DETECTION AND TYPING METHODS OF METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS

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Summary

Staphylococcus aureus is one of the most important pathogens in hospital infections and its importance in the community is also prominent, especially since the 1990's. Today methicillin-resistant *Staphylococcus aureus (*MRSA) is one of the most common causes of hospital infection, causing 40-70% of staphylococcal infections in intensive care units. Data on the prevalence of MRSA isolates differ in various parts of the world, as well as between hospitals, from 0.6% in Norway to 66.8% in Japan, and in 2008 a trend was noticed of a fall in the number of MRSA infections in various European countries. It is possible to isolate MRSA from clinical isolates, as part of the procedure of diagnostics of the infection, or as isolates from the site of colonization through MRSA screening procedures. Identification of *S. aureus* is based mainly on production of catalase, coagulase and DNA-se and with latex agglutination test based on detection of specific *S. aureus* antigens (e.g. protein A). Methicillin-resistance can be detected by susceptibility testing to cefoxitine or by using molecular tests to confirm the presence of *mec*A gene, responsible for methicillin-resistance. There are also some automated laboratory systems that can combine identification and susceptibility testing of MRSA and other bacteria. Detection of MRSA in screening samples is challenged by presence of other bacterial flora and can be overcome by use of different selective broth for initial (overnight) incubation and / or different selective agar plates (e.g. chromogenic plates). Screening can also be performed with molecular tests and new, third generation PCR detects the whole region of genome between SCC*mec* fragment and *orf*X (not only *mec*A gene).

Typing is important for MRSA in order to investigate the correlation between dif-

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ferent isolates, are they same, similar, do they origin from the same pathogen source etc. One group of methods is phenotypic typing, which is very much substituted with genotypic methods. Among genotypic methods, the most widely used are Pulse–Field Gel Electrophoresis (PFGE), Multi-Locus Sequence Type (MLST) analysis and sequencing of protein A region of *Staphylococcus aureus*. It is very important for laboratory and hospitals to be able to detect MRSA in samples, accurately and reasonably fast, in clinical samples or as a part of screening strategy. It is up to laboratory to choose which method to use, following guidelines and recommendations and depending on ability to afford different tests.

Keywords: MRSA; detection; typing methods

INTRODUCTION

Staphylococcus aureus is one of the most important pathogens of hospital infections and its importance in the community is also prominent, especially since the 1990's. It causes a wide range of illnesses, from benign skin infections to life-threatening deep abscesses, osteomyelitis, sepsis and endocarditis [1]. It causes infections which are a challenge for treatment, and its transmission within a ward, between patients, members of staff and households, enables it to spread, colonize and infect new patients. Infection may be caused by its direct presence in the organism, but also it causes illness through toxin secretion [2].

The heterogeneous nature of these illnesses and the unique ability of *Staphylococcus aureus* to develop resistance to every newly-discovered antibacterial drug shows it ability to adapt and survive in a variety of conditions; MRSA can colonize the nose, throat, perineum and skin, the large intestine, the urino-genital systems of men and women etc. Although most colonized people have no symptoms, MRSA may cause local invasive infections, penetrating through sensitive skin. The genetic basis of methicillin resistance is found in the presence of the *mec*A gene [3], which is found on mobile genetic elements of the chromosome (staphylococcal chromosomal cassette *mec*, SCC*mec*).

THE EPIDEMIOLOGY OF METHICILLIN-RESISTANT
STAPHYLOCOCCUS AUREUS

Methicillin-resistant *Staphylococcus aureus* first appeared amongst hospital isolates in 1961 [4] as a blood isolate. The first MRSA isolate, originating from the United Kingdom in 1961, was SCC*mec* I and it was a typical representative of the archaic clone which spread around the world in the 1960's. Methicillin, originally called celbenin, was introduced into treatment in Europe in 1959-1960. Although methicillin

is no longer used for treatment of staphylococcus infections, the acronym MRSA has remained, and relates to *Staphylococcus aureus* which is resistant to all beta-lactam antibiotics, including cephalosporins and carbapenems. The first MRSA epidemic was described in 1963 [4].

