

Isolation of Methicillin-Resistant *Staphylococcus aureus* (MRSA) from HIV Patients Referring to HIV Referral Center, Shiraz, Iran, 2011-2012

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Abstract

Extension of drug resistant *Staphylococcus aureus* strains is one of the problems of modern society. Presence of methicillin-resistant *Staphylococcus aureus* (MRSA) in HIV-infected individuals is an important cause of severe infections. Therefore, the main goal of this study was to determine the prevalence rate of MRSA carriage rate among HIV patients referring to the Shiraz HIV referral center (Shiraz, Iran) during 2011-2012. Nasal swabs were obtained from HIV positive patients and were cultured on differential and selective media to isolate *Staphylococcus aureus*, which was confirmed by standard biochemical tests. For isolation of MRSA isolates, bacterial suspensions were cultured on Muller-Hinton Agar containing NaCl and Oxacillin. Finally, data were analyzed by the SPSS software. Of 180 HIV patients, MRSA was isolated from nasal cavity of 23 (12.8%) patients. Most of the isolates were recovered from male subjects who were under 40 years old. No variables such as skin disease, history of hospitalization or infectious disease had significant association with the MRSA colonization rate. The presence of MRSA isolates in the nasal cavity of HIV patients in such a rate warns us about the potential spreading of MRSA among HIV patients in our society and emphasizes on establishing better prevention strategies.

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Introduction

Staphylococcus aureus is an opportunistic pathogen, which causes a wide range of infections such as skin lesions, abscesses, endocarditis, septicemia, and toxic shock syndrome.¹ In 1960s, the first case of methicillin-resistant *Staphylococcus aureus* (MRSA) was reported as a high rate nosocomial pathogen.² Based on the statistics, 29-35% of all clinical bacterial isolates in the United State are *Staphylococcus aureus* and about 40% of *Staphylococcus aureus* isolates are methicillin resistant.^{1,3}

MRSA infections in HIV-infected patients are considered as an important morbidity factor.⁴ Host immune dysfunction in these patients causes higher risk for MRSA bacteremia

infections.⁵ Although this bacterium colonizes more in the anterior nares of HIV-infected patients, some types like MRSA pulsed-field gel electrophoresis (PFGE) type USA300 with higher priority colonizes in the buttocks, genitals, and perineum.⁶ This population is at risk for CA-MRSA more than HA-MRSA infections because of overlapping community networks.⁷ These patients show *Staphylococcus aureus* infections and colonization in particular skin and soft tissue infections (SSTIs).⁸

Management of drug resistant bacteria in such patients is a serious concern. MRSA strains are a potential risk for people who have acquired immune deficiency syndrome. Since there are no data about this issue in Shiraz, the objective of this study was the isolation of MRSA from HIV-infected persons referring to the HIV referral center. This study could help us to understand the prevalence rate of MRSA carriers among HIV positive patients to establish preventative strategies.

Patients and Methods

This cross-sectional study was performed at Shiraz University of Medical Sciences in collaboration with Shiraz HIV referral center during 2011-2012. The participants were HIV-infected patients who referred to this center for therapeutic and medical purposes during that year. From all participants (180 patients) that were over 20 years of age, a written informed consent was taken. Patients were excluded from the study if they had received treatment with intranasal antibiotic treatment in the previous 14 days. The socio-demographic information, history of hospitalization, time of affliction with HIV, etc. were recorded for supplemental study.

During this time, nasal swab samples (right and left) were collected from 180 HIV positive patients. For each patient a specimen collected from the nares with a dry, un-moistened sterile swab. The tip of the swab was inserted approximately 2.5 cm into the nares and rolled three times in each nostril. Swabs were then put in Stuart transport medium and immediately sent to a microbiology laboratory to isolate *Staphylococcus aureus* bacteria. In cases of transportation delay, the samples were kept at 4°C.

