SENSITIVITY TO ANTIBIOTICS OF STRAINS OF STAPHYLOCOCCUS AUREUS ISOLATED FROM GENITAL INFECTIONS

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Keywords:
Staphylococcus aureus, genital infections, antibiotics resistance

Abstract: *Objectives: Genital infections caused by Staphylococcus aureus are common in medical practice and therefore, knowing the resistance phenotypes, namely the sensitivity to antibiotics of the circulating strains, as well as the emergence of methicillin-resistant strains, is a major goal motivating this study to start an appropriate early therapy. Material and method: I studied the antibiotic sensitivity of 420 strains of Staphylococcus aureus isolated from vaginal secretions during the period June 2010 - December 2011. The strains were isolated and identified in the laboratory of Filantropia Hospital of Craiova. The isolates were from patients hospitalized in the Department of Obstetrics and Gynaecology I of the Filantropia Hospital of Craiova. Specimens were seeded on selective and nonselective culture media and the identification was based on culture and morpho-dyeing characteristics, on the presence of coagulase and on biochemical properties. Antibiotic susceptibility testing was performed by two methods: diffusimetric - Kirby-Bauer and the determination of minimum inhibitory concentrations between two break points. Results and discussions: Of the 420 studied strains, 121 (28.8%) were methicillin-resistant, and 299 (71.2%) methicillin-sensitive. The tested strains showed reduced susceptibility to macrolides and lincosamides, the dominant resistance phenotype was not inducible. Regarding aminoglycosides, 38.09% of the analyzed strains were resistant to kanamycin, 13.09% to tobramycin, and 10.95% to gentamicin. The strains showed high resistance to tetracycline (45.95%), relatively low resistance to ciprofloxacin (10.95%) and low resistance to trimethoprim-sulfamethoxazole (3.09%). Conclusions: Methicillin-resistant strains represented 28.8% of the tested strains. All strains tested were sensitive to vancomycin and linezolid. In case of drug-resistant strains, trimethoprim-sulfamethoxazole may be a therapeutic option.*

Cuvinte cheie:
Staphylococcus aureus, infecţii genitale, rezistenţă la antibiotice

Rezumat: *Obiectiv: Infecţiile genitale, determinate de Staphylococcus aureus sunt frecvent întâlnite în practica medicală şi de aceea, cunoaşterea fenotipurilor de rezistenţă, respectiv a sensibilităţii la antibiotice a tulpinilor circulante, precum şi emergenţa tulpinilor meticilino-rezistente, reprezintă un deziderat major care motivează scopul acestui studiu pentru iniţierea unei terapii precoce adecvate. Material şi metodă: În perioada iunie 2010 - decembrie 2011 am studiat sensibilitatea la antibiotice a 420 tulpini de Staphylococcus aureus, izolate din infecţii genitale. Tulpinile au fost izolate şi identificate în Laboratorul Spitalului Filantropia Craiova. Izolatele au provenit de la paciente spitalizate în secţia de Obstetrică-Ginecologie I a Spitalului Filantropia Craiova. Produsele patologice au fost însămânţate pe medii de cultură selective şi neselective, iar identificarea s-a făcut pe baza caracterelor de cultură, morfo-tinctoriale, prezenţei coagulazei şi a proprietăţilor biochimice. Testarea sensibilităţii la antibiotice s-a efectuat prin două metode: difuzimetrică - Kirby-Bauer şi de determinare a concentraţiilor minime inhibitorii între două puncte de ruptură. Rezultate şi discuţii: Din cele 420 tulpini studiate, 121 (28,8%) au fost meticilino-rezistente, 299 (71,2%) meticilino-sensibile. La macrolide şi lincosamide tulpinile testate au prezentat sensibilitate redusă, fenotipul de rezistenţă dominant fiind cel inductibil. Faţă de aminoglicozide, din totalul tulpinilor analizate, 38,09% s-au dovedit rezistente la kanamicină, 13,09% la tobramicină şi 10,95% la gentamicină. Tulpinile au arătat o rezistenţă crescută la tetraciclină (45,95%), relativ redusă la ciprofloxacin (10,95%) şi redusă la trimetoprim-sulfametoxazol (3,09%.) Concluzii: Tulpinile meticilino-rezistente au reprezentat 28,8% din tulpinile testate. Toate tulpinile testate au fost sensibile la vancomicină şi linezolid. În cazul tulpinilor multirezistente, trimetoprim-sulfametoxazolul poate constitui o opţiune terapeutică.*

INTRODUCTION

Genital infections are common in medical practice, their severity ranging from mild to potentially life-threatening forms.

