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Nasal Carriage of *Staphylococcus aureus* in Healthy Adults and in School Children

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SUMMARY

Staphylococcus aureus* (*S. aureus*) is a microorganism that colonizes the skin and mucosal surfaces of healthy individuals, but it is also one of the most common causes of community-acquired and hospital infections. Nasal carriage of *S. aureus* represents a major risk factor for the development of infection with this bacterium. A special therapeutic problem are methicillin-resistant isolates of *S. aureus* (MRSA). The aim of this study was to assess the nasal carriage of *S. aureus* in healthy individuals in the local community, and the sensitivity of the microorganism to antibiotics. The study enrolled 56.868 healthy individuals aged 19 to 65 years, and 2.040 healthy school children aged 15 to 19 years. Specimens to be studied were obtained from anterior nares. We used the disk diffusion method (Kirby-Bauer) on Mueller-Hinton agar to assess the sensitivity of isolated *S. aureus*. *S. aureus* was isolated in 1.381 (2.34%) respondents. Positive findings were obtained in 2.33% of adult examinees, and in 2.59% of studied school children. We found a low level of susceptibility only to penicillin (5.36%). The susceptibility of *S. aureus* isolates to all other tested antibiotics was present in a high percentage, with the lowest percentage of susceptibility to doxycycline (71.54%) and erythromycin (86.09%). The highest percentage of susceptibility of tested isolates was reported for fusidic acid (99.27%). In relation to the total number of *S. aureus* isolates from nasal swabs in adults, MRSA was present in 8.96% (119 isolates), while there were 4 MRSA isolates from nasal swabs in school children. In this study, we established a low percentage of nasal carriage of *S. aureus* in the population of healthy individuals, but a high percentage of MRSA.

Key words: **nasal carriage, *Staphylococcus aureus*, healthy population**

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INTRODUCTION

Staphylococcus aureus (*S. aureus*) is a microorganism that colonizes the skin and mucosal surfaces of healthy individuals, but it is also one of the most common causes of community-acquired and hospital infections. The bacteria can cause infections of all tissues and organs, starting from superficial skin infections, to severe, life-threatening infections, such as bacteriemia and sepsis. The most common community-acquired infections are skin and soft tissue infections. *S. aureus* causes invasive infections mostly in immunocompromised and hospitalized patients (1). In healthy individuals, *S. aureus* can colonize the skin and mucosa of any part of the body, with the anterior nares being the most commonly colonized site (2). The nasal carriage rates of *S. aureus* vary depending on the population observed. A large study conducted by Mainous et al. in the USA showed that 32.40% of the examined population aged from 1 to over 65 years are colonized by *S. aureus* (3). Although the exact mechanisms of occurrence and persistence of nasal carriage of this microorganism have not been fully elucidated, it is believed that a microorganism-host interaction is a prerequisite (4). Nasal carriage of *S. aureus* represents a major risk factor for the development of infection with this bacterium. Community-acquired infections are principally of endogenous origin. Moreover, infections occurring in hospitalized patients in various hospital settings are often associated with nasal carriage. The studies involving surgery patients, those on hemodialysis or CAPD, have shown that hospital infections with *S. aureus* can be caused by the same strain that had already colonized the patient (5). The study by Wertheim et al. demonstrated that 80% of cases of hospital bacteriemias are of endogenous origin. In these patients, genotyping of *S. aureus* microorganisms isolated from the nose and blood demonstrated that the identical strain was in question (6).

Most strains of *S. aureus* produce penicillinase, which makes them resistant to penicillin. A special therapeutic problem are methicillin-resistant isolates of *S. aureus* (MRSA), resistant to all beta-lactam antibiotics. In addition to MRSA being the principal cause of hospital infections, the cases of community-acquired MRSA -caused infections have been reported lately, and these strains have been termed community-acquired methicillin-resistant *S. aureus* (CO-MRSA). Nose colonization with the CO-MRSA strains has been associated with endogenous soft tissue infections. Ellis et al. studied nasal carriage in the USA soldiers and found that out of 53% with the nasal carriage status, in 29% there occurred skin infections with the same strain of *S. aureus* during the following year (7-9). From the literature data, differences in the presence of nasal carriage of *S. aureus* in local populations have been decribed. More recent studies have identified the need for screening of healthy population in order to establish the overall presence of colonization with MRSA (10-12). The aim of this study was to assess the nasal carriage of *S. aureus* in healthy individuals in the local community, and the sensitivity of the microorganism to antibiotics.

