THE PREVALENCE OF *STAPHYLOCOCCUS AUREUS* NOSE AND THROAT CARRIAGE BY HEALTHY ADULTS

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Abstract

Purpose. To determine the prevalence of *Staphylococcus aureus* carriage and its associated potential risk factors in healthy adult population in Vilnius; to estimate the presence of the Panton-Valentine leukocidin gene and to evaluate resistance patterns of isolated strains.

Methods. A prevalence study involving 537 healthy adults was performed. Swabs from anterior nares and throat were taken to determine the presence of *S. aureus*. Antibiotic susceptibility testing was performed using the disk diffusion method according to the Clinical Laboratory Standards Institute guidelines. Polymerase chain reaction was used to detect the PVL gene in all isolated *S. aureus* strains. A questionnaire was obtained to identify potential risk factors for *S. aureus* colonization.

Results. The prevalence of *S. aureus* carriage in Vilnius adult population was 50,8 %. A total of 298 different *S. aureus* strains were isolated. Antimicrobial susceptibility testing revealed that 65,4 % of the isolated strains were resistant to penicillin. All tested strains were sensitive to oxacillin and cefoxitin. The following parameters were significantly associated with colonization: antibiotics used within the last 2 years (OR = 1,48; 95 % Cl 1,03 to 2,12; p = 0,027), visit to outpatients' clinics during the past 2 years (OR = 1,56; 95 % Cl 1,09 to 2,24; p = 0,011), family members' admittance to the hospital (OR = 1,49; 95 % Cl 1,01 to 2,19; p = 0,035) and their suffering from chronic diseases (OR = 1,65; 95 % Cl 1,03 to 2,66; p = 0,028), whereas a job in kindergarten (OR = 0,39; 95 % Cl 0,18 to 0,81; p = 0,006) was inversely associated to colonization.

Conclusion. *S. aureus* carriage rate is quite high in the healthy part of adult population in Vilnius, but the resistance rate to antimicrobials is low.

Keywords: *Staphylococcus aureus*, colonization, methicillin-resistant *Staphylococcus aureus*, CA-MRSA, resistance to antibiotics, Panton-Valentine leukocidin.

INTRODUCTION

Staphylococcus aureus is one of the most important human pathogenic bacteria and causes a large proportion of hospital and community associated infections. Six months after methicillin was marketed (in 1960s), methicillin-resistant *S. aureus* (MRSA) strains were identified in the United Kingdom and in the United States hospitals [1, 2]. Since then, MRSA has become a common cause of nosocomial infections worldwide [3, 4, 5]. Within the last 20 years MRSA was furthermore reported as a community originating infection. Community-acquired MRSA (CA-MRSA) was first described in 1980–1981 in injecting drug users in Detroit, USA [6]. Since 1989 CA-MRSA strains were reported as present from other parts of the world [7, 8, 9, 10]. *S. aureus* and MRSA are known as a frequent cause of complicated skin and skin-structure infections acquired in the community, which often requires surgical intervention and prolonged treatment [11]. The increased virulence of this organism has been associated with the Panton-Valentine leukocidin (PVL) virulence factor. PVL is more frequently found in the CA-MRSA but is more seldom identified in MSSA [10, 12].

Numerous sites may be colonized with this microorganism; including skin, nasopharynx, perineum, vagina, gastrointestinal tract and the axillae, but the anterior nares are the main ecological niche for *S. aureus*.

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Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of *S. aureus* infections [12]. Patients with nasally colonized *S. aureus* have a significantly higher risk of developing staphylococcal wound infection after a surgical procedure than those who are not colonized [13]. Studies of the *S. aureus* carriage rate in Lithuania are lacking. Two studies, one on the prevalence of *S. aureus* among pre-school and school-aged children [14] and one on *S. aureus* carriage prevalence among hospitalized patients [15] were performed in Kaunas city, Lithuania. However, research on the prevalence of *S. aureus* in healthy adult population in Lithuania has not been carried out yet. The primary aim of this study was to determine the prevalence of *S. aureus* carriage and its associated potential risk factors in healthy adult population in Vilnius; secondary aims were to estimate the presence of the virulence factor (PVL) and to evaluate susceptibility patterns of isolated strains.

