

Genotypic Characteristics of Outpatient Antibiotic Resistance and Nosocomial *Staphylococcus aureus* Strains

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Abstract

The article presents the results of a study of antibiotic resistance of 191 methicillin-sensitive (MSSA) and methicillin-resistant (MRSA) strains *Staphylococcus aureus* (*S. aureus*), isolated from outpatient and hospital patients. The data of molecular-genetic typing of strains of *S. aureus* and established types of SCCmec cassettes. There were significant differences in the sample genotype MRSA strains isolated from patients and hospital outpatient profile. Isolates from outpatients, contained in its composition genetic elements SCCmec IV a, c, d types. MRSA, isolates from hospital patients profile were identified only type II SCCmec cassettes. Vancomycin, fusidic acid, mupirocin antistaphylococcal possessed high activity against MRSA and MSSA. MRSA were more resistant to antimicrobial drugs compared to MSSA. Azithromycin and clindamycin demonstrated moderate anti-staphylococcal activity. Revealed low activity of chloramphenicol, tetracycline and erythromycin against *staphylococci* strains.

Keywords: Methicillin-sensitive; Methicillin-resistant; *Staphylococcus aureus*; Genotyping SCCmec; Health-care associated MRSA; Community-acquired MRSA

Introduction

The incidence of health-related infections (health-care associated infections) remains high. In addition to socially-mediated factors associated with the sanitary and epidemiological situation, "socially independent" factors play a role, such as the selection of highly virulent strains of microorganisms with multiple resistance to antimicrobial agents-antibiotics, antiseptics, disinfectants [1,2]. In the etiological structure of ISMP, *Staphylococcus aureus* remains relevant. In the epidemiological aspect, methicillin (oxacillin) resistant *staphylococci* are significant; first of all, *Staphylococcus aureus* (Methicillin resistant *Staphylococcus aureus*-MRSA) [3]. There are nosocomial and out-of-hospital strains of MRSA. Strains acquired as a result of hospitalization or associated with the provision of medical care are recorded as HA-MRSA (health-care associated MRSA). Patients who do not seek medical care in recent months are characterized by "out-of-hospital" strains of MRSA (community-acquired MRSA, CA-MRSA) [4]. Strains CA-MRSA and HA-MRSA differ in phenotypic and genotypic characteristics. Methicillin resistance of *staphylococci* depends on the presence of the *mec* complex in the chromosomal cassette (SCCmec). The main elements of this complex are: *mecA*, a structural gene encoding the production of an additional penicillin-binding protein (PSB-2a); *mecI* and *mecR1* are the components responsible for the transcription of *mecA4*, *mec*-associated DNA [5].

The genetic characteristic of all MRSA, regardless of the genotypes of the SCCmec element, is the presence of the *mecA* gene that causes the resistance of these strains to oxacillin and beta-lactam antibiotics

and *ccr*-complex genes that encode proteins that excise and site specific integration of *mecA* into the *staphylococcus* genome [6]. Mobile genetic elements SCCmec which include cassettes IV and V types can be transferred from some *S. aureus* strains to others fairly freely, in comparison with SCCmec cassettes of I, II and III types, usually not having mobility [7]. It should be noted that strains containing SCCmec IV-V types in their genome, due to pronounced mobility and ability to horizontal transfer, pose a major epidemiological hazard in the spread of MRSA and the transmission of this trait between *staphylococci* colonizing the human body. Mobile genetic elements of SCCmec I, II and III types are usually present in hospital clones of methicillin-resistant *staphylococci* (HA-MRSA), characterized by the presence of antibiotic resistance genes for other groups (tobramycin, kanamycin, tetracycline, erythromycin) [8].

