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Major Article

Prevalence of mupirocin and chlorhexidine resistance among methicillin-resistant coagulase-negative staphylococci isolated during methicillin-resistant *Staphylococcus aureus* decolonization strategies

Emad M. Eed MD [a,b,*], Mabrouk M. Ghonaim MD [a,b], Amany S. Khalifa MD [c,d], Khalid J. Alzahrani PhD [b], Khalaf F. Alsharif PhD [b], Aza A. Taha MD [e,f]

[a] Department of Medical Microbiology and Immunology, College of Medicine, Menoufia University, Menoufia, Egypt
[b] Clinical Laboratory Department, College of Applied Medical Sciences, Taif University, Taif, Saudi Arabia
[c] Department of Clinical Pathology, College of Medicine, Menoufia University, Menoufia, Egypt
[d] Department of Clinical Pathology, College of Pharmacy, Taif University, Taif, Saudi Arabia
[e] Department of Public Health and Community Medicine, College of Medicine, Menoufia University, Menoufia, Egypt
[f] Department of Family and Community Medicine, College of Medicine, Taif University, Saudi Arabia

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Background: The widespread of methicillin-resistant *Staphylococcus aureus* (MRSA) antimicrobial decolonization in the clinical setting may lead to an increase in the prevalence of multiresistance to coagulase-negative staphylococci (CoNS) owing to their selection. This study aimed to investigate the impact of MRSA decolonization strategies, using mupirocin and chlorhexidine, on their CoNS susceptibility.

Methods: A total of 312 CoNS isolates were collected before starting the decolonization protocols "baseline strains" (BLS) group, 330 isolates were collected after application of the targeted decolonization protocol "targeted decolonization strains" group, and 355 isolates were collected after application of the universal decolonization protocol "universal decolonization strains" group. Methicillin-resistant CoNS (MR-CoNS) were identified and tested for mupirocin and chlorhexidine susceptibilities. Heptaplex polymerase chain reaction assay was applied for simultaneous screening for chlorhexidine (CHX-R) and mupirocin resistance (Mu-R) genes.

Results: Mu-R prevalence of MR-CoNS among the BLS group was considered moderate (9.1%); however, CHX-R in the BLS group was 5.8%, the rate of which significantly increased among the universal decolonization strains group.

Discussion: Both MRSA decolonization strategies have an additional benefit in reducing the prevalence of MR-CoNS. The prevalence Mu-R rate didn't change significantly during either of the MRSA decolonization practices that may be due to the local nature of mupirocin application on the nasal mucosa only. In contrast CHX-R that was found to be significantly higher among the UDS group.

Conclusions: Our findings indicate that both MRSA decolonization strategies have an additional benefit in reducing the prevalence of MR-CoNS. Although the universal MRSA decolonization has superior efficacy in decolonization of CoNS, it may increase the risk of selecting CHX-R and Mu-R. In addition, other potential resistance genes should be studied.

Coagulase-negative staphylococci (CoNS) is a heterogeneous group that is based on specific laboratory diagnostic procedures to differentiate from *Staphylococcus aureus*,[1] with *Staphylococcus epidermidis* and *Staphylococcus haemolyticus* representing the most significant species that constitute important candidates for health care–associated infections.[2] CoNS infections represent an increasing burden on health facilities worldwide; however, the problem is magnified, owing to the increasing number of susceptible patient groups (eg, newborns, diabetics, elderly, and immunocompromised patients) and the increasing use of prosthetic devices and inserted foreign bodies.[3,4] CoNS colonization of the skin and mucous membranes are the main source of endogenous infections. However, the hands of medical personnel and/or

* Address correspondence to Emad M. Eed, MD, Department of Medical Microbiology and Immunology, 55 Abo Bakr St, Taif, Saudi Arabia.
E-mail address: dremadeed@yahoo.com (E.M. Eed).