MATERIALS AND METHODS

Study population. The study was performed in the 10-month period from 22 October, 2007 to 2 September, 2008, in Vilnius city, Lithuania. A total of 537 individuals were recruited for this study. The study participants included 212 students from Vilnius University, Vilnius College and Agricultural School, 179 volunteers from the National Blood Center and 146 participants from non-medical institutions. All participants were ≥ 18 years old, not hospitalized within the last 3 years, and did not work in the health care service. The participants of this study were provided with the information about *S. aureus*, MRSA and the study itself and a written consent was obtained from each participant. A short questionnaire was completed to identify potential risk factors for *S. aureus* colonization: age, gender, hormone or antibiotic consumption (within 2 years), skin diseases, contact with pets, any family member working in a health care institution or kindergarten during the last 2 years, chronic illness, such as lung, gastrointestinal, kidney, liver diseases or cancer and etc. Bacterial culture samples were taken from the anterior nares and throat. This study was approved by the Lithuanian Bioethics Committee.

Samples and culture. Nasal and throat swabs were obtained with sterile cotton-wool swabs (Transwabs, Corsham, United Kingdom). Swabs were immediately placed in Stuart's transport medium (Tran-swab) and within 2 hours cultured on the mannitol salt agar (MSA) (Liofilchem, Italy) and sheep blood agar (Bio-Rad, France). The MSA and blood agar plates were incubated at 35 ^{0}C for 24 hours, plus 24 hours at room temperature. Identification of *S. aureus* was based upon the growth and mannitol fermentation on the MSA, colony morphology on blood agar, positive tube coagulase test results with rabbit plasma (Bio-Rad, France), DNase (bioMerieux sa, France) and latex agglutination positive tests (Pastorex, Staph-Plus, Bio-Rad, France).

Antimicrobial resistance testing and PVL detection. Resistance testing was performed by disc diffusion method using Mueller-Hinton agar (MH) (Bio-Rad, France). There were used 6 mm discs (Oxoid Limited, Hampshire, UK) impregnated with antimicrobials: oxacillin (1 μg), cefoxitin (30 μg) [16], rifampin (5 μg), kanamycin (30 μg), clindamycin (2 μg), erythromycin (15 μg), streptomycin (10 μg), norfloxacin (10 μg), fucidic acid (10 μg), penicillin (10 U), ciprofloxacin (5 μg), tetracycline (30 μg) and gentamicin (10 μg) and interpreted according to CLSI [17]. Isolates intermediate resistant to streptomycin were retested by agar diffusion method using NeoSensitabs tablets (Rosco, Taastrup, Denmark) on Danish blood agar (Statens Serum Institut) with semiconfluent growth [18]. Determination of MICs for ciprofloxacin, gentamicin and vancomycin with the E-Test strips (AB Biodisk, Solna, Sweden) were performed according to the manufacturer's recommendations [19]. Mecillinam (33 μg NeoSensitabs tablets) were used for detection of β-lactamase production on MH as described by Bruun B. and Gahrn-Hansen B. [20].

S. aureus ATCC 25923 and ATCC 29213 were used as the control strains. PVL gene detection was performed with polymerase chain reaction as described by Larsen AR. et al. [21].

Bacteriological cultures of *S. aureus* and their susceptibility testing was performed at Vilnius University Department of Infectious Diseases, Dermatovenereology and Microbiology and Microbiology Department at Statens Serum Institute, Denmark. Panton-Valentine leukocidin gene detection was performed at Microbiology Department at Statens Serum Institute, Denmark.

