

***Staphylococcus aureus* Nasal Carriage Among Injecting and Non-Injecting Drug Users and Antimicrobial Susceptibility**

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ABSTRACT

Staphylococcus Aureus (SA) is one of the most prevalent bacterial pathogens in human beings. Approximately 20% of healthy persons are persistent carriers and 60% are intermittent carriers of SA. Nasal cavity is one of the most important sites of its colonization. Intravenous (IV) drug abuse has been proposed as a risk factor for colonization of SA in the nasal mucosa. The goal of this study was to determine the frequency of SA carriers in nasal cavity among IV and non-IV drug abusers (addicts), as well as to assess the antimicrobial susceptibility pattern of the positive cases. In a cross-sectional analysis of 300 drug addicts (Group I: 100 non-injecting addicts, Group II: 100 IV injecting drug addicts in rehab, Group III: 100 IV injecting drug addicts not in rehab) in the infectious diseases clinics of Tabriz's Imam Reza and Sina teaching hospitals and the rehabilitation center of Razi hospital, were investigated. Hospitalized addicts, insulin-dependent diabetic cases, HIV positive patients and those on chronic hemodialysis were excluded. The nasal mucosal sample was prepared from each case for SA isolation and its antimicrobial susceptibility was investigated by antibiogram. Eighty-four cases (28%) were culture positive for SA, including 26 cases in group one, 32 cases in group two and 26 cases in group three ($p = 0.55$). There was only one MRSA isolate present in all the cases studied (1.2%). No resistance to linezolid, rifampin and vancomycin was observed. The resistance to erythromycin, cefoxitin, ciprofloxacin, clindamycin, co-trimoxazol and gentamicin were 3.6, 4.8, 2.4, 3.6, 1.2 and 2.4% respectively. No statistically significant differences existed between the three groups in antibacterial susceptibility pattern. Sensitivity to oxacillin using the E-test results and disc diffusion were completely consistent. The percentage of carries of SA in the anterior nasal mucosa among IV and non-IV drug addicts is not considerably higher than the general population. MRSA isolates were uncommon in our cases. However, given the importance of localized and systemic infections in this group, early detection and treatment is reasonable.

Keywords: *Staphylococcus Aureus*, Drug Addicts, Nasal Carriers, Antimicrobial Susceptibility

1. INTRODUCTION

Staphylococcus Aureus (SA) is Gram positive cocci which can asymptotically colonize in different parts of the skin and mucous membranes. In adults the most

common site of colonization is the anterior nares. Colonization of this organism is usually transmitted from person to person and rarely results in disease at the colonization site (Zanelli *et al.*, 2002). In healthy adults, 20% are permanent carriers and 60% are intermittent

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carriers of SA (Mainous *et al.*, 2006). Nasal carriage of *Staphylococcus aureus* in a healthy population studies in Italy was 30.5% and the prevalence of this condition in the USA between 2001-2002 was estimated at about 32.40% (Williams, 1963). Clinical importance of this colonization presents when distant or systemic infections occur especially with Methicillin-Resistant Isolates (MRSA). According to some studies, some population including intravenous drug users are more prone to have staphylococcal (especially MRSA) colonization in their skin and anterior nasal mucosa (Mulqueen *et al.*, 2007).

In this study we plan to determine and compare the prevalence of SA colonization in nasal mucosa among injecting and non-injecting drug addicts in North-West of Iran (East Azarbaijan) within period of time. We hope our study could yield in better understanding of SA colonization, the importance of considering MRSA infection in injecting drug addicts of the region, reasonable primary antibiotic selection in treating infections complications of addicts and importance of isolation of these patients in hospital setting. Since disc diffusion test is the most common laboratory test to determine antimicrobial susceptibility of bacterial organisms, we used this test to determine its sensitivity to differentiate Methicillin-Sensitive *Staphylococcus Aureus* (MSSA) from MRSA isolates.

2. MATERIALS AND METHODS

2.1. Patients and Methods

In a cross-sectional study, 300 drug addicts were divided into three groups: 100 using inhaling drugs, 100 Intravenous (IV) injecting addicts in rehab and 100 IV injecting addicts not in rehab. They evaluated for SA nasal carriage and its antimicrobial susceptibility.

