and hospital settings.

The statistical analysis carried out revealed that the P-value =2. Since the calculated p=2>0.005, we accept the null hypothesis and conclude that the presence of both MRSA and MSSA, as well as MR-CoNS and MS-CoNS, in sewage is not significant

CONCLUSION

This study revealed that *mecC*, not *mecA* as usual, is the primary methicillin-resistant driver in the study area (Gombe) hospital sewage, demanding revised AMR surveillance strategies. Hence, consistent UV wastewater treatment targeting *mecC*-bearing strains will reduce AMR dissemination and the threat it poses.

ETHICAL CLEARANCE

The study was carried out with the approval of the regulatory committee, reference number NHREC/25/10/2013.

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