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Genetic Antimicrobial Resistant *Staphylococcus* Coagulase Negative Isolates from Nasals of Health Workers in Selected Hospitals in Makueni County

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Abstract

Coagulase-negative staphylococci (CoNS) are common skin and mucous membrane flora but are increasingly recognized as opportunistic pathogens and reservoirs of antimicrobial resistance in healthcare settings. Nasal colonization among healthcare workers (HCWs) represents a potential source of transmission within healthcare facilities. This study assessed the prevalence, antimicrobial resistance patterns, and genotype characteristics of CoNS among HCWs at Makueni County Referral Hospital (MCRH) and Makindu Sub-County Hospital (MSCH). A total of 294 nasal swabs were collected from HCWs across different professional cadres. Isolates were identified by: inoculating on mannitol salt agar, followed by standard biochemical tests. Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method, and polymerase chain reaction (PCR) was used to detect the mecA gene among phenotypically confirmed methicillin-resistant CoNS (MRCoNS) isolates. Overall, 87.8% (258/294) of HCWs were colonized with CoNS, with carriage rates of 88.7% at MCRH and 85.0% at MSCH, though the difference was not statistically significant (p = 0.378). CoNS isolates exhibited extensive antimicrobial resistance, including 100% resistance to penicillin, 96.6% kjto ampicillin, 71.3% to cefoxitin, 65% to ciprofloxacin, 60% to trimethoprim-sulfamethoxazole, 53.2% to clindamycin, and 47.8% to tetracycline. Erythromycin showed the lowest resistance at 30%. Among phenotypically confirmed MRCoNS isolates, the mecA gene was detected in 87.6% (177/202), while 12.4% (25/202) were mecA-nega-

tive. These findings demonstrate a high prevalence of multidrug-resistant and mecA-positive CoNS among HCWs and emphasize the importance of routine surveillance, detection of circulating resistance determinants, and strengthened infection prevention and control measures to reduce nosocomial transmission.

Subject Areas

Agricultural Science, Entomology

Keywords

Coagulase-Negative *Staphylococcus* Species, Methicillin Resistance, mecA Gene, Health Care Workers

1. Background

Antimicrobial resistance (AMR) is a major global public health concern that threatens the effective prevention and treatment of infectious diseases. Antimicrobial resistance arises when microorganisms develop mechanisms that enable them to withstand the effects of antimicrobial agents, leading to treatment failure, prolonged illness, and increased mortality. The World Health Organization recognizes AMR as one of the top global health threats, with healthcare settings serving as key reservoirs for the emergence and spread of resistant pathogens.

CoNs are among the most abundant bacterial species colonizing the human anterior nares. While *S. aureus* is a well-known pathogen, CoNs are part of the normal flora but have increasingly been implicated in opportunistic infections, particularly in hospitalized and immunocompromised patients [1]. Nasal carriage of these organisms is clinically significant, as it serves as a reservoir for both endogenous infections and transmission to vulnerable patients.

Healthcare workers (HCWs) are at increased risk of colonization due to frequent exposure to patients and the hospital environment. Nasal carriage of CoNs among HCWs plays a critical role in the epidemiology of healthcare-associated infections, as colonized individuals can act as reservoirs and vectors for transmission within healthcare settings [1] [2]. Determining the prevalence of nasal carriage among HCWs is therefore essential for understanding the burden and potential transmission dynamics of these organisms, particularly in high-risk units such as neonatal and intensive care units.

The growing concern surrounding these organisms is largely driven by their ability to develop antimicrobial resistance. CoNS have demonstrated increasing resistance to commonly used antibiotics, limiting treatment options and complicating infection management [3]. CoNS, in particular, are recognized as important reservoirs of resistance genes that can be transferred to more pathogenic species, further amplifying the AMR problem [4]. Assessing the antimicrobial resistance profiles of these isolates is crucial in guiding appropriate therapy and informing

antimicrobial stewardship programs.

A key mechanism of resistance in staphylococci is mediated by the mecA gene, which confers resistance to methicillin and other beta-lactam antibiotics through the production of an altered penicillin-binding protein (PBP2a) [5]. The presence of this gene is associated with methicillin-resistant S.aureus (MRSA) and methicillin-resistant CoNS (MR CONs), both of which are of significant clinical concern. Detection of the mecA gene provides a more accurate understanding of the genetic basis of resistance compared to phenotype methods alone.