The study was done at the infectious disease clinics of the Imam Reza and Sina teaching hospitals and the rehab center of Tabriz Razi hospital. Due to lack of adequate IV injecting addicts visiting the study sites, some of the samples were taken in person from other rehab centers. Sampling was done over a period of 18 months from July 22, 2009 through January 20, 2011. Hospitalized addicts, insulin-dependent diabetic drug users, cases under hemodialysis and HIV positive addicts (based on medical records and past medical history) were excluded from study.

Samples were made using nasal cotton swab from the anterior nasal mucosa (anterior nares). We collected one sample from each participant. Samples were taken to the Lab in test tubes containing medium NaCl 6.5% and

nutrient broth. After 48 h incubation at 37°C, they were isolated in plates containing monitol salt agar and re-incubated for another 24-72 h at 37°C. Finally, the culture positive cases in yellow colonies were transferred to sheep blood agar medium. After 24 h. incubation, Gram stain, catalase and coagulase test were done from the colonies grown in this medium. Then, half Mac Farland turbid solution (in sterile physiologic serum) was prepared from the Gram-positive, catalase and coagulase positive Cocci. The samples were transferred to the medium containing Muller-Hinton agar and incubated at 37°C for 24 h. for antimicrobial susceptibility test. In the isolates showing resistance to Oxacillin disc, Oxacillin E-test was performed from the same sample in Muller-Hinton medium as gold standard. All of media and discs used in our study were from Highmedia brands made in India. Oxacillin E tests were also from bioMérieux and used based on manufacturer's instructions. Resistant isolates to oxacillin in E test method considered Methicillin Resistant *Staphylococcus Aureus* (MRSA) and sensitive cases considered to be Methicillin Sensitive *Staphylococcus Aureus* (MSSA). Oxacillin susceptibility was evaluated using both disc diffusion and E test methods. The sensitivity to gentamicin, linezolid, vancomycin, co-trimoxazol, cefoxitin, erythromycin, clindamycin and rifampin were evaluated using disc diffusion method alone.

Cases examined included age, number of injections per week, average duration of intravenous drug abuse, history of previous hospitalization or imprisonment in all of our participants.

This study has been approved by the Ethics Committee of Tabriz University of Medical Sciences.

2.2. Statistical Analysis

The collected data expressed as mean \pm SD, Frequency and percentage. The quantitative variables were compared using the t-test for independent groups. Comparison of qualitative variables has been made using chi-square test or Fisher exact test, depending on the circumstances. To determine the level of agreement, Kappa coefficient was determined. In all cases studied, the difference having $p \leq 0.05$ were considered statistically significant by SPSS soft ware (version 18).

3. RESULTS

In this study, 300 male drug addicts in 3 distinct groups were studied. There were 100 inhaling (crack) drug addicts, 100 Intravenous (IV) injecting addicts in

rehab and 100 IV injecting addicts not in rehab. Among these, 84 cases (28%) were SA nasal carriers.

These carriers were 26 cases (26%) of those inhaling addicts, 32 cases (32%) of those IV injecting addicts in rehab and 26 (26%) of those IV injecting addicts not in rehab. Chi-square test results showed that in this respect there is no significant difference among the three groups ($p = 0.55$). The average age of non SA carrier addicts was 34.9 ± 10.30 (18-59) years. In SA carrier addicts it was 32.40 ± 9.75 (18-56) years. t test for independent groups showed that there is no significant statistical difference between two groups ($p = 0.06$).

In group 3 (IV injecting addicts), the average frequency of injection among non SA carriers was 4.23 ± 2.35 (1-14) times/week and 4.52 ± 2.25 (1-12) times/week for SA carriers. T test results showed that in this respect there is no significant statistical difference among the two groups ($p = 0.42$). In above mentioned group the average time of IV addiction among non SA carriers was 3.61 ± 1.96 (1-10) years and for SA carriers were 4.2 ± 3.55 (1-23) years. t test for independent groups showed that in this respect there is no significant statistical

difference ($p = 0.013$). 87 cases (36.1%) of non SA carriers and 71 cases (87.5%) of SA carriers have been hospitalized or imprisoned previously (not during last month). Chi-square test for independent groups showed that in this respect there is significant difference between the two groups ($p = 0.001$).

