

Methicillin-Resistant *Staphylococcus aureus* Carriage in Hemodialysis Vicinity: Prevalence and Decolonization Approach

Abstract

Hemodialysis (HD) patients are at risk for developing serious infections. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most prevalent pathogens in healthcare facilities with a major threat to the medical community. We aimed to determine the prevalence of MRSA colonization among patients and medical staff members in a HD Unit and determine efficacy of mupirocin as a decolonizing agent. This cross-sectional study enrolled 250 patients and 35 health care providers of a HD unit. Nasal and hand swabs were collected to assess the prevalence of MRSA carriage. Those exhibiting MRSA phenotype were subjected to conventional Polymerase chain reaction (PCR) assay for detection of *mecA* gene. Colonized patients and medical personnel with MRSA were prescribed mupirocin ointment (2%) for decolonization. The screening approach identified 54/285 (18.9%) nasal MRSA carriers (41/250 of HD patients and 13/35 of the medical staff members). Concomitant extranasal MRSA colonization of the hands was observed in 10 (18.5%) of these 54 MRSA carriers. In relation to PCR results the sensitivity, specificity, and diagnostic accuracy of cefoxitin disk test were 98.2%, 75%, and 93.9% respectively and for MRSA Select II agar screening method, the sensitivity, specificity, and diagnostic accuracy were 92.6%, 66.7%, and 87.9% respectively. Decolonization approach using mupirocin ointment revealed an overall success rate up to 77.8% (42/54) and failure rate of 16.7% (9/54), while 5.6% (3/54) of decolonized carriers showed recolonization. There is still high prevalence of MRSA colonization in HD vicinity. Implementation of strict infection control measures is essential in dialysis units to avoid MRSA cross-transmission and invasive infections.

Keywords: Colonization, HD unit, MRSA, mupirocin

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most important bacterial pathogens in both community and hospital settings.^[1] As a nosocomial pathogen, it is involved in a diverse array of life-threatening diseases such as bacteremia, skin and soft tissue infections, pneumonia, endocarditis, osteomyelitis, and toxin-mediated syndromes with significant morbidity and mortality.^[2]

The general appearance and spread of MRSA harboring multiple resistance genes have critical importance as it renders management of infections less effective and makes their clinical outcomes worse, especially in health care facilities.^[3] It has become evident that the expression of low-affinity penicillin-binding protein (PBP2a) genes namely *mecA* is responsible for methicillin resistance. These genes are present on a part of the

staphylococcal cassette chromosome (SCC) and 11 types of SCC *mec* have been universally characterized.^[4]

Infections in hemodialysis (HD) patients represent the second most common cause of morbidity, hospitalization, and mortality after cardiovascular diseases and *Staphylococcus aureus* (*S. aureus*) represents the most common bacterial infections in these patients.^[5] The risk of MRSA infection among dialysis patients is a 100-time higher than that in the general population.^[6] HD patients have high susceptibility for colonization and infection with MRSA because of repeated hospitalization, their frequent and long-term use of antibiotics and immunosuppression. Exposure to invasive procedures and regular contact with other colonized patients and healthcare workers are also considerable risk factors.^[7,8]

The primary site of *S. aureus* colonization in humans is the nose and extranasal colonization sites results from

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contamination from nasal picking.^[8] Spread of MRSA from their carriers in health care facilities can lead to infections and use of decolonization agents can eliminate MRSA carriage. Trustworthy screening techniques are fundamental to block the dissemination of MRSA from carriers of infection by the enforcement of satisfactory contact and sanitary measures.^[9,10]

The prevalence of staphylococcal infections is largely dependent on nasal and hand colonization and a critical proportion of these infections are of endogenous source (from hospital and dialysis vicinity),^[8] so the aim of the current study was to evaluate the prevalence of MRSA colonization among HD patients as well as the medical staff at HD unit by different phenotypic methods, to identify the antimicrobial resistance profile of these isolates, to verify the presence of the *mecA* gene by PCR as well as to evaluate the performance of mupirocin ointment (2%) for eradication of MRSA nasal colonization.

