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TYPING OF *STAPHYLOCOCCUS AUREUS* IN ORDER TO DETERMINE THE SPREAD OF DRUG RESISTANT STRAINS INSIDE AND OUTSIDE HOSPITAL ENVIRONMENT

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ABSTRACT

Staphylococcus aureus is one of the most important etiological factors of both nosocomial and community-acquired infections. Multidrug-resistant *S. aureus* is frequently isolated nowadays. Antibiotics used on the hospital ward exert a selective pressure on the strains and favor resistant strains. Multidrug-resistant and highly virulent strains can spread not only within the hospital but also between hospitals. Numerous studies show a predominance of one clone on a specific territory. The spread of such dangerous clones to neighboring countries and the entire continent is possible. Typing methods are very useful in infection control and prevention. Modern methods which are based on sequencing are necessary in rationalizing of infection control programs. Typing of *Staphylococcus aureus* includes methods that allow to determine the spread of drug-resistant pathogens. 'Gold standard' is pulsed-field gel electrophoresis (PFGE), which relies on separating the DNA fragments after restriction cutting. MLST (Multi Locus Sequence Typing) is based on a comparison of "housekeeping" gene sequences controlling the basic cell functions. With the MLST method, it is possible to demonstrate a broad, international spread of the specific clones. However, for epidemiological investigations, MLST seems to be too time-consuming and expensive to be used as a basic typing tool. The complementary method is *spa* typing, based on the sequencing of short repetitive sequences of the polymorphic X region from the gene encoding protein A. This method can be used for studying molecular evolution of *S. aureus*, as well as for testing for hospital outbreaks. It is faster and cheaper than MLST. It is also necessary to subtype the elements responsible for methycillin resistance (SCC*mec*), which allows to distinguish MRSA (*Methicillin-resistant Staphylococcus aureus*) clones with a common ancestor, but different epidemiological origin. All of those methods have their specific advantages and disadvantages and there is no single method efficient and suitable in any case.

Keywords*: Staphylococcus aureus, MRSA, typing, PFGE, MLST*

Staphylococcus aureus constitutes one of important microorganism which forms the human commensal flora and is potentially infectious. *S. aureus* colonizes mainly warm and moist region of mucous membranes, especially the nasal vestibule, where its receptors are located. From the literature data show that the colonization of nasal vestibule (reported in 58% of patients and residents) is mainly associated with temporary colonization of pharynx (1,2). The past history of *S. aureus* colonization is an important risk factor of infection progress. Different strains produce diverse toxins and virulence agents which enables the bacteria the invasion and have a negative effect on the immunology system of the host (3). Many strains are resistant to numerous antibiotics which limits the treatment options and result in spreading of the resistance genes on sensitive strains (3).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important cause of community (CA-MRSA) and health care-associated MRSA (HA-MRSA) infection (4). The strains of CA-MRSA as opposed to HA-MRSA strains, remain usually resistant to majority of antibiotics, excluding beta-lactams (5). According to *Munckhof,* the criterion of CA-MRSA diagnosis should be sensitiveness to more than two drugs of non-beta-lactams (6). CA-MRSA strains are usually more virulent and many of them can produce Panton-Valentine leukocidin (5).

From the perspective of hospital epidemiology and surveillance of infections among the most important are

skin and soft tissues infections, blood stream infection and pneumonia. These infections may occur in hospitalized patients and residents in the long-term care facilities as the endemic infection and it may contribute to epidemic. It is estimated that *S. aureus* is the most frequently identified etiologic agent of infections (7) and accounts for 1% of all hospital-acquired infections (*nosocomial infections*). Approximately half of *S.aureus* infections in hospitalized patients are hospital-acquired infections (8,9). In the United States, the incidence of *S. aureus* infections amounts to 9.13/1,000 admissions, of which 43% are the cases associated with MRSA (8). *S. aureus* is usually the most important pathogen observed in the Intensive Care Units (ICU), in adults (10), as well as in newborns (11).

