

PREVALENCE OF NASAL CARRIAGE OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* AMONG HEALTHCARE WORKERS IN THE DEPARTMENTS OF OTORINOLARYNGOLOGY AND DENTISTRY IN KYIV, UKRAINE

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ABSTRACT

The aim: To obtain the first estimates of the current prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* (MRSA) among healthcare workers (HCWs) in the departments of Otorhinolaryngology and Dentistry and to determine of genes virulence factors (Panton Valentine Leukocidine (PVL) genes).

Materials and methods: We performed a multicenter cross-sectional study. The susceptibility to antibiotics was determined by disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing. The virulence factor encoding genes, *mecA*, *lukS-lukF*, were detected by Polymerase Chain Reaction (PCR).

Results: Incidence rate of *S. aureus* nasal carriage among HCWs was 36.2%, whereas MRSA carriage was 17%. Prevalence of MRSA carriage rate was 34.9% in Otorhinolaryngology departments and 9.7% in Dentistry. PCR testing confirmed that all MRSA strains were *mecA* gene-positive. The virulence factor encoding genes were detected in 82.3% of the *S. aureus* isolates from HCWs. Among *S. aureus*, the *lukS-lukF* genes were detected in over 59% of the strains. The *lukS-lukF* genes were detected in 55.5% of MRSA and in 58.9% of MSSA strains. *LukS-lukF* genes were most commonly co-present in MRSA strains. No significant difference was detected between the occurrences of *lukS-lukF* genes ($P > 0.05$).

Conclusions: Personnell in otorhinolaryngology and dentistry departments have a high rate of nasal colonization of MRSA. This carrier state may be an important risk factor for transmission MRSA from physicians and nurses to patients and vice-versa. Screening for MRSA nasal carriage of HCWs is a key element in enabling infection control measures and early therapeutic decisions.

KEY WORDS: *Staphylococcus aureus*, nasal carriage, MRSA, healthcare workers, Otorhinolaryngology, Dentistry, Panton Valentine Leukocidine

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INTRODUCTION

Staphylococcus aureus is a very common bacterium that is both a normal flora and main human pathogen of healthcare-associated, and community acquired infections resulting to widespread morbidity and mortality. [1-5]. In Europe, the proportion of methicillin resistance in strains of *S. aureus* isolates in infected patients varied in 2011 from less than 0.5% to more than 50% [6]. In the United States, the proportion of methicillin resistance in *S. aureus* strains approached almost 60% in 2003, with an average rate of resistance over the period 1998–2002 of around 50% [7].

Approximately 30% of the world human population is carriers of *S. aureus* [8]. The prevalence of *S. aureus* nasal carriage in Ukraine was 40.4%. We found that 9.1% of the strains were classified as methicillin resistance *S. aureus* (MRSA) [9]. A recent review estimated prevalence of MRSA in healthcare workers (HCWs) to be 4.6%. However, MRSA carriage in HCWs in non-outbreak settings is thought to be higher than in an outbreak situation, due to increased hygiene awareness in outbreaks, but valid data are missing [10].

In Ukraine nasal carriage of MRSA in HCWs was 16.6%. The frequency of MRSA carriage also varied according to the department/ward. The highest prevalence of nasal carriage of MRSA was in the surgical wards [11]. However, the burden of MRSA colonisation seems to extend beyond the hospitals to Dentistry setting and outpatient Otorhinolaryngology care.

The pathogenicity of *S. aureus* results from its ability to produce specific toxins and hydrolytic enzymes. However, the studies regarding the prevalence of MRSA strains and distribution of genes encoding virulence factors, which have colonized patients and HCWs in Ukraine, is limited.

THE AIM

The aim of this study was to obtain the first estimates of the current prevalence of nasal carriage of MRSA among healthcare workers in the departments of Otorhinolaryngology and Dentistry and to determine of genes virulence factors.

MATERIALS AND METHODS

STUDY DESIGN AND SETTING

This cross-sectional multicenter study was conducted between January 2020 and June 2020. The study population consisted of healthcare workers in the 7 Dentistry setting and 8 outpatient Otorinolaryngology departments in Kyiv, Ukraine. We have included Dentistry setting and Otorinolaryngology departments that are similar in terms of medical equipment, personnel, and laboratory facilities.

