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Methicillin-resistant Staphylococcus aureus (MRSA) – ,,neverending story''

Metycylinooporne gronkowce złociste (MRSA) – „niekończąca się historia"

Staphylococcus aureus is a Gram-positive bacteria discovered by Sir Alexander Ogston in 1883 [10]. Nowadays, it is one of the most important pathogen responsible for hospital or community-acquired, local and systemic infections, e.g. skin and soft tissue infections or blood infections. In addition, it also causes the catheter-related infections as the result of biofilm formation and also toxin-mediated diseases, like toxic shock syndrome, scalded skin syndrome and staphylococcal foodborne disease [7, 11]. It is important to know that about 20–30% of people are persistent carriers of *S. aureus*, while 30% are colonized transiently (intermittent carriers); *S. aureus* preferably colonizes the anterior nares of the carriers [11].

From the beginning of the antibiotic era, selective pressure exerted by antibiotics has very soon resulted in resistance development in staphylococci. Antibiotic resistance is a major worldwide public health problem as the result of improper use or overuse of the antimicrobials [6, 13].

MECHANISMS OF *S. AUREUS* RESISTANCE TO BETA-LACTAMS

The most important antimicrobial agents with potential antistaphylococcal activity are beta-lactams. For a long time they were used as the drug of choice for treatment of *S. aureus* infections due to good safety profile of beta-lactams and their bactericidal activity against staphylococci. The target for these antibiotics are penicillin binding proteins (PBPs), which are the bifunctional enzymes involved in peptidoglycan synthesis – the main compound of bacterial cell wall responsible for its flexibility and strength. Staphylococci possess four PBPs (PBP1-PBP4). These enzymes are involved in elongation of the glycan strands (glycosyltransferase activity) and in interconnection of the glycan strands by peptide cross-linking (transpeptidase activity). Beta-lactam antibiotics are the structural analogs of the natural substrate for PBPs, such as dipeptide D-alanyl-D-alanine, an important component of the peptide cross-linkage between the glycan strands. The beta-lactam antibiotics covalently bind to PBPs, followed by inactivation of their enzymatic activity [8, 11].

The first beta-lactam antibiotic introduced to medicine in 1942 was penicillin G. One year later, in 1943, the first strain of S. aureus resistant to this antibiotic was detected, possessing the ability to produce beta-lactamases, called penicillinases, responsible for enzymatic inactivation of penicillin G due to cleavage of beta-lactam ring, essential for antibacterial activity of beta-lactams. These enzymes are determined by blaZ gene usually located on penicillinase plasmids. Staphylococcal beta-lactamases are able to inactivate natural penicillins, like penicillin G and penicillin V and also aminopenicillins (ampicillin, amoxicillin) [11]. Modification of penicillins resulted in obtaining the semi-synthetic penicillins like methicillin, oxacillin and nafcillin. These antibiotics are resistant to the activity of penicillinases. However, in 1961 the first strain resistant to methicillin was discovered. Moreover, methicillin-resistant strains express resistance to all beta-lactam antibiotics, like penicillins, cephalosporins and carabapenems. They are also insensitve to a combination of beta-lactam with beta-lactamase inhibitors (e.g. amoxicillin with clavulanic acid). The mechanism of methicillin-resistance is quite different from that described above and is based on the presence of mecA gene encoding an alternative, modified PBP protein – PBP2a with low affinity for beta-lactams, allowing undisturbed peptidoglycan synthesis, despite the presence of antibiotic in the environment [8, 11].

THE GENETIC ORGANIZATION OF MECA OPERON

The *mecA* gene is present in the unique segment of DNA called the staphylococcal chromosome cassette (SCC*mec*); the scheme of SCC*mec* organization is presented in Fig. 1. SCC*mec* is a genetic element with conserved and variable regions located within the chromosome of MRSA strains near the origin of replication (*orfX*). The conserved region contains the *mec* operon composed of *mecA* gene and the regulatory *mecI*, *mecR1* genes as well as the cassette chromosome recombinase complex *ccr* (*ccrA, ccrB* or *ccrC* genes). The *ccr* genes are responsible for integration of the cassette into the chromosome and its precise excision from the chromosome. The variable region (J-region) can contain the following elements: plasmids (pT181, pUB110, p1258), transposons (Tn554) or insertion sequences (IS431, IS1272, IS256). Eight types of SCC*mec* (I-VIII) were described on the basis of the existence of several variants of *ccr* locus (allotypes). Moreover, some SCC*mec* types (e.g. type II) can contain resistance genes to other antibiotics (macrolides, tetracyclines) or heavy metal ions (mercury, cadmium) [8, 11].