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nursing procedures may transmit the infection as well.. Similar to methicillin-resistant *Staphylococcus aureus* (MRSA), insufficient hand hygiene, inadequate instruments sterilization, and surfaces disinfection are the assumed factors enhancing the spread of CoNS.[5,6]

In contrast to MRSA, the epidemiology of CoNS has been much less investigated in health care facilities.[7] Multidrug-resistant CoNS in hospitals may be selected primarily owing to antibiotic overuse. However, pathogen-related factors may also contribute to successful intra- and inter-hospital spread, including both its colonization and biofilm production abilities.[8,9] Hill et al[10] reported that CoNS can colonize and infect all kinds of inserted or implanted foreign bodies. Most CoNS infections are characterized by a sub-acute or chronic course with a nonspecific and mild clinical picture.[11] CoNS constitute common causative pathogens of foreign body-related health care–associated bacteremia. After bacteremia, localization of the infections may result in endocarditis, meningitis, infective arthritis, bone abscesses, or other metastatic infections. Local infections at the insertion sites of devices and wound infections are also reported.[12] CoNS developed resistance against most of the available antibiotics, including penicillin, oxacillin, clindamycin, ciprofloxacin, erythromycin, and aminoglycosides.[13,14] Many previous studies reported methicillin-resistant CoNS (MR-CoNS) in hospitals as well as community environments that carried various *SCCmec* types harboring the *mecA* gene.[5,15,16] In 2006, vancomycin-resistant CoNS were characterized through harboring van genes (*vanA*). Many of the genetic elements responsible for these resistances are mobile by nature, which enables their transfer to more pathogenic staphylococcal species.[17] Mupirocin is a topical antimicrobial agent that was approved in the late 1980s.[16,17] It acts through competitive inhibition of bacterial isoleucyl-tRNA-synthetase, leading to the prevention of bacterial protein and RNA synthesis.[18] Currently, it is widely used for MRSA decolonization as 2% nasal ointment.[19] This widespread use for MRSA decolonization may be claimed for the development of mupirocin resistance (Mu-R) by both *S aureus* and CoNS.[20] Mu-R is mediated by either of 2 conjugative plasmids that carry the *mupA* or the *mupB* genes that encode for a novel isoleucyl-tRNA-synthetase and mediate high-level Mu-R(HLMu-R), with minimum inhibitory concentration (MIC) ≥ 512 mg/L.[21] These 2 conjugative plasmids have the ability to enable cross-transmission between *S aureus* and the CoNS species.[22] In addition, a low-level Mu-R (LLMu-R) with (MIC 8-256 mg/L) may develop, owing to mutations in the chromosomal isoleucyl-tRNA-synthetase gene. However, these mutations are typically stable and nontransferable.[23]

Notable, the prevalence of CoNS resistance to mupirocin shows regional variations. In previous German and Brazilian studies, [24] the HLMu-R rate among CoNS was 11.7% and about 8%, respectively. However, higher prevalence rates (up to 61%) of Mu-R in CoNS have been reported. This high rate should be considered as an alarming sign, owing to the widespread use of mupirocin in MRSA decolonization protocols.[25] Chlorhexidine is a broad-spectrum bactericidal agent that acts through binding to the bacterial cell membrane, leading to disruption of its integrity and subsequent cell death.[21] In MRSA decolonization strategies, chlorhexidine is often used in the form of a whole body bath as a skin antiseptic. Bacterial resistance to chlorhexidine has been reported since 1995.[26] Bacterial chlorhexidine resistance (CHX-R), as well as other quaternary ammonium compounds, is mediated by 3 genes; *qacA/B* that confers high-level resistance and *smr* that confers low-level resistance.[27] Each of those genes is carried on conjugative plasmid that encodes for proton-dependent multidrug efflux pumps.[28,29] Little is known about the resistance of CoNS to antiseptics. In a recent French study, 12% CoNS isolates recovered from a neonatal intensive care unit exhibited decreased susceptibility to chlorhexidine, owing to transferable plasmids and mostly associated with other antibiotic resistance.[25] Recently, many health facilities have applied the global recommendations regarding MRSA decolonization. The aim of this study was to investigate the impact of the application of these MRSA decolonization strategies by mupirocin and chlorhexidine on their resistance development among CoNS.

