Original paper

Staphylococcus aureus carriage state in healthy adult population and phenotypic and genotypic properties of isolated strains

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Adv Dermatol Allergol 2020; XXXVII (2): 184–189
DOI: https://doi.org/10.5114/ada.2020.94837

Abstract

Introduction: At present, infections induced by staphylococci, especially methicillin-resistant *Staphylococcus aureus* (MRSA) are one of key therapeutic and epidemiological problems.
Aim: The assessment of *Staphylococcus aureus* carrier state occurrence among a healthy adult population as well as determination of phenotypic and genotypic properties of the isolated strains.
Material and methods: The study included 100 healthy individuals. Material for bacteriological evaluation was collected from the posterior pharyngeal wall and tonsils, nasal vestibule and the skin of anterior nares using a sterile swab. The isolates identified as *Staphylococcus aureus* were analysed further, towards slime-forming capacity and the presence of genes mecA and nuc.
Results: The analysis included 300 samples obtained from the posterior vault of the pharynx and tonsils, nasal vestibule and the skin of nares. Pharyngeal and vestibular *S. aureus* carriage was determined in 20% of the examined adults, whereas in 11 people with recognized positive throat colonization, the concurrent presence of golden staph was detected in the vestibule of the nose and on the skin, in the nose region. Identification process indicated the occurrence of strains defective in *clumping factor* synthesis (5% of isolates) and lack of the *coa* gene (11% of examined isolates). The PCR technique used to screen for the presence of the *mec* gene, did not confirm it in any of the strains under study. All the isolates had the gene encoding the thermostable nuclease *nuc*.
Conclusions: *S. aureus is* a pervasive pathogen in community settings with constantly changing trends.

Key words: *Staphylococcus aureus*, carriage, polymerase chain reaction.

Introduction

Over the last decade, *Staphylococcus* genus has undergone rapid evolutionary changes and as a consequence, highly specialized pathogenic strains, i.e. *coagulase-positive Staphylococcus* (CPS) and its *coagulase-negative* counterpart (CNS) developed. Both demonstrate resistance to a number of antibiotics and antimicrobial agents. At present, infections induced by staphylococci, especially methicillin-resistant *Staphylococcus aureus* (MRSA) are one of key therapeutic and epidemiological problems [1, 2].

Generally, *S. aureus* makes an asymptomatic constituent of the natural bacterial flora, yet under favourable conditions can cause a number of local infections, mostly involving skin and skin structures (wounds and soft tissues) as well as invasive infections, e.g. pneumonia, osteomyelitis or endocarditis [3]. Particularly severe strains are isolated in hospital settings and may lead to life-threatening conditions, such as sepsis [4, 5].

The recent increase in the incidence of staphylococcal infections has been associated not only with increasing resistance to many common antibiotics (including vancomycin) but prevalent colonization as well. According to Yang *et al.* [6], colonization favours the maintenance of the microorganism in a population, whereas asymptomatic carriage, especially chronic, serves as a major reservoir for the strain assisting its spread in environment.

It is estimated that 70–90% of the general population are at least intermittent (transient) carriers of *S. aureus* [7]. Chambers [8] reports that approximately 20% of the population are persistent carriers, while in about 60%

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Received: 18.04.2018, **accepted:** 22.09.2018.

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colonization occurs intermittently. Only 20–30% of populations are non-carriers who never harbour *S. aureus*.

Staphylococcus aureus predominantly colonizes the mucous membranes of the upper respiratory tract, including the throat and nose, rarely perineum and anus [9].

Pathogen carriage is associated with individual predisposition of the host and notably, most frequently colonization is acquired by hospitalized patients and in direct contact with healthcare settings [6, 10]. The main factors predisposing to the staph infection development include age, chronic disease, immunodeficiency and a genetic predisposition [7].

The clinical and epidemiological implications of *S. aureus* carriage have directed attention towards rapid and accurate microbiological diagnostics. Early identification of carriers is the crucial step in preventing staphylococcal infections. It allows not only for determination of its potential role in the pathological process but also helps identify the potential source of infection thus, prevents reinfection [11].

