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MEDICAL STUDENT AS A POTENTIAL SOURCE OF STAPHYLOCOCCAL INFECTION

SHABAN ZHANNA GEORGIEVNA,

PhD, associate professor

**KHAZAAL MOHAMAD SAMIR,
ROZOV ALEKSANDR SERGEEVICH**

students

Belarusian State Medical University

Abstract: The prevalence and massiveness of tonsillar and nasal carriage of lecithinase+ staphylococci in medical students and the factors determining it have been studied. High resistance of isolated staphylococcal strains to penicillins and cephalosporins has been revealed. Epidemiologically significant strains of staphylococci resistant to oxacillin and vancomycin have been identified. Criteria for mandatory screening for staphylococcal carriage and diagnostic recommendations increasing the efficiency of detection of staphylococcal carriers have been developed.

Key words: staphylococcal carriage in a medical student, tonsillar carriage, nasal carriage, oxacillin-resistant staphylococci, vancomycin-resistant staphylococci.

СТУДЕНТ-МЕДИК КАК ПОТЕНЦИАЛЬНЫЙ ИСТОЧНИК СТАФИЛОКОККОВОЙ ИНФЕКЦИИ

Шабан Жанна Георгиевна,

кандидат медицинских наук, доцент

Хазааль Мохамад Самир,

Розов Александр Сергеевич

студенты

Белорусский государственный медицинский университет

Аннотация: Изучена частота и массивность тонзиллярного и назального носительства лецитиназо+ стафилококков у студентов-медиков и факторы, её определяющие. Выявлена высокая устойчивость изолированных штаммов стафилококков к пенициллинам и цефалоспоринам. Выделены эпидемически значимые штаммы стафилококков, устойчивые к оксациллину и ванкомицину. Разработаны критерии для обязательного скрининга на стафилококковое носительство и диагностические рекомендации, повышающие эффективность выявления стафилококковых носителей.

Ключевые слова: стафилококковое носительство у студента-медика, тонзиллярное носительство, назальное носительство, оксациллин резистентные стафилококки, ванкомицин-резистентные стафилококки.

Staphylococcus aureus is a frequent cause of infections in both the community and hospital. The nose and the throat are the main ecological niches of *S. aureus*, and colonization is a risk factor for infection. Staphylococcus carriers are major sources of exogenous infection, including healthcare-associated infection. At the same time, carriers of *S. aureus* have an increased risk of acquiring an infection with this pathogen [1, p. 751].

Endogenous staphylococcal infection most often affects the nasopharynx or skin. Nasal staphylococcal colonization is well described. However, we have limited knowledge about *S. aureus* throat colonization [2, p.

1–2]. It is known, that *S. aureus* is one of the most frequent pathogens in the etiology of tonsillitis and its relevance is due to its antimicrobial resistance and persistence in the internal tissues of the tonsils. Tonsillectomy is indicated in cases of recurrent tonsillitis after several failures of antibiotic therapy. In the removed tonsils from patients who had a history of recurrent tonsillitis, were isolated *S. aureus*, that showed high level resistance to penicillin (83.6%), and 18.0% were considered multidrug resistant [3, p. 12].

Worldwide, the problem of effective elimination of *S. aureus* remains relevant. The increasing resistance of this pathogen to various antibiotics complicates treatment of *S. aureus* infections. Effective measures to prevent *S. aureus* infections are therefore urgently needed. Multidrug resistant isolates colonizing tonsils even without infection, demonstrate persistence of the bacterium and possibility of antimicrobial resistance dissemination and recurrence of infection [1, p. 760].

At the same time, screening for staphylococcal carriage is not included in the program of mandatory medical examination of medical students, despite the fact that they may be potential sources of infection. Effective detection and eradication of pathogenic staphylococci from carriers is a preventive strategy that pursues 2 goals: preventing infection in medical students and ending their role as a source of infection in medical care.

Purpose of the study: to investigate the prevalence of tonsillar and nasal carriage of staphylococci in 2nd and 3rd year students of the Medical Preventive Faculty of Belarusian State Medical University (BSMU) and the epidemic significance of isolated strains, to develop a methodology for effective detection of students who are carriers of pathogenic staphylococci.

The study design included an examination of 49 students of the Medical Preventive Faculty of BSMU aged 18–25 years, of whom 40 were female and 9 were male, which is due to the specifics of education. At the same time, 25 students (20 of them are women and 5 are men) were examined in the 2nd year and 24 students (20 of them are women and 4 are men) in the 3rd year.

Material and methods. The material for the study was the discharge from the mucous membrane of both nasal passages and both palatine tonsils. The material was taken in the afternoon, a few hours after eating and oral hygiene. The students were not informed about the planned examination, which increased its diagnostic significance. Separate disposable sterile cotton swabs for each biotope were used. The swabs were pre-moistened with sterile saline solution.

Research methods. The material was inoculated immediately after collection onto 4 sectors of a dish with yolk-salt agar (YSA). The cultures were incubated for 2 days at 37°C. The intensity of colonization of biotopes with lecithinase+ staphylococci was assessed by the number of grown typical colonies with a corolla of opacity, in which Gram+ cocci in the form of grape-like clusters were detected by microscopy with Gram staining.

