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Peculiarities of antibiotic resistance of *Staphylococcus aureus* strains isolated from nosocomial infections

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Abstract

Aim. To study of the antibiotic resistance of *S. aureus* strains isolated from nosocomial infections (pneumonia, surgical site infections and sepsis) in a multidisciplinary surgical clinic.

Methods. Microbiological testing of sputum in 41 patients with pneumonia, of samples (wound, abscess, drainage) obtained from 40 patients with surgical site infections (SSI) and of blood from 46 patients with signs of sepsis was performed. The obtaining cultures were identified by conventional methods (including morphological, cultural, biochemical features, etc.). Disc diffusion method was used to detect methicillin-resistant *S. aureus* (MRSA) strains. Inducible clindamycin resistance (ICR) of *S. aureus* strains was determined by double disk approximation test (D-test).

Results. Methicillin-resistant *S. aureus* was found in 14.3% (2 of 14) of the patients with surgical site infections, in the sputum 27.3% (3 of 11) of the patients with pneumonia, and in the blood 50.0% (7 of 14) of the patients with sepsis (p >0,05). The rate of inducible clindamycin resistance of isolated *S. aureus* strains in patients with surgical site infections (2 of 14 cases, 14.3%) and with pneumonia (2 of 11 cases, 18.2%) did not statistically significant difference with the rate of methicillin resistance (p >0.05). However the rate was significantly lower septic infections — 7.1% and 50.0% respectively (p=0.0328).

Conclusion. Among *S. aureus* strains isolated from nosocomial infections, the rate of methicillin-resistant *S. aureus* had not depended on the type of nosocomial infection; the rate of inducible clindamycin resistance in septic infections was lower than resistance to methicillin.

Keywords: nosocomial infections, Staphylococcus aureus, methicillin-resistant Staphylococcus aureus (MRSA), inducible clindamycin resistance (ICR).

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Background

Staphylococcus aureus, the most widely known *Staphylococcus* species, plays a prominent role in the development of various pathological conditions in humans. Passing into almost all organs and systems of the body, *S. aureus* generally causes pyoinflammatory processes. Living on the surface of the skin and mucous membranes, these bacteria can cause not only local but also systemic infections (e.g., sepsis). This depends on the availability of numerous pathogenicity factors of *S. aureus*, such as cellular components that cause an inflammatory reaction, toxins that damage tissues, and aggressive enzymes that promote the spread of the pathogen and protect it from the action of body defense factors. Recently, an increase has been observed in the role of this bacterium in the development of healthcare-associated infections (HAI) [1–3].

Moreover, based on the sensitivity to methicillin, *S. aureus* can be divided into two groups: methicillin-sensitive *S. aureus* (MSSA–from the English methicillin-sensitive *S. aureus*) and methicillin-resistant *S. aureus* (MRSA–from the English methicillin-resistant *S. aureus*) strains. While the MRSA is mainly responsible for HAI, it is known that resistance to methicillin is associated with changes in the structure of penicillin-binding protein (PBP1; transpeptidase enzyme) in the *S. aureus* strains. Thus, compared to the natural strains, MRSA strains synthesize PBP2 instead of PBP1, which has a weak connection with penicillin. The PBP2 synthesis is encoded by the *MecA* gene [4,5].

The Staphylococci resistance to macrolides, lincosamides, and streptogramins B (MLSB resistance) is also of clinical significance and can be both constitutive and inducible. Erythromycin

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and clindamycin are antibiotics from the macrolide group and lincosamide groups, respectively. Antibiotics, by joining the 50S subunit of ribosomes in a bacterial cell disrupt protein synthesis, thus giving an antimicrobial effect.

The ribosome zona (ribosomal ribonucleic acid) that fixes to the antibiotic is methylated and remains inactive due to the inability of the antibiotic to interact with this zona. A constitutive resistance to clindamycin and erythromycin occurs due to the presence of a constant methylated zona of the ribosome which is provided by the erm gene class of the bacterial genome. However, if the MLSB resistance is caused by an inducing factor (for instance, erythromycin is an effective factor in the induction of MLSB-type resistance), then the inducible resistance develops. Despite the isolates being resistant to erythromycin, they show sensitivity to clindamycin in in vitro testing. However, in staphylococcal infections, especially those caused by MRSA, treatment with clindamycin is unsuccessful; although in vitro, these strains show sensitivity to clindamycin. The reason for this lack of the effect from the treatment is associated with the presence of *Staphylococcus*-inducible resistance to clindamycin (ICR–from the English inducible clindamycin resistance) [6, 7].

Aim

To study the features of antibiotic resistance in *S. aureus* strains isolated from HAI [pneumonia, surgical site infections (SSI), and sepsis] for patients admitted in the educational and surgical clinic of the Azerbaijan Medical University in 2014–2018.