Statistical analysis. Statistical Package for the Social Sciences (SPSS) for Windows (Version 13,0; SPSS, Chicago, III, USA) software was used for the statistical analysis of the data. Frequency and percentage were presented for categorical data. Pearson's chi-squared, Fisher's exact, crude odd ratio (OR), 95 % confidence interval (CIs) were applied to determine significant associations among risk factors and

S. aureus carriage. The level of significance was set at 0,05 using two-tailed method.

RESULTS

Demographic information on the 537 participants is given in table 1. The prevalence of *S. aureus* carriage in the study population was 273 people (50,8 %) [95 % Cl 46,52–55,14]. Colonization was higher in males than in females (56,8 % and 43,2 %, respectively), but the difference was not significant ($p = 0,116$). The *S. aureus* carriage rate was significantly higher among students (62,4 %) than among other community residents (43,2 %) ($p < 0,0005$).

S. aureus was found in the nose only in 39,2 % of colonized persons, in the throat – only in 31,5 % and both in the nose and the throat – in 29,3 % of cases, respectively (Table 2). If the nose had been the only screening site, 34,8 % (187/537) instead of 50,8 % (273/537), of *S. aureus* carriers would have been identified. PVL was identified only in 9 different isolates (Table 2).

Table 3 shows the results of the analysis of risk factors for *S. aureus* carriage. Being a student, usage of antibiotics or visit to outpatients' clinics in the two years period, family members' admittance to the hospital or their suffering from chronic diseases, were significant risk factors for *S. aureus* colonization. In contrast, job in kindergarten was associated with the reduced carriage whereas presence of skin or chronic underlying diseases, use of oral contraceptives or hormonal therapy, presence of pets, living together with family members, who work in a health care institution, or having children, who go to kindergarten, were not significantly associated with *S. aureus* carriage in our study.

Antimicrobial susceptibility. Based on the susceptibility pattern a total of 298 different *S. aureus* strains were isolated. In 21 participants different *S. aureus* strains in the anterior nares and throat was found and four volunteers had two different *S. aureus* strains at the same site. Sixty-five percent of the isolates were resistant to penicillin. Low resistance rates to other antibiotics were also detected: tetracycline (8,1 %) and < 3 % of the isolates were resistant to kanamycin, gentamicin, erythromycin, clindamycin and streptomycin. All isolates were sensitive to oxacillin, cefoxitin, rifampin, norfloxacin, ciprofloxacin, fucidic acid and vancomycin. Ninety-nine (33,2 %) of the isolated *S. aureus* strains were susceptible to all antibiotics. Among the isolates resistant to erythromycin and clindamycin, resistance was inducible in 6 (75 %) isolates, and the M-phenotype (resistance to erythromycin but

Table 1. Demographic characteristics of the *S. aureus* carriage study participants

Variable	No. (%) of subjects (n = 537)	Age				SD
		Minimum	Maximum	Mean	Median	
Location						
Students:	**212 (39,5)**	**18**	**23**	**20**	**20**	**1,168**
Vilnius University	177 (33,0)					
Vilnius College	15 (2,8)					
Agricultural school	20 (3,7)					
Others:	**325 (60,5)**	**18**	**72**	**40**	**40**	**11,130**
National Blood Center	179 (33,3)					
Other non-medical institutions	146 (27,2)					
Sex						
Male	287 (53,4)	18	72	33	32	13,583
Female	250 (46,6)	18	71	31	27	12,874

Table 2. Frequency of sites colonized with *S. aureus* and presence of PVL gene

Colonized site	No. (%) of carriers (n = 273)	Presence of PVL virulence factor n = 9 (%)
Nose	107 (39,2)	6 (5,6)
Throat	86 (31,5)	1 (1,2)
Nose and throat	80 (29,3)	2 (2,5)
Staphylococcus aureus strains in nose and throat (n = 80)		
The same strains n (%)	59 (73,75)	
Different strains n (%)	21 (26,25)	