From the 1960's, strains of MRSA spread gradually throughout most hospitals all over the world. In the 1970's MRSA isolates appeared in the USA and in Japan and Australia. In 1982 MRSA SCC*mec* type II was discovered in Japan, and the New York/Japan clone, to which it belongs, also spread, after which the isolation of the MRSA strain SCC*mec* type III followed, in New Zealand. The first MRSA isolates of SCC*mec* type IV appeared in the 1990's in the USA. The first isolate of SCC*mec* type V was found in Australia in 2000 [5].

Today MRSA is one of the most common causes of hospital infection, causing 40-70% of staphylococcus infections in intensive care units [6]. In Croatia the first report of the importance of MRSA strains was published in 1997 [7].

Data on the prevalence of MRSA isolates differ in various parts of the world, as well as between hospitals, from 0.6% in Norway to 66.8% in Japan [6], and in 2008 a trend was noticed of a fall in the number of MRSA infections in various European countries [8].

Methicillin-resistant *Staphylococcus aureus* has already for many years been linked with hospital stay, homes for the elderly and infirm and similar institutions, and it was only from the 1990's that community acquired MRSA appeared, with a large number of characteristics which differentiate it from the previously known hospital acquired MRSA. Hospital acquired MRSA is mainly multi-resistant, and the choice of antibiotics for treating infections caused by hospital-acquired MRSA is limited to vancomycin and linezolid. Furthermore, hospital acquired MRSA mainly causes serious infections in patients who are predisposed in some way: those with a weak immune system, after long-term hospitalization, long-term use of antibiotics, a progressive underlying illness etc. Infection by MRSA strains in hospital conditions is usually preceded by colonization of differing duration. Only a few epidemic MRSA clones are responsible for a large percentage of infections caused by this agent [9].

DETECTION OF MRSA ISOLATES

It is possible to isolate MRSA from clinical isolates, as part of the procedure of diagnostics of the infection, or as isolates from the site of colonization through MRSA screening procedures. Clinical samples are processed according to current microbiology procedures, with minor variations within each laboratory. In order to

control the spread and prevent the entry of MRSA into a hospital, MRSA screening is performed in some wards (those most at risk) or in all (universal screening) or only for certain groups of patients, where MRSA carriers are actively sought. The samples from which there is the greatest likelihood of isolating MRSA are usually swabs from the nose, throat, underarm, perineum and rectum, and several samples are usually combined in order to increase the sensitivity of the screening itself.

Clinical samples

Blood, liquor, respiratory samples, urine, swabs or aspirates from wounds etc., are usually placed on primary non-selective agar plate and after overnight incubation at 35° C there is visible growth of colonies that appear to be *S. aureus*. Samples of stools and rectum and perineum swabs are placed on selective media so that the normal flora from those areas do not inhibit the growth of MRSA. By staining colonies and the catalase test, staphylococcus is shown to be definitely present in the sample, but it is important to determine whether it is *S. aureus* or some other, coagulase-negative staphylococcus (CNS).

In demonstrating *S. aureus* we use coagulase tests in a test tube, on a glass slide or the latex agglutination test, and by testing products of DNA-se or thermostable nuclease. The coagulase test in a test tube shows the presence of free (extra-cellular) coagulase, which coagulates rabbit plasma after 4 or 24 hours. A test which is negative after 4 hours, [10] is re-examined after 24 hours due to the fact that a small percentage of strains needs more than 4 hours to coagulate. Some other forms of staphylococcus, such as *S. schleiferi* and *S. intermedius*, may also give a positive test although they are not frequent isolates in human bacteriology. Also, rare strains of *S. aureus* are coagulase negative.

Coagulase testing on glass slides is undertaken over a relatively short period of time, but up to 15% of *S. aureus* isolates are negative, so that a negative test related to coagulase must be confirmed by other tests [11]. Isolates which show positive coagulase on glass slides or DNA-se, need to undergo confirmation testing for definitive proof of *S. aureus*.

Latex agglutination tests are also used to identify *S. aureus* and are mainly based on the presence of antibodies to protein A and/or coagulase and more recent formulations also use various surface antigens, specific for *S. aureus*, which contributes to an increase in the sensitivity of the tests. That is to say, some isolates of *S. aureus* produce relatively small quantities of coagulase or protein A.