MATERIAL AND METHODS

The study enrolled 56.868 healthy individuals aged 19 to 65 years, and 2.040 healthy school children aged 15 to 19 years. Specimens to be studied were obtained from anterior nares with sterile swabs and plated on Columbia agar. Agar plates were incubated at 37°C for 24 hours. The colonies that were yellow or gold pigmented were subcultured onto mannitol salt agar and selected for the tube coagulase test using rabbit plasma (BioMerieux). Mannitol fermenting and tube coagulase positive isolates were identified as *S. aureus*. We used the disk diffusion method (Kirby-Bauer) on Mueller-Hinton agar to assess the sensitivity of isolated *S. aureus* to penicillin, cefoxitin, erythromycin, clindamycin, gentamicin, trimetoprim/sulphametoxazole, doxycycline, and fusidic acid (Rosco, Danmark). The interpretation of inhibition zones was performed observing the guidelines of the Clinical and Laboratory Standards Institute (CLSI). A double disk diffusion test (D-test) was used to detect inducible resistance to clindamycin. Erythrtomycin and clindamycin disks were placed at the distance of 15 mm. The test was considered positive if the inhibition zone around clindamycin disk was „D“ shaped. The isolates in which inducible resistance was detected were considered resistant to clindamycin. The isolated with inhibition zone ≥21 mm around the cefoxitin disk were designated as methycillin-resistant isolates (MRSA). The study took place at the Public Health Institute Niš.

RESULTS

Out of the total number of 58.908 studied nasal swabs of healthy individuals, *S. aureus* was isolated in 1.381 (2.34%) samples. Positive findings were obtained in 2.33% of adult examinees, and in 2.59% of studied school children (Table 1).

Out of the total number of adult examinees with positive findings, there were 668 women (50.30%) and 660 men (49.70%), while among the school children there were 17 cases (32.07%) of female gender and 36 (67.93%) of male gender. The lowest percentage of positive findings was obtained during the examination in February (1.43%), and the highest percentage was found in June (3.56%).

Reviewing the susceptibility of all tested isolates to antibiotics, we found a low level of susceptibility only to penicillin (5.36%). The susceptibility of *S. aureus* isolates from healthy individuals to all other tested antibiotics was present in a high percentage, with the lowest percentage of susceptibility to doxycycline (71.54%) and erythromycin (86.09%). The highest percentage of

susceptibility of tested isolates was reported for fusidic acid (99.27%).

The sensitivity of 1.328 isolates of *S. aureus* from nasal swabs in adults and 53 from nasal swabs in school children to the tested antibiotics is shown in Table 2.

All the isolates in which the inhibition zone around the cefoxitin disk was over 21 mm represented MRSA. In relation to the total number of isolated *S. aureus*, MRSA was found in 8.91% (123 isolates). In relation to the total number of *S. aureus* isolates from nasal swabs in adults, MRSA was present in 8.96% (119 isolates), while there were only 4 MRSA isolates from nasal swabs in school children. In relation to 53 isolates of *S. aureus* from the swabs in this studied group, MRSA was present in 7.54%. In relation to the total number of examinees, the proportion of MRSA isolates was 0.21%.

MRSA susceptibility to the tested antibiotics is shown in Table 3. The highest percentage of susceptibility was observed for fusidic acid (96.75%), while slightly over half of the isolates were susceptible to erythromycin (52.84%). There were 65.85% of isolates susceptible to clindamycin, and a high percentage of susceptibility was detected to gentamicin and trimethoprim/sulphametoxazole.

Table 1. *Nasal carriage of S. aureus in healthy population*

Studied population	Positive findings	
	Number	%
Adults (n=56.868)	1328	2,33
School children (n=2040)	53	2,59

Table 2. *Antimicrobial susceptibility of S. aureus isolates (n=1381)*

Antimicrobial agent	Adult population (n=1328)		School children (n=2040)	
	susceptible no.	%	suscptible no.	%
Penicillin	73	5,49	1	1,88
Cefoxitin	1209	91,04	49	92,45
Erythromycin	1132	85,24	49	92,45
Clindamycin	1204	90,66	50	94,33
Gentamicin	1267	95,40	53	100
Trimethoprim/sulphametoxazole	1252	94,28	51	96,23
Doxycycline	953	71,76	53	100
Fusidic acid	1318	99,24	53	100

Table 3. *Antimicrobial susceptibility of MRSA isolates (n=123)*

Antimicrobial agent	Susceptibility (%)
Erythromycin	52,84
Clindamycin	65,85
Gentamicin	82,93
Trimethoprim/sulphametoxazole	82,93
Doxycycline	71,54
Fusidic acid	96,75

DISCUSSION

S. aureus is a pathogenic microorganism able to cause a large number of diseases, starting from superficial infections of the skin and soft tissues, to severe, life-threatening infections. These infections can be endogenous or they occur after the transmission from carriers or infected individuals. The infections are commonly endogenous, i.e. caused by the strain that has already colonized the patient. The most common site of colonization by *S. aureus* is the nasal mucosa. Nasal carriage of *S. aureus* thus represents the source of infection both in the community and hospital settings (1, 5).