In the laboratory, first the samples were codified, cultured on selective and differential media (Mannitol Salt Agar, Phenol red Mannitol Salt Broth and Columbia Blood Agar, Himedia, India), and incubated at 37°C for 48 hours. Grown colonies on these media were identified as *Staphylococcus aureus* with morphology,

gram staining, and catalase, coagulase, DNase tests. Then, disk diffusion test based on Clinical and Laboratory Standard Institute (CLSI) method was used for the isolates to evaluate their susceptibility to methicillin.⁹ For MRSA detection, first bacterial suspensions equal to 0.5 McFarland tube were made from *Staphylococcus aureus* isolate which were grown on TSB medium (Himedia, India). The suspension was cultured with swab on Muller-Hinton Agar (Himedia, India) that contained 4% NaCl and 6 µg/mL oxacillin (Himedia, India), and then incubated at 35°C for 18 to 24 hours and examined for evidence of growth.

Collected data were analyzed using the SPSS (version 21) statistical software for Windows. The Chi-square test was used for the analysis of categorical variables between MRSA colonized and un-colonized patients.

Results

During the study period, a total of 23 (12.8%) MRSA were isolated from 180 HIV positive patients, most of them were male and between 20-40 years old. There were differences among patients' time of affliction with HIV; 10 persons (5.6%) for 1 year or less, 78 persons (43.3%) for about 1 to 5 years and 71 persons (39.4%) between 5 to 10 years were HIV positive. No one was on antibiotic treatment at the time of study. The majority of the participants (144 persons) did not finish high school education and all of them were Iranian race. About 82.6% of MRSA were isolated from the male patients. Characteristics of the study population are shown in Table 1.

Among the patients, 2 had minor thalassemia as a background illness. Sixty-four patients had infectious diseases; Hepatitis C Virus (HCV) co-infection was in the majority of them (n=47), followed by 8 with tuberculosis, 4 with Hepatitis B Virus (HBV), HCV co-infection in 2 patients

Table 1: Characteristics of HIV positive patients participated in the study (n=180)

Characteristic	n (%)
Age	
20-40	113 (62.8)
40-60	65 (36.1)
60-80	2 (1.1)
Sex	
Male	120 (66.7)
Female	60 (33.3)
Education	
Under diploma	144 (80.0)
Diploma	27 (15.0)
Higher education	9 (5.0)

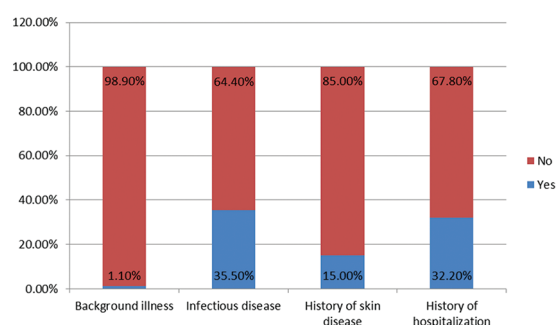


Figure 1: Shows medical history details of HIV patients participated in the study.

with wound infection, 2 with pneumonia and 1 with Hepatitis A Virus (HAV) infection. Details about patients' medical history are displayed in Figure 1.

Most of MRSA isolates were detected in patients with approximately 1 to 5 years of HIV infection (43.5%) and then in patients with more than 5 years of infections (30.4%) and 3 (13.0%) with less than 1 year history of seropositivity. Four MRSA isolates were from patients who had dermatological diseases compared with approximately half of MRSA carriers who had other infectious diseases and history of hospitalization. All MRSA isolates were from patients who had no background illnesses (Table 2). No significant relationships were detected between risk factors and prevalence of MRSA in HIV patient ($P < 0.05$).