Detection of deoxyribonuclease (DNA-se) may be used for identification screening, since different CNSs also produce DNA-se and a positive result needs to be confirmed by additional tests. Thermostable endonuclease may be used for identi-

fication in the form of the metachromatic agar-diffusion test, which is of moderate sensitivity, and latex agglutination tests are also described.

There are also many kits in existence, or panels of biochemical tests, by which it is possible to identify staphylococcus on the basis of the profile obtained, and they are read either manually, or visually, or automatically. The performance of these tests is satisfactory, apart from the fact that they take longer and are more expensive than coagulase and latex agglutination [12]. There are also tests which are relatively quick (2 hours) using prothrombin and protease inhibitors.

There are molecular tests based on the PCR method, which demonstrate the genes which code nuclease (*nuc*), coagulase (*coa*), protein A *(spa)*, *fem*A, *fem*B, Sa442, 16SrRNA and fibrinogen-binding proteins [13].

DEMONSTRATING METHICILLIN RESISTANCE IN *S. AUREUS*

At the same time as tests to show whether it is a case of *S. aureus* or some other *Staphylococcus*, susceptibility to antimicrobial drugs is also tested. Currently, the EU-CAST (European Committee on Antimicrobial Susceptibility Testing) standards are used to test the susceptibility and interpretation of results in Croatia [13].

We test methicillin resistance by placing a cefoxitin disc on Mueller-Hinton agar, the suspension is added and after incubation, susceptibility is established by measuring the zone of inhibition to beta-lactam antibiotics. If the inhibition zone around the cefoxitin is less than 22 mm, the tested isolate most probably contains the *mec*A gene and is resistant to all beta-lactam antibiotics. If minimum inhibitory concentration (MIC) cefoxitin is being tested, a value of 4 mg/L or more also indicates MRSA. The method to test the susceptibility to antimicrobial drugs and to cefoxitin was derived from the Kirby-Bauer method, which is aligned with the EUCAST standards used throughout Europe. The medium used as a base for testing is Mueller-Hinton [14] agar, without supplements, and incubation is conducted at a temperature of 35° ± 1° C for 18 ± 2 h in atmospheric conditions, and the time of application of the disc is 15 minutes from the addition of the suspension.

The latex agglutination method to demonstrate PBP 2a exists in several variations, and is produced by various companies. It is a quick method, including extraction of PBP 2a from suspect colonies and subsequent agglutination with monoclonal antibodies to PBP 2a. Some MRSA isolates are weakly positive or agglutinate slowly [15].

Identification of *S. aureus* may also be made using a number of automated systems of which the best-known are: Vitek 2 (BioMerieux), Phoenix (BD) and Microscan (Dade Behring) which have proved to be quite reliable. Automated systems

mainly combine identification and sensitivity tests for the pathogen being tested, and potential MRSA isolates are identified by showing their resistance to cefoxin, mainly by testing a certain range of MICs. There are also methods which require incubation for a certain length of time based on inhibition of growth in a broth, due to the action of oxacillin and with fluorescent indicators of the presence of acids.

MOLECULAR METHODS

The fact that methicillin resistance is undoubtedly related to the importance of the *mec*A gene which codes PBP 2a, the altered protein which binds penicillin, makes it possible to create molecular tests relatively quickly for definite proof of MRSA.

The oldest molecular methods were founded on tests of marked radioactive isotopes or digoxines, but this procedure is technically demanding and time consuming, with a delayed reading of results. Methods based on detection of the *mec*A gene, the PCR method are used routinely in many laboratories now, with the result that they discover even non-functional and non-expressed genes.

In general, PCR methods using only one target sequence, specifically *mec*A, are robust and simple. Of course, the weakness is that it is necessary to have a strain of *S. aureus* already grown and proved, and *mec*A positivity is useful and simple. For a more reliable method it was necessary to develop a test which would at the same time identify *S. aureus* and test for the presence of the *mec*A gene and also include an internal control.