Materials and Methods

Study population

The study was conducted at Menoufia University Hospitals in collaboration between Microbiology and Immunology Department and Nephrology Unit during the period from January to November 2017. The study population involved 285 individuals, including 250 patients undergoing HD (155 males and 95 females) and 35 members of the medical staff (10 doctors, 18 nurses, and 7 workers). Duplicate swabs (nasal and hand) were obtained from all participants after obtaining written informed consent. The study protocol was approved by the Ethical Committee of Menoufia University.

Collection, identification, and storage of *S. aureus* isolates

Samples from the anterior nares and hands were obtained by sterile cotton swabs (NaCl 0.9% wet cotton nasal swabs, which were circled in both nares of a participant) and subsequently inoculated into tubes containing tryptic soy broth with 6.5% NaCl (Oxoid-England), incubated at 37°C for 24 h. Tubes containing turbid broth were subcultured onto mannitol salt agar (Oxoid).^[8] After incubation at 37°C for 24–48 h, *S. aureus* isolates were identified by Gram staining, colony morphology, catalase, and standard tube coagulase tests.^[11] Confirmed *S. aureus* isolates were suspended in nutrient broth supplemented with 16% glycerol and stored frozen at –80°C.

Antimicrobial susceptibility profile was performed for all *S. aureus* isolates by the Kirby–Bauer disk diffusion method on Muller–Hinton agar plates (Oxoid) against different antimicrobial agents (Oxoid) as recommended by CLSI, 2017 including; 1 µg oxacillin, 30 µg cefoxitin, 10 µg gentamicin, 30 µg tetracycline, 10 µg penicillin G, 10 µg ampicillin, 15 µg azithromycin, 2 µg clindamycin,

1.25/23.75 µg trimethoprim/sulfamethoxazole, 30 µg doxycycline, 5 µg rifampin, 5 µg ofloxacin, 30 µg teicoplanin, and 30 µg linezolid. Zone diameters were interpreted according to CLSI 2017 guidelines.^[12]

Screening and phenotypic confirmation of MRSA colonization: Suspected MRSA phenotypes with cefoxitin zone diameter of ≤21 mm were inoculated onto MRSA Select II chromogenic agar plates (MSI; Bio-Rad, USA). Culture plates were incubated for 18 to 28 h at 37°C in ambient humidity and were examined for the presence of characteristic pink colonies indicative of MRSA phenotype. MRSA Select II agar plates that did not exhibit pink colonies after 28 h incubation were considered negative.^[13]

Genotypic detection of *mecA* gene by conventional PCR: All *S. aureus* isolates with reduced susceptibility to cefoxitin (zone diameter ≤21 mm) and/or positive MRSA Select II screening agar plates were tested for the presence of *mecA* gene by conventional PCR technique.

DNA extraction: Cellular DNA was obtained from *S. aureus* isolates grown overnight on blood agar plates using DNA extraction kit (Qiagen, Germany) according to the manufacturer's instructions. PCR assay: A set of specific primers used for *mecA* gene detection involved a forward primer 5'-AAAATCGATGGTAAAGGTTGGC-3', and reverse, 5' AGTTCTGGAGTACCGGATTTGC-3'. The PCR reaction mixture (25 µL) consisted of 1 µL of template DNA added to 10 µL Taq green PCR master mix, 0.7 µL of 0.8 µmol/L each primer and 12.6 µL of sterile distilled water. The PCR program was performed in the DNA amplification instrument thermal cycler gradient (Biometra, Germany). It involved an initial denaturation step at 95°C for 3 min, followed by 33 cycles of 94°C for 1 min, 53°C for 30 s and 72°C for 1 min, with a final extension step at 72°C for 6 min. The amplified products (533 bp) were visualized by 2% agarose gel electrophoresis [Figure 1] stained with ethidium bromide.^[14]

