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Evaluation of Antibiotic Susceptibility of Pathogen *Staphylococcus Aureus* Isolates

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Abstract In this article, the antibiotic susceptibility of Staphylococcus aureus strains isolated from clinical samples in March 2026 at the Bacteriology Laboratory of the Republican Scientific Center for Emergency Medical Care was assessed using the disk diffusion method (DDM). A total of 928 *Staphylococcus spp.* were isolated from 1785 clinical samples, of which 645 were *S. aureus* isolates. The susceptibility of the isolates to rifampicin, cefoxitin, linezolid, gentamicin, ciprofloxacin, and erythromycin was studied. The results showed that there was a significant difference in the level of antibiotic resistance between MRSA and MSSA strains.

Keywords *Staphylococcus aureus*, MRSA, MSSA, Antibiotic susceptibility, Disk diffusion method, Clinical isolate

1. Introduction

Staphylococcus aureus (*S. aureus)* infections are a global health problem. A number of antibiotics are used to treat infections caused by this bacterium, and they are aimed at affecting key bacterial intracellular processes such as cell wall synthesis, translation, transcription, and DNA synthesis. However, antibiotic resistance is an increasing problem, leading to treatment failures and high medical costs [2,9]. Antibiotic resistance arises through several mechanisms, such as targeted drug modifications, enzymatic drug inactivation, increased flux of antimicrobial compounds and altered drug entry, and the spread of resistance is facilitated by numerous mobile genetic elements. The resistance of this bacterium to antibiotics poses serious challenges in the effective treatment of infections [10,11]. In particular, MRSA (Methicillin-Resistant *Staphylococcus aureus*) strains limit the possibilities for treatment. Therefore, assessment of susceptibility to various antibiotics is important in selecting an optimal therapeutic approach [1,3]. *S. aureus* produces various enzymes, such as protease, lipase, and hyaluronidase, which degrade tissues. These bacterial products may facilitate the spread of infection to adjacent tissues, but their role in the pathogenesis of the disease is not well understood. β-lactamase, produced by the bacterial cell, is an enzyme that inactivates penicillin. Penicillin-binding proteins are enzymes located in the cytoplasmic membrane and are responsible for the resistance of *S. aureus* to penicillins and cephalosporins. The enzyme coagulase is a prothrombin activator that converts fibrinogen to fibrin [4,5].

Although resistance has been observed for almost all compounds, individual strains resistant to all drugs have not emerged. Nevertheless, resistance still poses a challenge to treatment. Fully resistant strains are rare, but despite a small increase in the prevalence of intermediate resistance, they are associated with more severe infections and longer duration of treatment than susceptible strains. This highlights that we have limited knowledge of how resistance genes and mutations affect the overall biology of resistant strains and the impact of resistance on pathogenesis [7,8]. In this article, we focus on the biology of antibiotic resistance in *S. aureus* and the behavior of resistant strains, and we conclude by describing some novel therapeutic approaches that may become future treatments for infections with antibiotic-resistant staphylococci.

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2. Materials and Methods

2.1. Hospital Setting

In accordance with the purpose of the microbiological study, the biological and pathogenic factors of these *S. aureus* strains were studied in detail in March 2026 using traditional methods in the “Adult Resuscitation Department”, “Children's Departments (I, II, somatic)” and “Emergency Cardiology Department” of the Republican Scientific Center for Emergency Medical Care of Uzbekistan (RSCEMCU). Biological materials from patients treated for acute respiratory infections, bronchitis, and unilateral and bilateral pneumonia were collected: nasopharyngeal swabs, sputum, venous blood, and urine. Collection of biological materials, bacteriological examinations of S. aureus by microscopy, cultivation in nutrient media, determination of sensitivity to antibiotics were carried out in the Bacteriology Laboratory of the RSCEMCU, MALDI–TOF–MS for species-specific identification in the Bacteriology Laboratory of the Department of Sanitary Epidemiological Control of the Main Medical Department under the Administration of the President of the Republic of Uzbekistan, and molecular genetic studies were carried out in the Laboratory of Special Devices of the National Research Institute of Biopharmaceutics.

2.2. Bacterial Strain and Culture Conditions

Microbiological characteristics of S. aureus were determined: morphological - by the Gram method, cultural - by fermentation of carbohydrates and production of carotenoid pigment of gold on MSA agar, and 5% blood agar was used to determine the hemolytic properties of the derivatives. Biochemical analyses of virulence factors - by the production of DNAse, hemolysin, plasmacoagulase, lecithinase enzymes were carried out.

2.3. MALDI–TOF–MS Analysis

MALDI–TOF–MS analysis for species-specific identification of isolated S. aureus strains with atypical biochemical characteristics. MALDI–TOF–MS was used to identify isolated colonies obtained on dense nutrient media. In order to confirm the results obtained by traditional cultural biochemical methods, identification was performed on a MALDI–TOF mass spectrometer "Microflex LRF" (Bruker Daltonics, Germany) in real-time mode using the Biotyper version 3.0 program, according to the manufacturer's instructions. The conclusion about the taxonomic affiliation of the microorganism was made based on the value of the similarity index (score - parameter C). The value of C from 1.7 to 1.99 indicates identification up to the genus, and C > 2 indicates identification up to the species and identification to the exact species. A score below 1.7 was scored as unreliable identification.