METHODS

CoNS isolates were collected from clinical samples referred to the microbiology laboratories of hospitals in the Taif region of Saudi Arabia, that have applied any MRSA decolonization programs (targeted decolonization ie, decolonization of patients after MRSA carriage confirmation and nontargeted (ie, decolonization of all hospital patients prior to intervention regardless of the MRSA colonization status). The studied isolates included 312 CoNS isolates that were collected prior to the application of any decolonization protocols and 685 isolates that were collected after starting the decolonization protocols, from December 2017 to September 2018. Staphylococcal isolates were identified by the standard microbiological methods as colony morphology, Gram's staining, and tube coagulase test.[30] Consequently, CoNS isolates were subjected to antimicrobial susceptibility and molecular testing. Methicillin resistance was performed according to CLSI by Kirby-Bauer disk diffusion method on Muller-Hinton agar using a 30-μg cefoxitin disk (Oxoid, Lenexa, KS).[31] Methicillin-resistant isolates were subjected to other antimicrobial susceptibility (mupirocin and chlorhexidine) and molecular assay. Mupirocin susceptibility was tested by the E-test method (bioMérieux, Durham, NC). HLMu-R was defined as MIC ≥ 512 mg/L, whereas LLMu-R was defined as MIC = 8-256 mg/L. Strains with MIC ≤ 4 mg/L are considered mupirocin susceptible.[32] Chlorhexidine susceptibility was tested by broth microdilution method (Sigma-Aldrich, St Louis, MO). Isolates with MIC ≥ 512 mg/L were considered CHX-R, whereas those with MIC ≤4 μg/mL were considered chlorhexidine-sensitive .[33] Staphylococcal isolates were tested by heptaplex polymerase chain reaction (PCR) assay, as previously described.[33] The assay enabled CoNS confirmation and differentiation from *S aureus* by detecting *Staphylococcus* genus-specific (*staph 16 rRNA*) and *S aureus* species-specific (*nuc*) to distinguish *S aureus* from CoNS. The assay allowed simultaneous screening for the antimicrobial resistance genes; the CHX-R genes (*qacA/B* and *smr*) and the Mu-R genes (*mupA* and *mupB*).Additionally, the methicillin resistance gene (*mecA*) was investigated; therefore, CoNS isolates were differentiated into MR-CoNS and methicillin-sensitive CoNS. Bacterial DNA was extracted by rapid method, in which the isolates were subcultured on tryptic soy agar (Becton, Dickinson and Company, Franklin Lakes, NJ). Colonies (1-5) were suspended in 50 μL of distilled water and held in a hot water bath (99°C) for 10 minutes, followed by centrifugation (20,000 rpm) for 1 minute. After treatment, extracted DNA (2 μL) was added to the PCR mixture and subjected to cycling conditions as previously described.[34] The PCR primer sequences and the expected product sizes are shown in Table 1.

RESULTS

A total of 997 CoNS isolates were recovered from specimens obtained from clinical infections that developed 3 days after hospitalization during the study period: 312 isolates were recovered before application of either decolonization protocol, "baseline strains" (BLS) group, 330 isolates were collected after application of the targeted decolonization protocol "targeted decolonization strains" (TDS) group, and 355 isolates were collected after application of the universal decolonization protocol "universal decolonization strains" (UDS) group. Most of the CoNS isolates were recovered from blood culture samples, local foreign body-related infections, and wound infections. Methicillin susceptibility testing revealed that MR-CoNS isolates were 121 (38.8%) in the BLS group, 103 (31.2%) in the TDS group, and 96 (26.3%) in the UDS group (Table 2). Mupirocin MIC testing of MR-CoNS (320 isolates) revealed that HLMu-R rates were 7.8% in the TDS

Table 1
The sequence of the used primers and the product size of the studied genes