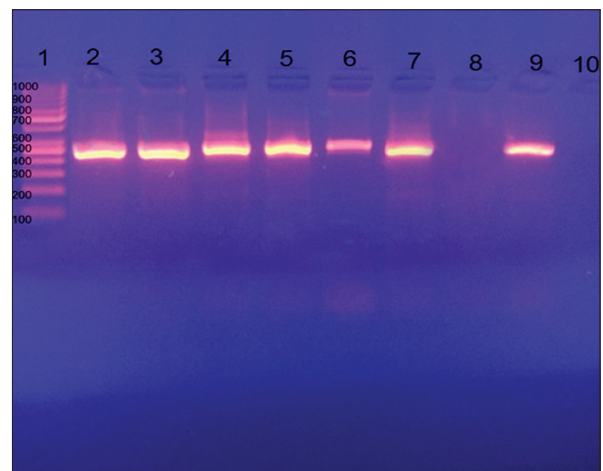


Figure 1: Agarose gel electrophoresis for PCR amplified of *S. aureus* *mecA* gene

Eradication of MRSA nasal colonization with 2% mupirocin ointment: All patients and medical personnel proved to be colonized with MRSA were informed to use intranasal mupirocin ointment (2%) twice daily on both the anterior nares for at least 4–7 successive days for decolonization.^[15] Nasal swabs were then taken and subsequently inoculated onto MRSA Select II agar plates after 1 week (at the end of treatment), 1, 3, and 6 month intervals to assess the success rate and the liability for recolonization. Outcomes were categorized as cure/success i.e., no *S. aureus* was recovered at the end of treatment and during the follow-up cultures, failure, i.e., persistence of *S. aureus* at the end of treatment and recolonization/relapse was further defined as recolonization by the previously colonizing *S. aureus* strain or acquisition of a new *S. aureus* strain during the follow-up intervals.^[16]

Statistical Analysis

The data collected were tabulated and analyzed by SPSS (statistical package for the social science software, SPSS Inc. Chicago, IL, USA) statistical package version 20 on an IBM compatible computer.

Results

A total of 250 chronic HD patients and 35 of medical staff members of the HD Unit were enrolled. A total of 570 nasal and hand swabs were collected from all participants and processed for screening of nasal and hand MRSA carriage. The mean age of the patients and medical staff members was 49.18 ± 14.26 years. The mean duration of dialysis was 3.78 ± 3.372 years. We had 92.8% of patients who started dialysis with a temporary catheter and only 7.2% were prepared for dialysis with permanent vascular access, which is mostly arteriovenous fistula. We found that about 4.4% current vascular accesses in studied patients were temporary and permanent catheters. There is no known history of previous mupirocin treatment.

The screening approach identified a total of 66 *S. aureus* isolates of which 54 (81.8%) isolates proved to be *mecA*-positive by the gold standard PCR assay. The prevalence of MRSA colonization was 16.4% (41 patients) for dialysis patients and 37.2% (13 individuals) for the medical staff members. Concomitant extranasal MRSA colonization of the hands was observed in 10 (18.5%) of these 54 nasal MRSA carriers [Table 1].

Regarding antimicrobial susceptibility profile, all *S. aureus* isolates were resistant to both penicillin and ampicillin followed by oxacillin and cefoxitin (84.8% for each). The resistance levels to trimethoprim/sulfamethoxazole, doxycycline, ofloxacin, gentamicin, rifampin, azithromycin, tetracycline and clindamycin, were 81.8%, 77.3%, 74.2%, 68.2%, 65.1%, 63.6%, 62.1% and 59.1% respectively. However, all isolates were susceptible to linezolid.