DATA COLLECTION

Nasal swabs were taken from 218 randomly selected HCWs (doctors, nurses and other workers). Only one isolate from each HCWs was included in the study. HCWs with a history of upper respiratory tract infection, fever, and recent nasal surgery, use of nasal medications or antimicrobial therapy were excluded. HCWs with a history of upper respiratory tract infection, fever, and recent nasal surgery, use of nasal medications or antimicrobial therapy were excluded. All personnel included in the study were full-time workers Dentistry setting and outpatient Otorinolaryngology departments. Personnel who worked part time were excluded from the study.

MICROBIOLOGICAL METHODS

Nasal swabs were collected from the anterior nares of the HCWs using a cotton swab in AMIES transport medium. The swabs were immediately transported to the microbiology laboratory for further processing. Microbial isolates were identified using standard microbiological techniques, including automated microbiology testing (Vitek-2; bioMérieux, Marcy l'Etoile, France).

The susceptibility pattern for several classes of antibiotics was determined by disk diffusion method according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. The tested antimicrobial agents were: cefoxitin, gentamicin, tetracycline, trimethoprim-sulfamethoxazole, erythromycin, clindamycin, ciprofloxacin, mupirocin, rifampicin, fusidic acid and penicillin. Isolates were classified as susceptible or resistant based on *S. aureus* epidemiological cut-off values issued by the EUCAST. Erythromycin-induced clindamycin resistance was detected by Disk approximation test (D-test).

POLYMERASE CHAIN REACTION (PCR)

DNA was extracted using the Genomic DNA Extraction kit (EURx, Gdansk, Poland) according to the manufacturer's guidelines. The cefoxitin-resistant isolates were analyzed for the presence of the *mecA* gene and *femA* endogenous control gene using PCR, as previously described. The primers used to amplify the *mecA* gene were (forward: 5'-AAGCGACTTCATCTATTAGGTTAT-3' and reverse: 5'-TATATTCTTCGTTACTCATGCCATAC-3'; [12]. The primers used to amplify the *femA* gene were forward:

5'-AACTGTTGGCCACTATGAGT-3' and reverse: 5'-CCAGCATTACCTGTAATCTCG-3'; [13].

Positive and negative controls were added in each run, we used reference strains that are *mecA* positive and negative. PCR, used to detect PVL (*lukS* and *lukF* genes), was performed as previously described [14]. The primers used to amplify the *lukS*-PV gene were 5'-AGTGAACT-TATCTTTCTATTGAAAAACACTC-3' and *lukF* gene were 5'-GCATCAASTGTATTGGATAGCAAAGC-3' [14].

ETHICS

The study was approved by the Ethics Committee of Shupyk National Medical Academy of Postgraduate Education (Kyiv, Ukraine). All study staff prior to specimen collection voluntarily agreed to participate in the study and signed an informed consent form. All study personnel data were anonymised prior to the analysis. Ethical considerations including privacy of personal data were considered during all steps of the research.

STATISTICAL ANALYSIS

The analysis of statistical data was performed using Excel (Microsoft Corp., Redmond, WA, USA). Results are expressed as median (range), mean standard deviation for continuous variables, and number and corresponding percentage for qualitative variables. Comparisons were undertaken using Student's t-test and Fisher's exact test for categorical variables. Statistical significance was defined as $P < 0.05$.

RESULTS

PREVALENCE OF MRSA CARRIAGE

The during study period a total 218 HCWs were screened for *S. aureus* carriage. The mean age of participants was 32.41 ± 8.29 years (range 19 -74 years) with a male-to-female ratio of 0.47. Overall, 79 bacterial isolates were phenotypically identified as *S. aureus*. All of the 79 isolates were confirmed as *S. aureus* by targeting the *femA* gene.

Accordingly, the rate of nasal carriage of *S. aureus* among HCWs is 36.2% (79/218). Among them 49 (62%) were identified as MRSA using the oxacillin disc resistance. However, using PCR targeting the *mecA* gene in *S. aureus*, only 37 (46.8%) isolates were confirmed as MRSA. The prevalence of *S. aureus* and MRSA carriage among the different HCWs is shown in Table I.

The overall nasal carriage rate of MRSA was 17% (37/218). The prevalence of nasal carriage rate of MRSA was 34.9% in Otorinolaryngology departments and 9.7% in Dentistry setting. *S. aureus* carriage rate was highest in Otorinolaryngology departments among doctors 62.5% (15/24), whereas carriage among nurses was 42.9% (9/21) and cleaners 38.9% (7/18).

