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Comparative antibiotic resistance of *S. aureus* strains isolated in surgical and therapeutic departments of hospitals

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Abstract

Aim – to identify the characteristics of resistance of clinical isolates of *S. aureus*, obtained in hospitals of various specialization, to beta-lactam antibiotics, and to improve recommendations for empirical etiotropic therapy prescribed to patients with staphylococcal infections.

Materials and methods. The subject of the study is the clinical isolates of *S. aureus* received from patients hospitalized to Vitebsk Regional Clinical Infectious Diseases Hospital "therapeutic" isolates, n=117) and purulent surgery departments and intensive care units of Vitebsk Regional Clinical Hospital ("surgical" isolates, n=111) in 2016–2017 years. Methods for the study of antibiotic resistance of clinical isolates of *S. aureus*: disk diffusion method, method for determining the beta-lactamase activity of bacterial suspension using the "BioLactam" test system.

Results. It was found, that 80.6% of the therapeutic isolates and 73.2% of the surgical isolates were resistant to the 1st generation penicillins. 5.4% of therapeutic isolates and 34% of surgical isolates were resistant to inhibitor-protected beta-lactams. 36.8% of surgical isolates and no therapeutic isolates were resistant to cephalosporins of the 3rd generation. No MRSA were detected among the therapeutic isolates, and among the surgical isolates the prevalence of MRSA was 30.5%. 93.3% of therapeutic isolates of *S. aureus* did not show any beta-lactamase activity. Among surgical isolates there were only 34.4% of such isolates. Direct Spearman's correlations of moderate strength were revealed between the levels of beta-lactamase activity of *S. aureus* isolates, the duration of hospitalization and febrile period, as well as between the isolation of MRSA and the duration of hospitalization, fever and diarrheal syndrome.

Conclusion. The use of inhibitor-protected penicillins and 3rd generation cephalosporins is recommended for empirical antibiotic therapy of "therapeutic" infections caused by *S. aureus*. Reserve antibiotics with selective antistaphylococcal activity (such as glycopeptides or oxazolidinones) are required for empirical antibacterial therapy of staphylococcal infections in surgical departments and resuscitation units

Keywords: clinical isolates of *S. aureus*, antibiotic resistance, MRSA, empirical etiotropic therapy, *S. aureus* antibiotic resistance mechanisms, therapeutic and surgical departments of hospitals.

Conflict of interest: nothing to disclose.

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Сравнительная антибиотикорезистентность изолятов *S. aureus*, выделенных в хирургических и терапевтических отделениях стационаров

И.В. Жильцов

Аннотация

Цель – выявить особенности устойчивости к бета-лактамным антибиотикам клинических изолятов *S. aureus*, выделенных в стационарах различного профиля, а также скорректировать рекомендации по эмпирической этиотропной терапии, назначаемой пациентам со стафилококковыми инфекциями.

Материал и методы. Объектом исследования являются клинические изоляты *S. aureus*, выделенные от пациентов, госпитализированных в Витебскую областную клиническую инфекционную больницу («терапевтические» изоляты, n=117) и отделение гнойной хирургии и ОРИТ Витебской областной клинической больницы («хирургические» изоляты, n=111) в 2016–17 гг. Методы исследования антибиотикорезистентности клинических изолятов *S. aureus*: диско-диффузионный метод, метод определения бета-лактамазной активности бактериальной взвеси с помощью тест-системы «БиоЛактам».

Результаты. Оказалось, что 80,6% терапевтических изолятов и 73,2% хирургических изолятов устойчивы к пенициллинам 1 поколения. К ингибитор-защищенным бета-лактамам были устойчивы 5,4% терапевтических изолятов и 34% хирургических изолятов. К цефалоспоринам 3 поколения были устойчивы 36,8% хирургических изолятов и ни одного терапевтического изолята. Среди терапевтических изолятов не было выявлено ни одного MRSA, а среди хирургических изолятов распространенность MRSA составила 30,5%. 93,3% терапевтических изолятов *S. aureus* не проявляли бета-лактамазной активности. Среди хирургических изолятов таких оказалось всего 34,4%. Выявлены прямые корреляции средней силы между уровнями бета-лактамазной активности изолятов *S. aureus*, длительностью госпитализации и продолжительностью лихорадочного периода, а также между выявлением MRSA и длительностью госпитализации, продолжительностью лихорадки и диарейного синдрома.