The phenotypic screening of MRSA carriage was performed by cefoxitin disk diffusion test [Figure 2]. The test was able to identify 53 out of 54 *mecA*-positive isolates; thus, the sensitivity, specificity, and diagnostic accuracy were 98.2%, 75%, and 93.9%, respectively. The chromogenic MRSA Select II agar was also evaluated as a phenotypic confirmatory test for identification of MRSA colonization [Figure 3]. The overall agreement between cefoxitin disk test and MRSA Select II screening methods was almost perfect [Table 2] (Kappa test = 0.891; $P < 0.001$). In relation to PCR results the sensitivity, specificity, and diagnostic accuracy were 92.6%, 66.7%, and 87.9%, respectively [Table 3].

Topical mupirocin ointment 2% was evaluated as a nasal decolonizing agent for all *mecA*-positive patients and healthcare providers. The overall rate of successful decolonization reached 77.78% (42/54) and was confirmed by successive negative cultures till 6 months after the end of treatment. However, 16.67% (9/54) of the colonized persons had persistent positive culture before and after nasal application of mupirocin. Three cases (5.56%) showed relapse/recolonization during follow-up cultures [Figure 4].

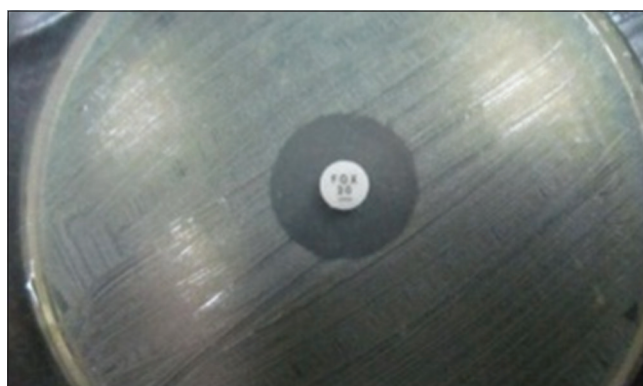


Figure 2: Cefoxitin disk diffusion test

Table 1: Distribution of Staphylococcal isolates among the collected specimens and study population

Study population (n=285)	Specimens (n=570)				Mec-A positive individuals of <i>S. aureus</i> isolates
	Nasal swabs (n=285)		Hand swabs (n=285)		
	<i>S. aureus</i>	CoNs	<i>S. aureus</i>	CoNs	
Patients (n=250)	37	26	9	15	41
Doctors (n=10)	4	5	0	6	3
Nurses (n=18)	7	10	3	4	4
Workers (n=7)	4	3	2	3	6

Total No. of *S. aureus* isolates=66, Total No. of CoNs isolates=72, mec-A positive individuals=54



Figure 3: MRSA SelectII medium showing pink colonies

Table 2: Results of the phenotypic tests used for detection of MRSA isolates

Methods	Cefoxitin disk diffusion		Total	Symmetrical measurement
	+ve	-ve		
MRSASelectII agar Screening	+ve 54 (96.4%)	0 (0.0)	54	Kappa test=0.891 P<0.001
	-ve 2 (3.6%)	10 (100)	12	
Total	56	10	66	
Kappa < 0				Interpretation
0.0-0.20				Poor agreement
0.21-0.40				Slight agreement
0.41-0.60				Fair agreement
0.61-0.80				Moderate agreement
0.81-1.00				Substantial agreement
				Almost perfect agreement

Discussion

HD patients are particularly vulnerable to life-threatening MRSA infections.^[2] Therefore, continuous epidemiological surveillance for MRSA, including genotypic analysis and implementation of adequate decolonization strategies, is crucial as eradication of MRSA carriage will reduce the possibility of autoinfection as well as disrupt transmission of multi-resistant isolates to others.^[1]

In the current study, a total of 66 *S. aureus* strains were isolated from HD patients as well as medical staff members of dialysis unit. All isolates were subjected to antimicrobial susceptibility testing against different antibiotics by disk diffusion test which revealed that, the resistance profile of *S. aureus* was 100% for both penicillin and ampicillin, 84.8% for both cefoxitin and oxacillin, 81.8% for trimethoprim/sulfamethoxazole, 77.3% for doxycycline, 74.2% for ofloxacin, 68.2% for gentamicin, 65.1% for rifampin, 63.6% for azithromycin, 62.1% for tetracycline and 59.1% for clindamycin, and 21.2% teicoplanin. However, all isolates were susceptible to linezolid. These