Currently, molecular genetic methods have established that there is a fairly clear relationship between the MRSA membership of a certain genetic "background" and the content of a certain type of SCCmec in DNA [9]. Strains containing SCCmec type IV are among the most numerous clonal group of CC8. This group includes such epidemic related strains and clones as EMRSA-2, -6, -12, -13, -14 prevalent in the UK, USA, Germany, France and the Netherlands [10]. It should be noted that SCCmec type IV is often detected in epidermal *staphylococci* (*S. epidermidis*), which are representatives of the normobiocenosis of the skin and mucous membranes, and is supposed to transfer this mobile element from *staphylococcus S. epidermidis* to coagulase-positive *S. aureus* within one biotope of the human body [11].

The aim of the study was to study the profile of antibiotic resistance and molecular-genetic typing of *Staphylococcus aureus* for the

presence of SCCmec complexes isolated in patients treated in outpatient and inpatient settings.

Materials and Methods

Objects of research

The study included 191 clinical strains of *S. aureus*, isolated in 2007-2014 from outpatient patients examined in the outpatient clinic of Kazan Scientific Research Institute of Epidemiology and Microbiology of Rospotrebnadzor and patients ("out-patients") receiving hospital treatment ("hospital") in Kazan. MRSA strains isolated from outpatients are sown from the nasal mucosa and nasal passages in patients with upper respiratory tract infections and from areas of affected skin in patients with atopic dermatitis. Hospital strains of MRSA are sown from wound detachable skin in patients with burn injuries; from the nasal cavity in patients with purulent rhinosinusitis, from the oral mucosa in patients with aphthous stomatitis, pericoronaritis, paradontal abscess.

Bacteriological methods

Cultivation of strains of *S. aureus* was carried out on 5% blood agar, mannitol-salt yolk agar (Columbia, Conda, Spain). Identification of *S. aureus* was carried out according to morphological features, biochemical methods in accordance with normative documents. The profile of sensitivity to antimicrobial agents was determined by the

disc-diffusion method using the Müller-Hinton medium according to MUK 4.2.1890-04 "Determination of the sensitivity of microorganisms to antibacterial drugs". Screening of methicillin resistance in *S. aureus* was performed using a disk with nitrocellin and then plated onto the chromogenic MRSA Agar chromomer Agar Biomerieux. Interpretation of antibiotic sensitivity data was carried out in accordance with NCLS 2008 standards.

Molecular-genetic methods

Genotyping of *S. aureus* was carried out according to the protocol presented in the guidelines of the Federal Center for Hygiene and Epidemiology of Rospotrebnadzor, 2006 [12]. Extraction of genomic DNA from the strains was carried out according to the protocols of commercial ZR Genomic DNA II Kit™ kits. Identification of SCCmec types was carried out by PCR amplification using 12 pairs of primers (oligonucleotides) synthesized in company "Sintol", Moscow, Russia. At the first stage, the specificity of the set of genes coding for the synthesis of recombinases (type ccr 1, 2, 3.5) was determined. Then, the set of genes belonging to the mec complex (class A or class B, using the primers MecI and IS272) was determined (Table 2). The amplification results with the determination of these sets of genes allowed identification of the presence of SCCmec I, II and III types. Identification of the presence of SCCmec IV a, b, c, d primers IS272, as well as T-IVa-T-IVd were used; in addition, primers TV and ccr-type 5 were used in the detection of SCCmec V type (Table 1).

Name primers	Type of identifiable SCCmec	Nucleotide sequence (5'-3')	Size of amplicon (bp)
T-IVa	SCCmecIVa	GCCTTATTCGAAGAAACCG CTA CTCTTCTGAAAAGCGTCG	776
T-IVb	SCCmecIVb	TCTGGAATTACTTCAGCTGC AAACAATATTGCTCTCCCTC	493
T-IVc	SCCmecIVc	ACAATATTTGTATTATCGGAGAGC TTGGTATGAGGTATTGCTGG	200
T-IVd	SCCmecIVd	CTCAAATACGGACCCCAATACA TGCTCCAGTAATTGCTAAAG	880
T-V	SCCmecV	GAACATTGTTACTTAAATGAGCG TGAAAGTTGTACCCTTGACACC	330
MecA147	Ген mecA	GTGAAGATATACCAAGTGATT ATGCGCTATAGATTGAAAGGAT	150
ccr-type 1	SCCmec I	ATTGCCTTGATAATAGCCTCT AACCTATATCATCAATCAGTACGT	700
ccr-type 2	SCCmec II	ATTGCCTTGATAATAGCCTCT TAAAGGCATCAATGCACAAACACT	1000
ccr-type 3	SCCmec III	ATTGCCTTGATAATAGCCTCT AGCTCAAAGCAAGCAATAGAAT	1600
MecI	Класс А mec	CAAGTGATTTGAAACCGCCT CAAAAGGACTGGACTGGAGTCCAAA	180
IS272	Класс В mec	ACCGCCACTCATAACATAAGGAA TATACCAACCCGACAAC	2000
ccr-type 5	SCCmecV	ATG-AAT TCAAAGAGGATGGC GATTTAGAATTGTCGTGATTGC	336