Surveillance of MRSA which is connected with a decrease in frequency of infections of this etiology may have an indirect effect on prevalence of MRSA in the general population, i.e. on risk of ward personnel colonization as the MRSA is equally frequently observed in community-acquired infections (12). It should be noted that the significant risk factor of MRSA infection is colonization with this strain (3).

It is observed that more countries are implementing the routine MRSA screening of hospital inpatients (excluding daily admissions, dermatology and others) (13). Compulsory surveillance of methicillin-resistant isolates of *S. aureus* is also conducted, which as the pathogen of significance in the hospital epidemiology is subject to compulsory monitoring within the frames of hospital-acquired infections control. Such surveillance is present in Polish hospitals and since many years in Finland, Norway, Sweden and The Netherlands (14).

Antibiotic treatment applied on the ward exerts pressure and contributes to selection of resistant strains. The strains which are multi-drug resistant and highly virulent, may clonaly spread inside hospital and between hospitals. On the territory of the given country, one specific clone is usually predominant. In the course of time, the clone may spread to the neighbouring countries and the whole continent. Due to the Multi Locus Seqence Typing (MLST), it is possible to demonstrate wide, often international range of specific clones. It may also be employed to examine the molecular evolution of *S. aureus* (15). It is a reference method for establishing the basic genetic structure of *S. aureus* population, which is dominated by several large clone complexes and includes several hundred sequence types (ST) (16). MLST consists in comparing conservative sequences of housekeeping genes which control the basic functions of each living cell. The first stage of sequencing is amplification of seven genes (*arcC, aroE, glpF, gmk, pta, tpi, yqiL*) with seven pairs of starters (16). The obtained nucleotide sequences are then compared to known allels for each locus by using the programme available at MLST database (http://www.mlst.net/). For each isolate, seven digital allels profiles are generated which define particular ST type. Due to the fact there are many allels in each analyzed loci, it is impossible that two isolates have the same profile accidently. The isolates of the same profile may be then classified to the same clonal type (17). The profiles obtained due to the MLST may be easily compared between laboratories. In the case of *Staphylococcus*, application of MLST may be time-consuming and requires the sequencing of large number of nucleotides (18). For the purpose of epidemiological investigation, MLST is found to be labour-intensive and costly to be employed as a basic tool of typing. So far, over 2,400 ST types for *S. aureus* have been described.

Spa typing is recommended as the method supplementing MLST. It consists in sequencing of short sequence repeats (SSR) from polymorphic X region of the protein A gene (*spa*) (18,19). In this region, the numerous spontaneous mutations (including loss and gains of sequence repeats) may occur (20). To date, approximately 600 different repeats and above 11,000 *spa* have been described (15). The diverse variations of SSR correlates with the pathogenicity and virulence of bacteria (21). Diversity of s*pa* gene, which is mainly composed of 24 repeats is the consequence of point mutation (both deletion and duplication) (22). The comparison of *spa* types within Europe and the world is possible due to the international database Ridom StaphType (Ridom GmbH, Germany, http://spaserver.ridom.de). The programme, which is available at the aforesaid webiste, automatically identifies the number of sequence repeats and assigns it to the appropriate *spa* type. *Spa* typing may be applied for the purposes of the analysis of molecular evolution of *S. aureus* and investigation of hospital epidemic. In comparison to MLST, it is less time- consuming and costly. However, it should be noted that discriminatory power of *spa* typing is lower than for PFGE. The most frequently identified *spa* type is t032, occurring with the prevalence of approximately 11% in many different European countries (excluding Poland), as well as in the United States and Australia. The successive types of global range are t003, t002 and t008, which are identified in Poland (23).