Table 3. Potential risk factors for *S. aureus* carriage among healthy adults

Variable	No. (%) of carriers (n = 273)	No. (%) of non-carriers (n = 264)	Odds Ratio (OR), (95 % CI), *p*
Occupation			
Students	132 (48,4)	80 (30,3)	OR = 2,15; CI (1,49–3,12); *p* < 0,0005
Others	141 (51,6)	184 (69,7)	
Use of antibiotics			
Absent	148 (54,2)	168 (63,6)	OR = 1,48; CI (1,03–2,12); *p* = 0,027
Present	125 (45,8)	96 (36,4)	
Skin disease			
Absent	232 (85,0)	233 (88,3)	OR = 1,33; CI (0,78–2,27); *p* = 0,265
Present	41 (15,0)	31 (11,7)	
Chronic illness			
Absent	242 (88,6)	228 (86,4)	OR = 0,84; CI (0,47–1,40); *p* = 0,424
Present	31 (11,4)	36 (13,6)	
Family members suffering from chronic diseases			
Absent	208 (77,9)	221 (85,3)	OR = 1,65; CI (1,03–2,66); *p* = 0,028
Present	59 (22,1)	38 (14,7)	
Family members' admittance to the hospital			
Absent	172 (64,4)	189 (73,0)	OR = 1,49; CI (1,01–2,20); *p* = 0,035
Present	95 (35,6)	70 (27,0)	
Family members working in a health care institutions			
Absent	166 (60,8)	180 (68,2)	OR = 1,38; CI (0,95–2,00); *p* = 0,074
Present	107 (39,2)	84 (31,8)	
Visits to outpatients clinics			
Absent	142 (52,0)	166 (62,9)	OR = 1,56; CI (1,09–2,24); *p* = 0,011
Present	131 (48,0)	98 (37,1)	
Hormone consumption			
Absent	257 (94,1)	254 (96,2)	OR = 1,58; CI (0,66–3,97); *p* = 0,263
Present	16 (5,9)	10 (3,8)	
Having children, who go to kindergarten			
Absent	257 (94,1)	237 (89,8)	OR = 0,55; CI (0,27–1,08); *p* = 0,062
Present	16 (5,9)	27 (10,2)	
Kindergarten employee			
Absent	261 (95,6)	236 (89,4)	OR = 0,39; CI (0,18–0,81); *p* = 0,006
Present	12 (4,4)	28 (10,6)	
Pets			
Absent	125 (45,8)	134 (50,8)	OR = 1,22; CI (0,86–1,74); *p* = 0,249
Present	148 (54,2)	130 (49,2)	

susceptibility to clindamycin) was present in 2 (25 %) isolates (Figure 1). Resistance to 2 or more antibiotics was noted in 30 (10,1 %) isolates. Twenty were resistant to 2 antibiotics (tetracycline-penicillin [18 strains], erythromycin-clindamycin [1 strain], and penicillin-streptomycin [1 strain]). Nine strains were resistant to 3 antibiotics (erythromycin-clindamycin-penicillin [4 strains], kanamycin-gentamicin-penicillin [2 strains],kanamycin-tetracycline-penicillin [1 strain], erythromycin-tetracycline-penicillin [1 strain], tetracycline-penicillin-streptomycin [1 strain] and one strain was resistant to 4 antibiotics (erythromycin, clindamycin, tetracycline and penicillin). A total of 103 (34,6 %) penicillin-susceptible *S. aureus* strains were found to have mecillinam zones ranged from 22 to 30mm [20]. All 195 (65,4 %) penicillin-resistant isolates were positive in the β-lactamase test, and all these strains were resistant to mecillinam (e. g. zones were from 9 to 13 mm).