For decades, identification of *S. aureus* has been based mainly on the evaluation of phenotypic, particularly biochemical and enzymatic characteristics. Nowadays, despite the application of many novel molecular biological techniques, phenotypic identification has still remained the major procedure used in routine microbiological diagnostics [12]. Occurrence of atypical *S. aureus* strains lacking specific factors, in that coagulase-negative (CNSA) or clumping factor (CFNSA) poses a serious risk for their possible incorrect identification [13]. Hence, genotypic methods are valuable tools of modern diagnostics as they facilitate precise identification of strains and their potential pathogenicity [14, 15].

Aim

The objective of the research was to assess the incidence of *Staphylococcus aureus* carriage among a healthy adult population and determine phenotypic and genotypic characteristics of the isolated strains.

Material and methods

The study included 100 healthy individuals aged 20–50 years who were evaluated in relation to the incidence of *Staphylococcus aureus* carriage state in the pharynx, nasal vestibule and the skin of anterior nares. All participants were not hospitalized within the last 3 years, and did not work in the health care service. Exclusion criteria were presence of skin lesions and current use of antibiotics. Each subject filled in a self-completion questionnaire to establish the body condition and hygiene habits. The questionnaire was devised using the following parameters:

1) age and sex;
2) health proneness factor, including:
   - incidence of upper respiratory tract disorders, frequency of;
   - antibiotic agent use, in that last month;
   - incidence of common cold last month;
   - skin disorders;
   - earlier diagnosed *S. aureus* in the nose or throat;
3) addiction to nicotine;
4) contact with hospital setting.

Material for bacteriological evaluation was collected from the posterior pharyngeal wall and tonsils, nasal vestibule and the skin of anterior nares using a sterile swab. The obtained material was inoculated into agar solid medium with 5% sheep blood plates (BioMaxima S.A., Lublin, Poland) and incubated under aerobic conditions for 48 h at 36 ±2°C temperature. The isolated microorganisms underwent the macro- and microscopic evaluation. The initial studies included performance of a Gram stain, catalase test and one for the presence of *clumping factor* CF (*Staphylococcus aureus* identification test, Biomed, Lublin, Poland). Microorganisms initially classified as *Staphylococcus aureus* were inoculated onto the Chapman medium and Baird-Parker medium (BTL, Lodz, Poland). After the 24 h-incubation at 36 ±2°C temperature, they were tested for their ability to degrade lipids and mannitol. The final species-level identification of the cultured strains was made using biochemical tests API Staph (bioMerieux, Inc., Lyon, France). The isolates identified as *Staphylococcus aureus* were analysed further, including towards slime-forming capacity and the presence of gene *mecA* and *nuc*.

Slime-forming capacity

Studies on the intensity of slime formation in all the *Staphylococcus aureus* strains isolated were conducted according to Freeman method [15] on the Congo Red Agar (Sigma-Aldrich, Saint Louis, USA). Aerobic incubation was carried out for 48 h at 36 ±2°C temperature. The readings were made twice after 24 h and 48 h incubation period, taking into account appearance, colour and the intensity of colony colour. The biofilm-producing strains formed dark brown to black colonies of dry crystalline consistency. As for moderate intensity of slime formation, colonies were red-brown with slightly wet surface and finally, the strains that do not produce biofilm constituted light red, wet and a little matt colonies.

Genetic studies

Isolation of genomic DNA from *S. aureus* followed the Extractme DNA Bacteria kit protocol (Gdansk, Poland). The research material comprised fresh cultures from the agar medium. The DNA purification protocol consists of 5 steps and utilizes spin minicolumns with membranes that selectively bind nucleic acids. In order to check efficiency of genomic DNA bacteria isolation, the isolation

Table 1. Number and percentage of identified *Staphylococcus aureus* as regards isolation site

Sampling site	Number of samples	Number of *Staphylococcus* sp. isolates	Number of *Staphylococcus aureus* isolates
Pharynx	100	60 (100%)	24 (40%)
Nasal vestibule	100	64 (100%)	18 (28%)
Nares	100	80 (100%)	2 (2.5%)
Total	300	204 (100%)	44 (22%)

products were separated using agarose gel electrophoresis under standard reaction conditions.

