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SENSIBILITATEA LA ANTIBIOTICE A TULPINILOR DE *STAPHYLOCOCCUS AUREUS* IZOLATE DIN INFECȚIILE DE PLAGĂ

SENSITIVITY TO ANTIBIOTICS OF *STAPHYLOCOCCUS AUREUS* STRAINS ISOLATED FROM WOUND INFECTIONS

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Rezumat
Introducere. Infecția de plagă, cauzată de *Staphylococcus aureus*, este o infecție frecvent întâlnită în practica medicală. Cunoaşterea fenotipurilor de rezistență, respectiv a sensibilității la antibiotice a tulpinilor circulante, precum şi emergența tulpinilor meticilino-rezistente, motivează scopul acestui studiu ca și suport al inițierii unei terapii precoce adecvate.
Material și metode. A fost analizată sensibilitatea la antibiotice a 183 tulpini de *Staphylococcus aureus*, izolate din infecțiile de plagă. Identificarea bacteriilor a fost efectuată folosind sistemul Vitek 2 Compact. Sensibilitatea la antibiotice s-a testat prin metoda difuzimetrică şi sistemul Vitek 2 Compact.
Rezultate. Din cele 183 tulpini testate, 108 (59,0%) au fost meticilino-rezistente, 75 (41,0%) meticilino-sensibile. La macrolide şi lincosamide tulpinile testate au prezentat sensibilitate redusă, fenotipul de rezistență dominant fiind cel inductibil. Față de aminoglicozide, din totalul tulpinilor analizate 45,9% s-au dovedit rezistente la tobramicină, 39,3% la gentamicină şi 13,7% la amicacină. Tulpinile au arătat o rezistență crescută la tetraciclină (48,2%) și ciprofloxacină (42,1%), și redusă la linezolid (1,6%). Toate tulpinile testate au fost sensibile la vancomicină.
Concluzii. Tulpinile meticilino-rezistente au reprezentat 59,0% din tulpinile testate. Vancomicina şi linezolidul sunt preparatele care rămân a fi indicate în tratamentul empiric al infecțiilor severe cauzate de S. aureus.
Cuvinte-cheie: *Staphylococcus aureus*, infecții infecții de flux sangvin, rezistență la antibiotice

Summary
Introduction. Wound infection, caused by *Staphylococcus aureus*, is frequently encountered in medical practice. Knowing the resistance phenotypes, respectively the sensitivity to antibiotics of circulating strains, as well as the emergence of methicillin-resistant strains, motivates the purpose of this study as a support for the initiation of appropriate early therapy.
Material and methods. The sensitivity to antibiotics of 183 strains of *Staphylococcus aureus,* isolated from wound infections, was analyzed. Bacterial identification was performed using the Vitek 2 Compact system. Sensitivity to antibiotics was tested by the diffusimetric method and the Vitek 2 Compact system.
Results. Out of the 183 strains tested, 108 (59.0%) were methicillin-resistant, 75 (41.0%) methicillin-sensitive. To macrolides and lincosamides, the tested strains showed reduced sensitivity, the dominant resistance phenotype being the inducible one. Regarding aminoglycosides, 45.9% of all analyzed strains were resistant to tobramycin, 39.3% to gentamicin and 13.7% to amikacin. The strains showed increased resistance to tetracycline (48.2%) and ciprofloxacin (42.1%), and reduced resistance to linezolid (1.6%). All strains tested were susceptible to vancomycin.
Conclusions. Methicillin-resistant strains represented 59.0% of the strains tested. Vancomycin and linezolid are the drugs that remain to be indicated in the empiric treatment of severe infections caused by S. aureus.
Keywords: *Staphylococcus aureus*, bloodstream infections, antibiotic resistance

Introduction

Staphylococcus aureus commensal to human skin and mucous membranes could cause nosocomial and systemic infections. *S. aureus* is also a leading cause of serious infections, such as bacteremia or infective endocarditis, which can have serious consequences for the patient. High morbidity and mortality are associated especially with the widespread occurrence of methicillin-resistant *S. aureus* (MRSA) strains [1].