MRSA carriage rate was significantly highest ($P = 0.001$) among doctors 41.7% (10/24) compared to other professions. 33.3% (7/21) of the isolates of *S. aureus* carried by the

Table I. Prevalence of *S.aureus* and MRSA carriage among the different HCWs in Kyiv, Ukraine

HCWs	Number of samples	Number of <i>S.aureus</i> carriers (n/%)	Number of MRSA carriers (n/%)	P-value
Otorinolaryngology	63	31 (49.2%)	22 (34.9%)	0.051
Doctor	24	15 (62.5%)	10 (41.7%)	
Nurses	21	9 (42.9%)	7 (33.3%)	
Cleaners	18	7 (38.9%)	5 (27.8%)	
Dentistry	155	48 (31%)	15 (9.7%)	
Doctor	50	21 (42%)	10 (20%)	
Nurses	62	18 (29%)	3 (4.8%)	
Cleaners	43	9 (20.9%)	2 (4.7%)	
Total	218	79 (36.2%)	37 (17%)	

HCWs – healthcare workers

MRSA – methicillin resistant *S. aureus***Table II.** Distribution of *S.aureus* and MRSA carriage among HCWs in the different Dentistry departments.

Department	Number of samples (n)	Number of <i>S.aureus</i> carriage (n/%)	Number of MRSA carriers (n/%)
Dental Surgery	34	18 (52.9%)	8 (23.5%)
Dental Therapeutic	71	20 (28.2%)	5 (7%)
Dental Orthopaedic	38	9 (23.7%)	2 (5.3%)
Dental Radiodiagnostics	12	1 (8.3%)	0
Total	155	48 (31%)	15 (9.7%)

HCWs – healthcare workers

MRSA – methicillin-resistant *Staphylococcus aureus*

nurses were MRSA. The carriage rate among cleaners was 27.8% (5/18). In Dentistry setting *S. aureus* carriage rate was highest among doctors 42% (21/50), whereas carriage among nurses was 29% (18/62) and cleaners 20.9% (9/43). MRSA carriage rate was significantly highest ($P=0.001$) among doctors 20% (10/50) compared to other HCWs. 4.8% (3/62) of the isolates of *S. aureus* carried by the nurses were MRSA. The carriage rate among cleaners was 4.7% (2/43) (Table I).

The frequency of *S. aureus* carriage also varied according to the Dentistry departments. The highest rate of *S. aureus* carriage was found in HCWs of the surgical departments (52.9%), followed by dental therapeutic and dental orthopaedic departments (28.2 and 23.7% respectively). On the other hand, the highest MRSA carriage rate was found in surgery wards (23.5%). Carriage of MRSA in other Dentistry departments was not high (Table II).

ANTIBIOTIC SUSCEPTIBILITY

All *S. aureus* strains isolated from HCWs were susceptible to fusidic acid. 11.4% (9/79) of the strains *S.aureus* were classified as resistance to mupirocin. The ability to produce beta-lactamases was detected in 69.6% (55/79) of the strains. MSSA and MRSA strains displayed no statistical difference in beta-lactamase secretion ($P>0.05$). 72.1% of MSSA iso-

lates were resistant to penicillin. Resistance to erythromycin was observed in 65.9%, to tetracycline in 14.5%, to gentamicin in 12.6%, to clindamycin in 16.5%, rifampicin in 18.3% and to trimethoprim-sulfamethoxazole in 11.4% of MSSA isolates. All MRSA strains were fusidic acid-susceptible. Seven out of 37 (18.9%) MRSA strains were trimethoprim/sulfamethoxazole-susceptible. 24 out of 37 (64.9%) MRSA were susceptible to mupirocin and gentamicin. 77.8% of those strains presented resistance to tetracycline, erythromycin, and clindamycin. Erythromycin-induced clindamycin resistance occurred in 31.4% MRSA strains and in 5.6% MSSA strains. The erythromycin-induced clindamycin resistance rate was significantly higher among MRSA strains, if compared with MSSA strains ($P<0.05$).

DISTRIBUTION OF VIRULENCE FACTOR ENCODING GENES

The virulence factor encoding genes were detected in 82.3% (65/79) of the *S. aureus* isolates from HCWs. Among the 79 strains *S.aureus*, the *lukS-lukF* genes were detected in over 59% of the strains. The *lukS-lukF* genes were detected in 55.5% of MRSA and in 58.9% of MSSA strains. *LukS-lukF* genes were most commonly co-present in MRSA strains. No significant difference was detected between the occurrences of *lukS-lukF* genes ($P>0.05$).

