Community-Acquired Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) Colonization and Infection in Intravenous and Inhalational Opiate Drug Abusers

AMANY EL-SHARIF* AND HOSSAM M. ASHOUR†,[1]

**Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt; and †Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Cairo, Egypt*

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a significant pathogen in hospital-acquired and community-acquired infections. We hypothesized that drug abuse is a risk factor for community-acquired MRSA (CA-MRSA) infection, and we employed a rapid multiplex PCR technique for MRSA identification. The study was conducted on MRSA isolates from 60 opiate addicts (intravenous and inhalational drug users) to detect the rate and location of MRSA colonization and infection among them in comparison to 60 non-addict patients and 15 healthy volunteer controls. The proportion of addicts with MRSA colonization (and/or infection) was significantly higher than non-addict patients with MRSA colonization. MRSA colonization was associated with infection in 58% of MRSA-colonized addicts. The MRSA nasal carriage in the addicts was significantly higher than MRSA carriage elsewhere, whether in the addicts group or in the non-addict patients group. Moreover, the nasopharyngeal carriage rate of MRSA among addicts was significantly higher than among the non-addict patients. Increasing the duration of addiction resulted in a significant increase in CA-MRSA colonization in opiate addicts. Both inhalational and intravenous drug use led to significant MRSA colonization in the addict population. In conclusion, this study demonstrated how drug abusers, a high-risk group for infections with MRSA, could be a source or a reservoir of CA-MRSA infection in the non-addict population. Exp Biol Med 233:874–880, 2008

Key words: MRSA, community, opiate, infection, colonization

[1] To whom correspondence should be addressed at Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Kasr El-Aini Street, Cairo, Cairo 11562, Egypt. E-mail: hossamking@mailcity.com

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Introduction

Staphylococcus aureus causes various infections in any organ system due to different virulence factors (1). *S. aureus* is second to coagulase-negative staphylococci (CNS) as a cause of nosocomial bacteremia (2). Serious *S. aureus* infections can cause 25% mortality (3). The availability of highly active antimicrobial agents has not solved the problem (3) due to the rapid evolution of antimicrobial resistance, such as methicillin resistance.

Methicillin-resistant *S. aureus* (MRSA) is a significant pathogen in nosocomial and community-acquired infections (4–7). Since the identification of nosocomial MRSA in the 1960s (8), strategies to prevent the spread of MRSA via hospitals has failed because most traditional antistaphylococcal agents are no longer highly effective against MRSA (9).

Community-acquired MRSA (CA-MRSA) strains have recently become more virulent (10), causing life-threatening diseases such as necrotizing pneumonia, necrotizing fasciitis, purpura fulminans, and pyomyositis (11, 12). CA-MRSA is showing a propensity not only to spread rapidly in communities but also to spread into hospitals. Thus, CA-MRSA strains can be a significant threat to the immunocompromised patients in hospitals.

Although MRSA infections have been described in patients without established risk factors (13), the association of multiple risk factors may increase the risk of MRSA infections (14). Injection drug users (IDUs) are at high risk for exposure to blood-borne bacterial and viral infections (15, 16). However, complications associated with drug abuse are frequently caused by factors other than the physiological effect of the drug, such as contamination of substances or usage of non-sterile syringes (17). There is a high colonization rate of *S. aureus* in IDUs (18). *S. aureus* causes soft tissue infections (19), endocarditis, bacteremias (20, 21), and septic deep-vein thrombosis in IDUs (22).

We tested the hypothesis that drug abuse was a risk

factor for CA-MRSA infection. MRSA specimens were collected from 60 opiate addicts (intravenous and inhalational) to detect the rate and location of MRSA colonization in comparison to 60 non-addict patients and 15 healthy volunteer controls. We also assessed the effect of duration of addiction and route of administration on MRSA acquisition rate.

Materials and Methods

Diagnosis of Drug Abusers. Diagnosis of drug abuse was done by psychiatrists using the DSM-IV criteria (23) and by examining the patients' urine for the major five classes of drugs: opiates, amphetamines, cannabinoids, benzodiazepines, and barbiturates. Each sample was double checked by enzyme-linked immunoabsorbent assay (ELISA) and enzyme-multiplied immunoassay technique (EMIT).

