The prevalence of methicillin-resistant *Staphylococcus aureus* among nursing home residents for the elderly in Slovakia

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ABSTRACT

The objective of our study was to examine the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) among residents of nursing homes for the elderly of selected institutions in two Slovak regions compared to non-institutionalized volunteers of the same age, as well as young volunteers (20–24 years old). Nasal swabs from all participants (n = 424) were processed using standard methods for the isolation and identification of *S. aureus* and MRSA. Statistically significant differences were found between nursing home residents and young volunteers (12% vs. 1.5%; OR 8.85; 95% CI 2.087–37.706; $p = 0.0007$), as well as between non-institutionalized seniors and young volunteers (11% vs. 1.5%; OR 8; 95% CI 1.888–33.901; $p = 0.005$) in the prevalence of MRSA. Our results suggest that nursing home residency and older age could be a risk factor for the occurrence of high-risk MRSA strains.

KEYWORDS

S. aureus – MRSA – elderly

SÚHRN

Kaiglová A., Melnikov K., Bárdyová Z., Kucharíková S.: Prevalencia meticilín-rezistentného *Staphylococcus aureus* medzi obyvateľmi domovov dôchodcov na Slovensku

Cieľom našej štúdie bolo zistiť prítomnosť *Staphylococcus aureus* rezistentného voči meticilínu (MRSA) medzi obyvateľmi vybraných domovov sociálnych služieb pre seniorov v dvoch krajoch Slovenska. Výsledky boli porovnané s neinštitucionalizovanými dobrovoľníkmi v rovnakom veku, ako aj s mladými dobrovoľníkmi (20–24 rokov). Výtery z nosovej dutiny od všetkých účastníkov (n = 424) boli spracované štandardnými metódami, ktoré sa bežne využívajú na izoláciu a identifikáciu *S. aureus* a MRSA. Štatisticky významné rozdiely v prevalencii MRSA boli zistené medzi obyvateľmi domovov dôchodcov a mladými dobrovoľníkmi (12 % vs. 1,5 %; OR 8,85; 95 % CI 2,087–37,706; $p = 0,0007$), ako aj medzi neinštitucionalizovanými seniormi a mladými dobrovoľníkmi (11 % vs. 1,5%; OR 8; 95% CI 1,888–33,901; $p = 0,005$). Naše výsledky naznačujú, že pobyt v domovoch sociálnej starostlivosti pre seniorov a vyšší vek by mohli byť rizikovým faktorom pre výskyt vysoko rizikových kmeňov MRSA.

KĽÚČOVÉ SLOVÁ

S. aureus – MRSA – seniori

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INTRODUCTION

Staphylococcus aureus occurs asymptomatically on the skin and mucous membranes of healthy individuals as part of the normal microbiota. However, in certain circumstances, it can cause a wide range of infectious diseases, such as skin and soft tissue infections, endocarditis, pneumonia, or bloodstream infections. Of great medical relevance is methicillin-resistant *S. aureus* (MRSA), which confers resistance to almost all beta-lactam antibiotics, therefore severely limiting therapeutic options. The mechanism of resistance to these antibiotics is mediated by the *mec*A gene (and its variants) localized on transferable genomic islands, called the Staphylococcal Chromosome Cassette *mec* region (SCC*mec*), integrated into the bacteria chromosome. The *mec*A gene encodes an alternative transpeptidase known as penicillin binding protein 2a, which shows low affinity for most beta-lactam antibiotics and does not inhibit the formation of bacterial cell walls even when antibiotics are present at inhibitory concentrations [1]. Since the 1960s, MRSA has proliferated around the world in both healthcare settings and communities [2]. MRSA strains possess the same virulence as methicillin-sensitive strains of *S. aureus* (MSSA) and cause a similar spectrum of disease. The main problem is that in healthy colonized elderly, nasal MRSA carriage can act as a potential endogenous reservoir for clinical infections [3]. A weak immune system makes the elderly more susceptible to all infections, including those caused by MRSA strains. In this study, we examine the prevalence of MRSA among residents of nursing homes for the elderly of selected institutions

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in two Slovak regions (Žilina and Trnava), compared to non-institutionalized volunteers of the same age, as well as young volunteers (20–24 years old). Based on our findings, MRSA can pose a threat to the elderly, especially those living in nursing homes.

MATERIALS AND METHODS

Sample Collection

This study included 424 participants. Two hundred forty-two samples were from seniors living in nursing homes in the Trnava and Žilina region (median age 72, range 59–100). Fifty samples were from non-institutionalized seniors living in the Žilina region (median age 69, range 61–86) and 132 samples from students of Trnava University in Trnava, Faculty of Health Care and Social Work (median age 22, range 18–25). Nasal swabs from all participants (one collection per person) were obtained using a sterile swab (Transport Amies Swab System, Copan, Italy), which was transported to the laboratory within 48 h after sample collection. Samples were stored in transport boxes at 4°C until delivery to the laboratory.