Results of antimicrobial susceptibility of isolates to different antibiotics are summarized in **Table 1**. Accordingly, there was no significant statistical difference among the three groups. In comparing the sensitivity test between oxacillin disc diffusion and oxacillin E-test, the results were completely similar ($\kappa = 1$ $p < 0.001$) statistical analysis reveals a meaningful but relative correlation results of cefoxitin disc diffusion and two oxacillin sensitivity tests ($\kappa = 0.09$ $p = 0.05$). In our study only one MRSA isolated that was resistant to oxacillin, gentamicin, erythromycin, clindamycin and ciprofloxacin, intermediate to cefoxitin and sensitive to vancomycin, linezolid, cotrimoxazol and rifampin.

Table 1. Antimicrobial susceptibility test of isolated SA stains in 3 distinct studied groups

Antibiotic	Susceptibility	Inhaling addicts	Injecting addicts in rehab	Injecting addicts non in rehab	Total	P value
Oxacillin (E-test)	Sensitive	25(96.2)*	32(100)	26(100)	83(98.8)	0.96
	Resistant	1(3.8)	0(0)	0(0)	1(1.2)	
Oxacillin (disc)	Sensitive	25(96.2)	32(100)	26(100)	83(98.8)	0.96
	Resistant	1(3.8)	0(0)	0(0)	1(1.2)	
Gentamicin (disc)	Sensitive	24(92.3)	32(100)	26(100)	82(97.6)	0.47
	Resistant	2(7.7)	0(0)	0(0)	2(2.4)	
Clindamycin (disc)	Sensitive	25(96.2)	30(93.8)	26(100)	81(96.4)	0.55
	Resistant	1(3.8)	2(6.2)	0(0)	3(3.6)	
Co-trimoxazol (disc)	Sensitive	25(96.2)	32(100)	25(96.2)	82(97.6)	0.53
	Intermediate	0(0)	0(0)	1(3.8)	1(1.2)	
	Resistant	1(3.8)	0(0)	0(0)	1(1.2)	
Erythromycin (disc)	Sensitive	25(96.2)	30(93.8)	25(96.2)	80(95.2)	0.64
	Intermediate	0(0)	0(0)	1(3.8)	1(1.2)	
	Resistant	1(3.8)	2(6.2)	0(0)	3(3.6)	
Ciprofloxacin (disc)	Sensitive	25(96.2)	32(100)	24(92.3)	81(96.4)	0.23
	Intermediate	0(0)	0(0)	1(3.8)	1(1.2)	
	Resistant	1(3.8)	0(0)	1(3.8)	2(2.4)	
Cefoxitin (disc)	Sensitive	24(92.3)	22(68.8)	20(76.9)	66(78.6)	0/81
	Intermediate	1(3.8)	10(31.3)	3(11.5)	14(16.7)	
	Resistant	1(3.8)	0(0)	3(11.5)	4(4.8)	
Linezolid (disc)	Sensitive	26(100)	32(100)	26(100)	84(100)	
Rifampin (disc)	Sensitive	26(100)	32(100)	26(100)	84(100)	
Vancomycin (disc)	Sensitive	26(100)	32(100)	26(100)	84(100)	

*; Data shown as frequency (%)

4. DISCUSSION

The prevalence of carrying SA in the anterior nasal mucosa was studied in different conditions. The range of SA nasal carriage is wide among general population, health center facilities staffs, hospitalized patients, diabetics, HIV positive cases, patients on chronic hemodialysis, drug addicts. In the general population, based on various studies, carriers ranged from 10 to 40% (Derakhshanfar *et al.*, 2009; Naeini *et al.*, 2005; Ziasheykh *et al.*, 2009; Fridkin *et al.*, 2005). The prevalence of nasal carrying SA in health care workers in different studies done in Iran varied between 13.8 to 71.4% (Derakhshanfar *et al.*, 2009; Naeini *et al.*, 2005; Ziasheykh *et al.*, 2009; Ghasemian *et al.*, 2004; Saderi *et al.*, 2004; Rashidian *et al.*, 2001). In other countries this rate was between 10.2 to 85% (Akoua *et al.*, 2004; Kluytmans *et al.*, 1997). However, there have been a few studies to evaluate the prevalence of SA nasal carriage among injecting and non-injecting drug addicts. Kazakova *et al.* (2005) and McDougal *et al.* (2003) reported the prevalence of SA carriers in the nasal mucosa of the injecting addicts in the United States ranged from 34 to 61%. Atkinson *et al.* (2009) studies between 2000-2003 and 2006-2008 revealed the prevalence of SA carriers among IV injecting addicts in Britain as 7 and 49% respectively. In another study in Fridkin *et al.* (2005) reported the prevalence of SA carriers in the nasal mucosa among IV and non-IV injecting drug users to be 55.2 and 25.9% respectively. In a meta-analysis, Kluytmans *et al.* (1997) reported the above mentioned prevalence among non IV injecting addicts varying between 9.1 to 49%. In summary, the prevalence of SA nasal carriers in the nasal mucosa among IV and non-IV injecting drug users varies between 7-61 and 9.1-49% respectively. Our findings (26% for non injecting addicts, 32% for IV injecting addicts in rehab and 26% for IV injecting drug users not in rehab), falls within the same range. It does not appear that SA carrying among addicts in our study to be higher than those in general population or other groups mentioned earlier. Certainly this idea should be evaluated in a case-control study. Furthermore, it should be noted that drug addicts are at higher risk for severe or even fatal SA infections originating from nasal mucosa, hence, this signifies the importance of early consideration and preventative measures (Tuazon, 1984; Tuazon and Sheagren, 1975; Bassetti and Battegay, 2004).