Primer name	Primer sequence	Product size (bp)
Staph-16SrRNA	F-AACTCTGTTATTAGGGAAGAACA R-CCACCTTCCTCCGGTTTGTCACC	756
Nuc	F-GCGATTGATGGTGATACGGTT R- AGCCAAGCCTTGACGAACTAAAGC	279
MecA	F-GTGAAGATATACCAAGTGATT R-ATCAGTATTTCACCTTGTCCG	112
MupA	F-TATATTATGCGATGGAAGGTTGG R- AATAAAATCAGCTGGAAAGTGTTG	456
MupB	F-CTAGAAGTCGATTTTGGAGTAG R-AGTGTCTAAAATGATAAGACGATC	674
qacA/B-	F-GCAGAAAGTGCAGAGTTCG R-CCAGTCCAATCATGCCTG	361
Smr	F-GCCATAAGTACTGAAGTTATTGGA R-GACTACGGTTGTTAAGACTAAACCT	195

group and 9.4% in the UDS group, with no statistically significant differences compared with the BLS group ($P = .890$ and .452, respectively). Chlorhexidine susceptibility testing showed that CHX-R among MR-CoNS was significantly more prevalent in the UDS group ($P = .03$) compared with the BLS group, although isolates in the TDS group showed no significant increase ($P = .367$) in CHX-R (Table 3). PCR results confirmed the phenotypically identified MR-CoNS isolates (all of the isolates were positive for *Staph-16S rRNA* and *mecA* genes, whereas the isolates were negative for the *nuc* gene) (Fig 1). The *mupA* gene was the most prevalent among HLMu-R in the 3 studied groups (Table 4). Although *mupB* was detected only in 2 isolates in both BLS and UDS groups. Additionally, the *qacA/B* gene was the only responsible for chlorhexidine resistance, whereas the *smr* gene was undetected. Only 2 isolates in the USD group were PCR-negative for *qacA/B*, although both were phenotypically CHX-R.

Table 2
The infection type of the isolated CoNS

	BLS		TDS		UDS	
Infection	CoNS	MR-CoNS	CoNS	MR-CoNS	CoNS	MR-CoNS
Bacteremia/ septicemia	97	25 (25.8%)	110	31 (30.9%)	93	26 (27.1%)
Local FBRIs	74	30 (40.5%)	82	27 (32.9%)	95	22 (22.9%)
Wound/burn	72	28 (38.9%)	92	31 (33.7%)	72	23 (24.0%)
Others	69	38 (55.1%)	46	14 (30.4%)	95	25 (26.0%)
Total	312	121 (38.8%)	330	103 (31.2%)†	355	96 (26.3)*

BLS, baseline strains; CoNS, coagulase-negative staphylococci; FBRIs, foreign body-related infections; MR-CoNS, methicillin-resistant CoNS; TDS, targeted decolonization strains; UDS, universal decolonization strains.
*$P = .001$.
†$P = .044$.

Table 3
The mupirocin and chlorhexidine susceptibility of the MR-CoNS isolates.

	BLS n = 121	TDS n = 103	UDS n = 96
Mu-S	110 (90.9%)	90 (87.4%)	85 (88.5%)
LLMu-R	3 (2.5%)	5 (4.9%)	2 (2.1%)
HLMu-R	8 (6.6%)	8 (7.8%)	9 (9.4%)
CHX-S	114 (94.2%)	94 (91.3%)	82 (85.4%)
CHX-R	7 (5.8%)	9 (8.7%)	14 (14.6%)*

BLS, baseline strains; *CHX-R*, chlorhexidine-resistance; *CHX-S*, chlorhexidine-sensitive; *HLMu-R*, high-level mupirocin resistance; *LLMu-R*, low-level mupirocin resistance; MR-CoNS, methicillin-resistant coagulase-negative staphylococci; *Mu-S*, mupirocin-susceptible; *TDS*, targeted decolonization strains; *UDS*, universal decolonization strains.
*$P = .03$.