Table 1: Primers used to identify SCCmec types.

PCR was carried out using the standard composition of the reaction mixture, the volume of one sample was 20 µl. Primers were introduced at a final concentration of 300 nmol, the concentration of the DNA template to 10 ng. Detection of primer-specific amplicons was carried out in a 2% agarose gel by horizontal electrophoresis using intercalating ethidium bromide dye and markers (Fermentas, DNA Ladder). Visualization of the results was carried out by the transilluminator (Vilber Lourmat, SCX-15 M, France).

The reference strains were used: for intralaboratory quality control of antibiotic resistance-*Staphylococcus aureus* ATCC 25923 (State Collection of Pathogenic Microorganisms, Moscow); for control of molecular-genetic typing SCCmec-cassettes isolated DNA from *Staphylococcus aureus* M307, *Staphylococcus aureus* M 258, *Staphylococcus aureus* 252, *Staphylococcus aureus* 720, *Staphylococcus aureus* E84/5528/1, *Staphylococcus aureus* I-78. (State Collection of Pathogenic Microorganisms, Moscow).

Results

From outpatient patients 166 strains of *S. aureus* were isolated. In this group, the occurrence of MRSA strains according to phenotypic identification was 27.1%.

A characteristic feature of the strain isolated from this group of patients was the prevalence (72.9%) of methicillin-sensitive strains (MSSA), compared with the group of hospital patients (44.0%). In hospital patients strains of *S. aureus* (n=28) were characterized by a predominance (56.0%) of methicillin-resistant isolates (MRSA).

It should be noted that some strains isolated from patients, both outpatient and hospital profile, were phenotypically moderately resistant to oxacillin, while molecular genetic analysis of DNA obtained from these strains revealed primer specific amplicons of the *mecA* gene in 30.1% of cases for outpatient and 64.0% for hospital isolates (Table 2).

Patient groups	Strains, n,%		
	Phenotype		Genotype
	MRSA	MSSA	gene <i>mecA</i> ⁺
Outpatient profile patients (n=56)	45 (27,1%)	121 (72,9%)	49 (30,1%)
Total	166 (100%)		
Hospital patients (n=28)	14 (56,0%)	11 (44,0%)	16 (64,0%)
Total	25 (100%)		

Table 2: The results of determining the sensitivity to oxacillin and the identification of the *mecA* gene in strains of *S. aureus* isolated from patients of two groups.

At the first stage of the study, the sensitivity of MRSA and MSSA strains to antimicrobial agents was determined.

The results of antibiotic susceptibility testing showed that glycopeptides (vancomycin) are highly active against *S. aureus* clinical isolates, all isolates were absolutely sensitive to this drug. The dominant numbers of strains were sensitive to fusidic acid: 98.02% MSSA *S. aureus* and 94.6% MRSA. A high anti-staphylococcal activity showed mupirocin (94.6% of MRSA isolates and 100% of MSSA strains). Clindamycin proved to be a less effective antistaphylococcal drug (84.6% for MSSA isolates and 75.7% for strains of MRSA); the proportion of gentamicin-sensitive isolates was 88.5% of MSSA and 70.27% of MRSA cultures. There was also a low activity of MRSA to erythromycin (54.1%); to tetracycline 54.0% and to chloramphenicol 56.7% of sensitive crops. MSSA was more sensitive to these antimicrobial agents. Thus, 77.5% of the strains were sensitive to chloramphenicol, 76.9% to tetracycline and 71.2% to erythromycin. Macrolides remained active drugs against the dominant majority of MSSA (78.8% of azithromycin susceptible cultures); and MRSA in only 67.5% of cases are susceptible to azithromycin.