Collection of Samples. Non-duplicative samples of sputum, venous blood, throat swabs, nasal swabs, pus from abscesses and/ or skin ulcers, and wound aspirates were collected from each of 60 opiate addicts within 48 hrs of admission into the hospital (before possibly acquiring any nosocomial infections), and from each of 60 non-addicted patients (patients admitted to the hospital for reasons other than addiction) at Al-Hussein Academic Hospital in Cairo, Egypt. Data collected on each patient consisted of demographic infromation including age, gender, admission date, ward, hospitalization duration, recent exposure to healthcare facilities, route of drug abuse, addiction duration, any previous antibiotic administrations, and sites of positive culture. Patients of the control and drug-user group who had a medical history of MRSA infection or colonization or had exposure to healthcare facilities (hospitalization, dialysis, surgery, residence in a long-term healthcare facility, or working in a medical specialty) in the previous 5 years were excluded from the study. Also, patients who were imprisoned or had a household MRSA contact in the previous 5 years were excluded from the study. The duration of our definition of a previous potential exposure to MRSA was arbitrarily set to 5 years (a relatively long duration of time) to take into account possible prolonged duration of MRSA colonization. We employed all the of standard Centers for Disease Control and Prevention (CDC) criteria for distinguishing CA-MRSA from HA-MRSA (24), except that we prolonged the duration of previous healthcare exposure into 5 years (CDC recommends at least 1 year) as a criterion for subject exclusion. As a control, similar samples were also collected from each of 15 healthy volunteers. All samples were cultured on the appropriate media. CDC criteria were used to determine whether the patient was infected or colonized with MRSA (25). Ethical approval to perform the study was obtained from the management board of the hospital and from the Egyptian Ministry of Health and Population.

Identification of Isolated Microorganisms. Identification of *S. aureus* was based on morphology and biochemical reactions according to standard laboratory criteria (26). Phenotypic characterization to the species level was performed using the Analytical Profile Index for the identification of *S. aureus* (API Staph) (Biomerieux, Marcy-l'Etoile, France). A reference strain of MRSA ATCC 12692 was used as a standard. The methods we used for identification were comparable in efficiency to the CLSI method. MRSA infection was defined as an infection with a strain of *S. aureus* bacteria that is resistant to antibiotics known as β-lactams, including methicillin, amoxicillin, and penicillin.

Detection of MRSA. All staphylococcal isolates were tested for oxacillin resistance by Disc diffusion method and Oxacillin agar screen plate.

Disc Diffusion Method (Kirby–Bauer Technique). To test for resistant *S. aureus* isolates, oxacillin discs (1 μg) were placed on the surface of Muller-Hinton agar medium with 4% NaCl (27), followed by incubation at 35°C (28). Reading of the plates was carried out after 24 hrs using transmitted light by looking carefully for any growth within the zone of inhibition (7). Appropriate control strains were used to ensure the validity of the results.

Oxacillin Agar Screen Plate. Muller-Hinton agar plates with 4% NaCl and 6 μg/ml oxacillin were prepared and inoculated as streaks in three directions by using a cotton swab dipped into a direct colony suspension of 0.5 McFarland standard in tryptic soy broth (29). As a control, the same medium containing 4% NaCl without oxacillin was inoculated first. Plates were incubated at 35°C for 24 hrs. Any growth indicated that the isolate was resistant to oxacillin.

Multiplex PCR. *DNA Purification.* Single colonies of the isolates were cultured in tryptic soy broth and incubated for 16 hrs at 37°C. DNA purification was carried out according to Vannuffel *et al* (30).

Oligonucleotides. Based on the DNA sequences of the *mecA* and *femA* genes, the oligonucleotides in Table 1 were designed to amplify staphylococcal DNA according to Vannuffel *et al*. (30). Overlapping sequences of staphylococcal insertion element IS431 were used as a positive control for the PCR.

