

Nasal Carriage of Methicillin-resistant and Methicillin-susceptible *Staphylococcus aureus* in Nursing Home Residents in Bolu, Turkey

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ABSTRACT

Background: This study aimed (a) to provide information on methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* colonization ratio in residents of nursing homes; (b) to determine the effect of hand-washing education given to nursing home residents and employees on nasal carriage of *Staphylococcus aureus* (NCSA) and (c) to obtain probable risk factors for MRSA colonization of residents in two nursing homes.

Methods: Seventy-nine volunteers (24 females and 55 males) from Bolu nursing homes were included in the study. Nasal samples were taken with sterile swabs from the anterior nares. *Staphylococcus aureus* strains were identified using classical methods and genotyping of methicillin resistant strains was done using Arbitrary Primed PCR (AP-PCR). Antibiotic susceptibilities were determined by disk diffusion methods according to NCCLS standards. After first nasal samples were taken, all employees and residents of nursing homes were educated about the methods of hand hygiene over two days.

Results: With hand-washing education, the decrease of NCSA rate (initially 43%; after education, 21%) was significant ($p < 0.05$) while decrease of MRSA carriage (initially 5%, after education 1%) was not ($p > 0.05$). MRSA carriage was significantly correlated with presence of skin lesions, prior hospitalization within the last six months, and antibiotics usage within the last six months. AP PCR results suggested that residents' carriage of MRSA was the result of the same source.

Conclusion: MSSA and MRSA colonization rates were found to be 38% and 5% in nursing homes, respectively. These ratios can decrease with simple precautions like hand-washing after a short education period.

Portación Nasal de *Staphylococcus Aureus* Resistentes a la Meticilina y Susceptible a la Meticilina en los Residentes del Hogar de Ancianos en Bolu, Turquía

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RESUMEN

Antecedentes: Este estudio tiene como propósito: (a) ofrecer información sobre la tasa de colonización *Staphylococcus aureus* resistentes a la metilina (SARM) y el de *Staphylococcus aureus* susceptibles a la metilina (SASM) de residentes en hogares de ancianos; (b) determinar el efecto de la educación del lavado de manos brindada a los residentes y empleados, sobre la portación nasal de *Staphylococcus aureus* (PNSA); y (c) obtener los factores de riesgo probables por la colonización de SARM en los residentes de dos hogares de ancianos.

Métodos: Setenta y nueve voluntarios (24 hembras y 55 varones) de los hogares de ancianos Bolu fueron incluidos en el estudio. Se tomaron muestras nasales con hisopos estériles, de 1/3 orificios nasales anteriores. Se identificaron cepas de *Staphylococcus aureus* usando métodos clásicos; y la genotipificación de las cepas resistentes a la metilina se realizó por medio de la técnica de la reacción en cadena de la polimerasa con iniciadores arbitrarios (AP-PCR). Las susceptibilidades antibióticas se determinaron mediante métodos de difusión por disco de acuerdo con los estándares del NCCLS. Después que se tomaron las primeras muestras nasales, todos los empleados y los residentes de los hogares de ancianos recibieron instrucción sobre métodos de higiene de las manos durante dos días.

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Resultados: Con la educación del lavado de manos, la disminución de la tasa de PNSA (inicialmente 43%; después del entrenamiento 21%) fue significativa ($p < 0.05$), mientras que la disminución de la tasa de portación de SARM (inicialmente 5%; después del entrenamiento 1%) no fue significativa ($p > 0.05$).

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BACKGROUND

Staphylococcus aureus infections are associated with considerable morbidity and mortality (1). Methicillin-resistant *Staphylococcus aureus* (MRSA) were first identified in Europe and the United States of America in the late 1960s. Recently, even community-acquired *S aureus* strains have shown resistance to methicillin (2). In recent years, decreasing susceptibility of MRSA to glycopeptides (such as vancomycin) has been reported. Due to an increasing number of infections caused by MRSA strains, which are most often multi-resistant, therapy has become problematic (3). Methicillin-resistant *Staphylococcus aureus* presents a major problem for elderly patients, especially those in nursing homes. People colonized with MRSA are at increased risk of MRSA infection. Nasal carriage of *Staphylococcus aureus* (NCSA) in the nose appears to play a key role in the epidemiology and pathogenesis of infection (4). Rate of infection is higher in carriers than in non-carriers (5). Therefore, prevention of staphylococcal carriage is now more important than ever. Elderly people have a lot of factors for colonization such as frequent hospitalization, antibiotic usage and organ failure (4).

Colonized people with MRSA also have a higher risk of death from MRSA infections (6). Therefore nursing homes and other institutional settings must be especially careful to prevent the spread of infection caused by this organism. Although, prevalence of *S aureus* in nursing homes is well-known in many countries, there is a lack of information in Turkey about this topic. Bolu province is in the Western Black Sea Region of Turkey and its population is 270 654 according to population census of year 2000. There are two nursing homes (Izzet Baysal and Neziha Baysal Nursing Homes) in this province. Although the total residential capacity of both nursing homes is 120 (60 each), 79 residents were staying in them during the study period.