DIRECT DETECTION OF MRSA FROM BLOOD CULTURES

In most microbiological laboratories, after noting positivity in blood culture in automated incubators with an indicator, as the first step, a direct smear is prepared and stained according to Gram. After that, different tests are performed to identify the bacteria in sample and, if it is *Staphylococcus aureus,* susceptibility to oxacillin or cefoxitin are neeeded to identify MRSA strain. There are studies in which the value of the use of molecular methods was tested for direct detection of MRSA in haemocultures, so that a decision may be made in good time about the use of appropriate and effective antimicrobial therapy. It is also possible to detect MRSA directly from respiratory and other samples.

DETECTION OF MRSA FROM SCREENING SAMPLES

Screening samples can be tested by the classical methods listed above, that is by the bacteriological or molecular methods to identify *S. aureus* and note the presence

of the gene which is responsible for methicillin resistance. Since it is possible for patients to be carriers of MRSA and have a small number of bacteria in themselves, an enriched broth is often used for screening, and it is also possible to pool samples, that is, to use one broth for several swabs or DNA extracts from swabs together at the same time. A liquid base for enrichment is used for incubating the samples overnight before they are implanted on a solid base.

Detection of MRSA by culture-agar plates

Many media use indicators on the basis of which the characteristics of *S. aureus* are distinguished from other types, and it is also possible to incorporate inhibiting substances for screening of MRSA, such as oxacillin and cefoxitin. Inhibiting substances also include: NaCl, ciprofloxacin, polymixin, aztreonam, tellurium and desferioxamin. Most screening methods use a system which contains carbohydrates, most often manitol and a pH indicator, and phenol red dye to show the MRSA colony. Chromogene methods are also in wide use, for which the time and conditions of incubation vary. Various chromogene agars with certain inhibiting substances are commercially available and have various levels of sensitivity and specificity, such as for instance: MRSA Select, CHROMagar, MRSA ID [16] which mainly most often use cefoxitin.

There is no single medium which will enable absolute detection of all MRSA and it is necessary to find a formulation which will function well in a local epidemiological situation and which is possible to finance.

Detection of MRSA by culture-enriched broth

The broths that are usually used to increase the sensitivity of screening allow a small number of MRSA to be expressed before sub-cultivation on a solid medium. Increased sensitivity using a broth is desirable, especially in endangered groups of patients and in monitoring the success of decolonization of MRSA. Enriched broths are also known as multiple broths, since several swabs are incubated in them, making savings possible in relation to the amount for processing individual swabs.

Mainly methicillin, oxacillin or cefoxitin are used in broths as the component for selection of staphylococcus. There is a large amount of literature recommending various concentrations of NaCl, but most often a concentration of 6.5% is used, although some MRSAs tolerate higher and some strains lower concentrations of NaCl. The other ingredients are mainly Soy-Tryptose broth, MH broth, BHI and Robertson medium. If an indicator is incorporated in the base, it may show the growth of MRSA

after overnight incubation, and if the medium has high sensitivity, no subculture will be necessary on a solid base in a large number of test tubes.

The use of a liquid base for enrichment, if no indicator is used, may extend the time of processing and increase costs, but if several samples are inoculated in the same liquid base, the costs are reduced so the broth has a role in MRSA screening [16].

MOLECULAR SCREENING METHODS

Various methods have been used for direct detection of MRSA in screening samples over the past 15 or so years. Most molecular tests have multiplex PCR as their basis, by which the gene specific for *S. aureus* and the *mec*A gene are identified at the same time. These methods may only be used with satisfactory effect for purified staphylococcus cultures and cannot be used with great certainty for direct detection. Commercial tests include an appropriate control. The first generation of MRSA molecular tests used this combination, and the weakness in detection directly from samples was the relatively high percentage of false positive results insofar as methicillin resistance coagulase-negative staphylococcus and *S. aureus* sensitive to methicillin were present in the same sample.

In order to eliminate these false positive results, various strategies are used. The best strategy has been shown to be where part of the SCC*mec* elements from the *S. aureus* are amplified at the same time as orfX, which is the basis of the latest PCR tests for direct detection of MRSA from screening samples. The time needed to run the tests, together with preparation of samples is 1.5 to 2 hours and systems are also being developed which may be used by medical staff, not from the laboratory, using applications containing isolation and detection [17]. The costs of molecular tests are significantly greater than using conventional cultures. In the coming years, it is thought that microchips will be the foundation of diagnostics and screening, and tests are being developed which record the use of ATP, bio-luminescence etc.