PCR Amplification. Each DNA sample (10 μl) was added to 90 ml of the PCR mixture consisting of 10 m*M* Tris HCl (pH 8.8), 1.5 m*M* $MgCl_2$, 50 m*M* KCl, 0.1% Triton X-100, 0.25 m*M* deoxynucleoside triphosphates, 100 pmol of each primer, and 1.25 units of DNA polymerase. Initial denaturation was carried out for 3 mins at 92°C followed by 30 cycles of amplification (denaturation at 92°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min with an increment of 2 secs per cycle). The final extension was performed at 72°C for 3 mins. Amplification was carried out in a Bio-Rad thermal cycler. After amplification, 15 μl of PCR sample was loaded on a 2% (w/v) agarose gel, and electrophoresis was performed in 0.1 *M* Tris HCl (pH 8.6),80 m*M* boric acid, and 1 m*M* EDTA containing ethidium bromide (0.5 mg/ml). Amplified

Table 1. Multiplex PCR Primers Used for Identification of MRSA

Primer	Oligonucleotide sequence (5′ → 3′)	Amplified fragment
M_1	(885-[5′]TGGCTATCGTGTCACAATCG-904)	310-bp fragment of the *mecA* gene
M_2	(1194-[5′]CTGGAACTTGTTGAGCAGAG-1175)	
F_1	(217-[5′]CTTACTTACTGGCTGTACCTG-237)	686-bp fragment of *femA* gene
F_2	(902-[5′]ATGTCGCTTGTTATGTGC-884)	
C_1	(32-[5′]AGGATGTTATCACTGTAGCC-51)	444-bp fragment of the staphylococcal insertion element IS431
C_2	(476-[5′]GATGTACAATGACAGTCAGG-457)	

ethidium bromide-stained DNA fragments were then visualized on a UV source at 300 nm.

Statistics. Differences between the group of addict patients and the group of non-addict patients or between different categories within the same group of patients was determined using the chi-square test. All P values were based on two-tailed tests of significance. A P value of 0.05 or less was considered statistically significant.

Results

The clinical isolates were identified morphologically and biochemically. Different species of *Staphylococcus* were identified using API Staph. The disc-diffusion method and oxacillin agar screen plate were used to identify MRSA. In addition, we used a rapid and reliable molecular approach (Multiplex PCR) for the detection of MRSA to confirm the presence of the genetic determinants of methicillin resistance (mecA and femA) in *S. aureus* (30, 31). The M_1/M_2 primer set was used to confirm the presence of methicillin resistance in the staphylococcal isolates. The F_1/F_2 primer set was used to detect *S. aureus*. The C_1/C_2 primer set was used as a positive control of *Staphylococcus* species regardless of the susceptibility pattern.

In Figure 1A, different MRSA isolates were confirmed by comparing to a standard MRSA isolate. Both the standard isolate and other isolates contained the 310-bp *mecA* fragment and the 686-bp *femA* fragment. In Figure 1B, the multiplex PCR strategy was used to differentiate between methicillin-resistant and methicillin-sensitive staphylococcal isolates. A standard MRSA isolate expressing the three bands was used as a control (Lane 2). In Lane 4, only C_1 and C_2 primers were used with a standard MRSA isolate as a control for the PCR. In Lane 5, only M_1 and M_2 primers were used with a standard MRSA isolate as a control for the PCR. The amplified DNA from coagulase-negative staphylococci (CNS) (Lanes 6 and 7) expressed only the 444-bp band, indicating that it was a staphylococcal isolate regardless of its β-lactam susceptibility. However, it did not express the mecA or the femA bands, indicating that it was not methicillin-resistant and that it was not *S. aureus*. Therefore, Lanes 6 and 7 represented methicillin-sensitive CNS. Lanes 3 and 8 represented *Escherichia coli* (no expression of any of the three bands).

We compared results obtained using conventional methods with those obtained with PCR (Table 2). There was a high level of agreement between the two methodologies. PCR had sensitivity, specificity, and positive and negative predictive values of 94.0%, 100%, 100%, and 93.1%, respectively. The high negative predictive value calculated for the PCR assay indicated the usefulness of the assay in accurately ruling out false positive results.