Antimicrobial resistance (AMR) poses a significant threat to global public health and was estimated to cause nearly 5 million deaths; in 2019, it directly led to 1.27 million deaths worldwide [2].

Methicillin-resistant *S. aureus* (MRSA) frequently causes disease outbreaks and has become endemic in many regions, adding to the morbidity, mortality, and cost of care associated with hospital-acquired infections. In 2019, MRSA emerged as the leading pathogen-drug combination of AMR, causing 13 800 and 121 000 deaths in European countries and worldwide, respectively [3].

The excessive use of new antibiotics with increased antibacterial effectiveness, the increase in the number of immunosuppressed patients, prolonged hospitalization, strict non-compliance with infection control measures by the nursing staff, are the main risk factors for the increase of bacterial resistance to antibiotics. Considering the continuous evolution of the antibiotic resistance phenomenon, including MRSA, it is important to accurately establish the antibiotic sensitivity profile of circulating *S. aureus* strains. At the same time, the global surveillance of antibiotic resistance, through programs such as ICARE, SENTRY, MYSTIC, and EARSS, draws attention to the importance of implementing local studies or national surveillance programs to highlight circulating phenotypes, in order to guide empiric antibacterial therapy in clinical situations that require the initiation of an early antibacterial therapy [4, 5, 6, 7].

The aim of this study was to evaluate the antimicrobial susceptibility pattern of *S. aureus* isolated from wound infections.

Material and methods

A retrospective study was conducted in which we analyzed the antibiotic susceptibility results of 183 strains of *Staphylococcus aureus* isolated from infections. The isolates came from hospitalized patients with wound infections. Bacterial identification was performed using the Vitek 2 Compact system (bioMérieux, France). Antibiotic sensitivity testing was carried out by two methods: diffusimetric - Kirby-Bauer and determination of minimum inhibitory concentrations - automatically in the Vitek 2 Compact system. Antibiotic testing and sensitivity interpretation were standardized, following the EUCAST standard (European Committee on Antimicrobial Susceptibility Testing).

Results and discussions

In the early 1940s, immediately after the application of penicillin in the therapy of infectious diseases, less than 1% of *Staphylococcus aureus* strains showed resistance to this drug. The incidence of penicillin resistance of *S. aureus* increased significantly in the following years, so that in Great Britain, in 1946, 6% of strains were resistant to penicillin, and in the USA at the end of the 1950s, 40% of *S. aureus* strains isolates were resistant Since 1960, the incidence of penicillin resistance has increased to over 60% of the strains, currently reaching a percentage of over 90%. Later, 2 years after the introduction of methicillin in therapy, in 1961 the first MRSA strain was described in Great Britain, the first VISA strain in Japan in 1996, and in 2002 the first VRSA strain was isolated in the USA [7]. Resistance to streptomycin, tetracycline, chloramphenicol and erythromycin is described with their introduction in the therapy of infections caused by penicillin-resistant staphylococci. Multiple antibiotic resistance (MDR) recorded an increased prevalence in the 1950s; in 1959 in Seattle, more than 40% of hospital strains resistant to four or more antibiotics were described [8,9]. The appearance of resistance to aminoglycosides was recorded after 10 years of their excessive use, and in 1976 the first MRSA strain with associated resistance to gentamicin was isolated [9]. The antibiotic sensitivity of the tested S. aureus strains is shown in Figure 1.

