Antimicrobial resistance (AMR) represents a real burden for the modern medicine. One of the most frequently isolated hospital acquired (HA) pathogens worldwide, is Methicillin resistant Staphylococcus aureus (MRSA). Recently not only HA, but also community-acquired MRSA (CA-MRSA) infections have been reported. A prospective study was performed between February 2009 and October 2010, with the aim to investigate bacterial resistance of CA-MRSA and HA-MRSA. DNA microarray technology has been used for the detection of 4 AMR genes for the studied MRSA strains. A number of 218 HA- S.aureus strains have been isolated, from which 89 (40.82%) were MRSA. In the community, 1,553 S.aureus strains were isolated, out of which, 356 (22.92%) were MRSA. From these, a number of 17 HA and 12 CA-MRSA strains have been analyzed by DNA microarray technology. From 100% phenotypically described HA-MRSA, we identified mecA gene in 10 (100%) strains. From these, 33% (10 strains) had mecA, only one (8.33%) was erm(A) positive and 4 (33.33%) were erm(C) positive. DNA microarray is a method allowing the concomitant scan of multiple genes and has been described for typing resistance genes in Gram positive and Gram negative bacteria, but it has not become a commonly used diagnostic method due to prohibitive costs of reagents, machines and lack of qualified personnel [12-15]. The aim of the present study was the molecular diagnosis of MDR-MRSA by DNA microarray technology in both HA and CA-MRSA strains in Western Romania.

**Experimental part**

**Bacterial strains collection and microbiological method**

A prospective study was performed between February 2009 and October 2010, with the aim to investigate bacterial resistance of CA-MRSA, with strains provided by S.C. Bioclinica S.A. and HA-MRSA, with strains provided by the intensive care unit (ICU) of Pius Branzeu Emergency Clinical County Hospital Timisoara (PBECCHT). PBECCHT is the biggest regional county hospital from the Western Romania, with more than 1100 beds, having a 27 beds ICU, which provides healthcare assistance for both medical and surgical cases.

**Keywords:** MRSA, mec A, erm(A), erm(C), tet(O), microarray
Only the strains identified after at least 48 h of hospitalization were included as HA pathogens (all strains identified upon admission were discarded); in both HA and CA infections, we only included the first clinically relevant strain, in order to avoid duplication and phenotypic changes induced by antibiotic selection pressure. No age, gender, infection site or prior antibiotic use exclusion criteria were applied.

HA- *S. aureus* strains have been identified especially from bronchial aspirates, blood, urine samples, wound secretions, catheter tps. Identification was done using the VITEK 2 Compact (BioMerieux®, France) automated system with VITEK 2 GP cards. The sensitivity of bacterial strains was analysed by the microdilution method (AST cards) and interpreted by the VITEK 2 Compact System according to the minimum inhibitory concentration (MIC) breakpoints set by the National Committee on Clinical Laboratory and Standards Institute Inc. (CLSI M100-S16, 2010). The following quality control strains were used: *S. aureus* ATCC 43300 and *S. aureus* ATCC 29213.

MDR was defined as acquired resistance to at least one agent from three or more antimicrobial categories and XDR as sensitivity to a maximum two antimicrobial categories.

Genomic DNA isolation and labeling
DNA was extracted from fresh (24 h from inoculation) colonies, grown on sheep blood agar medium. The total bacterial DNA was extracted from half a loopful of bacterial cells suspended in 200 µL PBS using High Pure PCR Template Preparation Kit (Roche Applied Science, cat. no. 1179682800). The quality and concentration of DNA was determined with the NanoDrop ND1000 spectrophotometer. DNA was labelled with Alexa Fluor 3/5 by a randomly primed polymerization reaction and purified using BioPrime Total Genomic Labeling System (Invitrogen, cat. no. 18097-011) according to the manufacturer’s directions.

Oligonucleotide design and microarray construction
Oligonucleotide probes were designed according to Frye [16] and represented 4 genes of the following classes of antimicrobials: ß-lactam antibiotics, tetracycline and macrolides. The kit was designed to determine resistance genes for *S. aureus*. The oligonucleotides were manufactured and spotted in triplicate by ArrayIt Corporation (Sunnyvale, CA, USA) in an 18 well subarray format on standard glass slide (25 x 76 x 0.96 mm).

Hybridisation, scanning and analysis
Dye-labelled DNA was dried, re-suspended in HybII 2 hybridisation buffer (ArrayIt Corporation, cat. no. HHS2) and applied to a specific well subarray prepared according to the manufacturer’s directions. Hybridisation was performed in 3 h at 42°C. Protocols suggested by the manufacturer were used for post-hybridisation washing procedures. Microarrays were scanned with SpotLight CCD Scanner (ArrayIt Corporation, Sunnyvale, CA, USA). Images were analysed using GenePix Pro 7 (Molecular Devices, Sunnyvale, CA, USA) (fig.1).

Statistical analysis and ethics
The 6.04.version of the EPI-INFO program was used for statistical analysis. Percent values were compared by contingency tables, using the chi-squared test and Fisher correction. All the statistical tests were calculated with two extremities and the value of p statistical significance was considered at ≤ 0.05.

The study was approved by the Ethical Committee of the Victor Babes University of Medicine and Pharmacy Timisoara (No.10/11.10.2008), and by the partner hospital involved in the project.