Isolation and identification of *S. aureus*

The biological material from the swabs was applied directly to blood agar and to a selective diagnostic medium, mannitol salt agar (MSA), which is commonly used to detect the presence of *S. aureus* in the examined samples. Plates were further incubated for 24–48 h at 37 °C. For rapid identification of the clumping factor (bound coagulase) and protein A, a commercially produced latex agglutination test The Prolex™ Staph Latex Kit was used. Positive isolates of *S. aureus* were further confirmed by PCR. Synthetic oligonucleotide primers were designed based on previously published sequence information (forward primer: 5´-GCG ATT GAT GGT GAT ACG GTT-3´, reverse primer: 5´-AGC CAA GCC TTG ACG AAC TAA AGC-3´) [4]. PCR was used to amplify a specific sequence (270 bp) of the *nuc* gene, which encodes the thermostable endonuclease of *S. aureus*. The PCR program used was as follows: 1. Initial denaturation – 4 min (94 °C); 2. DNA denaturation – 45 s (94 °C); 3. Annealing – 45 s (50 °C); 4. DNA polymerization – 60 s (72 °C); 5. Final DNA polymerization – 2 min (72 °C). Steps 2, 3 and 4 were repeated thirty times. The PCR product was detected by agarose gel electrophoresis. GelRed dye (manufacturer: Biotium Inc. USA) was used to label the DNA.

Identification of MRSA

To identify MRSA, *S. aureus* isolates were further tested using the disc diffusion method for their susceptibility to cefoxitin (30 µg) according to The European Committee on Antimicrobial Susceptibility Testing (EUCAST). After incubation at 37 °C for 18 h, the diameters of the inhibition zone were measured and interpreted according to the EUCAST breakpoint tables. Strains were considered resistant when the diameter of the inhibition zone was less than 22 mm and sensitive when the diameter of the inhibition zone was greater than 22 mm. The *S. aureus* ATCC 33591 (MRSA) and *S. aureus* ATCC 29213 (MSSA) strains were used as controls.

Regarding the fact that there is no *mecA* gene in *S. aureus* strains sensitive to methicillin, the detection of this gene in any *S. aureus* isolate is indicative of MRSA. The *mecA*-specific primer pairs used for the amplification of the fragment of 310 base pairs were prepared according to Geha et al. (forward primer 1: 5´-GTA GAA ATG ACT GAA CGT CCG ATA A-3´; reverse primer 2: 5´-CCA ATT CCA CAT TGT TTC GGT CTA A-3´) [5]. The PCR reaction was performed as previously described for the *nuc* gene.

Statistical analysis

For statistical analyses, the Vassarstats program calculator was used. Categorical data were examined using the chi-square test (x^2-test), with a significance level of $\alpha = 0.05$, and the odds ratio (OR) with 95% confidence intervals. Fisher's exact test was used if at least one of the values in the cells of the contingency table was less than five. P values below or equal to 0.05 indicated statistically significant differences.

RESULTS

The carriage of *S. aureus* was detected in 45 samples (34%) of young volunteers (n = 132); in 123 samples (51%) of residents of nursing homes (n = 242) and in 9 (18%) samples of non-institutionalized seniors (n = 50).

Among those identified as carriers of MRSA strains, we found 2 students (i.e., 4% out of 45 positive samples for *S. aureus* and 1.5% out of 132 samples examined), 29 nursing home residents (i.e., 24 % out of 123 positive samples for *S. aureus* and 12% out of 242 samples examined), and 3 non-institutionalized seniors (i.e., 33% out of 9 positive samples for *S. aureus* and 6% out of 50 samples examined) (Table 1).

Table 1. Prevalence of MRSA carriers among participants of the study

	Number of participants	*S. aureus* carriage *n* (%)	MRSA carriage *n* (% out of *S. aureus* posit. samples; % out of all samples examined)
Nursing home residets	242	123 (51%)	29 (24%; 12%)
Non-institutionalized seniors	50	9 (18%)	3 (33%; 6%)
Young volunteers (students)	132	45 (34%)	2 (4%; 1.5%)

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We determined the statistically significant difference between the prevalence of MRSA among nursing home residents and among students. The odds of contracting MRSA among residents of nursing homes were eight times higher than those of students (12% vs. 1.5%; OR 8.85; 95% CI 2.087-37.706; $p = 0.0007$). When comparing the prevalence of MRSA between the nursing home groups in the Trnava and Žilina region, we did not find statistically significant differences between these groups ($p = 0.105$). Although the prevalence of MRSA in the non-institutionalized group of seniors was lower than in the group of nursing home residents, the difference was not significant (6% vs. 12%; OR 2.13; 95% CI 0.624–7.297; $p = 0.319$). However, the prevalence of *S. aureus* was statistically significantly higher in nursing home residents than in non-institutionalized seniors (51% vs. 18%; OR 4.709; 95% CI 2.193–10.111; $p < 0.0001$), suggesting a potential risk factor for the development of methicillin – resistant strains in this group of elderly people.