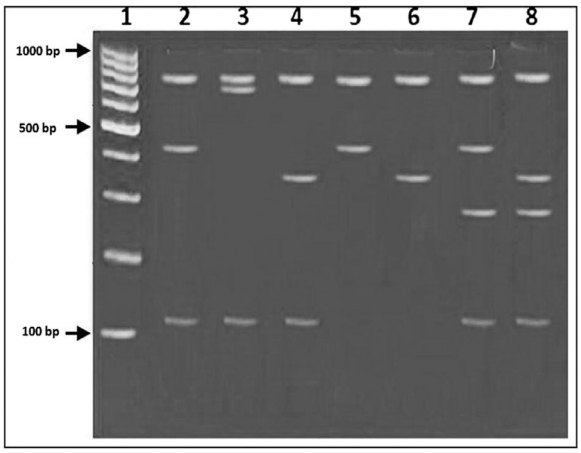

Fig 1. PCR results of the studied genes.(Lane 1) DNA ladder. (Lane 2) *mupA* positive MR-CoNS. (Lane 3) *mupB* positive MR-CoNS. (Lane 4) *qacA/B* positive MR-CoNS. (Lane 5) *mupA* positive methicillin-sensitive CoNS. (Lane 6) *qacA/B* positive methicillin-sensitive CoNS. (Lane 7) *mupA* positive MRSA. (Lane 8) *qacA/B* positive MRSA. *MR-CoNS*, methicillin-resistant coagulase-negative staphylococci; *MRSA*, methicillin resistance *Staphylococcus aureus; PCR*, polymerase chain reaction.

Table 4
The positive PCR results of the investigated genes among MR-CoNS isolates

The investigated gene	BLS n = 121	TDS n = 103	UDS n = 96
Staph-16S rRNA	121 (100%)	103 (100%)	96 (100%)
Nuc	0 (0%)	0 (0%)	0 (0%)
mecA	121 (100%)	103 (100)	96 (100%)
mupA	6 (5.0%)	8 (7.8%)	7 (7.3%)
mupB	2 (1.7%)	0 (0%)	2 (2.1%)
qacA/B	7 (5.8)	**9** (8.7)	14 (14.6%)
Smr	0 (0%)	0 (0%)	0 (0%)

BLS, baseline strains; *MR-CoNS*, methicillin-resistant coagulase-negative staphylococci; *PCR*, polymerase chain reaction; *TDS*, targeted decolonization strains; *UDS*, universal decolonization strains.

DISCUSSION

CoNS constitute important pathogens of nosocomial infections, with increasing rates of antimicrobial resistance.[35,36] Compared with MRSA, the epidemiology of CoNS in health care facilities has been much less investigated.[25,37] The notable capacity of CoNS for biofilm production together with modern medicine practices as insertion of foreign body devices and antimicrobial overuse are strong factors for their selection.[38] MR-CoNS were reported since the early 1980s, with increasing rates over the years.[39] In the present study, the overall MR rate among the isolated CoNS was 32.1%. Similar results were reported by Bhatt et al,[40] in which MR-CoNS accounted for 32.7% by PCR detection of *mecA* gene. Although other studies[41,42] reported higher rates up to 75.2%. In contrast, a previous study from Sweden reported a near absence of MR-CoNS among hospital-associated clones.[7] Both of the MRSA decolonization protocols (targeted and universal decolonization) include application of mupirocin and chlorhexidine on the patient's skin and mucosal surfaces as 5-day regimen.[43,44] Mupirocin (2% ointment) is applied intranasally, twice daily, along with a chlorhexidine daily bath. MRSA eradication is confirmed after 3 consecutive negative swabs.[11] Both strategies were found to be effective in reducing the MRSA cross-transmission rate in the hospital environment.[45] However, the effects of these decolonization protocols on other pathogenic microorganisms circulating in hospitals have not yet been well -studied.

