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Major article

Prevalence and molecular characteristics of methicillin-resistant *Staphylococcus aureus* strains isolated in a multicenter study of nursing home residents in Croatia

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Background: Residents of nursing homes (NHs) are often hospitalized and could present a potential reservoir for methicillin-resistant *Staphylococcus aureus* (MRSA). The aim of the study was to determine the prevalence for MRSA carriage in residents and staff in Croatian NHs and to characterize MRSA strains using genotyping techniques.

Methods: A cross-sectional study was performed among 877 residents and staff of 7 NHs representing 3 major Croatian regions. Nasal swabs from residents and staff and other samples from residents with invasive devices were obtained. Identified isolates were submitted to susceptibility testing and genotyping with SCC*mec* typing, *S aureus* protein A (spa) locus typing, and pulsed-field gel electrophoresis (PFGE).

Results: The overall prevalence of MRSA colonization was 7.1% (95 confidence interval, 5.4%-8.8%), ranging from 0% to 28.8%. Four MRSA isolates were found in NH staff. All MRSA isolates were negative for Panton-Valentine leukocidin–encoding genes. SCC*mec* type II was found in 32 MRSA strains; SCC*mec* IV, in 27 strains; SCC*mec* I, in 3 strains. The predominant spa type was t008, found in 49 strains; PFGE analysis revealed 2 major clonal groups.

Conclusions: MRSA strains were found to be colonizing residents and staff of 7 NHs in Croatia. Our study demonstrates the spread of 2 clones within and among Croatian NHs. The data presented here provide an important baseline for future surveillance of MRSA in NH.

With the aging of modern society, more and more people are living in residential institutions and nursing homes (NHs). Many residents need only assistance in daily living, whereas others have conditions requiring medical attention.[1,2] There are 229 NHs in Croatia, most of which are financed by the county administration or the national government.[3]The average size of a Croatian NH is 88 beds, with single-, double-, and multiple-bed rooms. Multiple-bed rooms are especially common in NHs with bedridden residents or wheelchair-bound individuals are present.

Residents of long-term facilities and NHs are often hospitalized and could present a potential reservoir for multiresistant bacteria. One of the most important multiresistant bacteria is methicillin-resistant *Staphylococcus aureus* (MRSA). *S aureus* is a part of the normal flora in 10%-30% of the population and is more prevalent among people with a chronic disease or receiving, among other conditions. *S aureus* is carried on the skin and mucosa of healthy individuals without causing harm, but in some persons can cause severe infections and bacteremia with high mortality. Infection with MRSA is associated with increased morbidity, mortality, and length of hospital stay compared with infection with methicillin-susceptible *S aureus* (MSSA), and patients colonized with MRSA are at greater risk for MRSA infection compared with noncolonized patients.[4]

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At the beginning of the 21st century, many countries reported the presence of community-acquired MRSA (CA-MRSA), which had been considered a typical "hospital bacterium" for the previous 3-4 decades until the appearance of these reports.[5]

Methicillin resistance is encoded by the *mec*A gene, which is situated on a larger genetic element, the SCC*mec* cassette, on the staphylococcal chromosome. SCC*mec* types I-III are commonly related to hospital strains.[5-7]

CA-acquired MRSA strains are genetically different from the MRSA strains typically associated with hospitals (ie, hospital-acquired [HA]-MRSA). These emerging CA-MRSA strains usually have different SCC*mec* elements (types IV-XI), and several produce Panton-Valentine leukocidin (PVL), a pore-forming cytotoxin.[5,6]

Because numerous hospitals have reported high MRSA colonization rates in elderly patients, and because *S aureus* colonization has been shown to increase with age, there are concerns about the introduction of MRSA into NHs by MRSA-positive patients discharged from the hospital. NHs provide an ideal reservoir for the acquisition and spread of MRSA, because residents are at increased risk for colonization owing to chronic illness and disability, multiple exposures to antimicrobial agents, and the presence of pressure ulcers and indwelling devices. The prevalence of MRSA within NHs is likely increasing as a result of the increased prevalence of MRSA within hospitals.[8] There are several other potential institutional reservoirs in addition to NHs, including jails and animal farms.[5,6]

The present study was conducted to determine the prevalence of MRSA carriage in residents and staff of Croatian NHs and to characterize MRSA strains using genotyping techniques in Zagreb, Čakovec, Osijek, and Split. We screened all consenting residents and several members of the nursing and other staff.

METHODS

Study design

A cross-sectional prevalence survey was conducted in 7 Croatian NHs (3%; 95% confidence interval [CI], 0.79%-5.21%) with a total of 1529 beds (9.5%; 95% CI, 8.03%-10.97%; median, 282 beds; range, 57-387 beds). The selected NHs were represented equally by region (northwestern, Adriatic, and Panonic Croatia). Residents were accommodated in rooms of 1-3 beds.