In our study, involving healthy adults and school children, *S. aureus* was isolated in only 2.34%, with a small difference in the presence of carriage status in adults and in school children (2.33% vs 2.59%, respectively). The prevalence of nasal carriage in healthy population varies depending on the region and age of the studied population. Pathak et al. examined nasal mucosa colonization in healthy children in India, reporting for regular vaccination, aged from 1 month to 59 months. *S. aureus* was isolated in 6.3%, with a higher percentage of positives among the children staying in institutions and those from large families (13). Significantly higher percentages of positive findings were shown in the studies from India too, involving the children aged 5 to 15 years. Ramana et al. reported positive findings in 16% of children in this age range (14), while Chatterjee et al. isolated *S. aureus* in as high as 52.3% of their examinees, with a similar prevalence of nasal carriage of *S. aureus* in children from urban areas (48.4%) and those from rural areas (56.1%) (15). The studies involving healthy children of up to 7 years of age, done in other parts of the world, similarly reported different prevalences of nasal carriage. While in Germany Fluegge et al. isolated *S. aureus* in 25.8% of their examinees (12), Lo et al. in 25% in Taiwan (16), Oguzkaya-Artan et al. reported positive findings in 18% of their examinees in this age range (17). Creech et al. compared the prevalence of nasal carriage of *S. aureus* in a population of healthy children aged up to 18 years from the same community in the USA, identified in the studies done in 2001 and 2004. The authors observed an increase of positive findings in 2004 (36.4%) compared to the situation in 2001 (29%) (18).

Studies of the prevalence of nasal carriage of *S. aureus* involving healthy adults also demonstrated a significantly higher percentage of positive findings compared to our study. Out of 274 individuals from Birmingham, Great Britain, Abudu et al. isolated *S. aureus* from nasal swabs in 23% (19). Similar results can be found in other studies worldwide. In healthy adults in Italy, Zanelli et al. obtained positive findings in 25.9% (20), Regev-Yochay et al. in 23.6% in Israel (21), Kumari et al. in 23.5% of studied students in Malaysia (22), O' Donoghue et al. in 23% in Hong Kong (10). A large proportion of individuals colonized by *S. aureus* was demonstrated by Manious et al. in the USA. A huge population-based study of these authors, involving 86.9 million examinees of all ages, revealed the prevalence of nasal carriage of *S. aureus* of 32.4%, with the prevalence of 30.67% in those over 20 years of age (3). A low percentage of positives in our study compared to the literature data could possibly indicate sampling and transport errors. In some studies, transport media were used (10, 12, 13, 15, 17, 19) or the swabs pre-wetted with sterile saline were used (13, 16), while Creech et al. used the enrichment broth to isolate *S. aureus* (18).

The mortality of invasive infections caused by *S. aureus* has been significantly reduced after the advent of penicillin in the fourties of the last century; however, after just several years, staphylococcus becomes resistant to this antibiotic via the production of beta lactamase, so that today over 95% of the isolates are resistant to this antibiotic. In the sixties of the last century, the first synthetic penicillin resistant to beta lactamases, methicillin, was introduced. However, a couple of years after the introduction the cases of resistance were encountered and reported, and the isolates resistant to this antibiotic were designated as methicillin-resistant *S. aureus* (MRSA). The original detection of MRSA was associated with hospital infections in large hospitals and in patients treated in intensive care units; nowadays, it is one of the most common, widely present causes of infection in both hospital in-patients and out-patients (5).