As an argument for the advantage of molecular tests in relation to conventional ones, the speed of detection and prevention of spread of MRSA are mentioned, due to the early use of measures of isolation and possible decolonization. However, there are many studies dealing with this issue, and their conclusion is that it cannot be said with certainty that introducing quick MRSA testing will lead to a reduction in the incidence of MRSA [18].

PHENOTYPING METHODS

In order to detect similarities between isolates and their relationship in an epidemiological sense, in examination of their pathogenesis and similar characteristi-

cs, the following have had the most important place: biotyping, phagotyping, multi-locus enzyme electrophoresis (MLEE), and serotyping of capsular polysaccharides. These methods have certain weaknesses.

Serotyping has limited application because a large number of unrelated isolates belong to a small number of capsular serotypes [19]. Phagotyping also has limited application since a significant number of isolates are not susceptible to bacteriophages and it is not possible to apply this method to them [20]. MLEE is a technically demanding method [20], and few centres have access to it.

Phenotyping methods also include examination of susceptibility to antimicrobic medication, which, apart from typing, also has the practical value in recommending treatment for the infection. Routinely in laboratories the disc-diffusion (the Kirby-Bauer method) and the micro-dilution method or the E-test method (AB Biodisk, Solna, Sweden) are used to determine the minimal inhibitor concentration. Phenotyping methods have mainly been replaced today by genotyping methods.

Confirmation of the genetic relationship between MRSA isolates is important for defining the occurrence of an epidemic and the use of the appropriate measures in a local environment. In order to examine more thoroughly the molecular evolution of MRSA and its spread in world terms, several molecular typing techniques have been developed.

GENOTYPING METHODS

Typing of bacteria pathogens may be divided into methods based on bands or electrophoretic gels or images, and methods based on sequencing. Imaging typing methods may be based on amplification, or PCR, or restriction, or a combination of these two types of methods. Of the methods based on sequencing we differentiate between multilocus sequence typing - MLST, and single locus sequencing (e.g. spa-Staphylococcal protein A typing).

TYPING THE SCC*MEC* REGION

The *mec*A gene codes penicillin binding protein, PBP, 2a, size 78 kDa which is structurally altered in relation to PBP 2 in methicillin sensitive strains, which is why beta-lactam antibiotics do not bind to it, and as a result there is uninterrupted synthesis of bacterial walls and resistance to beta-lactam antibiotics. The existence of the *mec*A gene on the *S. aureus* chromosome has been known since 1975 [21] but the regulatory genes *mec*I and *mec*R1 were determined and sequenced later, along with the remaining SCCmec elements. SCCmec is found in a fixed location on the chromosome within orfX, of unknown function, but it is in the immediate vicinity

of the area in which gene transcription begins, which explains its rapid expression, very soon after transcription [22].

To date 11 SCC*mec* types are known [23] between 20.9 and 66.9 kb in size, along with several sub-types. So far only methods to type SCCmwc from I-VII have been developed, based mainly on multiplex PCR systems, of varying sensitivity and simplicity.

SCC*mec* typing

In typing the SCC*mec* gene segment, the method of Oliveir et al is the most used and cited, which uses the multiple PCR method to detect six gene loci and the *mec*A gene in the SCC*mec* complex [24]. By small modifications of the method, using the same starters, it is also possible to type SCC*mec* type V [25]. There are some other methods for SCC*mec* typing [27-29]. Zhang et al. suggested a new multiplex PCR, using new starters, which makes it possible to differentiate between sub-types of SCC*mec* IV (a, b, c, d) .

OTHER TYPING METHODS FOR MRSA

Nucleic acid electrophoresis is the basic method of: ribotyping, Pulsed Field Gel Electrophoresis (PFGE), restriction analysis of plasmid DNA [30,31], restriction analysis of PCR products of the coagulase gene (*coa*), restriction fragment length polymorphism, PCR analysis of «inter-IS256 spacer» polymorphism, randomly amplified polymorphic DNA method (RAPD), binary typing, and typing on the basis of VNTR (variable number tandem repeats) and other methods based on PCR.