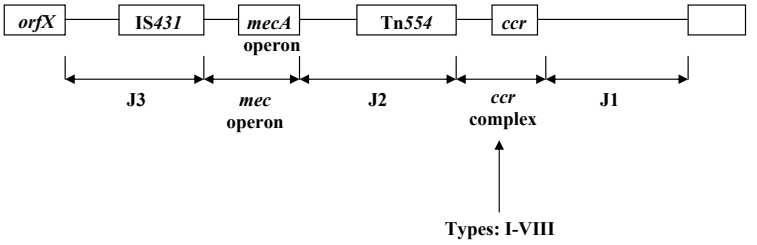

Fig. 1. The structure of *mec* staphylococcal chromosome cassette (SCC*mec*); *orfX* – open reading frame of bacterial chromosome; J1-3 – variable regions containing plasmids, inserted sequences (IS) and/or transposons (Tn); *mecA* operon – locus containing *mecA* gene with regulators like *mecR1* and *mecI*; *ccr* complex – locus containing variable allotypes of *ccr* genes

TYPES OF MRSA STRAINS

Nowadays, on the basis of the differences in epidemiology of MRSA, they have been categorized into three groups:

- HA-MRSA – hospital-acquired MRSA,
- CA-MRSA – community-acquired MRSA,
- FA-MRSA – farm-associated MRSA (also called as LA-MRSA – livestock MRSA).

The definition of HA-MRSA strains describes them as isolates from patients that had been MRSA-negative at the beginning of hospitalization and MRSA were isolated at least 48 hours after hospitalization [1]. On the basis of molecular typing HA-MRSA strains have been grouped into clonal complexes. The clones of HA-MRSA responsible for several outbreaks all over the world were described, like: Iberian clone (CC247, Spain, 1989), Brazillian clone (CC239, Brasil, 1992), EMRSA-15 clone (CC22, United Kingdom, 1993), Pediatric clone (CC5, Portugal, 1992), New York/Japan clone (CC5, USA, 1998). These clones are also called pandemic because of their ability to disseminate internationally [14]. The HA-MRSA usually infects patients with immunosupression, especially hospitalized in intensive care units. The most frequent HA-MRSA infections are: infections of postoperative wounds, pneumonia and also bacteremia together with sepsis. The typical characteristic of HA-MRSA is the multidrug resistance, i.e. insensitivity to at least three antibiotics belonging to different chemical groups, as a result of the presence of additional resistance genes within SCC*mec*; usually HA-MRSA contains SCC*mec* type II. Moreover, it was found that HA-MRSA very often are able to produce at least one superantigen toxin (e.g. TSST-1 – toxic shock syndrome toxin). These extracellular enzymes are strongly mitogenic towards T cells and do not require proteolytic processing prior to binding with MHC type II on antigen-presenting cells. Moreover, they can have a direct toxic effect on endothelial cells and may lead to capillary leakage, hypotension and shock [11].

In the years 1997–1999 another group of MRSA called community-acquired MRSA (CA-MRSA) became a worldwide emerging problem in the community [2]. They are defined as MRSA isolated from outpatients with no history of hospitalization within the past year. These patients do not show any established risk factors for MRSA infections, like surgery, residence in a long-term care facility, dialysis, the presence of indwelling medical devices or catheters. It has to be noted that very often young and healthy people are predicted for the infections of CA-MRSA [1]. In most cases this pathogen causes skin and soft tissue infections (70–80%) and, to a lesser, extent urinary tract infections, sinusitis or the most dangerous – necrotizing pneumonia. Interestingly, CA-MRSA are quite often associated with pneumonia as a result of co-infection of influenza patients; fatal pneumonia caused by CA-MRSA was recognized in patients infected with the virus of swine flu (H1N1v). In contrast to HA-MRSA, CA-MRSA strains usually contain the SCC*mec* type IV or V, without any additional antibiotic resistance genes. In Europe CA-MRSA strains are usually resistant to clindamycin and tetracycline. The characteristic of some of these strains, especially involved in skin and soft tissue infections, is the ability to produce Panton-Valentine leucocidin (PVL) [2, 14]. The significance of PVL in pathogenesis of CA-MRSA infections remains disputed (unclear). Some data suggest that this toxin may be an important virulence factor in pulmonary infections. On the other hand, it has also been described that PVL may be only a marker for CA-MRSA [2, 14]. Similarly to HA-MRSA, the population of CA-MRSA strains expresses clonal character; several

clones are described, e.g. CC1 (clone USA400), CC8 (clone USA300) in USA and Canada and CC80 clone in Europe and also in South Africa. Characteristic of CC80 clone is resistance to fusidic acid, as a result of the presence of one of the following genes: *fusA, fusB* or *fusB*. Strains belonging to this clone may be also highly resistant to mupirocin, which in consequence makes eradication of MRSA difficult [14].

A new group of MRSA, called as FA-MRSA, was recognized in the Netherlands in 2003. All of these strains belong to clonal complex CC398 [3, 5, 12]. It was found that this clone colonizes pigs and may spread to other farm animal species; it is now prevalent in farm animals in some countries of Central and Northern Europe. The FA-MRSA strains were isolated from animal infections and also from human and animal carriers [9]. Also, some infections in humans can be caused by this pathogen, mostly skin and soft tissue infections. Previous data [12] showed that a direct contact with pigs and veal calvesis was associated with an elevated risk of MRSA carriage. It was found that healthcare workers are involved in transmission of FA-MRSA between patients and there have been several reports strongly pointing to them as a source of nosocomial MRSA infections. Moreover, transmission between people, for example between family members has been demonstrated. However, it was also found that this transmission is quite slow, so clone CC398 seems to be primarily adapted to animals. The FA-MRSA are usually sensitive to antibiotics; in most cases the resistance to tetracycline is detected as a result of its usage in veterinary [12].