Genotyping of *S. aureus* strains was conducted on the basis of the polymorphism of restriction fragments of the variable region of the *coa* gene using the commercial diagnostic kit *S. aureus* Coag made by DNA Gdansk (Poland). The material for examination comprised the previously isolated DNA. Detection was based on amplification of the *coa* gene fragment. Afterwards, the PCR product obtained was digested using the restriction enzyme of high cutting frequency – Csp61. The amplified fragment was 500–600 bp in size.

Detection of genes *mecA* and *nuc* was performed with the assay for genotyping methicillin-resistant strains, *S. aureus* MRSA-Screen test kit (DNA Gdansk, Poland). Polymnerase chain reaction (PCR) was performed individually for each gene according to the manufacturer's instructions. A standard 25 µl PCR reaction mix contained the following components: a pair of primers (1 µM each), concentrated reaction buffer, 4 mM $MgCl_2$, 1.6 mM deoxyribonucleotide triphosphates (0.4 mM each, 0.04 U/µl thermostable DNA polymerases *Taq* and 1 ng DNA template (target)).

The PCR assay was performed in a thermocycler PTC-200 DNA Engine Cycler (BioRad Laboratories Inc., USA) through the following amplification steps: initial denaturation – 1 min, 94°C; denaturation – 30 s, 94°C; primer annealing – 60 s, 55°C; elongation – 60 s, 72°C; final elongation – 4 min, 72°C.

The PCR product underwent agarose gel electrophoresis and after the ethidium bromide staining, the reading was taken. Positive control was indicated for PCR product by its presence at 533 bp in size for the gene *mecA* and 280 bp for the gene *nuc*.

Statistical analysis

The obtained results were tabulated, presenting the number and percentage of the identified isolates. The results of the questionnaire including population health proneness and hygiene habits of the studied subjects were analysed statistically. A Pearson *r* correlation coefficient was estimated for the binary variables. The analysis was conducted using statistical package Statistica 8.0 (StatSoft Inc. Tulsa, USA).

Results

A total of 300 samples obtained from the posterior vault of the pharynx and tonsils, nasal vestibule and the skin of anterior nares were analysed (Table 1). The research material served as growth media for 204 *Staphylococcus* genus strains, in that *S. aureus* was recovered from 32 (32%) individuals (Figure 1). As many as 24 *Staphylococcus* isolates originated from the posterior pharyngeal wall. Among 80 strains cultured on the material collected from the anterior nares, *S. aureus* accounted for 2.5% (Table 1).

Staphylococcus aureus carriage in the throat and vestibule of the nose was determined in 20% of the examined adults (Figure 1), whereas 11 people with recognized positive colonization in the pharynx showed the concurrent presence of staph aureus in the nasal vestibule (10 people) and on the skin in the nose region (1 person). In only one person, the swabs taken from the skin of the nose identified the presence of *S. aureus*. In this case, positive colonization was also established in the nasal vestibule.

Most frequently, *S. aureus* carriers were patients aged 30.7 (Table 2) and the pathogen was isolated at an equal rate from male and female carriers. Among the respondents, 52 people admitted frequent airway diseases and positive colonization was observed in 16 subjects. Perma-