It is believed that with time the prevalence of MRSA increases in healthy carriers, so that in addition to the surveillance of nasal carriage of *S. aureus* in healthy individuals, the prevalence of MRSA has also been investigated. The study by Creech et al. demonstrated that compared to all *S. aureus* isolates, MRSA was present in 25% in 2004, while the corresponding percentage was as low as 3% in 2001 (18). Although a rise of prevalence of MRSA in healthy carriers has been suggested, the data from different parts of the world vary. In the study by Fluegge et al., only one out of 403 isolates (0.2%) of *S. aureus* was MRSA (12). One MRSA isolate was also detected by Zanelli et al. (0.4%) (20). However, other authors reported higher MRSA prevalences: Ozaki et al. 3.7% (11); Chatterjee et al. 3.89% (15); Oguzkaya-Artan et al. 5.6% (17); Lo et al. 13.2% (16), Pathak et al. 16.3% (13), Ramna et al. 19% (14). Since these studies involved pediatric populations as well, lower prevalence of MRSA tended to be reported by the authors studying nasal carriage in adults. Kumari et al. did not isolate any MRSA in 162 examinees (22). Manious et al. reported MRSA in 2.03% of examinees aged from 1 to 64 years (3), Abudu et al. in 6% of examinees aged from 16 to over 85 years (19), O'Donoghue et al. in 4.1% (10), and Alghaithy et al. in 5.1% (23). The prevalence of MRSA compared to other *S. aureus* isolates in our study was 8.91%, which was a high percentage in view of the literature data. Future studies sho-

uld address the risk factors associated with nasal carriage of MRSA, such as the data on previous hospitalizations and use of antibiotics.

Since nasal carriage of *S. aureus* represents a possible source of infection with this microorganism, the studies of its susceptibility to antibiotics could have an impact on empiric therapy of infections. Except for a high percentage of resistance to penicillin, the isolates of *S. aureus* in our study demonstrated a rather good susceptibility to the tested antibiotics, which agreed with the available literature data (12, 15, 17, 20). The lowest susceptibility to doxycycline (71.54%) can be related to the wide availability and low price of this antibiotic in our country. However, approximately half of MRSA isolates were resistant to erythromycin, and 34.15% to clindamycin. Clindamycin is an antibiotic commonly used to treat community-acquired infections caused by MRSA. The resistance to clindamycin of MRSA isolates of 37.5% was reported by Chatterjee et al. (15), and resistance of 26% by Creech et al. (18), while only 8.3% of MRSA isolates were resistant to this antibiotic in the study by Oguzkaya-Artan et al. (17).

CONCLUSION

In this study, we established a low percentage of nasal carriage of *S. aureus* in the population of healthy individuals, but a high percentage of MRSA. Using some better sampling methods, future studies should try to determine the risk factors of influence regarding the colonization by this bacterium.

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NALAZ STAPHYLOCOCCUS AUREUS U SLUZOKOŽI NOSA KOD ZDRAVIH ODRASLIH OSOBA I ŠKOLSKE DECE

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Sažetak

***Staphylococcus aureus* (*S. aureus*) je mikroorganizam koji kolonizuje kožu i sluzokože zdravih osoba ali i jedan od najčešćih uzročnika vanbolničkih i bolničkih infekcija. Kolonizacija sluzokože nosa *S.aureus*-om predstavlja glavni faktor rizika za razvoj infekcija ovom bakterijom. Poseban terapijski problem predstavljaju meticilin-rezistentni sojevi *S. aureus* (MRSA).**

Cilj ovog istraživanja bio je utvrđivanje kolonizacije sluzokože nosa *S. aureus*-om u zdravoj populaciji u lokalnoj sredini i njihove osetljivosti prema antibioticima.

Istraživanjem je obuhvaćeno 56 868 zdravih odraslih osoba starosti od 19 do 65 godina i 2 040 zdrave školske dece uzrasta od 15 do 19 godina kod kojih su uzorkovani brisevi sluzokože nosa. Ispitivanje osetljivosti izolovanih sojeva *S. aureus* izvršeno je disk difuzionom metodom (Kirby-Bauer) na Mueller-Hintonovom agaru. *S. aureus* je izolovan kod 1381 ispitanika ili 2,34%. Pozitivan nalaz dobijen je kod 2,33% odraslih ispitanika i kod 2,59% ispitivane školske dece. Nizak nivo osetljivosti detektovan samo prema penicilinu (5,36%). Osetljivost *S. aureus* izolata prisutna je u visokom procentu prema ostalim testiranim antibioticima, sa najmanjim procentom osetljivih izolata prema doksiciklinu (71,54%) i eritromicinu (86,09%). Najveći procenat osetljivosti ispitivanih izolata je prema fusidinskoj kiselini (99,27%). U odnosu na ukupan broj izolata *S. aureus* izolovanih iz briseva odraslih osoba MRSA je zastupljen sa 8,96% (119 izolata), dok je iz briseva školske dece izolovano samo 4 MRSA izolata.

Ovim istraživanjem utvrdili smo da je kliconoštvo *S. aureus* u populaciji zdravih osoba zastupljeno u malom procentu, ali je visok procenat zastupljenosti MRSA.

***Ključne reči:* kolonizacija nosne sluzokože, *Staphylococcus aureus*, zdrava populacija**