TREATMENT OF MRSA INFECTIONS

As stated above, the MRSA possess insensitivity to all beta-lactam antibiotics. The general sensitivity of CA-MRSA and FA-MRSA strains to tetracyclines, macrolides, clindamycin, trimethoprim/sulfamethoxazole, fluoroquinolones, rifampicin (always in combination with other antibiotics) makes them useful in the treatment of infections caused by these strains [2]. The problem of multidrug resistance MRSA is usually associated with HA-MRSA isolates. In this situation quite often the drugs of choice are glycopeptides such as vancomycin. Similarly to beta-lactams, vancomycin is the inhibitor of peptidoglycan cell wall synthesis as a result of noncovalent binding with the D-alanylo-D-alanine termini of peptidogylcan precursors [1,8]. However, the side-effects of vancomycin limit its usage. Another problem is the appearance of MRSA strains expressing intermediate sensitivity to vancomycin (VISA – vancomycin intermediate-resistance *S. aureus*) or even resistance (VRSA – vancomycin resistance *S. aureus*) [4].

The newly described drugs with activity against MRSA were introduced at the beginning of 21st century, e.g. linezolid and daptomycin [1, 14]. Linezolid belongs to a new class of antibiotics – oxazolidinones, inhibitors of protein synthesis as a result of binding with 23 S rRNA in 50S ribosomal subunit. The important characteristic of linezolid is better penetration into tissue than that of vancomycin. However, the first strain of *S. aureus* resistant to linezolid has been detected. Moreover, the bacteriostatic effect limits the usage of this drug [14]. One of the newest antibiotic active against MRSA is daptomycin. In contrast to linezolid, it expresses bactericidal effect. It is a cyclic lipopeptide with unique mechanism of action with no cross-resistance to glycopeptide-resistant strains. Daptomycin was registered to the treatment of skin and soft tissue infections and right-sided

endocarditis. Unfortunately, it cannot be used in pneumonia since daptomycin is inactivated by the surfactant.

A new group with potential anti-MRSA activity expresses ceftobiproles, also called the fifth generation of cephalosporins. Interestingly, they are able to bind with modified PBP2a. At this time, these antibiotics have been registered in Switzerland and Canada for the treatment of complicated skin and soft tissue infections, including diabetic food infections. Moreover, the new antibiotics with potential activity against MRSA in vitro are under studies like new glycopeptides (telavancin), oxazolidinone (RX-1741, TR-701), streptogramins (flopristin/linopristin), tetracyclines (PTK0796) and cephalosporins (ceftaroline) [14].

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SUMMARY

Staphylococcus aureus is one of the most common human pathogens causing hospital- and community-acquired infections. Methicillin-resistant strains of *S. aureus* (MRSA) make a therapeutic problem due to their resistance to all of the beta-lactam antibiotics, often accompanied by resistance to other groups of antibiotics (called as multiresistance). The mechanism of methicillin-resistance is associated with an acquisition of mecA gene encoding modified enzymatic protein (PBP2a), which is responsible for biosynthesis of peptidoglycan in bacterial cell wall, despite the presence of beta-lactams in the environment. For many years the problem with MRSA has been restricted to hospitalized patients; strains isolated in the hospital are called as HA-MRSA (hospital-acquired MRSA). However, in recent years new groups of MRSA called as CA-MRSA (community-acquired MRSA) and FA-MRSA (farm-associated MRSA) have been isolated in the community. Recently, new antibiotics with potential activity against MRSA such as linezolid and daptomycin have been introduced for treatment of infectious diseases.

STRESZCZENIE

Staphylococcus aureus jest jednym z najpowszechniej występujących patogenów człowieka, wywołującym zakażenia szpitalne i pozaszpitalne. Problem terapeutyczny stanowią metycylinooporne szczepy *S. aureus* (MRSA), ponieważ wykazują oporność na wszystkie antybiotyki beta-laktamowe, a często również na inne grupy antybiotyków (tzw. wielooporność). Mechanizm metycylinooporności jest związany z nabyciem genu mecA, kodującego zmodyfikowane białko enzymatyczne (PBP2a) odpowiedzialne za biosyntezę peptydoglikanu ściany komórkowej bakterii, nawet w obecności beta-laktamów w środowisku. Przez wiele lat problem MRSA był ograniczony do pacjentów szpitalnych; w tym środowisku izolowano HA-MRSA (hospital-acquired MRSA). W ostatnich latach zaobserwowano pojawienie się nowych grup MRSA związanych ze środowiskiem pozaszpitalnym – CA-MRSA (community-acquired MRSA) oraz FA-MRSA (farm-associated MRSA). Po roku 2000 do lecznictwa wprowadzono nowe leki aktywne wobec MRSA, takie jak linezolid czy daptomycyna.