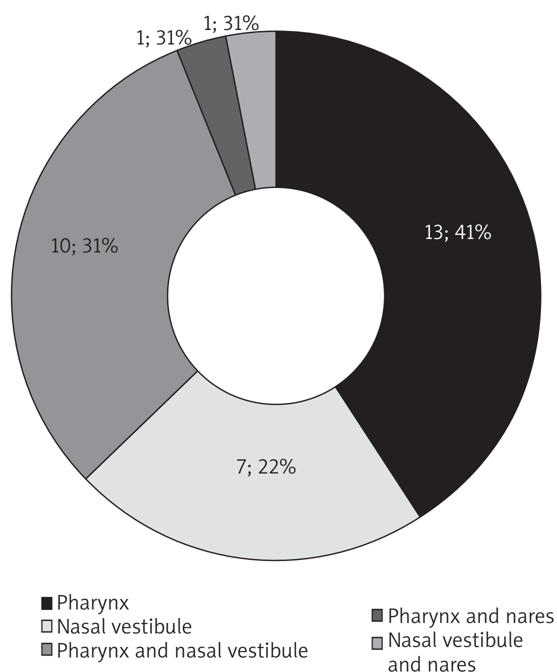

Figure 1. Number of adults with *Staphylococcus aureus* positive colonization

Table 2. Questionnaire results with respondents' health proneness factor

Characteristics	Total	*S. aureus* colonization		*r*	*P*-value
		Negative	Positive		
Number of subjects	100 (100%)	68 (100%)	32 (100%)	–	–
Age, mean ± SD	32.8 ±10.4	33.9 ±10.9	30.7 ±9.2	0.142	0.158
Gender (female)	55 (55%)	37 (54%)	18 (56%)	0.017	0.865
Nicotine addiction	28 (28%)	20 (29%)	8 (25%)	–0.046	0.651
Dietary supplementation	28 (28%)	19 (28%)	9 (28%)	0.002	0.985
Frequent incidence of upper respiratory tract diseases	52 (52%)	36 (53%)	16 (50%)	–0.027	0.786
Earlier diagnosed pharyngeal *S. aureus*	27 (27%)	19 (28%)	8 (25%)	–0.031	0.760
Earlier diagnosed nasal *S. aureus*	17 (17%)	12 (18%)	5 (16%)	–0.025	0.804
Contact with hospital setting	15 (15%)	11 (16%)	4 (13%)	–0.048	0.635
Frequent antibiotic agent use	27 (27%)	21 (31%)	6 (19%)	–0.127	0.206
Common colds last month	25 (25%)	15 (22%)	10 (31%)	0.099	0.327
Antibiotic agent used last month	1 (1%)	1 (1%)	0	–0.069	0.495
Skin problems (oily/mixed)	78 (78%)	51 (75%)	27 (84%)	0.106	0.296

Table 3. Characteristics of *Staphylococcus aureus* isolates

Sampling site	Number of subjects	Phenotypic properties				Genotypic properties		
		Lipid degradation	Mannitol degradation	Catalase presence	CF factor	Gene nuc	Gene mecA	Gene coa
Pharynx	24 (100%)	12 (50%)	24 (100%)	24 (100%)	22 (92%)	24 (100%)	0	22 (92%)
Nasal vestibule	18 (100%)	8 (44%)	18 (100%)	18 (100%)	18 (100%)	18 (100%)	0	16 (89%)
Nares	2 (100%)	0	2 (100%)	2 (100%)	2 (100%)	2 (100%)	0	1 (50%)
Total	44 (100%)	10 (23%)	44 (100%)	44 (100%)	42 (95%)	44 (100%)	0	39 (89%)

Table 4. Assessment of slime-formation ability of *Staphylococcus aureus* strains

Sampling site	Number of subjects	Strong production	Moderate production	No production
Pharynx	24 (100%)	9 (38%)	5 (21%)	10 (41%)
Nasal vestibule	18 (100%)	8 (44%)	3 (17%)	7 (39%)
Nares	2 (100%)	1 (50%)	1 (50%)	0
Total	44 (100%)	18 (41%)	9 (20%)	17 (39%)

nent contact with hospital environment was confirmed by 15 respondents and the rate of *S. aureus* colonization for them was slightly lower. The questionnaire demonstrated that the subjects who claimed overuse of antibiotics accounted for a much lower carriage percentage (19%) compared to those free from *S. aureus*. As for recurrent common colds reported over a month preceding the study, 25 respondents complained about it and for 10 of them, the *S. aureus* presence was identified in the swab samples. The statistical analysis performed did not indicate any significant ($p < 0.05$) correlation between the parameters included into the questionnaire and *S. aureus* colonization.

All the isolates under study degraded mannitol and showed the positive-catalase test result (Table 3). Among the isolates recovered from the throat, 2 (8%) strains lacked clumping factor synthesis as well as the *coa* gene. The *S. aureus* strains defective in the *coa* gene were also isolated from the nasal vestibule and the skin. The PCR technology used did not confirm the presence of the *mec*A gene in any of the investigated strains. All the isolates had thermostable nuclease *nuc*.

