Inducible clindamycin resistance and nasal carriage rates of Staphylococcus aureus among healthcare workers and community members.

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Abstract

Background: Nasal carriage of Staphylococcus aureus is becoming an increasing problem among healthcare workers and community individuals

Objectives: To determine the prevalence of methicillin-resistant S. aureus (MRSA) nasal colonization and inducible clindamycin resistance (ICR) of S. aureus among healthcare workers at Soba University Hospital and community members in Khartoum State, Sudan.

Methods: Five hundred nasal swabs samples were collected during March 2009 to April 2010. Isolates were identified using conventional laboratory assays and MRSA determined by the disk diffusion method. The D-test was performed for detection of ICR isolates with Clinical Laboratory Standard Institute guidelines.

Results: Of the 114 S. aureus isolated, 20.2% represented MRSA. The occurrence of MRSA was significantly higher among healthcare worker than community individuals [32.7% (18/55) vs. 6.9% (5/59)] (p=0.001). Overall the 114 S. aureus isolates tested for ICR by D-test, 29 (25.4%) yielded inducible resistance. Significantly higher (p=0.026) ICR was detected among MRSA (43.5%) than methicillin-susceptible S. aureus (MSSA) (20.9%).

Conclusion: MRSA nasal carriage among healthcare workers needs infection control practice in hospitals to prevent transmission of MRSA. The occurrence of ICR in S. aureus is of a great concern, D- test should be carried out routinely in our hospitals to avoid therapeutic failure.

Keywords: S. aureus nasal carriage, healthcare workers, community members, inducible clindamycin resistance

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Introduction

Nasal carriage of Staphylococcus aureus plays an important role in the epidemiology and pathogenesis of infection and is becoming an increasing problem among healthcare workers and in the healthy community individuals[1,2]. General populations with persistent S. aureus nasal carriage rates at 10% to 20%,[2,3] and up to 50% are intermittent carriers[3]. Furthermore, carrier levels of 25% have been reported among hospital healthcare workers[3]. S. aureus nasal colonization has been determined as an important risk factor for the development of different types of infections ranging from skin infection to serious conditions[4,5]. The severity of these infections is mainly due to the presence of methicillin-resistant S. aureus (MRSA), which defined as multi-drug resistance bacteria[6]. The treatment of infections caused by multi-drug resistance bacteria, especially MRSA has become a health problem due to limitation of therapeutic choice[7].

Clindamycin, the macrolide-lincosamide-streptogramin B (MLSB) antimicrobial group is an alternative treatment option for S. aureus infections[9,8]. The use of this antimicrobial agent in the presence of erythromycin resistance is of a great concern, since there is a possibility of induction of cross-resistance mechanism

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among members of the MLSB[10]. The most common mechanism of macrolide resistance is mediated by erm genes which encode enzymes that confer constitutive or inducible resistance to MLSB agents in the presence of either a macrolide or a lincosamide inducer [7,9]. Clindamycin resistance among S. aureus isolates appear to be susceptible to clindamycin in the absence of erythromycin disk during routine antimicrobial susceptibility testing. Reporting of such results indicated to poor laboratory identification of these isolates[11,12]. Thus inducible resistance of such isolates can be detected by the D-test, a disk diffusion test in which an erythromycin disk will induce clindamycin resistance[11,13]. This study aimed to determine the prevalence MRSA nasal colonization among healthcare workers at the Soba University Hospital and community members in Khartoum State, Sudan. In addition, to detect inducible clindamycin resistance (ICR) among MRSA and methicillin- susceptible S. aureus (MSSA) isolates.

Materials and methods

Study design and settings

This descriptive comparative study was carried out during the period from March 2009 to April 2010. Five hundred nasal swab samples were collected equally from the healthcare workers, including doctors, nurses and medical technologists in the Soba University Hospital and from the adult community members in Khartoum State, Sudan. Each adult participant was selected randomly and asked if he or she agreed to participate in the study before obtaining samples. The study was approved by the Research Council Board of Faculty of Medical Laboratory Sciences, Khartoum University. The criteria was designed to exclude hospitalized community members, while the inclusion criteria was: Community members who were apparently healthy individuals.

Sampling procedures

Nasal swab samples collected from each subject by rotating four times inside each anterior nares using sterile cotton wool swab. The samples were transported immediately to the Microbiology Laboratory at the Faculty of Medical Laboratory Sciences, University of Khartoum and were processed within two hours.

Isolation and identification of S. aureus

Each nasal swab was inoculated onto Manitol salt agar plate (Oxoid, Basingstoke, England). All cultured plates were incubated at 37 ºC over night. Identification of S. aureus isolate was determined on the base of colony morphology, Gram stain, catalase production, coagulase test and DNase test[14].