In Table 3a, MRSA was isolated from addict and non-addict patients. No MRSA was detected in a control group of 15 healthy volunteers. In the opiate addicts group, MRSA was isolated from 31 addicts, 13 of whom were asymptomatic carriers with MRSA nasal colonization but no infection. Eighteen patients had MRSA colonization that led to infection. In the non-addict patients group, MRSA was detected in 10 patients, 4 of whom were asymptomatic carriers with MRSA colonization but no infection. Six patients had MRSA colonization as well as infection. Chi-square tests showed that the proportion of addicts with MRSA colonization (and/or infection) was significantly higher than non-addict patients with MRSA colonization and/or infection (31 of 60 vs. 10 of 60, $P < 0.01$). Although MRSA colonization was asymptomatic in 42% (13 of 31) of the MRSA-colonized addicts, it was associated with infection in 58% (18 of 31) of these cases. Similarly, MRSA colonization was associated with infection in 60% (6 of 10) of the MRSA-colonized non-addict patients.

Carriage of *S. aureus* was shown to be an important risk factor for infection with this organism (32). In addition, carriage rates were high in patients who repeatedly puncture the skin, as in the case of IDUs (18). Thus, it was important to determine the carriage rates of MRSA. In Table 3b, MRSA carriage was compared in different specimens isolated from addicts and non-addict patients. Of 60 nasal swabs collected from addict patients, 31 (52%) were positive for MRSA. Thus, nasal carriage in addicts was significantly higher than the carriage of MRSA in any other location, whether in the addicts group or non-addict patients group, as indicated by the chi-square test ($P < 0.05$). In addition, the nasopharyngeal carriage rate of MRSA among addicts was significantly higher than that among the non-addict patients. Moreover, 24% (72 of 300) of the isolates collected from addicts (one isolate per carriage site per patient) showed positive MRSA. Since 5 isolates were collected from different sites in each patient, a 24% percent positive MRSA rate means that, on average, every addict patient will have at least one isolate that is positive for