Заключение. Для эмпирической антибактериальной терапии терапевтических инфекций, вызванных *S. aureus*, рекомендовано использование ингибитор-защищенных пенициллинов и цефалоспоринов 3 поколения. Для эмпирической антибактериальной терапии стафилококковых инфекций хирургических и реанимационных отделений необходимы антибиотики резерва с избирательной антистафилококковой активностью, например, гликопептиды или оксазолидиноны.

Ключевые слова: клинические изоляты *S. aureus*, антибиотикорезистентность, MRSA, эмпирическая этиотропная терапия, механизмы антибиотикорезистентности *S. aureus*, терапевтические и хирургические отделения стационаров.

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ОРИТ – отделение реанимации и интенсивной терапии;
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INTRODUCTION

Staphylococcus aureus (S. aureus) is one of the most well-known pathogenic microorganisms that causes various human diseases. This microorganism is equally one of the most common causative agents of various hospital-acquired infections, primarily responsible for the development of numerous complications of invasive medical interventions. In the United States, up to 500,000 diseases and up to 50,000 deaths are associated with S. aureus infection annually [1, 2].

S. aureus is known for a variety of mechanisms of resistance to beta-lactam antibiotics. It is capable of producing classical type A-D beta-lactamases [3] and can acquire modifications of penicillin-binding proteins (PBP2a), thereby reducing significantly their affinity for beta-lactams and at the same time creating resistance to the so-called methicillin resistance. This can be seen in the total resistance of the corresponding strain to all beta-lactam antibiotics and revealed by the presence of resistance to indicator antibiotics (methicillin and oxacillin) [4]. The proportion of methicillin resistant *S. aureus* (the so-called MRSA) ranges from 17 to 40% [5].

The ratio of different mechanisms of resistance of *S. aureus* to beta-lactam antibiotics can vary significantly in different countries, depending on the approach adopted there for empirical prescription of antibiotics against purulent-inflammatory diseases. The origin of S. aureus isolates is also important. As with out-of-hospital infections, the proportion of MRSA is much lower than with hospital-acquired infections thereby making the detection rate of out-of-hospital pneumonia caused by MRSA 2.4% in average [6], while the frequency of registration of hospital-acquired pneumonia caused by MRSA is up to 10.5% of the total number of such pneumonia (up to 20–30% hospital-acquired pneumonia are caused by S. aureus, and up to 32–37% of the total number of isolates of *Staphylococcus aureus* isolated are methicillin-resistant [7, 8]). The figures given are averages because of the great variations noticed in various studies. The percentage of MRSA in the total number of clinical isolates of S. aureus seems to be a variable with multiple unknowns, and it is incorrect to extrapolate automatically the data obtained for other regions, even neighboring ones, to a certain region.

In addition, almost all studies analyzing the aspects of *S. aureus* antibiotic resistance were conducted abroad although most of the Russian researches on this issue were conducted in the 1990s or early 2000s, making the information gotten during that time outdated. Nevertheless, knowledge of the prevailing mechanism of antibiotic resistance of hospital-acquired and out-of-hospital *S. aureus* strains isolated in a certain region is critically important in determining the local approach of empirical etiotropic therapy for patients with suspected staphylococcal infections under corresponding conditions [9].

AIM

The work aimed to reveal the aspects of resistance to beta-lactam antibiotics in clinical isolates of *S. aureus* isolated in hospitals with various specialities, and to adjust the recommendations for empirical etiotropic therapy prescribed to patients with staphylococcal infections.

MATERIAL AND METHODS

The object of this study is *S. aureus* clinical isolates isolated from patients hospitalized at the Vitebsk Regional Clinical Infectious Diseases Hospital (VRCIH) (so-called "therapeutic isolates," n = 117), in the department of purulent surgery and especially in the resuscitation and intensive care unit (ICU) of the Vitebsk Regional Clinical Hospital (VRCH) (the so-called "surgical isolates," n = 111) in 2016–2017.