Despite the clinical importance of staphylococcal colonization and resistance, there is limited data on the prevalence, antimicrobial resistance patterns, and genetic determinants such as mecA among HCWs in many low- and middle-income settings, including Makueni County. Most available studies in Kenya have focused on phenotypic resistance, with minimal emphasis on molecular characterization [6]. This gap in knowledge limits the ability to fully understand the role of HCWs as reservoirs of resistant organisms and to implement targeted infection prevention and control strategies.

2. Materials and Method

2.1. Study Design

This was a descriptive cross-sectional study conducted among healthcare workers at MCRH and MSCH between April 2024 and September 2024. The study included the following cadres: nurses, medical laboratory staff, medical officers and interns, clinical officers and interns, and ward assistants. Participants were recruited using a convenience consecutive sampling approach, whereby all eligible HCWs available during the study period were invited to participate, and only those who provided written informed consent were included in the study.

2.2. Ethical Considerations

Scientific approval letters were obtained from the JKUAT Scientific Steering Committee and approved by the National Commission for Science, Technology and Innovation, license number 872090. To reach the MCRH and MSCH health care workers, permission was sought from the Makueni County Health Department, and an approval letter was given from the unit of research.

2.3. Sample Population

The minimum calculated sample size for the study was 288 healthcare workers. However, a total of 294 healthcare workers were recruited, slightly exceeding the minimum required sample size. Of these, 214 participants were from MCRH and 80 from MSCH.

Nasal swab samples were collected from consenting healthcare workers drawn from the following cadres: nurses, medical laboratory staff, medical officers and interns, clinical officers and interns, and ward assistants. The swabs were placed in Army transport medium and transported to the laboratory for processing within

2 hours of collection to preserve organism viability.

2.4. Laboratory Analysis

2.4.1. Culture and Identification of Bacteria

Samples were inoculated onto Mannitol Salt Agar (MSA) and incubated at 35˚C for 18 - 24 hours. After incubation, all growth suggestive of staphylococci was examined. Colonies showing yellow coloration on MSA were considered presumptive *Staphylococcus* aureus, while pink or red colonies with no mannitol fermentation were considered presumptive CoNS.

All isolates were subjected to Gram staining to confirm the presence of Gram-positive cocci in clusters, followed by the catalase test to differentiate staphylococci (catalase-positive) from streptococci and enterococci (catalase-negative). Catalase-positive Gram-positive cocci were then tested using the coagulase test. Isolates that were coagulase-negative were identified as coagulase-negative staphylococci.

2.4.2. Susceptibility Testing

Isolates were tested for antibiotic susceptibility using the Kirby-Bauer method. The following antibiotic disks were used: erythromycin (E, 30 µg) ampicillin (AMP, 10 µg), Cefoxitin (FOX, 30 µg), penicillin G (P, 10 µg), gentamicin (CN, 10 µg), clindamycin (CLD, 2 µg), ciprofloxacin (CIP, 5 µg), tetracycline (TE, 30 µg), Linezolid (LZD, 30 µg) and Trimethoprim/sulphamethoxazole (SXT, 25 µg). Susceptibility testing was done on Mueller-Hinton agar, and samples were incubated at 37˚C for 18 - 24 hours. *S. aureus* ATCC25923 and Escherichia coli ATCC25922 were included for quality control (The Clinical and Laboratory Standards Institute, 2024).

2.4.3. Genomic DNA Extraction

DNA was extracted using the boiling method. Briefly, 200 µl volume of DNase-free water solution was added to fresh sterile Eppendorf tubes. To each Eppendorf tube filled with 200 µl volume of DNase-free water, a loopful of 24-hour-old bacterial colonies was emulsified. The tubes were then vortexed for 5 - 10 seconds, after which the tubes were transferred to a heating block at 95˚C for 20 minutes. After boiling for 20 minutes, the tubes were left to cool for 10 minutes before centrifugation at 14,700 rpm for 10 minutes. Thereafter, 50 µl of the supernatant was transferred to fresh Eppendorf tubes refilled with 450 µl of RNase DNase-free PCR water. All the tubes were labeled carefully using the respective isolate codes and stored at −20˚C until further analysis.