This study aim (a) to provide information on MSSA and MRSA colonization ratio in residents of nursing homes, (b) to determine the effect of hand-washing education given to nursing home residents and employees on NCSA and, (c) to obtain probable risk factors for MRSA colonization of residents in two nursing homes.

SUBJECTS AND METHODS

Subjects

The study consisted of an elderly population in nursing home residents who volunteered for study and an informed consent was obtained from each subject. A total of 79 subjects were evaluated for age, gender, existing illness, urinary catheterization, intravenous catheterization, hospitalization, wound

and other skin lesions, and residential conditions after they were approved for participation in this study.

Bacteriologic procedures

Nasal specimens were collected using two sterile cotton-wool swabs for each subject. Swabs were rotated three times clockwise and three times anticlockwise in the anterior nares (7). The swabs were immediately placed in Stuart's transport medium and kept at 4°C before being inoculated onto mannitol salt agar (Chapman Medium, BioMérieux, Marcy l'Etoile, France). The plates were incubated at 37°C for 48-hours. All mannitol-positive colonies were subcultured onto 5% blood agar and *S aureus* isolates were defined as catalase-producing gram-positive cocci which were positive for tube coagulase and confirmed by a rapid *S aureus*-specific latex agglutination test (Staphylofect plus (Oxoid, Hampshire, UK). Cases in which nasal cultures yielded more than three colonies for *S aureus* were accepted as carrier (8). *In vitro* susceptibility testing was performed by disc diffusion method, according to NCCLS guidelines (9).

Arbitrary Primed PCR (AP-PCR)

Bacterial DNA extraction and AP-PCR application were performed as described by Betino *et al* (10) with some modifications. Single colony was taken into 100 µL DNase – RNase free distilled water from which *S aureus* isolates were identified by standard methods, and kept at 100°C and 80°C for 10 minutes. Twenty µl lysostaphyne (1 µg/µl, Sigma Chemical Co, St Louis, Mo), 20 µl proteinase K (10 µg/µl, Sigma Chemical Co, St Louis, Mo) and 20 µl lysozyme (10 µg/µl, Sigma Chemical Co, St Louis, Mo) were added and the mixture was incubated at 55°C for 20 minutes for enzymatic inhibition. After waiting at 100°C for 10 minutes it was centrifuged at 14 000 rpm and the supernatant was taken into eppendorf tubes. Five µl suspension was added to 50 µl PCR mixture (75 mM Tris-HCL (pH:8.8) 20 mM (NH₄)₂ SO₄ %0.01 Tween 20, 3 mM MgCl₂, 0.2 mM dNTP's, 1.5U/µl taq polymerase enzyme, 0.1 µM primer, 70 ng DNA) and was amplified with 5'-CGGCAGCGCC-3' primer and 40 cycles of one minute denaturation at 94°C, 1.5 minute annealing at 30°C, 3 minute extension at 55°C and 5 minute final extension at 55°C were performed. Products were run in agarose gel concentration.

Education

After the first nasal samples were taken, all employees and residents of the nursing homes were educated about the methods of hand hygiene over two days. Importance of hand-washing in the prevention of infections was explained

to them. Furthermore, informative illustrated brochures were hung above lavatories. All nursing home employees were asked to wash their hands with soap for 15 seconds under the following conditions: before patient care, after patient care, after wound care, after handling medical equipment, after taking off their gloves, when their hands got dirty, after going to the bathroom and after handling permanent urinary catheter.

All nursing home residents were asked to wash hands with soap for 15 seconds under the following conditions: when their hands got dirty, after going to bathroom, before and after touching another person, after touching a patient with affixed permanent urinary catheter and before and after meals. It was asked of all nursing home residents that during this time interval, usage of antibiotics and local pomades with antibiotic be avoided, so NCSA would not be directly affected.

Exclusion

Subjects having active infections and a history of antibiotic usage in the last 15 days were excluded from the study.

Statistical analysis

Quantitative variables were compared between the two groups using non-parametric test (Mann-Whitney test). The data of the other parameters were compared by χ^2 or Fisher's exact tests where appropriate. Epi-info 6.0 (Centers for Disease Control, Atlanta) was used to perform the analysis. *p*-value of < 0.05 was considered significant.