Taking into account an α value of 0.05, to achieve an absolute precision of estimate of $\pm$2% with a confidence level of 95%, and an expected prevalence of 10% (according to literature and preliminary results), a sample size of 821 residents was calculated.

In each facility, skilled medical personnel coordinated the survey. The study protocol was approved by Ethical Committee of the University of Zagreb School of Medicine as part of an ongoing project ("Genotypes and Virulence Factors of Hospital-Acquired Pathogens"). Written informed consent was obtained for each resident enrolled in the study or from a legal representative for those residents with a cognitive disorder. All data were reported anonymously with regard to patient and NH identification. Microbiological results were confidentially reported to each resident's family doctor.

Each resident's nostrils were swabbed with a cotton swab moistened with saline and then placed in tryptic soy broth with 6.5% NaCl. After overnight incubation, all swabs were subcultivated on blood agar plates and mannitol salt agar plates, and suspected *S aureus* colonies were tested with coagulase and Slidex Staph tests. Susceptibility testing was performed following Clinical and Laboratory Standards Institute guidelines using the disk diffusion method and the following antibiotics: penicillin, oxacillin, cefoxitin, erythromycin, clindamycin, azithromycin, gentamicin, amikacin, tetracycline, rifampicin, trimethoprim/sulfamethoxazole, linezolid, ciprofloxacin, and vancomycin. The results were checked using European Committee on Antimicrobial Susceptibility Testing standards.

For *S aureus* strains with a zone diameter $<$22 mm, *mec*A detection was performed. SCC*mec* typing was done using the multiplex polymerase chain reaction (PCR) technique described by Oliveira et al.[9]

Typing of the S aureus *protein A locus*

For amplification of the *S aureus* protein A (spa) repeat region, PCR was performed in a total volume of 50 μL with HPLC-cleaned. The primers spa-1113f (5′-TAAAGACGATCCTTCGGTGAGC-3′) and spa-1514r (5′-CAGCAGTAGTGCCGTTTGCTT-3′) were used for amplification.

The spa types were assigned using Ridom StaphType version 1.4 (http://www.ridom.de). Because the results of spa typing, together with the BURP algorithm, has been shown to be in accordance with the typing results obtained by multilocus sequence typing (MLST) and pulse-field gel electrophoresis (PFGE), the associated MLST clonal complex was allocated through the Ridom SpaServer (http://spaserver.ridom.de).[10]

PFGE

Digestion of chromosomal DNA with SmaI and PFGE were performed as described previously.[11] The band patterns were analyzed using Dice comparison and unweighted pair-group matching analysis settings with GelCompar II (Applied Maths, Sint-Martens-Latem, Belgium) according to the scheme of Tenover et al.[12] MRSA isolates with a similarity index of $\geq$80% were classified as a clonal group.[11]

RESULTS

A total of 1044 samples were obtained from NH residents and staff. Colonization was investigated in the nasal vestibule. Of the 1002 nasal swabs analyzed, 877 were obtained from residents, and 125 were obtained from staff members, mainly nurses. The vast majority (80.7%; 95% CI, 78.09%-83.31%) of the NH residents were women.

The percentage of residents with an invasive device was 2.6% (95% CI, 1.55%-3.65%); 23 of the 877 residents had a urinary catheter, stoma, or cannula. Along with samples the from nasal cavity, during the study we analyzed also 24 decubital swabs, 14 catheter urines, and 4 samples from gastric stoma, colostomy, and tracheal cannulas.

MSSA was isolated from 346 participants (39.4%; 95% CI, 36.17%-42.63%). The prevalence of *S aureus* carriage in residents varied among NHs, ranging from 28% to 53% (Table 1).

In the 125 nasal swabs collected from nursing and auxiliary staff, 4 MRSA strains were identified (3.2%; 95% CI, 0.11%-6.29%).

A total of 62 MRSA isolates were found in residents, and 4 MRSA isolates were found in NH staff. Methicillin resistance was discovered and genetic mechanisms were detected in 62 of 408 *S aureus* strains isolated from 877 nasal swabs from NH residents (15.2%; 95% CI, 11.72%-18.68%). The overall prevalence of MRSA colonization was 7.1%, ranging from 0% in NH 2 and NH 6 to 28.8% in NH 3. Beyond the nose, MRSA was isolated from a tracheal cannula,[1] wound/decubital swabs,[7] stoma,[2] and urine.[1]

The results of the susceptibility testing grouped our MRSA strains into 7 groups (Table 2). Only 3 isolates were considered non-multiresistant; all other strains were resistant to 2 or more antibiotic groups.