DISCUSSION

We found that the carriage rate of *S. aureus* as high as 50,8 %. This was similar to the carriage rate found in other community-based studies in Lithuania [14, 15]. Even higher rates have been found in various studies [22] but most studies report a carriage rate of 20–30 %

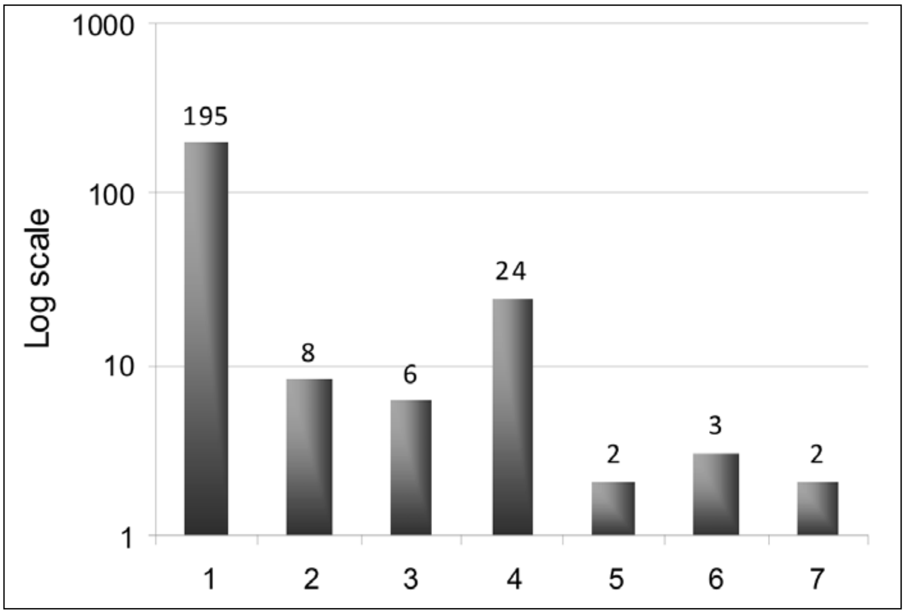

Figure 1. Resistance rate of isolated *S. aureus* strains. 1-penicillin, 2-erythromycin, 3-clindamycin, 4-tetracycline, 5-streptomycin, 6-kanamycin, 7-gentamicin

[23]. The carriage rate varies depending on the different investigated population groups, the use of culture methods and sample sites. The high prevalence of *S. aureus* colonization in this study could at least in part be related to the use of both nasal and throat swabs for screening of *S. aureus* carriage. An examination of the nares only, which is done alone in most studies of *S. aureus* carriage, would have given a prevalence of *S. aureus* carriage of 34,8 %.

Prior studies have identified that the anterior nares are the classic reservoir for *S. aureus* carriage and development of staphylococcal infections. We found that 39,2 % of the participants carried *S. aureus* in anterior nares and in 31,5 % of the participants the throat was the only site from where *S. aureus* could be isolated. Our data corresponds to the previous studies [24] claiming that the throat is an important site of *S. aureus* colonization. Therefore, we believe that the future studies evaluating *S. aureus* colonization should include cultures of both: the anterior nares and the throat, to optimize the estimate of colonization frequency.

Our results indicate that the prevalence of MRSA in the healthy adult population of Vilnius is low (< 0,5 %). The proportions of participants carrying CA-MRSA differ from the mentioned studies in Lithuania, where the prevalence ranged from 1,6 % to 2,2 %.

Rates of MRSA colonization remain low among healthy people worldwide. Rates of carriage among children with no risk factors for MRSA colonization have ranged from 0,8 % to 3,0 % [25, 26]. The prevalence of CA-MRSA in adults is currently low, but appears to be increasing. In the United States, the prevalence of *S. aureus* and MRSA in the general population reached 31,6 % and 0,84 % respectively [27]. Analysis of 57 studies on CA-MRSA prevalence among hospitalized patients and community members suggested that the prevalence of CA-MRSA among people without risk factors is 0,24 % [28]. Tiemersma et al. state that the prevalence of CA-MRSA in Europe is 0,03–1,5 % [29]: 0,7 % – in Portugal, 0,1 % – in Switzerland and 0,03 % – in the Netherlands. Findings in our study have demonstrated that all isolated *S. aureus* strains were methicillin susceptible (MSSA). Further studies must be carried out to determine the carriage rate of MRSA, covering large population and different groups of subjects.