In our study, both targeted and universal decolonization protocols resulted in a significant reduction in the MR-CoNS rate ($P = .0443$ and .0012, respectively) compared with the baseline rate before protocols application. This reduction may be secondary to the significant reduction in MRSA as *mecA* gene was found to be transmissible between staphylococcal species.[43] In addition, in the universal decolonization, the indiscriminate use of disinfectants to all patients may constitute another explanation for MR-CoNS reduction. However, a major expected drawback of this decolonization is the significant increase in mupirocin and/or chlorhexidine resistance among both of MRSA and MR-CoNS. Therefore, screening for the increased rates of resistance to these 2 agents is recommended.[29] Although CoNS were considered as less important, these pathogens were found to be a reservoir of resistance genes that can be transferred to other virulent pathogens, such as *S aureus*.[46] This may justify the necessity for more effective screening of its antimicrobial susceptibility during the interventional strategies. In this study, our aim was to investigate the impact of those MRSA decolonization protocols on the MR-CoNS susceptibility to both mupirocin and chlorhexidine. Our findings indicated Mu-R prevalence of MR-CoNS in the BLS group was considered moderate (2.5% LLMu-R and 6.6% HLMu-R). This prevalence rate did not change significantly during either of the MRSA decolonization practices. A reasonable explanation for this nonsignificant change may be owing to the local nature of the mupirocin application on the nasal mucosa, in which a large proportion of CoNS colonizing the patient's skin was not exposed to the drug. This explanation may be supported by previous studies,[45,47] reporting significantly higher rates of Mu-R and attributing that rate to the widespread and sustained use of mupirocin to the extra-nasal sites as on the vascular catheter exit sites and wound infections.

The results of our study are consistent with Hetem et al,[48] who studied the prevalence of MuR, after universal decolonization, and concluded that no significant change had occurred among both CoNS and *S aureus*. However, another longitudinal study showed a significant increase in Mu-R (CoNS isolates carrying the *mupA* gene increased from 8%-22%) during preoperative MRSA decolonization.[49] Our findings also indicated that the *mupA* gene was the main gene responsible for Mu-R, where it was detected among 21 isolates (7.5%), whereas the *mupB* gene was detected only in 4 isolates. This may be in accordance with a French study that reported a higher rate of Mu-R (10.3%) among CoNS. Moreover, all of these isolates were harboring the *mupA* gene.[50] In this study, a relatively low prevalence of *qacA/B* and CHX-R was detected among the BLS group (5.8%). CHX-R was found to be significantly higher ($P = .0295$) among the UDS group, whereas there was no significant increase ($P = .368$) among the TDS group.However, Taheri et al[51] reported a higher rate of the *qacA/B* gene (47.1%) among CoNS and attributed their findings to the selective pressure imposed by the various quaternary ammonium compounds (including chlorhexidine) used in their hospitals and to the location of the *qacA/B* genes on widespread multiresistance plasmids.[51] The results in our study show there was some variation between phenotypic CHX-R and the PCR results of the *qacA/B* gene especially in the UDS group (2 isolates were PCR negative, whereas both were CHX-R by MIC test). This variation may be attributed to another efflux pump mediated by genes other than *qacA/B*.[52] Moreover, our results revealed a rare co-existence of mupirocin and chlorhexidine resistance. Only 2 strains were positive for both the *mupA* and *qacA/B* genes. This co-existence—if present—represents a crucial risk factor for decolonization failure.[20] These findings are in accordance with a previously published report,[34] in which the co-existence of CHX-R and Mu-R among MR-CoNS was detected in only 1 isolate. Again, this discrepancy between Mu-R and CHX-R during application of decolonization protocols may be owing to the difference in the mode of application, in which the first is used locally on the nasal mucosa, the second is used as a whole body wash, and a large proportion of the body is exposed to it.

CONCLUSIONS

MR among CoNS was found to be moderate in our hospitals. Both MRSA decolonization strategies have an additional benefit in reducing the prevalence of MR-CoNS. Universal MRSA decolonization is more cost-effective than targeted decolonization. In addition, it has superior efficacy, not only in MRSA decolonization, but also in decolonization of CoNS. However, these benefits should be balanced against the risk of selecting chlorhexidine and Mu-R. In conclusion, these results should be interpreted with caution, and other resistance genes should be studied in larger national-scale studies. Periodic surveillance is also recommended for health care facilities that have applied any decolonization protocols.

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