2.4.4. Amplification of the mecA Gene

The mecA gene was tested to confirm methicillin resistance among the MRSA isolate. PCR amplification and detection of the mecA gene was done on all MRSA isolates using forward primers 5'AAA ATC GAT GGT AAA GGT TGG C3' and reverse primers 5'AGT TCT GCA GTA CCG GAT TTG C3'. In every PCR tube, 5 µl of the Taq 5x Master Mix, 1 µl of both forward and reverse primers, 16 µl DNase-free water, and 2 µl of sample DNA. Amplification reactions were initiated with an initial denaturation at 95˚C for 30 seconds. Thereafter, the preparation

was subjected to 30 cycles of denaturation at 95˚C for 30 seconds, annealing at 54 for 30 seconds, extension at 68˚C for 1 minute, and final extension at 68˚C for 5 minutes. A holding temperature of 4˚C was maintained. One control strain was incorporated in the DNA amplification process. The expected PCR product encoding the mecA gene was 533 bp. Quality control was done by including mecA-positive *S. aureus* ATCC 43300 and mecA-negative *S. aureus* ATCC 29213 for each PCR run.

2.5. Data Analysis

Statistical analysis was performed using IBM SPSS Statistics version 20 and WHONET. It was specifically used to calculate the prevalence of *S. aureus*, its distribution among the departments, cadre, and years of experience; it was also presented in 95% confidence interval (CI). It was also used to give proportions of MRSA with 95% CI. Chi-square was used to test the association between the mecA gene and methicillin resistance.

3. Results

3.1. General Participant Characteristics

A total of 294 healthcare workers were included in the study, of whom 214 were from MCRH and 80 were from MSCH. The participants comprised diverse healthcare worker cadres, with nurses forming the largest group (39.1%), followed by clinical officers (26.9%), medical laboratory officers (17.0%), medical officers (12.9%), and ward assistants (4.1%). With respect to working experience, most participants had relatively short to mid-term professional exposure, with the highest proportions reporting 6 months - 5 years (32.7%) and 6 - 10 years (31.3%) of experience, 11 - 15 years, while fewer had 16 - 20 years (20.7%) or more than 20 years (5.4%) of service. The mean working experience was 2.35 years, indicating that most participants had approximately 2.7 years of professional experience. The standard deviation of 1.323 indicated moderate variability in years of service across the different cadres. These findings are illustrated in **Table 1**. This distribution suggests a workforce largely composed of early- to mid-career healthcare workers, who represent a critical group for infection prevention and antimicrobial stewardship. These groups are typically more actively engaged in routine patient care activities, including frequent patient contact, specimen handling, medication administration, and bedside procedures. Consequently, they have high exposure to patients and the clinical environment, where transmission of infectious agents and antimicrobial misuse are common. Their central role in day-to-day service delivery positions them as key healthcare workers in preventing the transmission of healthcare-associated infections (HAIs) and promoting the appropriate use of antimicrobials in the hospital setting.

3.2. Prevalence of Coagulase-Negative *Staphylococcus* Species among Health Care Workers

The prevalence of CoNs nasal carriage among healthcare workers was high at both

study sites, with 88.7% (190/214) at MCRH and 85.0% (68/80) at MSCH. Overall, 87.8% (258/294) of healthcare workers were colonized, reflecting the role of CoNs as skin and mucous membrane flora. There was no statistically significant difference in carriage between the two hospitals ($p = 0.378$). Table 2 provides a summary of the study findings.

Table 1. General characteristics of healthcare workers recruited (N = 294).