Antibacterial susceptibility testing

Antimicrobial susceptibility testing of S. aureus isolates was performed by the Keby-Bauer disk diffusion method following the CLSI recommendations.[13] In brief, a suspension equivalent 0.5 McFarland standard turbidity was prepared for each isolate and inoculated onto Mueller-Hinton agar plate (Difco Laboratories, Detroit, USA), using a sterile cotton swab by streaking the swab over the entire sterile agar surface 3 times. Then antimicrobial disks of cefoxitin (30µg), erythromycin (15µg) and penicillin (30µg) were placed at the recommended distance. All cultured plates were aerobically incubated at 37ºC for 18 hours before the zone sizes were recorded. S. Areas ATCC 29213 (susceptible) and S. aureus ATCC 33591 (resistant) were used as control strains. The test result was only validated in the cases where inhibition zone diameters of the control strains were within the performance range in accordance with the CLSI guidelines.[13]

Detection of MRSA

A disk diffusion method with cefoxitin (30 µg) was used to detect MRSA strains as previously described[15]. This test was carried out immediately along with each susceptibility testing of the isolate being performed. All the S. aureus isolates that showed cefoxitin inhibition zone diameter of ≤ 20 mm were reported as MRSA strains and ≥24 mm was considered as MSSA strain[16].

D- test performance for screening of inducible clindamycin resistance isolates

Each S. aureus isolate found to be resistant to erythromycin was tested for inducible resistance by 'D test' as per CLSI guidelines[16]. Suspension of the isolated organism equivalent to 0.5 McFarland standard turbidity was inoculated onto Mueller Hinton agar plate (Difco Laboratories, Detroit, USA). Clindamycin (2ug) and Erythromycin (15ug) antimicrobial disks (Oxoid, Basingstoke, England) were placed at a distance of 15mm (edge to edge) from each other. Quality control was performed by S. aureus ATCC 25923. Following overnight incubation at 37^0C, a D-shape zone around the clindamycin in the area between the two disks, the isolate was positive for inducible resistance[17].

Statistical analysis

Collected data was analyzed using Statistical Package for Social Sciences program (SPSS Inx., Chicago, IL., USA) Version 16. The Chi-square test was used to compare between every two variables. All p-values less than 0.05 were considered as statistically significant.

Results

The Prevalence of MRSA among healthcare workers and community individuals

Out of the 500 nasal swab samples examined, S. aureus was detected in 22.8% (114) of the total samples. Of these 114 positive samples, 55 isolates were collected from the healthcare workers, while 59 isolates from the community members. The results of antimicrobial susceptibility test of the S. aureus isolated from community members (n=55) and healthcare workers (n=59) are given in Table 1.

Table 1: Antimicrobial susceptibility of *S. aureus* isolated from community members and healthcare workers

Antibiotic	*S. aureus* isolates	
	Community member (n=59) (% of resistant)	Healthcare worker (n=55) (% of resistant)
Cefoxitin	4 (6.8%)	18 (32.7%)
Erythromycin	18(30.5%)	21(38.2%)
Penicillin	56(100%)	55(100%)

Overall, the 114 S. aureus isolates screened for the presence of MRSA strains, 20.2% were found to be MRSA with 32.7% among health care workers and 8.5 % among community individuals isolates) (Table 2). The occurrence of MRSA isolates were significantly higher among the healthcare workers than in the community individuals [32.7% (18/55) vs. 8.5% (5/59)] (p = 0.001).

Table 2: Frequency of MRSA and MSSA isolates from hospital healthcare workers and community members at Khartoum State, Sudan

Source of isolates	Number of isolates	Frequency	
		MRSA	MSSA
Healthcare worker	55	18 (32.7%)	37
Community individual	59	5 (8.5%)	54
Total	114	23 (20.2%)	91

Detection of inducible clindamycin resistance in MRSA and MSSA

One hundred fourteen S. aureus isolates (23 MRSA and 91 MSSA) tested for ICR by D-test, 29 (25.4%) yielded inducible resistance. Of these 29 isolates, 10 were MRSA whereas 19 were MSSA (Table 3). Inducible clindamycin resistance was found to be significantly higher among MRSA than MSSA isolates [43.5% (10/23) vs. 20.9% (19/91)

Table 3: Distribution of inducible clindamycin resistance in MRSA and MSSA isolates

Type of isolates	Number	No. (%) of inducible clindamycin resistance
MRSA	23	10 (43.5%)
MSSA	91	19 (20.9%)
Total	144	29 (25.4%)

Discussion

The presence of S. aureus nasal colonization among healthcare personnel and healthy community members known to be as a major risk factor for the development of both community-acquired and nosocomial infections including MRSA[1,7]. However, determination of colonization prevalence provides a useful estimate of the potential for development of S. aureus infections[4]. This study estimates the S. aureus nasal carriage rates among healthcare workers in a university hospital and among community members at Khartoum State, Sudan. In the present study, the prevalence of S. aureus nasal colonization among healthcare workers at the Soba University Hospital was 32.7% and that of healthy community individuals was 6.8%. These findings are almost similar to that previously reported in the Soba University Hospital during the period from the 1996-1997 by Ahmed et al. (1998)[18]. These authors have estimated nasal carriage among patients and staff personnel at 26.8%. Worldwide studies have been documented S. areas nasal colonization. In Turkey[1], the nasal carriage rates of S. aureus were 27.5% among hospital personnel and 24.0% normal healthy subjects. In Iran[19], reported as 31.1% among healthcare workers, in France[20], among hospital employees was 33.4% prevalence, in Spain[21], among medical students was 39.3%, among Libyan health care workers was 22%[5], in Nigeria[22] , reported as 14 % among medical student, in Jordan[23], the nasal carriage rate in healthy volunteers was 7•5% .