It should be noted that the difference between hospital-acquired and out-of-hospital *S. aureus* isolates is largely conditional, since neither laboratory nor epidemiological studies enable to establish with absolute certainty the hospital-acquired or out-of-hospital origin of a particular isolate. A hospital-acquired pathogen causes hospital infections and therefore differs fundamentally from an out-of-hospital pathogen, while hospital-

acquired infections are those that develop during hospitalization or when visiting a healthcare facility for treatment within 48 hours after hospitalization or visit [10]. In this case, the possibility of colonization of the patient's skin and mucous membranes with hospital-acquired strains of *S. aureus* during the previous hospitalization must be considered; as out-of-hospital staphylococcal infections in strict sense may actually be caused by hospital strains of *Staphylococcus aureus* [11]. Staphylococcal infections, which were formally hospital-acquired, may be associated with autoinfection with out-of-hospital flora persisting on the skin and mucous membranes of hospitalized patients [12].

S. aureus isolates from VRCIH were obtained from mainly pediatric patients with acute infectious gastroenteritis, enterocolitis, and upper respiratory tract infections of formal non-hospital origin. Isolates of *S. aureus* from the Department of Purulent Surgery and VRCH ICU were isolated from patients with infectious complications of surgical interventions and invasive manipulations that developed directly in a hospital and, according to formal criteria, were hospital-acquired. Nevertheless, neither in the first nor in the second case can it be stated with certainty that the isolates of *Staphylococcus aureu*s isolated in the infectious diseases hospital are unambiguously out-of-hospital, just as it is impossible to state unequivocally that the isolates of *Staphylococcus aureus* isolated from patients with surgical hospital infections, are undoubtedly hospital-acquired, for reasons stated above. We therefore conditionally designated *S. aureus* isolates isolated from VRCIH patients as "therapeutic," and isolates obtained from VRCH as "surgical," as in the first case they are most likely out-of-hospital, and hospital-acquired in the second case.

To isolate isolates of pathogenic microorganisms from the biological material of patients and assess their properties, conventional methods of microbiological research were used; disc diffusion analysis being the main technique used to determine the antibiotic resistance profile of the isolated isolates, conducted in accordance with the current international (CLSI) recommendations [13].

In order to detect quantitatively the beta-lactamase activity of pure cultures of microorganisms caused by the production of beta-lactamases of all classes, we used a spectrophotometric technique based on the registration of the decay of the beta-lactam bond of nitrocephin [14]. This technique was implemented using the BioLactam test system (Sivital, RB). The β-lactam bond in the nitrocefin molecule is known to be destroyed under the influence of all known beta-lactamases, while the absorption maximum of the resulting reaction product changes from 390 nm to 486 nm, enabling spectrophotometric assessment of the beta-lactamase activity level by determining the degree of the reaction substrate loss [15].

In the present study, the resistance of microorganisms to first generation penicillins (benzylpenicillin, ampicillin), inhibitor-protected beta-lactams (amoxicillin/clavulanate, piperacillin/tazobactam), and third generation cephalosporins (cefotaxime, ceftazidime), as well as oxacillin, was evaluated. The ability of the isolated *S. aureus* isolates to produce beta-lactamases was equally assessed using the BioLactam test system. In the event that the bacteria were resistant to the first generation penicillins, but sensitive to inhibitor-protected beta-lactams, and produced beta-lactamases, their resistance was believed to be due to the production of class A beta-lactamases. If the bacteria turned out to be resistant to the third generation cephalosporins, with their proven production of beta-lactamases, they were believed to produce extended-spectrum beta-lactamases (ESBLs). If the analysis showed that the isolate under study is resistant to all beta-lactams used in the study, including oxacillin, but does not produce beta-lactamases, then it was considered that this variant of *S. aureus* has modified PBP, i.e., is the MRSA. The rare cases in which the analyzed isolates of S. aureus were resistant to oxacillin, but sensitive to any other beta-lactam antibiotics, were regarded as laboratory artifacts and were excluded from further analysis.