Results and discussions
In the studied ICU, a number of 218 *S. aureus* strains have been isolated, from which 89 (40.82%) were MRSA. We identified 145 (66.51%) MDR and 44 (20.18%) XDR *S. aureus* strains. In the community, we isolated a number of 1,553 *S. aureus* strains, from which, 356 (22.92%) were MRSA. Also, we identified 209 (13.45%) MDR and 20 (1.27%) XDR strains.

From these, a number of 17 HA and 12 CA-MRSA strains have been analyzed by DNA microarray technology. Almost all the 17 HA-MRSA strains associated other resistance phenotypes, being included in the group of MDR microorganisms (presenting wild phenotypes of resistance to only 2-3 antimicrobial classes: oxazolidinones, fusidic acid, rifampiclin). Fortunately, the 12 CA-MRSA strains were less resistant, with many other wild phenotypes of resistance, to other classes, like, fluoroquinolones, glycopeptides, sulfamides, etc. (table 1).

Regarding the DNA microarray technology, from 100% phenotypically described HA-MRSA, we identified mecA gene in 10 strains (58.83%), comparing with 83.33% CA (10 strains), with p = 0.234. In terms of erythromycin resistance, 6 HA strains (35.29%) were erm(A) positive, comparing with only one CA (8.33%), with p=0.187. Other 4 HA-MRSA strains (33.33%) were positive for erm (C) while, no erm(C) positive CA strains have been found (with statistical significance with p= 0.020). No tetracycline genotypic resistance was found in HA - MRSA strains, comparing with 4 CA strains (23.53%), with p=0.121.

A possible explanation for the small percentage of mecA gene identification should be eventually, the small amount of DNA. Because the culture media is inhibitor for DNA extraction, it is possible that it might not been enough for being marked with Cy3 or Cy5.

Nizami D. et al [8] reported a total of 16.5 per cent of *S. aureus* isolates showing resistance to methicillin and carrying mecA gene. Also, a total of 145 isolates were resistant to erythromycin, and contained at least one of the erythromycin resistance genes erm(A), erm(B), erm(C) and msr(A). The erm(A) and erm(C) genes have been detected in 77 isolates and erm(B) in 13 isolates. Eleven isolates carried both erm(A) and erm(B). A total of 121 isolates were resistant to tetracycline and carried either tet(K) or tet(M) or both resistance genes.
<table>
<thead>
<tr>
<th>No.</th>
<th>Provenience</th>
<th>Resistance phenotype</th>
<th>mecA</th>
<th>tetO</th>
<th>ermB</th>
<th>ermC</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Control strain</td>
<td>MRSA - positive control</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>0</td>
<td>Control strain</td>
<td>S. aureus ATCC 43500</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>HA</td>
<td>MRSA + beta-lactamases production + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>HA</td>
<td>MRSA + beta-lactamases production + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>9</td>
<td>HA</td>
<td>MRSA + betalactamases production + Resistant (APH (3') - LIII)/Resistant (ANT (4') - IV)/wild</td>
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<td>-</td>
<td>+</td>
<td>-</td>
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<td>10</td>
<td>HA</td>
<td>MRSA + beta-lactamases production + Resistant (APH (3') - LIII)/Resistant (ANT (4') - IV)/wild</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>HA</td>
<td>MRSA</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>HA</td>
<td>MRSA + PBP Modification + wild AMLC + Resistant</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + wild</td>
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<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>HA</td>
<td>MRSA + PBP Modification + Heterogen (APH (2') + AAC(6')) + resistant/partially resistant</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>HA</td>
<td>MRSA + PBP Modification + Resistant (APH (3') - LIII)/Resistant (ANT (4') - IV)/wild</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>HA</td>
<td>MRSA + PBP Modification + Resistant (APH (3') - LIII)/Resistant (ANT (4') - IV)/wild</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
In another study performed by Elhassan M. et al, 90.2% from all the MRSA studied strains were mecA positive, while the remaining 9.8% failed to produce the band of 310 bp specific for mecA gene. Bacterial DNA was isolated with the aid of ready kit from Thermo Scientific GeneJET Genomic, Lithuania [17].

Lim et al [18] reported that the erm(A) gene was more prevalent than the other erythromycin resistance genes in S. aureus isolates, and erm(C) gene was found mostly in coagulase negative staphylococcus (CoNS). Similarly, in a study performed by Martineau et al [19], the erm(C) gene has been reported to be more prevalent in CoNS.

In our study, more erm(A) genes were isolated in the hospital and erm(C) in the community.

In another study conducted by Spence R. et al, characterization of 43 S. aureus isolates by the microarray technology and pulsed-field gel electrophoresis demonstrated the ability of the array to differentiate between isolates representative of a spectrum of S. aureus types, including methicillin-susceptible, methicillin-resistant, community-acquired, and vancomycin-resistant S. aureus, and to simultaneously detect clinically relevant virulence determinants. The microarray technology was comprising 84 gene targets, including species-specific, antibiotic resistance, toxin, and other virulence-associated genes, capable of examining 13 different isolates simultaneously [20].

Conclusions

Our findings indicate a high prevalence of HA and also CA – MRSA, which represents a priority in terms of treatment and control. That’s why, rapid and reliable tests are important, to start an appropriate therapy. If MRSA strains identification and AST by conventional methods require a minimum of 48 hours, the detection of AMR genes by DNA microarray technology can be done within a few hours.

However, the absence of mecA gene in a considerable percentage of MRSA isolates requires technique improvement and also, more oligonucleotides probes needed to be designed for the identification of other antimicrobial resistance genes, by microarray technology.

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References


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