Variable	Overall, n (%)	Makueni County Referral Hospital, n (%) (n = 214, 72.8%)	Makindu Sub-County Hospital, n (%) (n = 80, 27.2%)
Cadre			
Clinical Officers	79 (26.9)	59 (75.6)	20 (24.1)
Nurses	115 (39.1)	78 (67.8)	37 (32.2)
Medical Laboratory Officers	50 (17.0)	32 (64.0)	18 (36.0)
Medical Officers	38 (12.9)	33 (86.8)	5 (13.2)
Ward Assistants	12 (4.1)	12 (100.0)	0 (0.0)
Working Experience			
6 Months - 5 Years	96 (32.7)	66 (68.8)	31 (31.2)
6 - 10 Years	92 (31.3)	61 (66.3)	31 (33.7)
11 - 15 Years	61 (20.7)	39 (63.9)	22 (36.1)
16 - 20 Years	29 (9.9)	21 (72.4)	8 (27.6)
Above 20 Years	15 (5.4)	13 (86.7)	2 (13.3)
Working Experience Score			
Mean	2.77		
Standard Deviation	1.323		

Note: Values are presented as frequency (n) and percentage (%). Percentages may not total 100 due to rounding.

Table 2. Prevalence of coagulase-negative staphylococci (CONs) among health care workers.

Variable	Overall, n (%)	Makueni County Referral Hospital, n (%)	Makindu Sub-County Hospital, n (%)	*p*-Value
CONs				0.378
Present	258 (87.8)	190 (88.7)	68 (85.0)	
Absent	36 (12.2)	24 (11.3)	12 (15.0)	

Note: Values are presented as frequency (n) and percentage (%). CONs = Coagulase-negative staphylococci.

3.3. Distribution of Coagulase-Negative Staphylococcus Species among Health Care Workers

CoNS carriage was observed across all professional cadres. The highest prevalence was among nurses (92.2%), followed by clinical officers (89.9%), medical officers (86.8%), and ward attendants (83.3%), while medical laboratory officers had the lowest carriage rate of (76.0%). Despite these apparent differences, the variation across professional cadres was not statistically significant ($p = 0.603$), indicating that CoNS colonization is generally high among HCWs regardless of professional role (Table 3).

Table 3. Association between cadre, working experience, and coagulase-negative *Staphylococcus* species positivity.

Variable	Category	Positive, n (%)	*p*-Value
Cadre	Clinical Officers	59 (74.7)	0.603
	Nurses	94 (81.3)	
	Medical Laboratory Officers	44 (88.0)	
	Medical Officers	28 (73.6)	
	Patient Care Takers	10 (83.3)	
Working Experience Category	6 Months - 5 Years	88 (91.7)	0.010
	6 - 10 Years	81 (88.0)	
	11 - 15 Years	53 (86.9)	
	16 - 20 Years	20 (69.0)	
	Above 20 Years	16 (100.0)	

Note: Values are presented as frequency (n) and percentage (%). Significant *p*-values are reported at $p < 0.05$.

CoNS colonization among HCWs varied by years of working experience as follows: 6 months - 5 years: 88/96 (91.7%), 6 - 10 years: 81/92 (88.0%), 11 - 15 years: 53/61 (86.9%), 16 - 20 years: 20/29 (69.0%), and >20 years: 16/16 (100%). These differences were statistically significant ($p = 0.01$), indicating that duration of work experience influences CoNS carriage. Overall, CoNS colonization was high across most groups, with a comparatively lower prevalence among participants with 6 - 20 years of experience, and complete carriage observed in those with more than 20 years of experience, suggesting a possible effect of cumulative exposure over time (Table 3).

Overall, these findings suggest that while CoNS colonization is widespread among HCWs irrespective of professional cadre, work experience influences carriage rates, possibly reflecting cumulative exposure over time.

3.4. Resistance Rate of Coagulase-Negative *Staphylococcus* Species

High resistance was observed across most antibiotics, with the highest in penicillin (100%) and ampicillin (96.6%). Moderate resistance was observed to ciprofloxacin (65%), gentamicin (63.5%), and SXT (61.1%), while erythromycin showed the lowest resistance (30%). Cefoxitin resistance was also high (71.3%), indicating MR-CoNS nasal carriage among healthcare workers. Overall, the isolates exhibited an MDR pattern. This is illustrated in Figure 1.