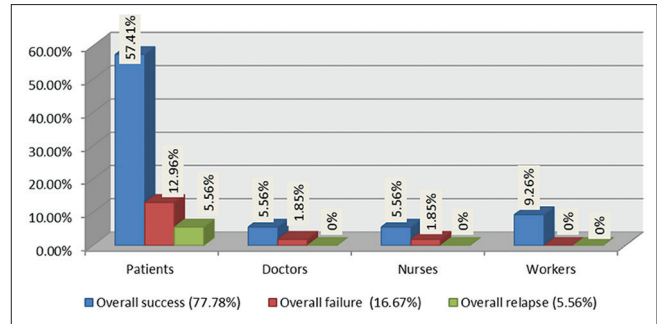


Figure 4: Results of nasal decolonization approach using mupirocin ointment 2%

results were in accordance with that of Alenizi, 2013^[17] who found that the highest rates of resistance of *S. aureus* were to penicillin and ampicillin followed also by high-resistance levels to other antimicrobials and that almost all isolates were still sensitive to linezolid.

As regards phenotypic screening of MRSA colonization, cefoxitin susceptibility test was applied. The test identified 56 (84.8%) out of 66 *S. aureus* isolates to be methicillin-resistant with cefoxitin zone diameters of ≤ 21 mm and that 10 isolates (15.2%) were reported as methicillin-sensitive. This finding agreed with the study of Ghoniem *et al.*, 2014^[18] who found that 28.3% of the isolated *S. aureus* to be methicillin-sensitive, and that 71.72% were identified as MRSA.

In this study, we also compared the performance of MRSA Select II chromogenic agar for MRSA identification with cefoxitin disk test. The overall agreement between the two methods was almost perfect (kappa test = 0.891; $P < 0.001$). Out of 56 methicillin-resistant isolates detected by cefoxitin disk test, 54 (96.5%) isolates produced pink colonies onto MRSA Select II agar. Nearly, the same observation was also obtained by Hernandez *et al.*, 2016^[13] who stated that the result of cefoxitin screening of colonies directly from MRSA Select II agar plates was 96.7%.

This study involved PCR assay as a gold standard for molecular characterization of MRSA isolates and to verify the presence of *mecA* gene. Out of 66 *S. aureus* isolates, 54 isolates (81.8%) were *mecA*-positive. Regarding PCR results, the sensitivity of cefoxitin disk diffusion method was 98.2%, specificity was 75% and diagnostic accuracy was 93.9%. These results were comparable with Sasirekha *et al.*, 2012 who found that the sensitivity and specificity for cefoxitin disk diffusion method were 100% and 99.1%, respectively.^[19] As regard MRSA Select II chromogenic agar screening method, the sensitivity, specificity, and diagnostic accuracy were 92.6%, 66.7%, and 87.9% respectively in relation to PCR results. These results were in accordance with that reported by Hernandez *et al.*, 2016^[13] who concluded that MRSA Select II agar is a simple, rapid, and robust method to routinely screen patients for MRSA colonization without the need for additional testing.

Table 3: Sensitivity, specificity, and accuracy of the phenotypic methods in relation to PCR as the gold standard for detection of *mecA* gene among *S. aureus* isolates (n=66)

Methods	PCR (n=66)						Sensitivity	Specificity	Accuracy
	+ve (n=54)		-ve (n=12)						
	No.	Percentage	No.	Percentage					
Cefoxitin Disk diffusion (n=66)	+ve (n=56)	53	94.6	3	5.4	98.2%	75%	93.9%	
	-ve (n=10)	1	10	9	90				
MRSA Select II agar (n=66)	+ve (n=54)	50	92.5	4	7.5	92.6%	66.7%	87.9%	
	-ve (n=12)	4	33.3	8	66.7				

Our screening approach identified 41/250 (16.4%) of HD patients as MRSA carriers. These results were comparable with that obtained by Schmid *et al.*, 2013^[20] who reported that the prevalence of MRSA colonization among HD patients was 11.7%. Giarola *et al.*, 2012^[8] found that, the rate of MRSA colonization among dialysis patients reached 49%. The authors explained these higher carriage frequencies by the fact that HD are continuously exposed to invasive procedures, repeated hospitalization, heavy pressure of antibiotic usage, and maintain contact with other colonized patients and health care professionals.