Excessive use of antibiotics, frequent or prolonged hospitalization, failure to strictly observe the infection control measures by the healthcare professionals are the main risk factors for increased bacterial resistance to antibiotics.

Given the continuous evolution of the phenomenon of resistance to antibiotics, including methicillin-resistant S. aureus (MRSA), it is important to accurately determine the antibiotic

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sensitivity profile of circulating S. aureus strains. At the same time, global surveillance of antibiotic resistance through programmes such as ICAR, SENTRY, MYSTIC, warns about the importance of implementing local studies or national surveillance programmes to highlight the circulating phenotypes in order to guide empirical antimicrobial therapy in clinical situations requiring the initiation of an early antibacterial therapy.(1)

PURPOSE

The purpose of the study is to establish the sensitivity to antibiotics of Staphylococcus aureus in order to improve the therapy of vaginal infections with this germ.

METHODS

I studied the antibiotic sensitivity of 420 strains of Staphylococcus aureus isolated from vaginal secretions during the period June 2010 - December 2011.

The strains were isolated and identified in CDT Plus Medica Laboratory, Craiova and the laboratory of Filantropia Hospital of Craiova. Specimens were seeded on selective and nonselective culture media and the identification was based on culture and morpho-dyeing characteristics, on the presence of coagulase and on biochemical properties. The antibiotic sensitivity tests were done by two methods: diffusimetric - Kirby-Bauer and the determination of minimum inhibitory concentrations between two break points. Antibiotic susceptibility testing and interpretation were done in a standardized manner, following the current CLSI guidelines (Clinical and Laboratory Standards Institute) for testing antibiotics.(2)

RESULTS

I evaluated the sensitivity to beta-lactams of strains of S. aureus isolates and I found the following: 121 strains, i.e. 28.8% were resistant to methicillin (MRSA) and 71.2%, i.e. 299 strains were susceptible to methicillin (MSSA).

To determine the antibiotic resistance phenotypes of S. aureus strains isolated, I determined the sensitivity of these strains to macrolides-lincosamides-streptogramins B (MLSB). I determined the resistance to macrolides-lincosamides-streptogramins B by D-test, a test that consisted in observing the antibiotic antagonism between the clindamycin disk and the erythromycin disc. I have categorized the strains where I found this phenomenon as MLSBi phenotype strains. Strains resistant to macrolides-lincosamides-streptogramins B and sensitive to ketolides have MLSBc phenotype. Strains resistant to erythromycin and susceptible to clindamycin were MLSBe phenotype.

The tested strains on which the MLSBi phenotype was noticed were reported as resistant to clindamycin. The prevalence of MLSB phenotypes is shown in table no. 1.

Table no. 1. Phenotype resistance at MLSB of the tested strains

Phenotype	Total strains (n=420)	MRSA (n=121)	MSSA (n=299)
MLSBi	96 (22,85%)	54 (44,62%)	42 (14,04%)
MLSBc	32 (7,61%)	35 (28,92%)	5 (1,67%)
MLSBe	34 (8,09%)	15 (12,39%)	21 (7,02%)

The MRSA strains had mostly MLSBi phenotype, followed by the constitutive one, thus indicating a high resistance to lincosamides - 71.9% resistance to clindamycin (table no. 2). Compared to erythromycin, the tested strains showed high resistance, especially the MRSA ones (81.81%).

Table no. 2. Antibiotics resistance of the tested strains

Antibiotics	Total strains (n=420)	MRSA (n=121)	MSSA (n=299)
Penicillin	386 (91,9%)	121 (100%)	275 (91,9%)
Oxacillin	113 (26,9%)	121 (100%)	0
Erythromycin	170 (40,47%)	99 (81,81%)	92 (30,76%)
Clindamycin	134 (32%)	87 (71,90%)	48 (16,05%)
Tobramycin	55 (13,09%)	52 (42,97%)	6 (2%)
Gentamicin	46 (10,95%)	47 (38,84%)	7 (2,34%)
Trimethoprim-sulphametoxazole	13 (3,09%)	9 (7,43%)	5 (1,67%)
Tetracycline	193 (45,95%)	100 (82,64%)	95 (31,77%)
Ciprofloxacin	46 (10,95%)	47 (38,84%)	9 (3,01%)

The dominant phenotype observed in MSSA strains was the wild phenotype, with sensitivity to all aminiglycosides. MRSA strains had increased resistance to aminoglycosides – 89.25% showed resistance to kanamycin and 42.99% related resistance to gentamicin, respectively KTG phenotype (table no. 3.)