The mechanisms by which staphylococci acquire resistance to β-lactams are known: the synthesis of penicillinases and the modification of the action target of β-lactams, with the synthesis of alternative PBPs - PBP 2a to which β-lactams will no longer bind, a consequence of the acquisition in the bacterial chromosome of the mecA gene. The phenotype associated with penicillinases is penicillin-resistant - methicillin-sensitive, confers resistance to narrow-spectrum penicillins and is inhibited by clavulanic acid, and the one related to the presence of PBP 2a penicillin-resistant - methicillin-resistant - causes cross-resistance to all β-lactams, being frequently associated with resistance to other groups of antibiotics. The BORSA phenotype (Borderline Oxacillin Resistant *Staphylococcus aureus*) - borderline resistance is the consequence of the excess production of penicillinases and has the associated less expressed resistance; In the treatment of infections caused by such strains, combinations of β lactams and β lactamase inhibitors can be used [10]. Currently, few strains have retained their sensitivity to penicillins sensitive to the action of penicillinase, with 10% of strains presenting the penicillin-S - methicillin-S phenotype. This phenotype, if not associated with resistance to other groups of antibiotics, describes the wild strain.

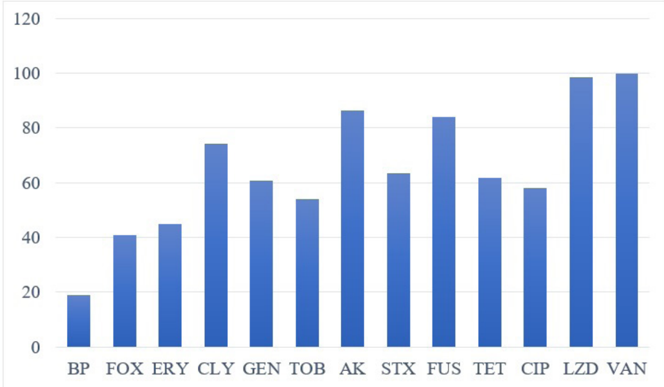

Figure 1. *Sensitivity of S. aureus strains to the tested antibiotics (BP – Benzylpenicillin, FOX – Cefoxitin, ERY – Erythromycin, CLY – Clindamycin, GEN – Gentamicin, TOB – Tobramycin, AK – Amikacin, STX – Trimethoprim-Sulfamethoxazole, FUS – Fusidic acid, CIP – Ciprofloxacin, LZD – Linezolid, VAN – Vancomycin).*

The beta-lactam sensitivity evaluation of the tested strains showed the highest sensitivity: methicillin-resistant strains (MRSA) - 108 (59.0%), methicillin-sensitive (MSSA) 75 strains (41.0%), the wild phenotype in 12 (6.6 %) stems.

Resistance to macrolides-lincosamides-streptogramins B (MLSB) is acquired through the synthesis of plasmidically transferred methylation enzymes or efflux mechanisms. Methylation enzymes are encoded by the erm genes. The phenotypic expression of this resistance can be inducible - coded ermC = MLSBi phenotype or constitutive resistance - coded ermB = MLSBc phenotype. Strains with MLSBc phenotype are resistant to macrolides, lincosamines, and streptogramin B and sensitive to ketolides. In strains with the MLSBi phenotype, the penetration of the macrolide induces the synthesis of methylation enzymes, which will methylate the RNA expressing the resistance; ketolides retain their sensitivity. It is determined in vitro by the D test, a test that consists of observing the presence of antibiotic antagonism between the bio-disc of clindamycin and erythromycin in strains with MLSBi phenotype. Efflux resistance (MLSBe) is encoded by the msrA genes, the phenotypic expression being the M phenotype, which confers resistance only to macrolides (erythromycin R, clindamycin S). This phenotype occurs in 1-2% of strains. Ignorance of the MLSBi phenotype identification method in bacteriology laboratory practice has the consequence of false reporting of sensitivity to clindamycin [11, 12, 13].

The strains tested showing the MLSBi phenotype were reported to be resistant to clindamycin. The majority of MSSA strains (81.3%) showed a wild phenotype with sensitivity to MLSB and a relatively low percentage of inducible resistance (10.7%). MRSA strains mainly had MLSBi phenotype (44.4%), followed by constitutive (23.1%). Compared to erythromycin, the strains tested showed a high degree of resistance, especially MRSA (81.5%).