All the strains identified as *S. aureus* were evaluated for slime-forming ability (Table 4). Among all the studied strains isolated from the collected material, 27 (61%) isolates showed the capacity to produce biofilm. Consid-

ering all the bacteria under investigation, the ability to form large amounts of slime applied to 41% of strains, while 39% did not produce it at all.

Discussion

Humans and animals are the primary natural reservoir for *S. aureus* and an asymptomatic carrier status is reported far more frequently than infections. It can be transient with short-term colonization and no need for therapy or persistent that can last for a couple of months or years. The estimates say that 25–70% of human population is colonized by *Staphylococci* [8, 16]. The present studies have demonstrated that every third adult (32%) was an asymptomatic carrier of *S. aureus*. The genetic studies excluded the presence of methicillin-resistant strains. *Staphylococcus aureus* was recovered from the pharynx more often than from the nose or skin. However, Mertz *et al.* [17] indicate that pharyngeal carriage occurs more rarely and recommend the control of both body sites at routine screening. According to Mainous *et al.* [18], the frequency of staphylococcal carriage in the nares was 10–45% and in approximately 3% of population the MRSA strains were detected.

These results partly agree with the present research ones where *S. aureus* carriage in the vestibule of the nose was determined in 18% of the examined people, however none of the isolated strains was found methicillin-resistant.

Velasco *et al.* [19] showed that the nasal carriage of *S. aureus* in healthy people was 7.6% and, in general, clones were genetically diverse. Similarly to our research, none of the *S. aureus* strains obtained from healthy people were mecA-positive.

Anwar *et al.* [20], in their studies on 1660 healthy adults, indicated colonization of the upper respiratory tract in 14% of people, mostly men. While, a percentage of *S. aureus* carriage was inversely proportional to age. The present research did not show such correlations.

The phenotypic and genotypic identification of the isolated strains proved the occurrence of strains defective for *clumping factor* synthesis (5% of tested isolates) and the *coa* gene (11% of the isolates). The studies by Garbacz *et al.* [21] conducted in healthcare settings showed that 7.2% of isolated *S. aureus* strains did not produce coagulase, and 3.8% were clumping factor CF-negative. This phenomenon may increase ability of the strain to recombinate and as a result, causes lack of some properties typical for *S. aureus*.

The present studies have indicated that as many as 61% of the isolated *S. aureus* strains produced slime at high or moderate intensity. The obtained results were consistent with those reported by Podbielska *et al.* [22]. A high percentage of biofilm-producing isolates was also determined by Votava and Woznicowa [23]. Kuthan *et al.* [24] informed that in their studies, all the isolated staphylococcal strains recovered from the skin, purulent exudate and nasal vestibule swabs exhibited the slime-forming capacity. Smith *et al.* [25] stated that the strains isolated from the skin surface, as compared to isolates collected from other body sites, had greater capacity to produce biofilms. The slime-generating ability is considered as one of factors enabling *Staphylococcus* colonization of the organism. The mucous substance surrounding cells facilitates bacterial adhesion to various types of surface, resists drugs and protects against phagocytosis, acts as a chemotaxis and opsonisation inhibitor and finally, induces inflammatory reaction [26].

Conclusions

It is generally accepted that asymptomatic colonization by *S. aureus* is common and is a prerequisite for the development of staphylococcal infection. The risk of the infection in nasal carriers is estimated to be 2–12 times higher than in non-colonized persons [27]. Layer *et al.* [28] report that MSSA can cause infection with the same severity and mortality as MRSA. MSSA has no β-lactam resistance but can be resistant to other classes of drugs and harbour different virulence genes.

Knowledge about importance and range of *S. aureus* carriage in a society as well as the serious threat posed by multi-resistant microorganisms to patients necessitate development of novel, rapid and efficient diagnostics. The presence of defective staph strains proves enhanced gene expression occurring within a given species and essentially, makes the diagnostics challenging which in turn, hinder bacterial eradication from the environment. In the case of *S. aureus* identification, especially strains of atypical phenotypic characteristics, application of the species-specific PCR should be the optimal solution. The screening tests increase the detection of MRSA and MSSA carriage and, as a result, prevent the pathogen transmission which contributes to a decreased carriage rate of multi-resistant strains in human population.

Conflict of interest

The authors declare no conflict of interest.

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