2.4. Statistical Analysis

The data obtained during the study were evaluated using parametric and nonparametric analysis methods. The data were collected and stored in an Excel database. The comparison of numerical data and the assessment of the significance of their differences were carried out using the Pearson $\chi 2$ test.

3. Results

3.1. A total of 1785 clinical samples analysis was done. A total of 1785 clinical samples were analyzed from various departments of the Republican Scientific Center for Emergency Medical Care. Among the patients, 48% were female and 52% were male, comprising the following age groups: 0–20 years (65.11%), 20–40 years (20.9%), 41–60 years (6.9%), and over 60 years (6.9%).

928 *Staphylococcus spp. from* 1785 samples was determined. An age-based analysis revealed that S. aureus infections occur predominantly in the age group up to 20 years. Complications are observed to be fatal, especially in young children.

3.2. 645 of them *S. aureus* formation during the study, a total of 645 biological samples were subjected to bacteriological analysis and the frequency of *S. aureus* was assessed across sample types (Figure 1). According to the results, this microorganism was most frequently detected in throat swab samples, with 420 samples, which was 65.12% of the total samples.

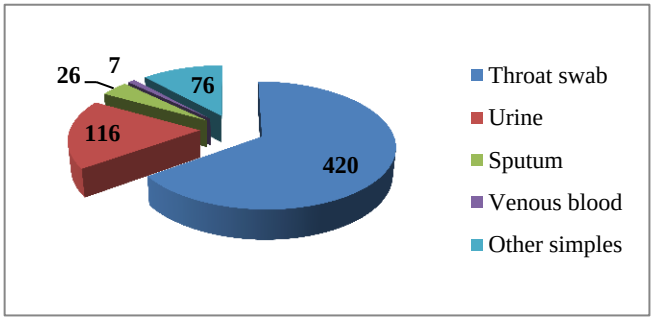

Figure 1. Rate of occurrence of *Staphylococcus aureus* in clinical samples

This indicator indicates that *S. aureus* is widespread in the upper respiratory tract, especially in the larynx and nasopharynx. This confirms the high colonization ability of this microorganism on human mucous membranes. Urine samples were in second place. *S. aureus* was detected in 116 cases, which was 18.0% of the total samples. This result indicates that *S. aureus* also has a certain significance in infections of the genitourinary system. Especially in patients treated in inpatient conditions, catheterization and other invasive procedures may cause an increase in this indicator. In the group of other samples, *S. aureus* was found in 76 cases, which was 11.8%. This group may include various clinical materials, such as wound swabs, skin and soft tissue samples, ear and nose swabs. This indicates the high ability of *S. aureus* to cause infectious processes in various anatomical loci. In sputum samples, the microorganism was detected in 26 cases, accounting for 4.0% of the total. This result indicates that *S. aureus* also plays a certain role in lower respiratory tract infections, including bronchitis and pneumonia. Although interest Although relatively low, this

pathogen in respiratory infections clinical significance important is considered. less to meet status venous blood in the examples observed - 7 cases, i.e. 1.1%. Blood *S. aureus* in samples of detection of bacteremia and septic conditions with related to be possible. Interest that the amount is low despite this situations high clinical risk birth with significant is considered.

3.3. 420 strains of *S. aureus,* identified by the classical method with atypical biochemical characteristics, were re-identified using MALDI–TOF–MS analysis. The protein profiles and species of *the S. aureus* bacteria studied by the MALDI–TOF–MS method were determined. The species identification of the isolates fully corresponded to their biochemical identification. **When assessing the susceptibility of** *S. aureus* to antibiotics by the disk diffusion method, we confirmed that strains resistant to cefoxitin (the growth inhibition zone was 22 mm) were MRSA (positive for the mecA gene 65.12% (420 isolates) of the 645 samples analyzed were MRSA and 34.88% (225 isolates) of the 645 samples analyzed were MSSA. The main results of assessing the susceptibility of phenotypically isolated MRSA and MSSA to antibiotics were as follows (Table 1).

MSSA strains are susceptible to most antibiotics, meaning that there are many effective options for treating these infections. The highest susceptibility rates in our studies were observed for Cefoxitin, Clindamycin, Tetracycline, and Rifampicin. However, the choice of antibiotic should also take into account the patient's general condition, the site of infection, and potential side effects.

The results of the analysis confirmed that all MRSA strains were completely resistant to the antibiotics Penicillin and Cefoxitin (0%). These results once again prove that these strains are resistant to methicillin (Table 2).

It serves as an important source of information for the selection of antibiotics for the effective treatment of MRSA infections. Linezolid, Rifampicin, Amikacin and Gentamicin are recommended as the most effective agents against MRSA. The conducted research has shown that traditional beta -lactam antibiotics are ineffective against MRSA and their use in treatment is not recommended.