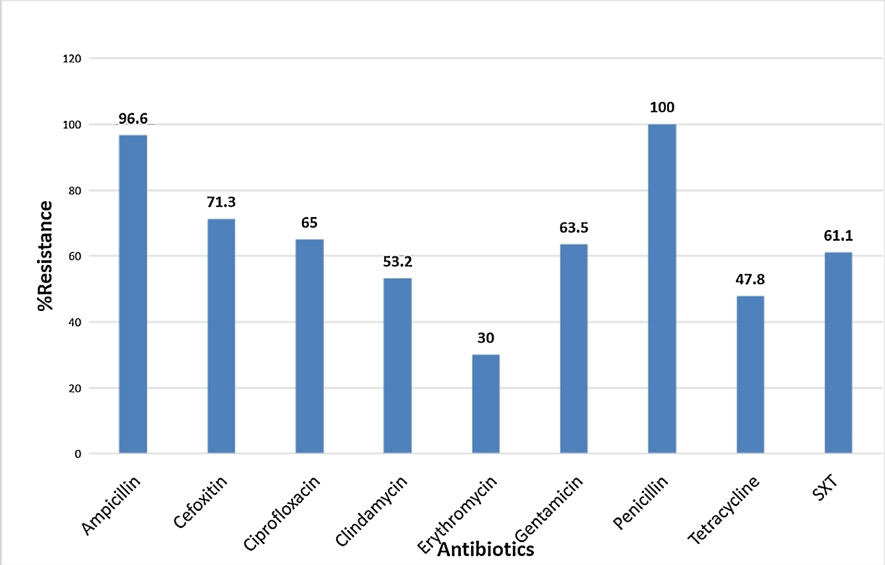

Figure 1. Resistance rate of coagulase-negative *Staphylococcus* species.

3.5. Resistance Pattern of Methicillin-Resistant Coagulase-Negative *Staphylococcus* Species

The MR-CoNS isolates in this study demonstrated a clear multidrug resistance pattern, with varying combinations of resistance across commonly used antibiotics. The most frequent profile was resistance to ciprofloxacin and penicillin (47.5%), followed by ciprofloxacin, penicillin, and gentamicin (29.7%). A considerable proportion also showed resistance to sulfamethoxazole, ciprofloxacin, penicillin, and gentamicin (19.8%), while the least common profile involved resistance to sulfamethoxazole, clindamycin, penicillin, and gentamicin (11.4%). Table 4 illustrates the resistance pattern.

Table 4. The resistance pattern of methicillin-resistant coagulase-negative.

Antibiotics Resistant	MR-CONS
Sulfamethoxaole/Clindamycin/Penicillin/Gentamycin	23 (11.4%)
Sulfamethoxazole/Ciprofloxain/Penicillin/Gentamycin	40 (19.8%)
Ciprofloxain/Penicillin/Gentamycin	60 (29.7%)
Ciprofloxain/Penicillin	96 (47.5%)

3.6. mecA Gene Carriage in Methicillin-Resistant Coagulase-Negative *Staphylococcus* Species

Out of the 202 MR-CoNS isolates analyzed, 177 contained the mecA gene, representing a prevalence of 87.6% mecA gene carriage among the MR-CoNS isolates. The presence of the mecA gene confirms the molecular basis of methicillin resistance in the majority of the phenotypically identified MR-CoNS isolates. However, 25 MR-CoNS isolates (12.4%) did not harbor the mecA gene, as shown in Figure 2.