RESULTS

Colonization prevalence and the Effect of Education on NCSA

Twenty-four women and 55 men, a total of 79 volunteers were taken into the study (mean age 74.9 ± 8.1 years). Before education, 34 of 79 (43%) cultures were positive for *S aureus* and four of 79 (5% of participating residents) had MRSA (Table 1). Three residents died before the education

Table 1: Comparison of the cases: detected and non-detected carriage of MRSA

Parameters	MRSA (+) n = 4 (%)	MRSA (-) n = 75 (%)	<i>p</i> value ^a
Gender (Male/Female)	3/1	52/23	NS
Age [Range]	75.5 [71–82]	74.4 [61–93]	NS
Diabetes mellitus	1 (25)	4 (5)	NS
Cardiac failure	3 (75)	25 (33)	NS
Renal Failure	2 (50)	11 (15)	NS
Hospitalization (last six months)	3 (75)	12 (16)	0.02
Presence of skin lesion	3 (75)	7 (9)	0.001
Antibiotic usage (last six months)	4 (100)	14 (19)	0.0001

^a Fisher exact test was used in statistical evaluations, NS = Not significant.

period was completed, one of them died from pneumonia (cultures were negative prior to death), the others died due to

myocardial infarction. Two of them were MRSA carriers (Table 2).

Table 2: Properties of MRSA colonized cases

Properties	Patient number			
	1	2	3	4
Nursing Home	IBNH	NBNH	NBNH	IBNH
Gender	Female	Male	Male	Male
Age	75	71	82	74
Diabetes mellitus	None	Present	None	None
Cardiac failure	Present	Present	Present	None
Renal Failure	None	None	Present	Present
Hospitalization (last six months)	Present	None	Present	Present
Presence of skin lesion	Present	None	Present	Present
Antibiotic usage (last six months)	CIP ^a AMP/SLB ^b	OFX ^c AMC/CLV ^d	CIP CFX ^e	TMP/SMX ^f AMP/SLB
MRSA colonization in second screening	None	NA	NA	Present
Outcome	Survival	Death by Myocardial infarction	Death by pneumonia ^g	Survival

IBNH = Izzet Baysal Nursing Home, NBNH = Neziha Baysal Nursing Home, ^aCIP = Ciprofloxacin, ^bAMP/SLB = ampicillin/sulbactam, ^cOFX = Ofloxacin, ^dAMC/CLV = amoxicillin/clavulanic acid, ^eCFX = cefuroxim/axetil, ^fTMP/SMX = trimethoprim/sulfamethoxazole, ^gcultures were negative prior to death, NA = Not applicable

After the education period, 16 of 76 (21%) cultures were positive for *S aureus*, one of them (1% of participating residents) had nasal carriage of MRSA. This MRSA carrier isolated in the second screening was also an MRSA carrier initially (Figure, Patient 4). The decrease of *MSSA* carriage

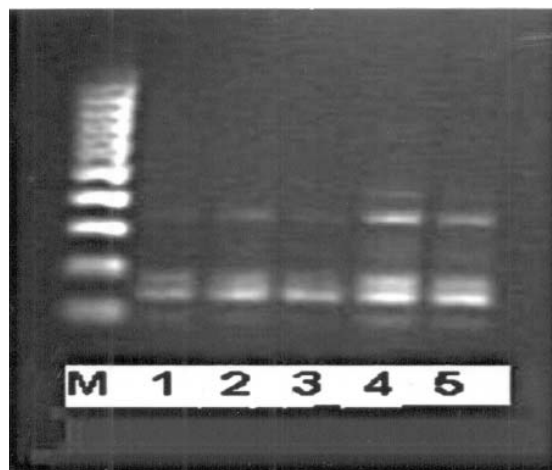


Figure: AP-PCR results of MRSA isolates.

- M*: Standard marker
- 1*: MRSA originated from Patient 1 (Izzet Baysal Nursing Home)
- 2*: MRSA originated from Patient 2 (Neziha Baysal Nursing Home)
- 3*: MRSA originated from Patient 3 (Neziha Baysal Nursing Home)
- 4*: MRSA originated from Patient 4 (Izzet Baysal Nursing Home)

(38% to 20%) was significant while decrease of MRSA carriage (5% to 1%) was not (Table 3).

Table 3: Ratio of nasal *S aureus* carriage before and after the hand-washing education

Parameters	Before education n = 79 (%)	After education n = 76 (%) ^a	P value
No detection of nasal <i>S. aureus</i>	45 (57)	60 (79)	0.003
MSSA	30 (38)	15 (20)	0.01
MRSA	4 (5)	1 (1)	0.195
<i>Staphylococcus aureus</i> (MRSA and MSSA)	34 (43)	16 (21)	0.03

MSSA = Methicillin susceptible *Staphylococcus aureus*

MRSA = Methicillin resistant *Staphylococcus aureus*

^aThree patient died during education period

Risk Factors for MRSA carriage

Four cases were determined as MRSA carriers in initial screening. Two of them were residents of Neziha Baysal Nursing Home while others were residents of Izzet Baysal Nursing Home. Cases with MRSA were compared with cases in which no MRSA was detected. There was no difference between groups regarding age, gender, diabetes mellitus, renal or cardiac failure ($p > 0.05$). However, there were significant differences with respect to skin lesions, hospitalization and antibiotic usage within the last six months ($p < 0.05$). The most frequently used antibiotics were quinolones in four cases in which MRSA was isolated. Specifications of MRSA carriers are shown in Table 2. A total of five (initial-four, after education-one) MRSA isolates had the same antibiotic resistance pattern and they had similar genotypes revealed by AP-PCR method (Figure).