The presence of membranotoxin was detected by inoculating the isolated pure culture of staphylococcus onto a dish with blood agar. The presence of a zone of complete β-hemolysis around the growth of colonies was regarded as the presence of pore-forming α-toxin.

Antimicrobial susceptibility of the lecithinase+ staphylococcal isolates to 16 antibiotics of different chemical groups with different mechanisms of action has been determined by disk diffusion test, when isolated pure cultures were cultured on Mueller Hinton agar.

A questionnaire was developed to assess the health status of a student carrier of pathogenic staphylococci, and risk factors for the development of carriage were identified.

The validity of the study results was determined by statistical methods.

Results and discussion. The following phenotypic characteristics of lecithinase+ staphylococcal strains isolated from medical student carriers has been described:

- gram+ cocci, arranged as bunches of grapes in the smear;
- are resistant to NaCl;
- produce carotenoid pigments of cream or yellow color;
- have an aggressive enzyme – α-toxin (8% of strains isolated from 2nd year students and 62.5% of strains isolated from 3rd year students);
- produce β-lactamases;
- are more likely to persist on the palatine tonsils.

Abundant confluent growth of lecithinase+ staphylococci prevailed in the majority of carriers (67.8%). The large number of colonies indicates a significant concentration of pathogenic staphylococci and indicates massive colonization of biotopes.

Carriage of lecithinase+ staphylococci has been detected in 81.6% of students. At the same time, 18.4% of students have been found to be carriers in all 4 biotopes, 10.2% – in three biotopes, 6.1% – in two biotopes.

Only nasal carriage of lecithinase+ staphylococci has been detected in 10.2% of students (one nasal passage – 4.1%, both nasal passages – 6.1%). Only tonsillar carriage of lecithinase+ staphylococci has been detected in 36.7% of students (one palatine tonsil – 24.5%, both palatine tonsils – 12.2%) (Fig. 1).

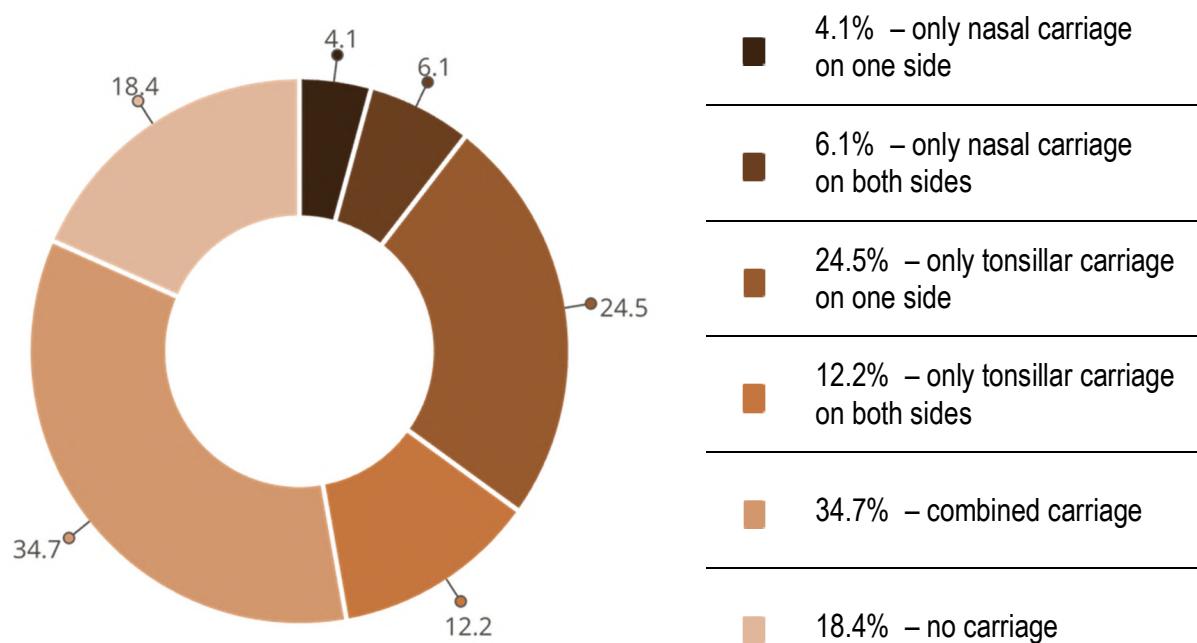


Fig. 1. Distribution of carriage of lecithinase+ staphylococci by biotopes

The overall probability of detection of lecithinase+ staphylococci carriage with a random selection of biotope for inoculation is only 62.5%. For the complete detection of carriers of pathogenic staphylococci, the material must be examined from all four biotopes.

Studying in a medical university is a risk factor for the development of staphylococcal carriage, since the percentage of student carriers increases significantly with the year of study. In the 2nd year, 76% of students are carriers of lecithinase+ staphylococci, in the 3rd year – 87.5% of students (Table 1). There is an increase in nasal carriage in connection with the year of study.