Statistical processing of the study results included calculation of the central tendency and scatter of quantitative parameters, frequencies of qualitative parameters, 95% confidence intervals for all calculated statistical indicators, calculation of correlation coefficients (Spearman's ρ), and analyzing the statistical significance of differences (Mann-Whitney *U*-test for quantitative features, chi-square test for qualitative features) [8]. In the course of statistical processing, only nonparametric methods of analysis were used, and the normal distribution of continuous quantitative characteristics was not tested. The calculations were performed using the Statistical 10.0 and MedCalc 15.6 software.

RESULTS

80.6% (95% CI: 71.1–90.1) of the "therapeutic" *S. aureus* isolates analyzed in this study were resistant to penicillins (ampicillin, benzylpenicillin). Among the "surgical" isolates, the proportion of those resistant to these antibiotics was 73.2% (95% CI: 63.6–82.8). The difference between these indicators turned out to be statistically insignificant (Chi-square test, $p = 0.18$).

5.4% (95% CI: 1.2–9.6) of "therapeutic" isolates of Staphylococcus aureus were resistant to amoxicillin/clavulanate. 31.4% (95% CI: 16.1–46.8) of "surgical" isolates were resistant to amoxicillin/clavulanate, and 34.0% (95% CI: 20.5–47.6) were resistant to piperacillin/tazobactam. The difference in the levels of resistance of "therapeutic" and "surgical" *S. aureus* isolates to inhibitor-protected

beta-lactams was statistically significant (Chi-square test, $p < 0.0001$).

Not a single case of resistance of the studied "therapeutic" isolates of Staphylococcus aureus to cefotaxime and ceftazidime was revealed and 36.8% (95% CI: 26.0–47.7) of the "surgical" isolates were resistant to these antibiotics. The difference between the levels of resistance of "therapeutic" and "surgical" isolates to third generation cephalosporins was also statistically significant (Chi-square test, $p < 0.0001$).

Among the "therapeutic" isolates of *S. aureus*, no methicillin-resistant isolates were identified. Among the "surgical" isolates, their amount was 30.5% (95% CI: 20.5–40.5). The difference between the indicated frequencies of MRSA occurrence is statistically significant (Chi-square test, $p < 0.0001$).

Beta-lactamase production was absent in 93.3% of "therapeutic" isolates of *Staphylococcus aureus*; and only two isolates identified among them had relatively low beta-lactamase activity (1.7%; 95% CI: 0–4.06). Among the "surgical" isolates, only 34.4% (17.9–50.8) were not beta-lactamase producers. High beta-lactamase activity was detected in 6 "surgical" isolates (5.4%; 95% CI: 1.2–9.6), with the average level of beta-lactamase production noted in 14 isolates (12.6%; 95% CI: 6.4–18.8). The difference between the frequency of beta-lactamase production by "therapeutic" and "surgical" isolates of S. aureus is statistically significant (Chi-square test, $p = 0.0008$). Correlation analysis (Spearman) revealed a correlation of moderate strength ($R = 0.538$; $p = 0.0006$) between the "surgical" origin of the isolates and their ability to produce beta-lactamases.

Among other correlations identified in the course of this study, direct correlations of the average strength between the levels of beta-lactamase activity of S. aureus isolates and the duration of hospitalization ($R = 0.447$; $p = 0.005$), as well as the total duration of the febrile period ($R = 0.434$; $p = 0.013$) are not significant.

Patients of the Department of Purulent Surgery and ICU of VRCH compared with VRCIH patients stayed longer in the hospital ($R = 0.261$; $p = 0.027$), had more pronounced anemia ($R = 0.245$; $p = 0.008$), higher ESR ($R = 0.348$; $p = 0.024$), and a longer febrile period ($R = 0.411$; $p = 0.017$). Among the clinical isolates of *S. aureus*, MRSA was more common ($R = 0.612$; $p < 0.0001$).