The southern blot hybridization of MRSA fragments after RFLP may contain genes specific for staphylococcus in the form of a probe, including the mec transposon Tn554, agr, aph(2``)-aac(6`) (gene resistance to aminoglycoside). Typing of insertion sequences is also conducted by RFLP fragments with probes for IS257/431 sequences.

RAPD (randomly amplified polymorphic DNA) analysis is used for typing *S. aureus* and from it the method of binary typing has been developed from which, with the use of more refined bases, probes, signalization and detection, and an increased number of target locations, micro array technology has been developed.

The most commonly used, with good discriminatory characteristics and standardized protocols, are: Pulsed Field Gel Electrophoresis (PFGE) [26], Multilocus sequence typing (MLST) [27], *spa* typing, and SCC*mec* typing [23].

PULSED FIELD GEL ELECTROPHORESIS (PFGE)

The genetic variety of staphylococcus may be examined by various methods, of which the most commonly used is pulsed field gel electrophoresis (PFGE), after restriction of the SmaI enzyme.

The method is based on splitting the genomes of bacteria by restriction enzymes (in the case of *S. aureus* it is the SmaI enzyme) after isolation of DNA in agarose molds and detection of fragments by pulsed field gel electrophoresis. PFGE, after it was first described in 1983, has developed into the reference method for typing of micro-organisms, the "gold standard" for *S. aureus.* Due to the size of DNA fragments obtained by restriction enzymes, which rarely split the DNA (10-800 kpb) their migration through the electrophoresis gel is slow since the mobility of DNA molecules of 50 kpb upwards does not depend on their molecular weight alone. Migration through the electrophoresis gel is improved by use of an electrical field which changes direction over graded time intervals, so that even such large molecules separate out both visually and physically.

The challenge met by the founders of this method was the preparation of intact DNA, or preventing the spontaneous autolysis of the DNA, whose fragments reach the agarose gel and prevent results being obtained. The optimal solution proved to be building the DNA into agarose blocks, into which the partially purified DNA enters, and the process of restriction and rinsing takes place in these blocks [28].

The DNA fragments obtained from the isolate being tested are compared with each other according to criteria describe by Tenover [29]. For application of these criteria it is necessary to have at least 10 bands obtained from the action of the restricted enzymes.

The similarity between the genomes of a large number of isolates is compared using a computer software system. For computer analysis of PFGE profiles it is vital to have evenly distributed, standardized bands on each electrophoresis gel. For comparison of the bands on the gel and between the gels, algorithms are used based on the principles of visual comparison of PFGE profiles, as described earlier in the text.

Regardless whether a computer system or visual comparison is used, it is up to the user to decide which value is for him a "significant difference". The basic weakness of the PFGE method is the relatively poor inter-laboratory reproducibility of the results. The advantage of the method is its application for all types of bacteria, its high power of resolution, and its great intra-laboratory reproducibility.

MULTILOCUS SEQUENCE TYPING (MLST)

MLST is a method developed for the needs of researching the population and evolution biology of important human pathogens, including *S. aureus*. Due to the specific nature, spread, clonal and other characteristics of *S. aureus*, MLST is an irreplaceable method in research of the clonal evolution of MRSA and MSSA, monitoring genetic changes over long periods of time and in different geographical areas, so giving rise to a global epidemiological picture [27]. The method evolved from MLEE and uses genes whose expression products are analysed by MLEE. The method is based on sequence analysis of fragments (0.5 kb) of seven *S. aureus* well conserved genes: *arcC, aroE, glpF, gmk, pta, tpi* and *yqiL*. They code the following enzymes respectively: carbamate kinase, shikimate dehydrogenase, glycerol kinase, guanilate kinase, phosphate acetyltransferase, Triosephosphate isomerase and Acetyl-Coenzyme A acetyltransferase.

By sequencing each gene, the allele profile or sequence type (ST) profile is obtained, which is defined by the alleles of seven genes. The Iberian clone, which is the most frequent, has the MLST profile 3-3-1-12-4-4-16, which belongs to ST247 (www.mlst.net). The nomenclature of MRSA is based on determination of the sequence type (ST) and the SCC*mec* type. Grouping *S. aureus* into clonal complexes (CC) is possible using the BURST computer program (based upon repeated sequences), developed at Bath University in the United Kingdom [30]. It is possible to use this program to analyse evolutionary events. *S. aureus* isolates are allocated to the same clonal complex when 5 of 7 genes have identical sequences.