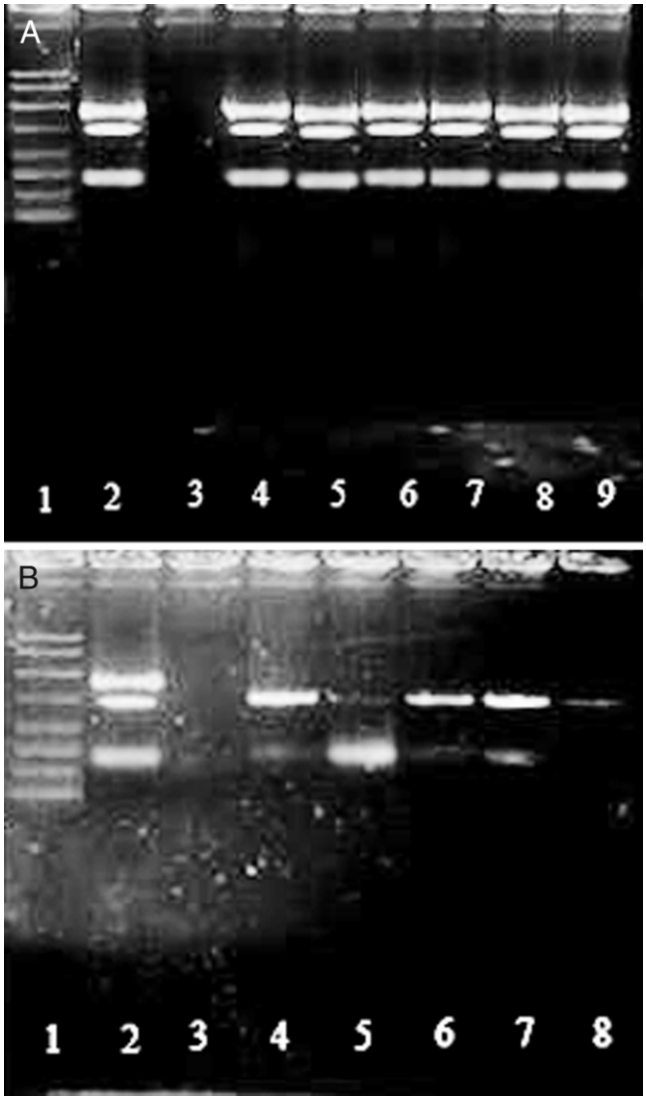

Figure 1. (A) Gel electrophoresis of DNA fragments generated by multiplex PCR for the identification of MRSA isolates. The amplified gene fragments (*mecA,* IS431, and *femA*) were detected at molecular sizes 310, 444, and 686 bp, respectively. Lane 1 represents the molecular size marker. Lane 2 represents the positive control (standard MRSA strain). Lane 3 represents the negative control (*E. coli*). Lanes 4–9 represent different MRSA isolates. (B) Gel electrophoresis of DNA fragments generated by multiplex PCR for the differentiation between methicillin-resistant and methicillin-sensitive staphylococcal isolates. Lane 1 represents molecular size marker. Lane 2 represents a standard MRSA isolate (positive control). Lanes 3 and 8 represent *E. coli* (negative control). Lane 4 is a control for the PCR (standard MRSA isolate; only C_1 and C_2 primers were used). Lane 5 is a control for the PCR (standard MRSA isolate; only M_1 and M_2 primers were used). Lanes 6 and 7 represent methicillin-sensitive CNS.

MRSA. Based on results shown in Table 3b, the most likely carriage site for such an isolate will be the nose. About 52% (31 of 60) of all nasal swabs from addicts were MRSA-positive. As indicated before, the control (healthy volunteers) group did not show any positive MRSA. It is noteworthy that any addict who was MRSA-positive for sputum, throat, blood, or pus specimen, had an associated MRSA-positive nasal swab.

Table 2. MRSA Identification: Multiplex PCR vs. Conventional Methods[a]

PCR test result	Number of specimens identified by conventional methods		Total number of specimens
	Positive	Negative	
Positive	31	0	31
Negative	2	27	29
Total	33	27	60

[a]PCR sensitivity, 94.0%; specificity, 100%; positive predictive value, 100%; negative predictive value, 93.1%.

By examining the relationship between the duration of addiction and CA-MRSA colonization in opiate addicts, we found that increasing the duration of addiction resulted in a significant increase in MRSA acquisition in opiate addicts (Table 4). We also investigated the relationship between the route of drug abuse and MRSA acquisition in opiate addicts. Results in Table 5 indicate that 35% (6 of 17) of addicts who administered drugs via injection were MRSA-positive, 55% (6 of 11) of addicts who administered drugs via inhalation were MRSA-positive, and 59% (19 of 32) of addicts who administered drugs via inhalation and injection were MRSA-positive. This emphasizes the role of the inhalational route of drug abuse as a risk factor in the CA-MRSA colonization.

Discussion

Due to the increasing number of infections caused by MRSA, prevention of staphylococcal infections is now more important than ever before. Without careful studies on CA-MRSA, we are by no means close to setting proper guidelines for prevention of MRSA-mediated infections. Detection of MRSA by standard clinical microbiological methods is a two-step process that involves first the identification of the isolated staphylococcal colonies and second the determination of their level of methicillin resistance. This is tedious, time-consuming, expensive, and less specific than multiplex PCR, a one-step process that can identify the isolates and assess their resistance to methicillin. Multiplex PCR has been successfully used to directly identify MRSA in nasal swabs and other specimens (33–35).

Staphylococcal infection in addicts could lead to serious health problems such as endocarditis (36). Therefore, we assessed the carriage rates of CA-MRSA (acquired before hospital admission) in drug addicts who were admitted into the hospital. Results presented in this study suggested that MRSA colonization could be the cause of, at least, some of these infections.

In the present study, 58 addicts reported the misuse of antibiotics and administered them without prescription (as a preventive measure against the spread of infections associated with intravenous or inhalational drug abuse). The high rate of MRSA colonization among addicts with a

Table 3a. Rates of Colonization and Infection of CA-MRSA in Opiate Addict Patients and Non-addict Patients

	Addict patients (IV and inhalational drug users)		Non-addict patients	
	No.	%	No.	%
Colonization and infection (MRSA)	18	30	6	10
Asymptomatic colonization (MRSA)	13	21.67	4	6.7
Total number of patients with MRSA isolates	31	51.67	10	16.7
Total number of patients free of MRSA isolates	29	48.33	50	83.3
Total number of patients	60	100	60	100