Colonization particularly with MRSA plays an important key factor for the development of different kinds of staphylococcal infections ranged from minor skin infections to soft tissue infections[18,2]. The carriage rate of MRSA nasal colonization varied significantly across different demographic features[28]. MSSA colonization appeared to be influenced more readily than MRSA colonization by many health and environmental factors in the univariate analysis. For instance, lower frequency of hand washing, influenza vaccination, upper respiratory tract infections, and use of antibiotics were associated with decreased incidence of MSSA colonization, but did not influence colonization by MRSA[29]. Different studies have described a high prevalence of MRSA colonization and infection among persons of low socio-economic status in the general community, may be associated with crowding, limited access to healthcare, or barriers to maintaining adequate hygiene[28]. Furthermore, the innate immunity of the host has been implicated in the mechanisms of S. aureus colonization[29].

In this study, our data showed that MRSA carriage rates were significantly higher (p=0.001) among healthcare workers than in healthy adults from the community. This finding is in-agreement with other studies[22,5], which have been documented that the MRSA nasal

carriage was higher among medical personnel than non-medical personnel. Yazgi et al. (2003)[1] proposed that the colonization of the resistant strains rather than the frequency of S. aureus colonization is more important in the hospital personnel. The primary mode of transmission of MRSA is by direct contact, usually with another person's hands. MRSA has also been isolated from people's hands after touching contaminated material or equipment. Lescure et al. (2006)[24] explained that MRSA infections seen in the community can be acquired either directly in hospitals or long-stay institutions or indirectly by contact with an MRSA carrier, such as a family member working in a hospital, a family member with a previous stay in the hospital, a general practitioner, or a community nurse .

Since MRSA infections could be on the rise in the hospital units through hospital personnel carriers, good hand hygiene practice of hospital staff is a primary important factor to avoid dissemination of multi-drug resistant organism in the hospital unit. In addition, implementations of infection control measures in our hospitals that is, understanding barriers of the spread and transmission of MRSA carriage, are necessary to reduce risk of subsequent infection.
Empirical therapeutic options for Staphylococcal infections in the hospital and community settings have become more limited due to increasing the prevalence of MRSA[7,6]. Clindamycin has long been the best choice option because of its efficacy action against both MSSA and MRSA[7]. Proper antimicrobial susceptibility data is important for appropriate therapy decisions; however, limited data is known about the prevalence of ICR in MRSA isolates[11].

In the present study, the overall prevalence ICR was 29.4 with 43.5% of MRSA and 20.9% of MSSA. In-agreement with other reports [9,8,25], our results revealed that there were significant differences ($p = 0.026$) of ICR rates between MSSA and MRSA. Elsewhere, studies have reported ICR between both MRSA and MSSA isolates. In a study conducted in South Africa, Shittu and Lin, (2006)[26] determined the inducible MLSB phenotype was detected in 10.8% of MSSA and 82% of MRSA respectively. Juyal et al. (2013)[13] reported among the inducible MLSB phenotypes, 13.3% isolates were MRSA and 28.9% were MSSA. In contrast, Patel et al. (2006)[7] found that the overall prevalence of ICR was 52%, with 50% of MRSA and 60% of MSSA isolates exhibiting ICR. Moreover,no statistically significant difference of ICR was observed between MRSA and MSSA strains ($p=0.434$) in the study by Eksi et al. (2011)[27]. These findings with our current results indicate the significant occurrence of ICR between MRSA and MSSA. Therefore, antimicrobial susceptibility data of ICR isolates should be evaluated routinely in each infections caused by S. aureus before starting the treatment.

Limitations

Firstly, information about estimation of variables related to demographics, past or current medical records such as exposure to antimicrobial agents, and lifestyle for the study groups was not available for analysis. Secondly, due to the limitation of our laboratory facilities, identification of MRSA was carried out only through applying a simple, reliable test that needs to be confirmed by a standardized molecular technique such as PCR amplification of the mecA gene, which was not applied in this study. Finally, some epidemiological factors influencing colonization of MRSA and MSSA nasal carriage rates may not have been collected. However, the identification of risk factors for nasal colonization may help in the development of strategies to prevent MRSA spreading.

Conclusion

S. aureus nasal colonization is more prevalent among healthcare workers than community member in particular, MRSA. Beside personal hygiene practices of medical staff, regular implementation of infection control practice, including screening of nasal carriages and microbial flora in our hospital are necessary to prevent spread of MRSA carriage. The occurrence of ICR between MRSA (43.5%) and MSSA (20.9%) is of a great concern, which contributed to the treatment failure of S. aureus infections. Since the D- test is a simple assay for the detection of ICR strains, therefore, it should be carried out routinely in our hospital to avoid clindamycin therapeutic failure.

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