When age was considered, we determined a significantly higher prevalence of *S. aureus* in the group of older responders (nursing home residents and non-institutionalized seniors) compared to the control group consisting of younger participants (students) (45% vs. 34%; OR 1.595; 95% CI 1.040–2.446; $p = 0.032$). There was also a significantly higher prevalence of MRSA in the group of older responders compared to students (11% vs. 1.5%; OR 8; 95% CI 1.888–33.901; $p = 0.005$), suggesting that older responders may have a higher risk of contracting MRSA.

DISCUSSION

MRSA currently represents a global burden because it is resistant to almost all beta-lactam antibiotics and may also be resistant to other groups of antimicrobials. It is an opportunistic pathogen that can cause a serious health problem with fatal consequences in immunosuppressed elderly people. The presence of MRSA in the nasal cavity puts colonized individuals at risk of developing a clinical infection or spreading of MRSA strains to others. Cuervo et al. (2015) documented that more than 40% of MRSA cases occurred in patients over 75 years of age [6]. In our study, 51% (n = 123) of the 242 samples examined among elderly residents of nursing homes were positive for *S. aureus* and 12% (n = 29) were identified as MRSA. Regarding the nasal carriage of MRSA, no significant differences were observed between the institutions examined in the Trnava and Žilina regions ($p = 0.105$). These results were consistent with the study conducted in Greece (Heraclion, Crete) between January and December 2015, in which the carriage of MRSA was observed in 33 out of 227 (14,5%) participants in the nursing homes examined [7]. Similarly, Chuang et al. (2015) monitored MRSA transmission in nursing homes in Hong Kong, China. In this study, 2776 residents from 36 nursing homes were included. The general prevalence of MRSA in this group was 20.4% (95% CI, 18.9–21.9%) [8]. Elderly people living in nursing homes are a high-risk group that can acquire these infections very easily. Various comorbidities, a weak immune system, and poor hygiene conditions contribute to the acquisition of infections, which can increase the prevalence rate of *S. aureus* in these facilities [9]. In Hong Kong, researchers O'Donoghue et al. (2012) studied the transmission rates of *S. aureus* and MRSA among nursing home residents and community-dwelling seniors (attending day care centers). Samples were taken from the nose and oral cavity. They found that MRSA colonization was 30.6% in nursing homes and 2.7% in seniors attending a day care center ($p < 0.001$). Based on their study, O'Donoghue et al. (2012) concluded that nursing home residents were clearly at risk for the spread of *S. aureus* and MRSA [10]. In our study, the prevalence of MRSA in the non-institutionalized senior group was lower than in the nursing home resident group as well; however, the difference was not significant (6% vs. 12%; OR 2.13; 95% CI 0.624–7.297; $p = 0.319$). We assume that our results may have been influenced by the relatively small group of non-institutionalized seniors (n = 50) from a single location (since this part of the study was carried out during the COVID-19 pandemic with limited transportation possibilities). Therefore, a further follow-up study would be necessary containing a larger group of elderly non-institutionalized study participants. However, when comparing the prevalence of *S. aureus* between the group of nursing home residents and non-institutionalized seniors, it was statistically significantly higher in nursing home residents (51% vs. 18%; OR 4.709; 95% CI 2.193–10.111; $p < 0.0001$), suggesting a possible risk factor for the development of methicillin resistant strains in this group of elderly people. Other limitations of the study include the fact that the identity of isolated MRSA strains was not established, nor was the possible horizontal clonal spread of MRSA strains among nursing home residents confirmed/excluded.

The risk of nasal MRSA carriage appears to be also related to age alone. A study by Brito et al. (2015) in Minas Gerais, Brazil, evaluated the prevalence of *S. aureus* and MRSA nasal carriage in 120 healthy elderly people (≥ 60 years), 83.1% of whom lived in households with 1 to 4 members [3]. According to this study, the prevalence of *S. aureus* and MRSA colonization among seniors was quite high: 17.8% and 19%, respectively. In line with these results, our study documented a significantly higher prevalence of *S. aureus* and MRSA in the group of older respondents (nursing home residents and non-institutionalized seniors) compared to students ($p = 0.032$; $p = 0.005$, respectively), suggesting that age alone may act as a higher risk of contracting MRSA.

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