DISCUSSION

Staphylococcus aureus is an important cause of nosocomial infection (11). In the 1960s within two years of the introduction of semisynthetic penicillinase-resistant penicillins, *S aureus* resistance to methicillin was seen (12). In the 1970s, MRSA infections were the main cause of hospital infections in many countries. Recently, MRSA has also been detected more often in the community (13). Infections caused by *S aureus* are a significant cause of morbidity and mortality in the older age groups (20).

Nursing home residents were described as a risk group for NCSA (14). In the present study, 79 nursing home residents were evaluated and 34 (43%) subjects were detected as NCSA and four (5%) of them were detected as MRSA carriage. In surveys of nursing homes, Lee *et al* (15) and Muder *et al* (16) reported point prevalence for NCSA as 35%, and 41% respectively, among the USA nursing homes. In another study, Hsu *et al* (17) found a prevalence of 24.4% for NCSA and 8.7% for MRSA. The authors' findings in the present study were compatible with results of prior reported studies.

There are a lot of studies about MRSA colonization among nursing home residents, but the authors could not find a study about the effect of hygiene education on *S aureus* col-

onization on nursing home residents. In the second part of this study a simple education about importance of hand-washing was given to residents and employees of nursing homes. The investigation was repeated 40 days after education. During this time interval, usage of antibiotics and local pomades with antibiotic was avoided. In the second screening, MSSA frequency decreased from 38% to 20% ($p < 0.01$). This indicated the efficacy of simple measures like regular hand-washing education in reducing colonization of *S aureus*. In the second screening, MRSA colonization was determined in only one case. However, it must be emphasized that two cases in whom MRSA was determined in the first screening died (Table 2, patients 2 and 3). There was a significant decrease in *S aureus* carriage but this was not detected in cases with MRSA carriage. This could be related to the small number of cases with MRSA. On the other hand, it can be related to the ineffectiveness of simple precautions like only hand-washing to decrease MRSA colonization.

This study also aimed to investigate risk factors causing the development of MRSA in the nursing homes. Diabetes mellitus, cardiac and renal failure were not established as risk factors for MRSA colonization in contrast to similar studies. Presence of skin lesion, presence of antibiotic usage within the last six months and presence of hospitalization within the last six months were found as a risk factor for MRSA carriage. These factors were described as risk factors for MRSA carriage with previous studies. This study has shown compatibility with previously reported studies (18–21).

The similar PCR and antibiotic resistance pattern of all MRSA isolates confirmed that the same strain was either spread between patients and/or from employees to patients (Figure). The authors believe that nursing home residents acquire MRSA during hospital stay rather than in the nursing home. In this study, hospitalization in the last six months has been determined as a risk factor for the carriage of MRSA. Three of four cases in whom MRSA was isolated had been hospitalized in the same hospital in the last six months. While two of them (patients 1, 3) stayed in the same nursing home, the others stayed in a different nursing home (patient 4, Table 2). Nevertheless, isolates in which both antibiogram and genotypic patterns were the same were obtained in cases staying in different nursing homes. These findings suggest that the same strain may circulate within different nursing homes in Bolu, probably reflecting the strain found in the same hospital.

This study had several limitations. The first limitation of this study is that nasal cultures could be taken only from nursing home residents willing to participate in the study. However, cultures of employees of nursing homes could not be taken because they refused to give nasal culture. If their cultures could have been obtained, the results would have been more reliable. Another limitation of this study is that four MRSA cultures were identified in initial screening and only one in the second screening. The MRSA carriage group

was small and thus might lack the power to distinguish between a true difference for MRSA colonization and related risk factors analysis. Further studies, especially larger well-designed ones are needed to determine NCSA and related risk factors in nursing homes.

In conclusion, MSSA and MRSA colonization rates were found to be 38% and 5% in nursing homes in this study. These rates can be reduced with simple precautions like hand-washing after a short education period. These centres must be observed with regular surveillance cultures and simple, inexpensive hygiene training such as hand-washing, must be given to residents and employees. The authors think that the early identification of patients colonized with MRSA by screening, and the subsequent use of contact precautions will reduce the likelihood of transmission to other patients and/or staff members. However, if an increase in the rate of MRSA or the existence of an epidemic is documented more intensive infection control measures should be implemented at these centres.

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