The weakness of MLST is that it is complex and time consuming, as well as its high cost.

SPA TYPING

Spa typing refers to the sequencing of short sequence repeat (SSR) regions where there is a spa gene, which codes protein A in *S. aureus* [31]. The polymorphic X region of the spa gene is built of a variable number of 24-bp repeating fragments. Differences occur between the fragments due to deletion, point mutations and duplication of nucleotide groups. The composition of the repeating fragments is presented in letters, in that a group of fragments in a certain isolate comprises the "spa repeat" code. The repeating fragments are also marked by a number, in view of their large number and for easier data processing. The spa typing method is suitable for research related to isolates within an epidemic in a hospital; it detects genetic microvariations and may be used in phylogenetic studies, where genetic macrovariations are key.

The basic advantage of this method is its simplicity, since as it only sequences one genetic locus [32] the probability of error is also smaller. The discriminatory ability is between MLST and PFGE [32].

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Sažetak

Metode detekcije i tipizacije meticilin-rezistentnih sojeva bakterije
Staphylococcus aureus

Staphylococcus aureus jedan je od najčešćih uzročnika bolničkih infekcija, a i njegova je uloga u izvanbolničkoj sredini također velika, osobito nakon 90-ih godina prošlog stoljeća. Danas meticilin-rezistentni *Staphylococcus aureus* (MRSA) uzrokuje 40 – 70% stafilokoknih infekcija u jedinicama intenzivnog liječenja. Podaci o prevalenciji MRSA-e razlikuju se u različitim dijelovima svijeta i po različitim bolnicama. Javlja se od 0,6% u Norveškoj do 66,8% u Japanu, a 2008. je zamijećen trend opadanja prevalencija incidencije MRSA-e koji se nastavlja u različitim europskim zemljama, pa i u Hrvatskoj. MRSA se izdvaja iz kliničkih uzoraka kao dio dijagnostičkog postupka, a iz nadzornih kultura kao dio programa nadzora nad kliconoštvom. Identifikacija vrste *S. aureus* temelji se uglavnom na dokazu katalaze, koagulaze i DNA-aze, a postoje i komercijalni lateks-aglutinacijski testovi koji se temelje na dokazivanju specifičnih antigena bakterije *S. aureus* (npr. protein A). Meticilinska rezistencija vrste *S. aureus* može se dokazati testiranjem osjetljivosti na cefoksitin ili dokazom prisutnosti gena *mecA* odgovornog za meticilinsku rezistenciju. Postoje i automatizirani sustavi koji se rabe i za identifikaciju bakterija i za određivanje osjetljivosti. Dokazivanje MRSA-e u nadzornim uzorcima otežano je zbog prisutnosti normalne bakterijske flore te se, radi sprječavanja rasta ostalih bakterija, rabe različiti selektivni bujoni za prekonoćnu inkubaciju i/ili različite selektivne podloge (npr. kromogeni agar). Probir MRSA-e može se izvoditi i uz pomoć molekularnih testova, među kojima su najspecifičniji i najosjetljiviji oni iz treće generacije, koji kao cilj detekcije imaju cijelu regiju između SCC*mec* fragmenta i *orfX* (ne samo *mecA* gen). Tipizacija je važna kod MRSA-e jer omogućuje uvid u povezanost izolata, pomaže utvrditi prijenos patogena i otkriti izvor iz kojeg se širi. Fenotipskim metodama tipizacije uspoređujemo fenotipska svojstva bakterija i umnogome su zamijenjene metodama genotipizacije. Najvažnije su i najčešće korištene i citirane genotipizacijske metode: elektroforeza u pulsirajućem polju (Pulse-field Gel Electrophoresis-PFGE), zatim višelokusno sekvencijsko tipiziranje (Multi-locus Sequence Type-MLST) i sekvencioniranje regije koja kodira za protein A (sequencing of protein A region of *Staphylococcus aureus* - spa typing). Potrebno je istaknuti važnost točne i relativno brze dijagnostike MRSA-e u kliničkim i probirnim uzorcima.

Ključne riječi: MRSA; detekcija; tipizacija