In second stage of the study, molecular-genetic typing of *mecA*-positive DNA strains was carried out and the types of SCCmec cassettes were established. The genotype of all isolated MRSA strains from patients of the outpatient and hospital profile in a one-stage study showed significant typical differences (Figure 1). An analysis of the distribution of the types of SCCmec cassettes present in the genome of

the investigated *S. aureus* strains showed (Figure 1) that strains isolated from outpatients, contained the genetic elements of SCCmec IV a, c, d types present in most isolates, the so-called community-acquired MRSA (CA-MRSA) [12]. In the MRSA strains isolated from patients in the hospital profile, only SCCmec cassettes of type II, as a rule, typical for hospital clones MRSA [12].

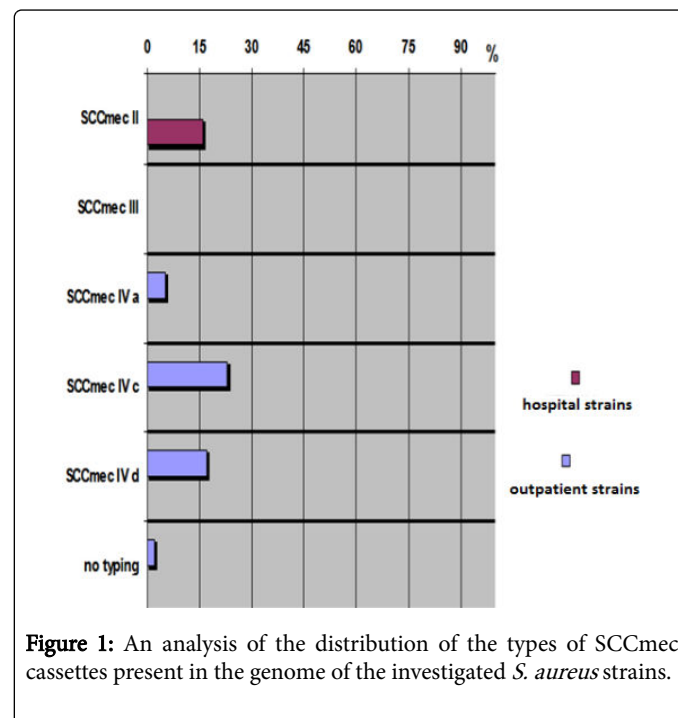


Figure 1: An analysis of the distribution of the types of SCCmec cassettes present in the genome of the investigated *S. aureus* strains.

Conclusion

The results of the study indicate that high antistaphylococcal activity against both MRSA and MSSA strains had vancomycin, fusidic acid and mupirocin. Methicillin-resistant isolates showed greater resistance to antimicrobial drugs than methicillin-sensitive cultures. Azithromycin and clindamycin have shown moderate antistaphylococcal activity. There was a low activity of chloramphenicol, tetracycline and erythromycin. Molecular-genetic typing of MRSA revealed significant differences in the structure of SCCmec cassettes. Isolates of methicillin-resistant strains of *S. aureus* isolated from patients of the hospital profile in its genome contained SCCmec of type II cassette, and strains from outpatients were type IV of SCCmec. There is a need to organize effective surveillance and control of the circulation of CA-MRSA and HA-MRSA strains in a particular region, especially at the local level-a medical and preventive institution, which will allow the development of measures to prevent the growth of multiple *S. aureus* resistance, conduct adequate antimicrobial therapy and sanitation-anti-epidemic measures.

Conflicts of Interest

The authors declare no conflicts of interest.

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