Weak and moderate direct correlations were also found between the MRSA isolation and the duration of hospitalization of patients ($R = 0.353$; $p = 0.00008$), as well as between the total duration of fever ($R = 0.375$; $p = 0.0002$) and the duration of diarrheal syndrome ($R = 0.334$; $p = 0.005$). These correlations were noticed in the analysis of the entire studied sample of patients without dividing into "therapeutic" and "surgical."

DISCUSSION

Analysis of the results obtained show that out-of-hospital ("therapeutic") isolates of *S. aureus* have a significantly higher sensitivity to antibiotics of the beta-lactam series than hospital-acquired ("surgical") ones, and this applies to antibiotics such as: penicillins, aminopenicillins, third generation cephalosporins, inhibitor-protected beta-lactams, acting against the frequency of occurrence of MRSA and beta-lactamase-producing strains. These results are generally expected and clearly correspond to global trends [17, 18, 19]. However, data on specific levels of local resistance of clinical isolates of *S. aureus* of various origins ("therapeutic" and "surgical") to beta-lactams, as well as data on the local correlation of the mechanisms of this resistance (MRSA/beta-lactamase production), are new and are of certain clinical interest.

The results obtained revealed that the frequency of MRSA among the studied isolates of *S. aureus* is significantly lower than the frequency of producers of beta-lactamases, and the difference indicated is statistically significant (Chi-square test, $p < 0.0001$) with both the production of beta-lactamases and methicillin resistance noted almost exclusively among the "surgical" isolates.

Based on the results of the correlation analysis, the level of beta-lactamase production by clinical isolates of *S. aureus* can be directly proportional to the severity of the disease in the corresponding patients. There may nevertheless be a logical error, since a high level of beta-lactamase production was much more often registered in "surgical" isolates of *Staphylococcus aureus*, and they were isolated from patients of a purulent surgical department with secondary infectious complications of surgical interventions, as well as from ICU patients whose diseases, were significantly more severe than in patients of an infectious diseases hospital.

The high level of resistance of "surgical" isolates of *Staphylococcus aureus* to inhibitor-protected beta-lactams (31–34%), third generation cephalosporins (≈37%) is not that important, and so is the high incidence of methicillin resistance in these isolates (30.5%) compared to very low (near-zero) values of the corresponding indicators in "therapeutic" isolates. This assertion casts doubt on the possibility of using the corresponding antibiotics as a starting empiric antibiotic therapy for the treatment of staphylococcal infections in patients with purulent surgery departments and intensive care units. Etiotropic therapy in appropriate cases should be started immediately with the use of rescue antistaphylococcal drugs, such as glycopeptides, cyclic lipopeptides or oxazolidinones.

CONCLUSIONS

1. Clinical isolates of *S. aureus* (first of all, isolated in surgical and intensive care units of hospitals) are capable of producing beta-lactamases and exhibiting

methicillin resistance, with the production of beta-lactamases registered 2.2 times more often than resistance to methicillin/oxacillin.

2. Considering the high level (73–81%) of resistance of both "therapeutic" and "surgical" *S. aureus* isolates to penicillins including aminopenicillins, the use of these antibiotics for the initial empirical etiotropic therapy against staphylococcal infections seems inappropriate.

3. Taking into account the high sensitivity of "therapeutic" *S. aureus* isolates to third generation cephalosporins (100%), the use of these antibiotics as initial therapy seems appropriate when prescribing empirical etiotropic treatment for patients with apparent out-of-hospital staphylococcal infections. Inhibitor-protected beta-lactams, to which 94.6% of the "therapeutic" isolates are sensitive, can be used as alternative drugs.

4. Taking into account the high level of resistance of "surgical" *S. aureus* isolates to inhibitor-protected beta-lactams (31–34%), third generation cephalosporins (36.8%), as well as the high probability of methicillin resistance in such isolates (30.5%), it seems reasonable to start empiric etiotropic therapy against staphylococcal infections of hospital origin immediately with the prescription of reserve antistaphylococcal drugs, such as glycopeptides, cyclic lipopeptides or oxazolidinones.

Conflict of interest. *The authors declare no conflict of interest.*

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