In this study 298 different *S. aureus* strains were identified using 1074 nasal and throat swabs.

Strong epidemiological associations exist between PVL and the pathogenicity of *S. aureus* infections. PVL is uncommonly found in MSSA and HA-MRSA isolates [30, 31]. Studies from Australia demonstrated a 0,5 to 16 % incidence of PVL detection rate in MSSA [10]. Perez-Vazques et al. recently found that in 36,3 % of all isolated MSSA PVL genes were detected [32]. In our study, the Panton-Valentine leukocidin gene locus was detected in 9 of 298 *S. aureus* isolates (3 %) and this result correlates with the mentioned studies. Our results demonstrate that PVL-positive *S. aureus* types were more commonly isolated from nose than from throat. The cause of this occurrence is unclear, but may relate to strain-specific characteristics such as adherence factors.

Previous studies in Lithuania and other investigators have reported penicillin resistance rates of *S. aureus* strains much high which reached 80,5–82,7 % [14, 15, 26]. Our data showed that only 65,4 % of the isolated *S. aureus* strains were resistant to penicillin. This low rate must be related to the comparatively low antibiotic consumption in primary health care of Lithuania [33]. Similarly for erythromycin resistance which was only 2,7 % in this study compared to the 16,8 to 34,6 % reported by Perez-Vazquez et al. [32] and Otsuka et al. [34]. The results of our study show that the investigated *S. aureus* strains were less resistant to clindamycin (2 %) as compared to some data in literature sources (8,3–26 %) [25, 32, 35, 36]. Lower clindamycin resistance levels in previous studies from Lithuania may be explained by the fact that the inducible clindamycin D zone test was not used [14, 15].

Our data showed that more than 8 % of the *S. aureus* were resistant to tetracycline and that corresponds to data of the previous studies [35, 37, 38].

According to the literature data, 0–2,6 % of *S. aureus* strains are resistant to aminoglycosides [14, 15, 25, 26, 32]. In comparison, our data is very similar. 0,7 % of *S. aureus* strains were resistant to streptomycin, gentamicin and 1 % to kanamycin.

In contrast, fusidic acid, vancomycin, norfloxacin, ciprofloxacin and rifampin were active against all the isolated *S. aureus* strains. Our results correlate with the results of other investigators [25, 26]. Although such a difference in resistance data could be related to the number of participants, differences in the investigated groups or discrepant techniques in the investigation of resistance to antimicrobials [39], we believe that the main explanation for the low antibiotic resistance rates detected stems from the fact that the antibiotic consumption in primary health care in Lithuania is comparatively low as compare with other European countries [33].

Another objective of this study was to determine risk factors for *S. aureus* colonization. The CDC, Atlanta, USA have suggested that a case of CA-MRSA is defined with none of the following health care risk factors present: hospitalization, surgery, dialysis, or residence in a long-term-care facility < 1 year before the onset of illness, permanent indwelling catheters or percutaneous medical devices or a previous positive MRSA culture [40]. These risk factors have already been identified; therefore, they were not investigated in this study and were, in fact, exclusion criteria for study participants. We found that being a student is an important determined risk factor for *S. aureus* colonization. It may be related with living conditions in students' dormitory, sharing of personal items, etc. Also antibiotics consumed in the last two years were another one of the major risk factor associated with *S. aureus* colonization and these data are in accordance with other studies [41, 42]. Our study revealed that kindergarten employees, people visiting outpatients' clinics in the last two years period, family members' admittance to the hospital and their suffering from chronic diseases were significant risk factors for *S. aureus* colonization. All these risk factors are related to *S. aureus* strains spread by direct and close contact. Children are known to have a high incidence of *S. aureus* and CA-MRSA carriage [1, 14]. Close contact with children in a kindergarten, family members' admittance to the hospital or their frequent hospital visits because of chronic diseases increased the probability of becoming a carrier of *S. aureus*.