Among medical staff members, the carriage rate of MRSA was 37.2% (13/35). These results were higher than that of Resić *et al.*, 2014^[21] and Lederer *et al.*, 2007^[22] who that, the prevalence of MRSA nasal carriage among medical staff members was 11.6% and 26%, respectively. This observation is probably due to overcrowding inside HD unit, inadequate infection control policies, and deficiency in personal protective equipments during the period of this study.

Concomitant extranasal (hand) MRSA colonization was observed in 10/54 (18.5%) of the study population (six patients, one nurse, and three workers). This observation signifies the liability for cross-contamination between the nose and hands of the patients, nursing and medical staff members and highlights the need for implementation of adequate infection control measures including proper hand hygiene, the usage of appropriate personal protective equipment including masks, gloves, and aprons to avoid MRSA cross-transmission.

Since colonization is often cited as the initial step in the pathogenesis of endogenous MRSA infection, and that when the anterior nares are topically treated, organism also disappears from other areas of the body,^[15] our study evaluated mupirocin ointment 2% as nasal decolonizer for all colonized subjects. The overall rate of successful eradication reached 77.78% (42/54) and was confirmed by successive negative cultures till 6 months after the end of treatment. However, nine (9/54; 16.67%) of colonized persons had persistent positive culture before and after nasal application of mupirocin. Three cases (3/54; 5.56%) showed relapse/recolonization during follow-up cultures. These observations were explained by Abad *et al.*, 2013^[15] who reported that usage of mupirocin as a decolonizing

agent comes with a considerable risk of resistance attributed to plasmid-mediated *mupA* gene encoding high-level mupirocin resistance in *S. aureus* and that poor patient compliance may also reduce its efficiency.

In another study by Mody *et al.*, 2003^[23] the authors concluded that mupirocin was effective in decolonizing MRSA; however, the effect was not sustained, probably because the factors promoting acquisition of MRSA are largely immutable. Nearly, the same results were also obtained by Wertheim *et al.*, 2005^[16] who addressed that the acquisition of exogenous MRSA strains after mupirocin treatment is a common phenomenon and that repeated exposure to mupirocin treatment increases the potential for development of resistance. Furthermore, patients treated with mupirocin should receive follow-up cultures to determine treatment failures.

The Centers for Disease Control and Prevention also does not recommend routine use of mupirocin for as a decolonizing agent and that its usage should be limited to outbreaks or high prevalence situations. Widespread use of mupirocin is offset by the necessity of surveillance cultures to identify candidates for decolonization, the likelihood of recolonization, and the potential for resistance.^[7]

Conclusion and Recommendations

There is high prevalence of MRSA colonization among HD patients and healthcare workers. Both cefoxitin disk diffusion test and MRSA Select II agar are rapid and simple methods for routine screening of MRSA colonization. Genotypic analysis should be applied to prove genetic relatedness between MRSA strains isolated from patients and those isolated from healthcare providers. Enforcement of adequate infection control policies and nasal decontamination is essential in dialysis units especially prior to high-risk procedures. Mupirocin ointment effectively decolonized MRSA, so we encourage use of mupirocin ointment for carriers' decolonization; however, reevaluation after adequate intervals is needed for the possibility of resistance and/or recolonization. New antibiotics are needed to decolonize the nose because bacterial resistance to mupirocin is rising.

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Conflicts of interest

There are no conflicts of interest.

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