The resistance of *S. aureus* to methicillin is the result of synthesis of transpeptidase PBP2a. This protein slightly binds to β-lactam antibiotics. As a consequence, even in the presence of these antibiotics, the cell wall synthesis is not disrupted. Thus, the bacteria may survive (15). The *mecA* gene, which encodes PBP2a, is located inside *mec* operon with regulatory genes (15). Due to the fact that there are more strains of *S. aureus* which have *mecA* gene, it is indispensible to subtype mobile elements responsible for the resistance to methicillin. These elements are defined as staphylococcal chromosomal cassette mec (SCC*mec*). It enables to differentiate MRSA clones having a common ancestor

but different epidemiological origins (16). The characteristic elements of SCC*mec* are:

1. presence of *mec* gene complex which is composed of resistance gene to methicillin *mecA* and regulatory genes (*mecR1* and *mecI*) and insertion sequences (IS*1272* and IS*431*),
2. presence of *ccr* gene complex which is composed of recombinase genes; these catalyze excision and integration of SCC*mec* elements to bacterial chromosome and as a consequence are responsible for mobility of SCC*mec* and surrounding elements,
3. presence of characteristic repeated sequences and inverted-complementary sequences at both ends (three regions J – J1 located at the right site of the cassette, J2 located between complexes *mec* and *ccr* and J3, located at the left site of the cassette). The regions may activate plasmids and transposons, carrying the determinants of resistance to antibiotics or heavy metal ions (24),
4. ability to integrate at 3'end of open reading frame (ORF).

For the first time, SCC*mec* elements were described and characterized in 1999 (25). So far, a total of eleven different SCC*mec* types have been identified (26). The epidemiological studies confirm that MLST, *spa* typing as well as SCC*mec* should be performed to classify the clone correctly (15,26). Three different approaches on SCC*mec* elements typing may be differentiated: methods based on polymerase chain reaction (PCR) and its type in real time (Real-time PCR) or methods of restriction digestion. However, the most valuable is the method proposed by *Kondo et al*. (27), based on six multiplex PCRs:

- M-PCR 1 amplifies *ccr* (1-4) together with *mec*A gene
- M-PCR 2 amplifies the classes *mec* A, B and C2
- M-PCR 3 amplifies ORF from region J1 SCC*mec* of I and IV types
- M-PCR 4 amplifies ORF of region J1 from SCC*mec* of II, III and V types
- M-PCR 5 and 6 amplify genes located in regions J2 and J3

However, this method is time-consuming and requires conducting of large number of reactions. In the majority of cases for the epidemiological purposes, M-PCR 1 and 2 are sufficient. M-PCR 3 and 4 are used for subtyping the differences in the region J1. M-PCR 5 and 6 identify integrated copies of transposons and plasmids. So far, the methods of typing SCC*mec* of types VII, X and XI have not been developed.

The analysis of genotype similarity of *S. aureus* may be performed by using pulsed field gel electrophoresis (PFGE). In the case of *S. aureus* typing, it is considered to be a gold standard. PFGE relies on electrophoretic separation in pulsed field of DNA fragments obtained from cutting bacterial genome by using selected restriction enzyme (*Sma*I for *S. aureus*). The separated DNA fragments are visible in the form of bands which form the banding pattern typical for a given strain. In the case of majority of bacteria, the separated fragments size ranges from 30Kb to 1Mb. The obtained restriction patterns may be analyzed by using the criteria proposed by *Tenover et al.* to differentiate the clonal groups (28) and with the usage of programme for analysis of strains relatedness (eg. GelCompar of Applied Maths, GeneProfiler and others). Additionally, in the case of insert on deletion of large mobile genetic elements to bacterial genome, the modifications will be visible in banding patterns (29). Furthermore, plasmid DNA of size amounting to 50Kb does not disrupt the electrophoretic separation and successive analyses due to the small size of plasmids (29). PFGE is employed in the analysis of genotype similarity between isolates, especially in epidemiological investigations. It is highly discriminating, however it is also time-consuming and requires special equipment. Moreover, the problematic could be the lack of restriction sites in particular strains. As a result, they are not subject to typing using this method (30). Furthermore, the results obtained in laboratories often cannot be compared.

Typing of microorganisms covers the methods which enable to reproduce the transmission routes of pathogens as well as compare them with global spreading of especially virulent strains. It enables to determine the infection of *S. aureus* etiology in diverse population of patients and contributes to its reducing. It should be noted that there is no one universal method of typing which is ideal in each case. All of them have both advantages and disadvantages. The successive important information is the fact that the differences identified by using one method, do not have to be confirmed with the usage of another technique. Thus, in some situations it is recommended to apply several typing methods simultaneously in order to achieve more detailed information.

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