Materials and methods

First, the microbiological study of sputum taken from 41 patients with symptoms of pneumonia was conducted. Morning sputum was used for the study. Second, a microbiological examination of the obtained materials (wound contents, drainage, and abscess) was made for 40 patients with SSI that occurred after a surgery. The drainage tube was treated with an antiseptic solution prior to the transfer of the contents of the drainage; a puncturing with a sterile syringe got the contents of the abscess. Samples were incubated for two days after seeding on various nutrient media, such as Muller-Hinton agar, blood agar, Endo medium or EMB agar, and Saburo media. The obtained cultures were then identified using generally accepted methods (taking into account morphological, cultural, biochemical, and other properties).

Next, a microbiological study of the blood of 46 patients with sepsis symptoms was made. For this study, the blood was immediately added to a liquid nutrient media (meat peptone broth) taken in a volume of ten times more than the volume of the test blood and incubated for a maximum of ten days at 37ºC. Samples were checked daily. When the broth turbidity appeared, it was transferred to the aforementioned nutrient media and incubated for two days, after which the resulting cultures were identified using common methods.

To determine the sensitivity of *S. aureus* to methicillin, the standard method of disk-diffusion on agar was used. The methicillin-resistant strains of *S. aureus* were evaluated as MRSA [5]. The ICR in *S. aureus* strains was determined by a phenotypic test (D-test) using two disks [7]. For this, a 2-μg disk with clindamycin and a 15-μg disk with erythromycin were placed 15 mm apart from each other on the surface of the Muller-Hinton agar with the inoculated *S. aureus* strain. The result was evaluated after one-day incubation at 37ºC in a thermostat. Accordingly, if the sterile zone around the clindamycin disk on the side of the erythromycin disk was restricted and a sterile zone in the shape of the letter D appeared, the presence of ICR in the *S. aureus* strains was established.

Besides, the statistical significance of differences between parts was determined using the Fischer's exact test [8] using an online calculator (https://www.socscistatistics.com/tests/fisher/default2.aspx).

Results

The frequency of *S. aureus* in various clinical forms of HAI did not vary significantly. *S. aureus* was one of the main etiological factors of pneumonia, SSI, and sepsis. Thus, 14 (35%) out of 40 patients with SSI, 14 (30.4%) of 46 patients with signs of sepsis, and 11 (26.8%) of 41 patients with pneumonia were found to have *S. aureus*.

Depending on the source, the isolated *S. aureus* strains also did not differ significantly in the frequency of resistance to methicillin. In SSI, the MRSA strain was detected relatively rarely: only 2 (14.3%) of the 14 *S. aureus* strains obtained were differentiated as MRSA. Likewise, among the *S. aureus* strains isolated from the sputum of patients with symptoms of pneumonia, MRSA was detected only in 3 (27.3%) of the 11 strains. However, MRSA was more common among *S. aureus* strains isolated from the blood of patients with sepsis symptoms: 7 (50%) out of 14 strains (Fig. 1).

Thus, a total of 12 strains of MRSA were obtained in pneumonia, SSI, and sepsis. Fig. 2 presents the spectrum of MRSA strains isolated in individual clinical forms of HAI.

Finally, summarizing the aforementioned results, it can be noted that depending on the source of production, *S. aureus* strains did not differ sig-

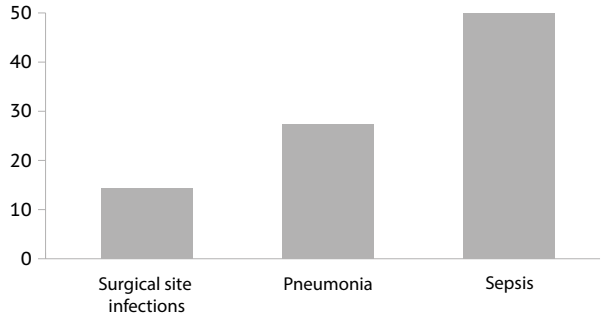

Fig. 1. Frequency of methicillin-resistant strains among all S. aureus strains in various clinical forms of surgical site infections (the ordinate axis indicates the number of strains as a percentage), $p > 0.05$.

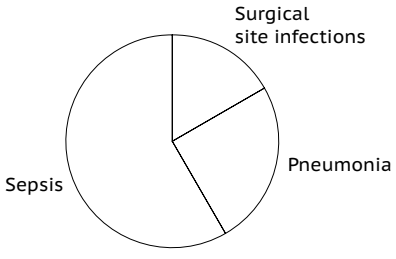

Fig. 2. A spectrum of methicillin-resistant strains isolated in various clinical forms of surgical site infections.

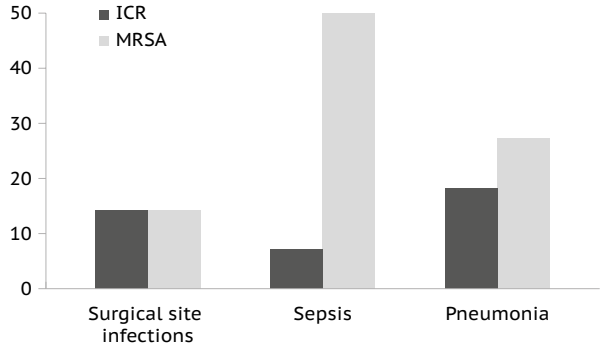

Fig. 3. Features of inducible resistance to clindamycin (ICR) in S. aureus strains (the percentage of strains is indicated on the ordinate axis); MRSA: methicillin-resistant S. aureus.

nificantly in their sensitivity and resistance to antibiotics and antimicrobials. Moreover, the MRSA strains were detected in 14.3% of the cases among the *S. aureus* strains isolated in SSI, 27.3% of those isolated in pneumonia, and 50% of those obtained from blood during sepsis ($p > 0.05$ compared to the *S. aureus* strains obtained in SSI and pneumonia). Thus, the methicillin resistance of the strains of *S. aureus* isolated in SSI and pneumonia was less common than in sepsis.