Various factors such as the status of the person (hospitalized or non hospitalized, IV or non-IV injecting addiction, underlying background problems), the tests

used and their accuracy, explain the wide range in reported prevalence of SA carrying in the nose of IV and non-IV injecting addicts (Kazakova *et al.*, 2005). Meanwhile, there is still no definite conclusion with regard to the risk factors.

Our study also revealed no significant correlation between ages, number of injection per week and the addiction time frame with carrying the SA in the nasal mucosa. However, the frequency of being hospitalized or imprisoned before, in the carriers was significantly higher than the non carrier group.

In our study, the MRSA prevalence among SA carriers was 1.2% (3.8% among the non-injecting drug addicts and none among either injecting addict groups whether active or in rehab). MRSA prevalence in Fathallah *et al.* (2010) study was 31.6%. Probably the main reason for this difference between results of the two studies, is the limiting of the latter study to those hospitalized IV injecting addicts whereas in our study none of cases were hospitalized at the time of study or even recently.

Antimicrobial susceptibility pattern of SA isolated from nose studied in various population in Iran. Derakhshanfar *et al.* (2009) reported the resistance to oxacillin, erythromycin, gentamicin and clindamycin in general population carrying SA in the nose in Hamadan, 35.7, 35.7, 7.1 and 71.4% respectively. They found no resistance to co-trimoxazol and vancomycin (Derakhshanfar *et al.*, 2009). Ziasheykh *et al.* (2009) study in Rafsanjan, the resistance of SA isolated from the nose of admitted patients to oxacillin, erythromycin, ciprofloxacin, co-trimoxazol, clindamycin and vancomycin was 77.3, 11.4, 6.8, 4.5, 2.3 and Zero percent respectively. In our study the resistance to erythromycin, cefoxitin, ciprofloxacin clindamycin, cotrimoxazol and gentamicin was 3.6, 4.8, 2.4, 3.6, 1.2, 2.4% respectively. We found no resistance to linezolid, rifampin and vancomycin.

Although there has not been a similar study in IV or non-IV drug users and we have not comparative study, but it does not appear that the antimicrobial resistance of SA isolated from nasal mucosa of non-hospitalized addicts, to be significantly higher than the general population.

According to present study, there was a complete correlation between results of oxacillin E test and Oxacillin disc diffusion methods. Whereas there was no such correlation between cefoxitin disc diffusion results and sensitivity to oxacillin using the above two methods. It should be noted that sensitivity or resistance to cefoxitin is at least as valuable as

oxacillin in differentiating MRSA from MSSA Cauwelier *et al.* (2004). This discrepancy first undermines the theory of total correlation of E-test and disc diffusion methods, second, it could be related to different quality of the antibiotic discs used in the study, hence it is important to continue the study with cefoxitin E-test, or sensitivity test to cefoxitin using tube dilution method on the isolated strains.

5. CONCLUSION

Although the prevalence of carrying SA and MRSA isolates among injecting and non-injecting addicts in our study was not significant based on one time random sampling, we propose precise diagnosis and prompt decision making given the importance of infection in addicts especially injecting ones. We recommended similar study in recently admitted injecting addicts, evaluation of stable (permanent) colonization using frequent programmed sampling. Also, further study of antimicrobial susceptibility of isolated strains using E test or tube dilution to cefoxitin, or even molecular study (PCR) of the isolated strains to determine the sensitivity or resistance to methicillin is proposed.

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