Discussion

The prevalence of MRSA colonization in HIV positive patients in the present study was 12.8 %. Various rates, ranging from 1.6% to 34.8% were previously reported. Such a variation might be due to distinct study groups or unique characteristics of each study.^{10,11}

Various risk factors were evaluated to find their relationships with MRSA isolation. It is notable that most MRSA isolation was in male subjects (15% vs. 6%, out of 180). Although no significant relationship was found between this factor and the rate of colonization ($P = 0.063$), other studies indicate a remarkable difference between the two genders. Popovich et al. observed 116 out of 162 MRSA strains in males and 46 in females. Also Furuno et al. reported 63.4% MRSA isolation in HIV positive male patients.^{12,13} Probably the reason is more exposure of men to various communities and person-to-person contacts.

Villacian et al. considered hospitalization as a risk factor for increased MRSA colonization in the nasal cavity of HIV patients.¹¹ This indicates

Table 2: Statistical relationships between risk factors and isolation of MRSA

Factors	MRSA count	Percent	P value
Time of affection with HIV			0.355
0-1	3	13.0	
1-5	10	43.5	
5-10	7	30.43	
>10	3	13.0	
Precedent hospitalization			0.072
No	12	52.2	
Yes	11	47.8	
Skin disease history			0.465
No	19	82.6	
Yes	4	17.4	
Infectious disease history			0.140
No	12	52.2	
Yes	11	47.8	
Background illness			*
No	23	100.0	
Yes	0	0	
Sex			0.063
Male	19	82.6	
Female	4	17.4	
Age			0.171
Less than 40	17	73.9	
More than 40	6	26.1	

*Could not be calculated because of too few patients (n=2) with background illness

that health care centers could be the potential transmission networks and such patients must receive excessive care during their hospitalization period to avoid morbidity and mortality or being a potential carrier for other members of the society after being discharged from hospital. However, our findings do not show a significant relationship between MRSA carriage and hospitalizations ($P = 0.072$), although approximately half of the isolates (n=11) were found in individuals who had a history of hospitalization. This is consistent with a report by Popovich et al.¹²

Similar to our study, Ramsetty et al. and Furuna et al. found no significant relationship between the existence of other infectious diseases and higher rates of MRSA colonization;^{13,14} however, in our study 11 out of 23 were isolated from patients who had other infectious diseases ($P = 0.140$). In fact, such diseases may be the result of immune system dysfunction and low CD_4^+ counts, which is one of the important risk factors for MRSA colonization. However, according to the literature until now no relationship has been documented.^{15,16}

Some studies indicate that skin diseases could increase the rate of MRSA colonization risk in HIV patients. Onorato et al. introduced

dermatologic diseases as an important risk factor.¹⁷ It is contrary to our results since we did not find such a relationship because MRSA were isolated from only 4 HIV patients who had a prior skin disease.

Kumar et al. performed a study on HIV patients in India with findings similar to our study and showed that the duration of HIV infection had no effect on the rate of MRSA colonization ($P=0.355$).¹⁸ It is in contrast with Shet et al.'s study who realized that MRSA colonization rate can increase over time.¹⁹ It might be because of more exposure to the community and health care systems or decreased CD_4^+ level due to disease progression.

We found no particular study that shows a specific correlation between age and bacterial colonization, but some studies have reported that younger people may be at higher risk for the bacterial colonization. The mean age of colonization in these studies is commonly 40 or 41 years.¹² This is similar to the present study that found 17 MRSA cases in a group younger than 40.

Since the number of HIV positive patients with background illness in our study was limited ($n=2$), a judgment about the relationship between this factor and MRSA carriage cannot be made. It was better to have a larger population for this study; however, gaining access to HIV patients is relatively difficult. In addition, some of these patients cannot be recruited to HIV center at proper times for any experiment. This problem was a limitation of the present study.

Conclusion

The presence of MRSA carriage in HIV-infected patients with the rate of 12.8% was confirmed by the results of our study. Failure to find a relationship between some risk factors and the rate of MRSA colonization could be due to the low number of patients in this study. Moreover, further studies are needed in different areas of Iran. However, MRSA presence in HIV patients, warns us about its potential spread among HIV patients in our society, which may cause a high morbidity or even mortality. Therefore, preventive measures should be organized based on universal instructions.

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Conflicts of Interest: None declared.

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