Table 3b. Carriage of CA-MRSA in Different Specimens Isolated from Addict Patients and Non-addict Patients

	Sputum no. (%)	Throat swab	Blood	Nasal swab	Pus in abscesses or wounds	Total
Isolates from addicts	13/60 (21.67%)	11/60 (18.34%)	9/60 (15%)	31/60 (51.67%)	8/60 (13.34%)	72/300 (24%)
Isolates from non-addicts	0/60 (0%)	3/60 (5%)	6/60 (10%)	1/60 (1.67%)	6/60 (10%)	16/300 (5.34%)

Table 4. Relationship Between Duration of Addiction and CA-MRSA Colonization in Intravenous and Inhalational Opiate Drug Abusers

Duration of addiction (year)	Addicts with positive MRSA	Addicts with negative MRSA
1–3	5/31 (16.13%)	13/29 (44.83%)
3–7	11/31 (35.48%)	10/29 (34.48%)
>7	15/31 (48.39%)	6/29 (20.69%)
Total	31/60 (51.67%)	29/60 (48.33%)

history of antibiotic misuse is consistent with studies that indicate that the previous use of non-prescribed antibiotics was a risk factor for MRSA colonization (20, 37). Only 7 patients in the non-addict group reported misuse of antibiotics. Other risk factors of colonization and infection included frequent needle injections (37, 38) and inhalational drug use, which could alter the integrity of the nasal mucosa and lead to nasal colonization (38). In our study, both intravenous and inhalational drug use were associated with MRSA colonization, especially in the nose.

Nasal colonization with *S. aureus* was reported to be one of the most important risk factors that could lead to infection in other parts of the body (39, 40). In this study, 31 of 60 (52%) nasal swabs collected from examined addicts were MRSA-positive. Interestingly, every addict who was found to harbor a positive MRSA isolate in sputum, throat, blood, or pus harbored a positive MRSA nasal isolate. This suggests that the nose could be a reservoir from which MRSA spreads elsewhere, colonizing and infecting other sites of the body. In addition to causing infections to themselves, drug abusers could cause the spread of MRSA infection in the surrounding community, and were thus considered a source of outbreak of MRSA (7). For example, one serious consequence of the colonization of CA-MRSA in drug addicts could be that they can act as carriers of the virulent strains of CA-MRSA into hospitals. In hospitals, patients would be particularly vulnerable due to their compromised immune systems.

Crane *et al.* reported that long-term addiction was associated with MRSA acquisition (20). Results presented in this study did not only indicate that addiction was an important risk factor for MRSA acquisition, but it also demonstrated that the duration of addiction played a role in the acquisition of MRSA. The frequency of MRSA infection increased with the the increase in the duration of addiction (Table 4).

The high prevalence of MRSA colonization and infection among addicts could be due to the impairment of their immune system. A number of studies reported immune dysfunction in drug abusers (not the one caused by coincidental infection with HIV) (41–43). Other factors might include non-sterile methods of drug administration, such as adulterants used to dilute IV drugs, and other drug administration habits that could contribute to colonization and infection.

In conclusion, this study demonstrated how drug abusers, a high-risk group for infections with MRSA, could

Table 5. Relationship Between Route of Drug Abuse and CA-MRSA Colonization in Opiate Drug Abusers

Route of drug abuse	Addicts with positive MRSA	Addicts with negative MRSA	Positive MRSA of total addicts
Inhalation	6/31 (19.35%)	5/29 (17.24%)	6/11 (54.54%)
Injection	6/31 (19.35%)	11/29 (37.93%)	6/17 (35.29%)
Inhalation and injection	19/31 (61.3%)	13/29 (44.83%)	19/32 (59.38%)
Total	31/60 (51.67%)	29/60 (48.33%)	31/60 (51.67%)

be a source of CA-MRSA infection in the non-addict population. The study calls for implementing policies for screening of patients (especially drug abusers and high-risk groups for carriage of MRSA) on admission into hospitals to reduce the risk of transmitting CA-MRSA into the hospitals. This is important, particularly in an era of highly transmissible, toxin- and superantigen-loaded strains of MRSA (CA-MRSA) arising *de novo* in the community (44, 45).

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