Further, as presented in Fig. 3, ICR was detected in 2 (14.3%) of the 14 strains of *S. aureus* isolated in the SSI ($p > 0.05$ relative to MRSA), 2 (18.2%) of the 11 strains from the sputum of patients with symptoms of pneumonia ($p > 0.05$ relatively to MRSA), and only 1 (7.1%) of the 14 strains from the blood of patients with sepsis, ($p = 0.0328$ relative to MRSA). Thus, no significant difference in the frequency was revealed by the study of ICR features in *S. aureus* strains obtained in various clinical forms of HAI.

Discussion

As noted, in our study, the frequency of *S. aureus* in different HAI was different, although insignificant. Depending on the source, the obtained *S. aureus* strains differed in their resistance to methicillin. The MRSA was detected in 14.3% of cases among the strains of *S. aureus* obtained in SSI, 27.3% among the strains isolated in pneumonia, and 50% among those obtained from blood in sepsis. Thus, the methicillin resistance of *S. aureus* strains isolated for SSI and pneumonia was less common than those isolated from the blood of patients with sepsis symptoms.

Likewise, according to the literature [9–11], the methicillin resistance of *S. aureus* strains, which is the main cause of HAI, varies within different limits. A study of 732 strains of microorganisms isolated from SSI reported that 28.3% of *S. aureus* were sensitive to methicillin, while 14.6% were MRSA [9]. In another research of 702 strains of microorganisms isolated from SSI, it was found that *S. aureus* was the main pathogen (20.4%), and the MRSA strain among all *S. aureus* strains was found in 72.0% of cases [10]. Among septic HAI and bacteremia, the number of bacteremia caused by MRSA strains increased annually by 7.6% prior to 2005, and the number of bacteremia caused by MSSA strains grew at 3.4% per year [11]. Therefore, the frequency of *S. aureus* detection, including MRSA strains in these studies can be compared with the results of this study.

The MRSA strains are usually not sensitive to macrolide antibiotics while being sensitive to non-β-lactam antibiotics. For this reason, we also analyzed the features of the ICR for *S. aureus* strains isolated during HAI. In isolated *S. aureus* strains, ICR was rarely found: in 2 (14.3%) of the 14 strains isolated for SSI, in 1 (7.1%) of the 14 obtained with sepsis, and in 2 (18.2%) of the 11 strains obtained from the sputum of patients with symptoms of pneumonia.

According to the literature, studies aimed at comparing resistance to methicillin and ICR in *S. aureus* strains are currently attracting attention [12, 13]. In a study of 190 *S. aureus* strains, the fea-

tures of the ICR were studied using a D-test, and a resistance to methicillin was determined by the Kirby Bauer's disc-diffusion method using the antibiotics oxacillin (1 mcg) and cefoxitin (30 mcg); 20 (10%) isolates had ICR, 18 (9%) had a constitutive resistance, and 16 (8%) had MSSA. The constitutive and inducible resistance in MRSA strains was more common than in the MSSA strains (20%, 16%, and 6%, 6%, respectively) [12].

In another study [12], we studied the features of ICR in *S. aureus* strains isolated from HAI and community-onset infections. Of the 402 *S. aureus* strains isolated, 52% strains had ICR. ICR was detected in 50% of MRSA strains and 60% of MSSA strains. However, among the *S. aureus* strains isolated from community-acquired infections, ICR was detected relatively rarely compared to hospital strains (33 and 55%, respectively).

Thus, it was found that among the strains of *S. aureus* isolated in HAI, the clinical form of hospital infection does not affect significantly the frequency of MRSA. The incidence of MRSA among *S. aureus* strains in septic infections is higher (50.0%) than in SSI (14.3%) and pneumonia (27.3%) ($p > 0.05$). The frequency of ICR in isolated *S. aureus* strains in SSI (14.3%) and pneumonia (18.2%) also did not differ from the frequency of MRSA ($p>0.05$); and in septic infections, ICR was less common than MRSA (7.1% and 50.0%, respectively, $p = 0.0328$).

Thus, the study of the features of antibiotic resistance of *S. aureus* strains isolated during HAI is of great interest and requires further in-depth research.

Conclusions

1. The frequency of methicillin-resistant strains does not depend on the clinical form of a hospital infection among *S. aureus* strains isolated from HAI.

2. The frequency of an inducible resistance to clindamycin in septic infections is lower than a resistance to methicillin.

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Conflict of interest. The authors declare no conflicts of interest.

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