Table no. 3. Phenotype resistance of aminoglicozide of the tested strains

Phenotype	Total strains (n=420)	MRSA (n=121)	MSSA (n=299)
K	96 (22,85%)	53 (43,80%)	51 (17,07%)
KT	8 (1,9%)	7 (5,78%)	3 (1%)
KTG	48 (11,42%)	52 (42,99%)	12 (4,01%)
Wild	268 (73,83%)	9 (7,43%)	233 (77,92%)

We tested only the sensitivity to tetracycline, 227 strains (54.05%) proved to be sensitive (table no. 2). Note the high level of resistance of MRSA strains (82.64%), compared to MSSA strains (31.77%) (table no. 2).

The lowest level of resistance was found in trimethoprim-sulfamethoxazole for both MRSA strains (7.43%) and MSSA (1.67%) (table no. 2).

After testing the susceptibility of S. aureus isolated strains to ciprofloxacin, as shown in table no. 2, only 9 (3.01%) MSSA strains were resistant to ciprofloxacin, unlike the MRSA where a resistance of 38.84% (47 strains) was noticed.

Sensitivity to vancomycin and linezolid was of 100%, meaning that all strains were susceptible to both antibiotics.

DISCUSSIONS

Two years after the introduction of methicillin in therapy, in 1961 the first strain of MRSA is described in the UK.(3,4) Resistance to streptomycin, tetracycline, chloramphenicol and erythromycin is described along with their introduction in the therapy of infections caused by staphylococci resistant to penicillin. Multiple resistance to antibiotics shows a high prevalence in the 1950s. Over 40% of hospital strains resistant to more than four antibiotics are described in Seattle in 1959.(5)

The emergence of resistance to aminoglycosides occurs after 10 years of excessive use, and the first strain of MRSA with associated resistance to gentamicin is isolated in 1976.(6,7)

The mechanisms by which staphylococci acquire resistance to β-lactams are: penicillinase synthesis and modification of target action of β-lactams. The phenotype

associated with penicillinases is penicillin-resistant - methicillin-susceptible and offers resistance to narrow-spectrum penicillins and the one related to target modification: penicillin-resistant - methicillin-resistant - causes cross-resistance to all β-lactams, being frequently associated with resistance to other groups of antibiotics.(8,9,10,11) The percentage of MRSA observed in our study fall in the range of 25-50% of the European EARS-Net report on antibiotic resistance surveillance in 2009 for Romania.(12)

The resistance to aminoglycosides is primarily enzymatic, by the action of enzymes that modify aminoglycosides. Of these, three types are mainly found in S. aureus: aminoglycoside-6'-N-acetyltransferase/2"O fosforyltransferase [AAC(6')/APH (2")] - encoded by the aac(6')-Ie-aph(2") gene, bifunctional enzyme that determines the KTG phenotype with resistance to gentamicin, kanamycin, tobramycin, neomycin, amikacin; the aminoglycoside-4'-O-nucleotidiltransferase I [ANT(4')-I] - encoded by the ant(4')-Ia gene inactivates kanamycin, tobramycin, neomycin, amikacin - KT phenotype, and the aminoglycoside-3'-O-fosforyltransferase III [APH(3')-III] - encoded by the aph(3')-IIIa gene determines resistance phenotype K by acting on kanamycin, neomycin.(13,14,15)

For tetracyclines, two mechanisms of resistance were described: by active efflux (tetK, tetL genes acquisition), and chromosomal resistance (encoded by tetM, tetO genes). Staphylococci acquire quinolone resistance by two mechanisms: punctiform mutations in the chromosomal genes encoding topoizomerases and through some efflux pumps mediated by the transport protein norA.(15) Opting for fluoroquinolone therapy is recommended according to the antibiogram.(16)

CONCLUSIONS

1. The most frequently observed phenotype in the S. aureus strains isolated from genital infections was methicillin-sensitive without associated resistance to other groups of antibiotics.
2. The MRSA strains identified and tested showed an increased associated resistance to macrolides, lincosamides, aminoglycosides and tetracycline.
3. Although clindamycin has increased antistaphylococcal activity, the high percentage of resistance observed in the tested strains limits the usefulness of clindamycin in the treatment of genital infections caused by S. aureus.
4. Aminoglycosides should be used in combined therapy because their use, as sole antimicrobial agent, predisposes to the occurrence of resistance.
5. Trimethoprim-sulfamethoxazole, whose clinical effectiveness is not fully proven, may be a therapeutic option for multidrug-resistant S. aureus strains, given the high sensitivity in vitro.
6. Although the literature cites the emergence of strains resistant to vancomycin and linezolid, all strains tested were found susceptible to these antibiotics.

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