The finding that men are at increased risk of *S. aureus* carriage has been shown previously by other researchers [10, 43]. No significant difference in isolation rates was observed between males and females in our study. Our ability to identify other important risk factors (apart from skin diseases, chronic diseases, living with family members, who work in a health care institution, or having children, who go to kindergarten) for *S. aureus* carriage of studied group was limited. Large community-based studies are needed to improve the understanding of the epidemiology and to confirm risk factors of *S. aureus* and CA-MRSA carriage. Such studies will guide the formulation of antibiotic policies and the development of preventive strategies against the spread of this microorganism and infections.

CONCLUSION

This study showed that despite the emergence of CA-MRSA infection as a cause of significant morbidity and mortality rate all over the world, there were no cases of CA-MRSA strains carriage in the Vilnius healthy adult population. In contrast, the *S. aureus* carriage rate was quite high, while the resistance rates were still very low. Screening for *S. aureus* should include swabs from the anterior nares and from the throat to improve the likelihood of detecting the carriers. During the research, low PVL prevalence was found among the isolated *S. aureus* strains. The findings conform with the data published in this field.

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Staphylococcus aureus nešiojimo nosyje ir gerklėje paplitimas tarp suaugusiųjų

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Santrauka

Tyrimo tikslas – nustatyti *Staphylococcus aureus* nešiojimo mastą, jį lemiančius rizikos veiksnius tarp Vilniaus miesto suaugusiųjų ir ištirti išskirtų padermių atsparumą antimikrobinėms medžiagoms bei PVL geno paplitimą.

Metodai. Tyrime dalyvavo 537 respondentai. *S. aureus* nešiojimui nustatyti mėginiai paimti iš nosies ir gerklės. Stafilokokų identifikavimas ir diskų difuzijos metodu nustatytas jautrumas antimikrobinėms medžiagoms atliktas remiantis CLSI rekomendacijomis. Visoms išskirtoms *S. aureus* padermėms atlikta polimerazės grandininė reakcija, siekiant nustatyti PVL geną. Rizikos veiksniams, galintiems turėti įtakos *S. aureus* kolonizacijai, išsiaiškinti naudotas klausimynas.

Rezultatai. 50,8 proc. tyrime dalyvavusių respondentų nustatytas *S. aureus.* Iš viso išskirtos ir identifikuotos 298 skirtingos *S. aureus* padermės. 65,4 proc. išskirtų *S. aureus* buvo atsparūs penicilinui. Visos padermės buvo jautrios oksacilinui ir cefoksitinui. Nustatyta, kad tokie rizikos veiksniai kaip antimikrobinių medžiagų vartojimas (ŠŠ = 1,48; 95 proc. PI 1,03–2,12; $p = 0{,}027$), apsilankymai poliklinikoje (ŠŠ = 1,56; 95 proc. PI 1,09–2,24; $p = 0{,}011$) per dvejus metus, šeimos narių hospitalizacija (ŠŠ = 1,49; 95 proc. PI 1,01–2,19; $p = 0{,}035$) ir jų lėtinės ligos (ŠŠ = 1,65; 95 proc. PI 1,03–2,66; $p = 0{,}028$) gali turėti įtakos *S. aureus* kolonizacijai.

Išvados. *S. aureus* nešiojimo dažnis tarp Vilniaus miesto suaugusiųjų yra gana didelis, tačiau atsparumas antimikrobinėms medžiagoms mažas. Šio tyrimo metu tarp išskirtų *S. aureus* padermių nustatytas mažas PVL paplitimas. Šie duomenys sutampa su skelbiamais literatūroje.

Raktažodžiai: *Staphylococcus aureus*, kolonizacija, meticilinui atsparus *Staphylococcus aureus*, CA-MRSA, atsparumas antimikrobinėms medžiagoms.

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