Table 1. Methicillin-susceptible Staphylococcus aureus (MSSA) N-225 indicator of antibiotic sensitivity

Name of antibiotics	Number of susceptible strains (n)	Sensitivity (%)	Zone diameter (mm)	Results reliability
Penicillin (10 μg)	73	32.59%	26.0 ±0.8	P ≤0.05
Cefoxitin (30 μg) (MRSA screening test)	225	100%	27.0 ±0.6	P ≤0.05
Ciprofloxacin (5 μg)	208	92.59%	52.1 ±1.4	P ≤0.05
Amikacin (30 μg)	212	94.44%	16.8 ±0.7	P ≤0.05
Gentamicin (10 μg)	196	87.03%	19.4 ±0.8	P ≤0.05
Erythromycin (15 μg)	192	85.18%	22.6 ±1.1	P ≤0.05
Clindamycin (2 μg)	220	97.77%	23.4 ±0.9	P ≤0.05
Tetracycline (30 μg)	223	99.25%	24.1 ±0.7	P ≤0.05
Linezolid (10 μg)	220	97.77%	23.2 ±0.6	P ≤0.05
Rifampicin (5 μg)	223	99.25%	31.2 ±0.9	P ≤0.05

$\chi 2 = 28.78$ p-value ≈ 0.0007 $p < 0.05$ There is a statistically significant difference between the susceptibility levels of MSSA strains to different antibiotics.

Table 2. Methicillin-resistant *Staphylococcus aureus (MRSA)* N-420 (%) indicator of antibiotic sensitivity

Antibiotics name	Sensitive number of strains (n)	Sensitivity (%)	Zone diameter (mm)	Results reliability
Penicillin (10 μg)	0	0%	6.2 ±0.2	P ≤0.05
Cefoxitin (30 μg) (MRSA screening test)	0	0%	6.1 ±0.1	P ≤0.05
Ciprofloxacin (5 μg)	365	86.95%	51.4 ±1.6	P ≤0.05
Amikacin (30 μg)	411	97.80%	17.2 ±0.7	P ≤0.05
Gentamicin (10 μg)	401	95.48%	18.6 ±0.6	P ≤0.05
Erythromycin (15 μg)	187	44.51%	16.4 ±1.2	P ≤0.05
Clindamycin (2 μg)	262	62.31%	17.1 ±1.1	P ≤0.05
Tetracycline (30 μg)	277	65.96%	21.2 ±0.9	P ≤0.05
Linezolid (10 μg)	412	98.17%	24.1 ±0.6	P ≤0.05
Rifampicin (5 μg)	414	98.65%	30.4 ±0.8	P ≤0.05

$\chi 2 = 297.62$ p-value $= 8.36 \times 10^{-59}$. $p < 0.05$ There is a statistically significant difference between the susceptibility levels of MRSA strains to different antibiotics.

Therapeutic approach. The therapeutic approach to diseases caused by *S. aureus* depends on the type of infection, the patient's age, clinical manifestations of the disease, concomitant pathology, the sensitivity of the pathogen to antibacterial drugs, and hospitalization. Various drugs are used for treatment, both as monotherapy and in combination. This situation once again confirms the importance of relying on local antibiogram data when choosing an empirical antibiotic.

4. Conclusions

The present study demonstrated a high prevalence of MRSA among the clinical *S. aureus* isolates investigated. Of the 645 *S. aureus* isolates identified, 420 (65.12%) were classified as MRSA, whereas 225 (34.88%) were identified as methicillin-susceptible *S. aureus* (MSSA). These findings indicate a substantial burden of methicillin resistance among clinical *S. aureus* isolates and highlight the importance of continuous antimicrobial susceptibility surveillance in hospital settings.

The disk diffusion susceptibility testing revealed distinct antimicrobial susceptibility patterns between MRSA and MSSA isolates. MRSA isolates demonstrated complete resistance to penicillin and cefoxitin, confirming the phenotypic characteristics of methicillin resistance. In contrast, MSSA isolates showed comparatively higher susceptibility to the tested antimicrobial agents. Among the MRSA isolates, the highest susceptibility rates were observed for linezolid, rifampicin, amikacin, and gentamicin, whereas greater resistance was observed to erythromycin and clindamycin.

Clinically, these findings emphasize that empirical treatment of suspected S. aureus infections should take into consideration the high local prevalence of MRSA and the antimicrobial susceptibility profile of circulating strains. Reliance on local antibiogram data, together with microbiological identification and susceptibility testing, is essential for rational antibiotic selection, optimization of antimicrobial therapy, and reduction of inappropriate antibiotic use.

The observed differences in susceptibility between MRSA and MSSA isolates were statistically significant, supporting the clinical relevance of differentiating these two phenotypic groups during laboratory diagnosis and therapeutic decision-making. Therefore, routine surveillance of antimicrobial resistance, early laboratory identification of MRSA, and continuous monitoring of changes in susceptibility patterns are important components of effective infection control and antimicrobial stewardship.

Overall, the findings indicate that *S. aureus* remains an important clinical pathogen, with a substantial proportion of isolates exhibiting methicillin resistance. Strengthening microbiological surveillance and implementing susceptibility -guided therapy may contribute to improved clinical outcomes and more effective management of staphylococcal infections.

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