Resistance to aminoglycosides (AG) is primarily enzymatic, through the action of enzymes that modify aminoglycosides (AME). Of these, three types are mainly found in staphylococci, having particular significance, because they inactivate the main therapeutically important AGs: aminoglycoside-6'-N-acetyltransferase/2"-Ophosphoryltransferase [AAC(6')/APH(2")] - encoded by the gene aac(6')-Ie-aph(2"), bifunctional enzyme that determines the KTG phenotype with resistance to gentamicin, kanamycin, tobramycin, neomycin, amikacin; aminoglycoside-4'- O -nucleotidyltransferase I [ANT(4')-I] - encoded by the ant(4')-Ia gene inactivates kanamycin, tobramycin, neomycin, amikacin - KT phenotype and aminoglycoside-3'- O - phosphoryl transferase III [APH(3')-III] - encoded by the aph(3')-IIIa gene determines the K resistance phenotype by acting on kanamycin, neomycin. In MSSA strains, the dominant phenotype observed was the wild type, with sensitivity to all AGs. MRSA strains proved to be more resistant to AG – 45.9% showed resistance to tobramycin, 39,3% resistance to gentamicin, and 13.7% resistance to amikacin. It is recommended to use AG in combined therapy because its use as a single antimicrobial agent predisposes to the emergence of resistance [8, 14].

Two resistance mechanisms have been described for tetracyclines: through active efflux (acquisition of the tetK, tetL genes) - minocycline remains active but with the possibility of inducible resistance to doxycycline and chromosomal resistance (encoded by the tetM, tetO genes), which determines resistance including to minocycline. In the present study, only sensitivity to tetracycline was determined, with 113 strains (61.78%) proving sensitive (fig. 1). Note the high level of resistance in MRSA strains (83.3%), compared to MSSA strains (30.7%).

To trimethoprim-sulfamethoxazole, 49.0% of MRSA strains showed resistance and 18.7% MSSA strains. Even if the Food and Drug Administration has not approved the use of trimethoprim-sulfamethoxazole in staphylococcal infections, the existence of numerous studies that prove sensitivity *in vitro* between 95-100%, underlines the fact that trimethoprim-sulfamethoxazole can be an important therapeutic option. The testing of strains to fusidic acid also showed a low level of resistance, respectively 29 strains (15.8%) of the total strains tested. In the British guideline for therapy and prophylaxis of infections caused by MRSA, a study is shown in which the percentage of resistance to MRSA is 9.3%, but the same guideline contraindicates therapeutic administration, due to high toxicity. Staphylococci acquire resistance to quinolones through two mechanisms: point mutations at the level of the chromosomal genes that encode topoisomerases, more rarely through efflux pumps mediated by the transport protein norA. Opting for fluoroquinolone therapy is recommended to be done according to the antibiogram [15, 16]. As shown in Figure 1, 42.1% of strains of S. aureus were resistant to ciprofloxacin. MRSA strains showed resistance to ciprofloxacin in 56.5% (61 strains), in contrast to MSSA, in which resistance was observed in 21.3% (16 strains). 100% sensitivity was obtained for vancomycin, and 98.4% for linezolid.

Conclusions

In *S. aureus* strains isolated from bloodstream infections, the most frequently observed phenotype was methicillin-resistant. MRSA strains represented 59.0% of the tested strains and showed an increased associated resistance to macrolides, lincosamides, aminoglycosides, and tetracycline. The wide use in medical practice of erythromycin and clindamycin explains the high percentages of resistance observed and raises the issue of their use in the therapy of staphylococcal infections. Although the strains tested showed increased sensitivity to fusidic acid, the proven high toxicity limits its applicability as a therapeutic agent. Although the specialized literature cites the emergence of strains resistant to vancomycin, all tested strains proved sensitive to this antibiotic.

Ethical considerations

The study was approved by the Ethics Committee of the Nicolae Testemitanu State University of Medicine and Pharmacy (approval number 3 from 14.04.2023).

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