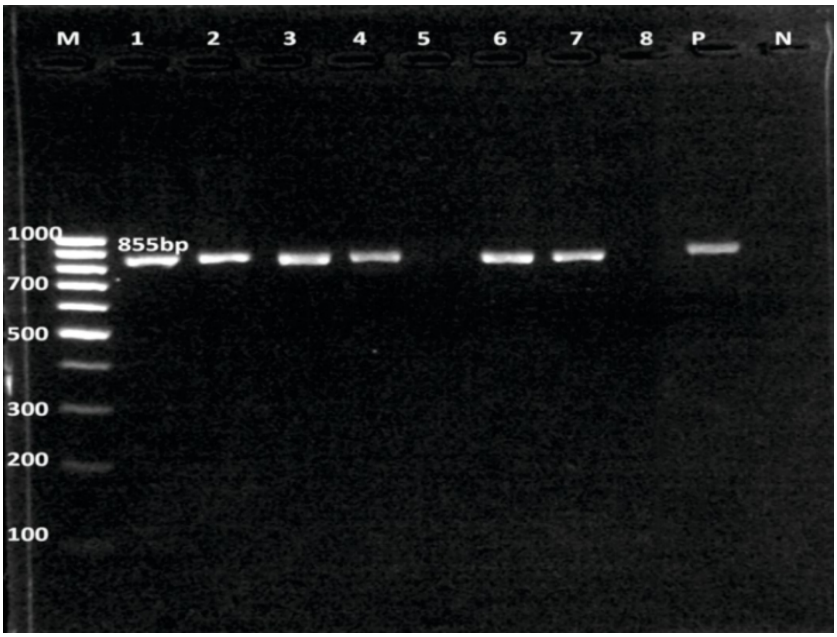

Figure 2. Agarose gel electrophoresis of the mecA gene amplification in MR CONs isolates.

4. Discussion

Numerous studies have extensively examined nasal colonization by S.aureus and the occurrence of methicillin resistance among healthcare workers (HCWs). However, CoNS has increasingly been recognized as a significant HAI pathogen in recent years, particularly in association with device-related and bloodstream infections. In addition to their pathogenic potential, CoNS serve as important reservoirs of antimicrobial resistance determinants, including the mecA gene, which mediates methicillin resistance and may be transferred to *S. aureus* [7]. The epidemiology of CoNS in healthcare settings is substantially less studied than it is for *S. aureus*. Furthermore, recent studies have demonstrated a high prevalence of nasal carriage of MR-CoNS among HCWs, with reported rates of approximately 45.9% in a tertiary care hospital setting [8]. Similar findings have been reported in other populations exposed to healthcare environments, where MR-CoNS colonization was found to exceed that of MRSA, highlighting their epidemiological importance [9]. In addition, evidence indicates that CoNS colonization is common in the nasal cavities of HCWs and plays a role in the transmission of multidrug-resistant organisms within healthcare settings

[10]. Therefore, in the present study, the nasal carriage of CoNS among HCWs was high, with 258 out of 294 HCWs (87.8%) colonized, and 71.3% were MR CONs. In comparison to a recent study in which 343 nasal samples (73.3%) were positive for CoNS, and only 10 (2.1%) were identified as MR-CoNS [8].

Furthermore, another study by Soma Kanta Baral *et al.* [8] reported a high prevalence of CoNS (66.2%) and MR-CoNS (45.9%), further supporting the widespread occurrence of resistant CoNS in healthcare settings. Similarly, a study by Neelima Kulshrestha *et al.* [11] reported CoNS in 148 (69.2%) and MR-CoNS in 76 (35.5%) of healthcare workers, which, although lower than the present study, still indicates a substantial burden of CoNS colonization and methicillin resistance in hospital settings.

The high prevalence of CoNS among HCWs is consistent with their status as normal commensals of the nasal mucous membrane and skin, which enables them to persist in the anterior nares. While CoNS are generally considered less virulent than *S. aureus*, they can act as opportunistic pathogens, particularly in immunocompromised patients or those with invasive devices. The high prevalence of MR-CoNS is of particular concern, as these organisms can serve as reservoirs of resistance genes that may be transferred to more pathogenic bacteria. Healthcare workers carrying MR-CoNS may inadvertently transmit these resistant strains to patients, highlighting the critical need for strict infection prevention and control measures and robust antimicrobial stewardship to limit the risk of HAIs, especially among vulnerable patient populations. The variations observed in the prevalence of nasal carriage of CoNS and MR-CoNS across different studies may be explained by differences in study populations, sampling techniques, or microbiological detection methods, geographical distribution of bacterial strains, disparities in infection prevention and control practices across healthcare settings, as well as differences in study periods and populations.

The distribution of CoNS and MR-CoNS was relatively uniform across different cadres and varying durations of work experience. This suggests that colonization is widespread and not limited to specific professional groups or levels of exposure within the healthcare setting. The findings may indicate that CoNS, being a normal commensal of the skin and nasal mucous membrane, are easily acquired and maintained regardless of role or experience. It also highlights the possibility that common environmental exposure and shared infection prevention practices within the hospital contribute to similar colonization patterns among HCWs.