Table 1
Carriage of lecithinase+ staphylococci in medical students depending on the year of study

Carrier type	% of students	
	2 nd year	3 rd year
NO CARRIAGE	24	12.5
ALL SITES, 4 biotopes (tonsils + nose)	24	12.5
3 different biotopes	12	8.33
2 different biotopes	–	12.5
One tonsil	24	25
Both tonsils	12	12.5
One nasal passage	–	8.33
Both nasal passages	4	8.33

Questioning of carrier students about their health condition showed that they have complaints of subfebrile fever (2.9% constantly, 52.9% sometimes), runny nose (8.8% constantly, 76.2% sometimes), nasal congestion (8.8% constantly, 67.6% sometimes), sore throat (2.9% constantly, 70.6% sometimes). Thus, carriage of pathogenic staphylococci is not asymptomatic. The medical doctor should be alerted by such complaints. Careful and attentive collection of patient history during a medical examination increases the chance of suspecting carriage of pathogenic staphylococci.

Isolated strains of lecithinase+ staphylococci showed high resistance to penicillins, including inhibitor-protected ones, and cephalosporins, and retained susceptibility to azithromycin and meropenem (Table 2).

Of major concern is that in recent years there has been a rise in the number of cases of *S. aureus* that are resistant to vancomycin. This means that the bacteria have developed a way to evade the effects of the antibiotic. It can lead to the spread of drug-resistant staphylococci, which can be difficult to treat.

Table 2

Susceptibility of lecithinase+ staphylococci to antibiotics

ANTIBIOTIC PANEL	ANTIBIOTIC	% of strains		
		Susceptible	Susceptible with increased exposure	Resistant
PENICILLINS	Amino	Ampicillin	—	—
	Wide spectrum	Ampicillin / sulbactam	43.3	6.7
	Anti-staphylococcal	Oxacillin	22.7	9.1
	Anti- pseudomonal	Ticarcillin / clavulanic acid	35.7	—
CEPHALOSPORINS	1 generation	Cefazolin	48.4	12.9
	II generation	Cefomandol	69	10.3
	III generation	Ceftriaxone	56.5	17.4
	IV generation	Cefepime	4.3	4.3
CARBAPENEMS		Meropenem	95.7	—
GLYCOPEPTIDES		Vancomycin	63.6	—
AZALIDES		Azithromycin	100	—

We have identified staphylococcal strains resistant to oxacillin and vancomycin in medical students (Table 2).

Therefore, it is necessary to sanitize students who are carriers of lecithinase+ staphylococci – potential sources of endogenous and exogenous infection.

Conclusions.

1. 81.6% of students of Medical Preventive Faculty of BSMU are carriers of lecithinase+ staphylococci. Students with multiple carriage of lecithinase+ staphylococci in 2-4 biotopes make up 34.7%. The virulence of staphylococci and the number of their carriers increases with the year of study, depends on the student group.
2. Screening for staphylococcal carriage is recommended in the medical examination program. It is mandatory in the presence of complaints of subfebrile temperature, chronic inflammation, and allergies.
3. Penicillins and cephalosporins cannot be used for sanitation and treatment in students carrying lecithinase+ staphylococci.
4. In the empirical selection of an antibacterial drug, the choice should be made in favor of azithromycin, to which 100% of the isolated strains of staphylococci are susceptible.
5. Epidemiologically significant strains of staphylococci such as ORS (68.2 %) and VRS (36.4 %) were isolated from carrier students. Students may spread resistant strains in the clinic during training, practice, and subsequent work, including as hospital epidemiologists.

We have developed the following diagnostic guidelines to identify staphylococcal carriers.

1. **Multiple biotopes.** It has been shown that for reliable diagnosis of staphylococcal carriage, material should be collected simultaneously and separately from four biotopes – both nasal passages and both palatine tonsils.

2. Given the possibility of staphylococcal carriage in one biotope, and to avoid transfer of staphylococci to another biotope during material collection, the material must be collected with different sterile swabs for each biotope. The use of a moistened swab makes the collection process more physiologic.

3. **Probability of detection.** If a single biotope is randomly selected, the probability of detection of staphylococcal carriage is only 62.5%. This significantly reduces the effectiveness of screening studies, may lead to false-negative test results of the study and miss potential sources of infection. To rule out the diagnosis of lecithinase+ staphylococcal disease, cultures should be taken from both nasal passages and both palatine tonsils.

4. **Incubation time.** The presence of lecithinase activity on YSA 24 hours after incubation was recorded in cultures from students with lecithinase+ staphylococci carriage in several biotopes. A definitive diagnosis of lecithinase+ staphylococci carriage can be performed only 48 hours after culture on YSA. Premature evaluation of results can lead to false negative conclusions.

5. **Integrated approach.** Combining microbiological diagnostic methods and examining all relevant biotypes significantly increases the reliability of diagnosis of pathogenic staphylococci carriage and allows for timely identification of potential sources of infection.

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