DISCUSSION

There is a paucity of information on the role of human carriage among HCWs, personnel that could easily carry and spread *S. aureus* strains to patients. There is no evidence of *S. aureus* susceptibility and occurrence of virulence encoding genes within the Ukrainian HCWs. This is the first study were to obtain of the current prevalence of nasal carriage of MRSA among healthcare workers in the departments of Otorinolaryngology and Dentistry and to determine of genes virulence factors. The present study reports that the prevalence of *S. aureus* nasal carriage among HCWs in Kyiv, Ukraine was 36.2%, whereas MRSA carriage was 17%. Prevalence of nasal carriage of *S. aureus* among healthcare workers in the departments of Otorinolaryngology and Dentistry were 49.2% and 31% respectively. The MRSA carriage rate was 34.9% in otorhinolaryngology departments and 9.7% in dentistry. MRSA carriage rate was significantly highest among doctors in surgical departments.

An overview of the published work highlights that carriage of MSSA or MRSA in HCWs occurs at a variable rate in countries with very different public health and social structures, however, there is no simple way to predict carriage rates on the basis of the mentioned variables. Also, differences in the prevalence of nasal carriage of *S. aureus* strains may be due in part to differences in the quality and size of samples and the use of different techniques and different interpretation guidelines. Data of our study are comparable to others reported in the literature [15-18].

Despite the possible role that HCWs may perform in dissemination of MSSA and MRSA strains, relatively few reports have addressed this issue. In our study, neither MSSA nor MRSA fusidic acid resistant strains were observed using the disk diffusion method. These data differ from the data concerning other countries [19, 20]. The absence of fusidic acid resistant strains can be explained by their limited use in Ukraine. Susceptibility to the other antimicrobials was also on a high level (tetracycline, gentamicin, clindamycin, rifampicin, clindamycin and trimethoprim/sulphamethoxazole). In our study MRSA came up to 17%. Thus, this is a major problem in the treatment of *S. aureus* infections. PCR testing confirmed that all MRSA strains isolated from our HCWs were *mecA* gene-positive.

In the present study, we found a high prevalence of PVL encoding genes. The virulence factor encoding genes were detected in 82.3% of the *S. aureus* isolates from HCWs. Over 59% strains isolated from nares in individuals with no staphylococcal infection symptoms, were *lukS-lukF*-positive (*lukS-lukF* genes). This evidence contrasts with previous reports. For instance, the prevalence of PVL-positive *S. aureus* nasal colonization in Dutch general practice patients was 0.6% [19]. Furthermore, a PVL prevalence of 38.9% was observed in *S. aureus* and it caused abscesses, arthritis, and soft-tissue infections [19]. The prevalence of PVL-positive *S. aureus* in nasal colonization was 2.4% in the United States [21]. According was estimated that PVL-positive *S. aureus* was more prevalent pathogen in the tropics and subtropics, if compared with European coun-

tries [22]. Travellers to tropical and subtropical countries are exposed to a higher risk of skin and soft-tissue infections. This phenomenon results from a higher PVL-positive *S. aureus* occurrence in tropical and subtropical countries [23]. Similarly, job seekers travelling from Ukraine could be a source of toxin-producing strains.

LIMITATIONS

The current study had several limitations. Most importantly, the small size limited the broad representative significance of the research. Second, sampling of only the nostrils without including other body parts may underestimate the frequency of MRSA colonization. Despite the above limitations, our results confirmed the nasal *S. aureus* colonization in a special population, Otorinolaryngology and Dentistry staff in local region.

A limitation of our study is that we studied isolates deriving only from Kyiv region of Ukraine and it cannot be representative of the overall Ukrainian situation. Furthermore, we did not perform spa typing and therefore we could not discriminate among different strains of *S. aureus*. Further studies are required to address those limitations. A systematic surveillance system can help prevent transmission and spread of drug resistant toxin producing *S. aureus* strains.

CONCLUSIONS

Health care workers in otorhinolaryngology and dentistry departments have a high rate of nasal colonization of MRSA. All MRSA isolates tested positive for the *mecA* gene. We also found a high prevalence of PVL- encoding genes among *S. aureus* nasal carriage strains. This carrier state may also be an important risk factor for transmission MRSA from physicians and nurses to patients and vice-versa. Screening for MRSA nasal carriage of HCWs in otorhinolaryngology and dentistry departments is a key element in enabling infection control measures and early therapeutic decisions. It is of importance to follow the evolution of resistance to antibiotics in this species, especially to β -lactams. A systematic surveillance system can help prevent transmission and spread of drug resistant toxin producing *S. aureus* strains.

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The Authors declare no conflict of interest

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