High resistance was observed across most antibiotics, with complete resistance to penicillin (100%) and very high resistance to ampicillin (96.6%). Moderate resistance was noted for ciprofloxacin (65%), gentamicin (63.5%), and co-trimoxazole (61.1%), while erythromycin showed comparatively lower resistance (30%). The high cefoxitin resistance (71.3%) further indicates a substantial burden of methicillin-resistant CoNS (MR-CoNS), and overall, the isolates demonstrated a multidrug resistance pattern. These findings are comparable to a 2016 Indian study, which also reported 100% resistance to penicillin and similar resistance to co-tri-

moxazole (63%) and erythromycin (56%) among MR-CoNS isolates [12]. However, resistance levels to ciprofloxacin (65%) and gentamicin (63.5%) in the present study are higher than those reported by Agarwal *et al.* [12], who documented 22% and 40% resistance, respectively. In addition, a study conducted at a tertiary care hospital by Baragundi Mahesh reported high resistance to penicillin (84.48%), co-trimoxazole (72.41%), ofloxacin (68.96%), and erythromycin (67.24%), with MR-CoNS strains showing higher resistance to most antibiotics, which is in agreement with the multidrug resistance pattern observed in the present study [13].

Overall, the dominance of ciprofloxacin and penicillin in most resistance combinations highlights strong selective pressure against β-lactams and fluoroquinolones, which are commonly used in clinical settings. The frequent addition of gentamicin resistance further indicates reduced effectiveness of aminoglycosides, limiting therapeutic options. Although sulfamethoxazole and clindamycin resistance were less common, their presence in multidrug combinations reflects progressive accumulation of resistance determinants among MR-CoNS isolates.

These findings demonstrate that MR-CoNS in this setting exhibit diverse but overlapping multidrug resistance profiles, with a core resistance backbone mainly driven by penicillin and ciprofloxacin. This pattern is concerning as it suggests continued selection and dissemination of resistant strains within the healthcare environment, emphasizing the need for strict antimicrobial stewardship and infection control measures.

mecA gene carriage was detected in 87.6% of MR-CoNS isolates, confirming that methicillin resistance is largely mediated by this gene. This prevalence is comparable to the 90% reported in the University of Nairobi study [14] and the 82% reported by Tluanpuii *et al.* [15], indicating a consistently high contribution of mecA to resistance across different settings. However, the absence of the mecA gene in 12.4% of phenotypically identified MR-CoNS in the present study suggests that alternative resistance mechanisms may be involved, such as the presence of mecA or other modifications in penicillin-binding proteins. This highlights that while mecA is the driver of methicillin resistance, reliance on phenotypic methods alone may not fully capture the underlying genetic mechanisms, emphasizing the importance of molecular characterization.

5. Conclusion

In conclusion, this study demonstrated a high prevalence of CoNS colonization among healthcare workers, with a substantial proportion identified as methicillin-resistant (MR-CoNS), indicating that these organisms serve as important reservoirs of antimicrobial resistance in healthcare settings. The resistance pattern showed widespread multidrug resistance, including very high resistance to commonly used antibiotics such as penicillin and ampicillin, thereby limiting therapeutic options. Furthermore, the high prevalence of the mecA gene among MR-CoNS isolates confirms its central role in mediating methicillin resistance, although its absence in a minority of isolates suggests the involvement of alternative resistance mechanisms.

Overall, these findings underscore the need for continuous surveillance, strengthened infection prevention measures, and rational antibiotic use to control the spread of resistant CoNS.

6. Recommendation

Based on the findings of this study, routine screening of healthcare workers for CoNS and MR-CoNS colonization should be considered to identify potential reservoirs of multidrug-resistant organisms. Strengthening infection prevention and control measures, including proper hand hygiene and adherence to standard precautions, is essential to limit transmission within healthcare settings. In addition, antimicrobial stewardship programs should be reinforced to promote the rational use of antibiotics and reduce selective pressure that drives resistance.

Conflicts of Interest

The authors declare no conflicts of interest.

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