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Investigation of Methicillin-Resistant *Staphylococcus aureus* strains from Satu Mare using Molecular Biology Techniques

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SUMMARY. *Staphylococcus aureus* is a clinically important bacteria. It causes mild to severe, often life-threatening infections. A total of 51 *Staphylococcus* strains were analyzed in this study using polymerase chain reaction. 33% of the strains were confirmed as *S. aureus*, and none of these were resistant to methicillin.

Keywords: *mecA*, MRSA, *nucA*, PCR, *Staphylococcus aureus*

Introduction

The *Staphylococcus aureus* is a sphere shaped, non-motile, Gram-positive bacterium, which forms grape-like clusters. Due to their antibiotic resistance, the methicillin-resistant *S. aureus* (MRSA) strains are widespread both in hospitals and ambulatory units. These strains were documented first in 1961 (Ito *et al.,* 1999).

The *mecA* gene encodes a modified penicillin-binding protein, the PBP2a, which has low affinity to penicillin antibiotics (Song *et al*., 1987). The *mecA* gene together with the *mecI* and *mecR1* genes form the *mec* operon, which is located on the SCC*mec* mobile genetic element (Ito *et al.,* 2004). This has several classes and sub classes (Ito *et al.*, 2001).

The aim of this study is to investigate the presence of MRSA is Satu Mare County. It is necessary to know what kind of antibiotics can be considered to be taken by the patients.

Materials and methods

The analysed *Staphylococcus* strains are isolated and determined using classical microbiological methods in the CityMed laboratory, from the Satu Mare county Public Health Department.

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Using the multiplex polymerase chain reaction (PCR) technique we amplified a 270 bp long region of the thermonuclease gene (*nucA*), which is specific to *S. aureus* strains, and a 533 bp long region of the *mecA* gene. In case of the *nucA* gene the presence of amplification products confirm that the analysed strains are *S. aureus*, and the *mecA* amplicon demonstrates that the studied strains are resistant to methicillin and other penicillin group antibiotics (cross-resistance) (Matthews and Tomasz, 1990). First we used a colony PCR method, which lacks DNA extraction and a suspension of bacteria were directly added to the PCR mix, and second the PCR was repeated with isolated DNA. Later the experiments were repeated using purified DNA from selected isolates. The PCR mix contained the following components:

10 µl 5x buffer
3 µl $MgCl_2$ with 1.5 mM final concentration
1 µl dNTP 0.2 final concentration
0.5 µl mecA I primer 1 µM final concentration
0.5 µl mecA R primer 1 µM final concentration
0.5 µl nucA R primer 1 µM final concentration
0.5 µl nucA I primer 1 µM final concentration
GoTaq polymerase 1.25 U

40 µl in a reaction tube + 10 µl sample DNA

We used the following PCR programme:

Initiative denaturation: 5 minutes 94º C
Denaturation: 30 seconds 92º C
Annealing: 30 seconds 52º C
Extension: 60 seconds 72º C
} 30 cycle
Final elongation: 5 minutes 72º C

Results and discussion

Using colony PCR we found seven MRSA suspect strains. Repeating the amplification with DNA from these suspect strains we could not confirm the presence of methicillin-reisitant *S. aureus*. From the total of 51 isolates only 33% were confirmed as *S. aureus* (Fig. 1). The investigated strains in majority were isolated from nasal discharge (Fig. 2).

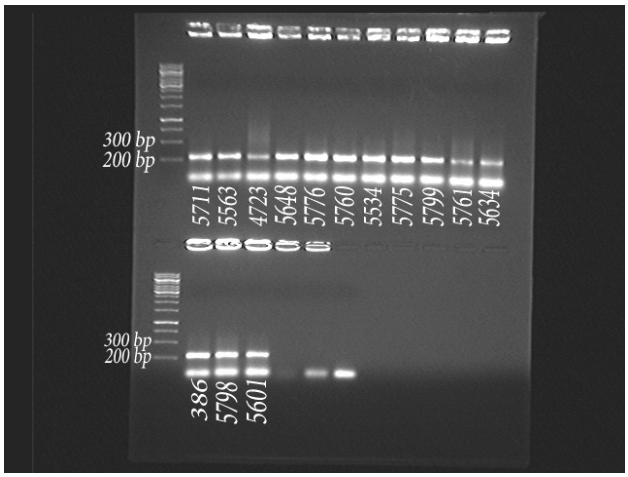

Figure 1. Separation by agarose gel electroforesis (1%) of multiplex PCR products. The first lane represents molecular size ladder (GeneRuler 100 bp DNA ladder, Fermentas). In case of *S. aureus*trains a clear band can be observed with an expected size of 270 bp. The last three lanes are negative controls without sample DNA.

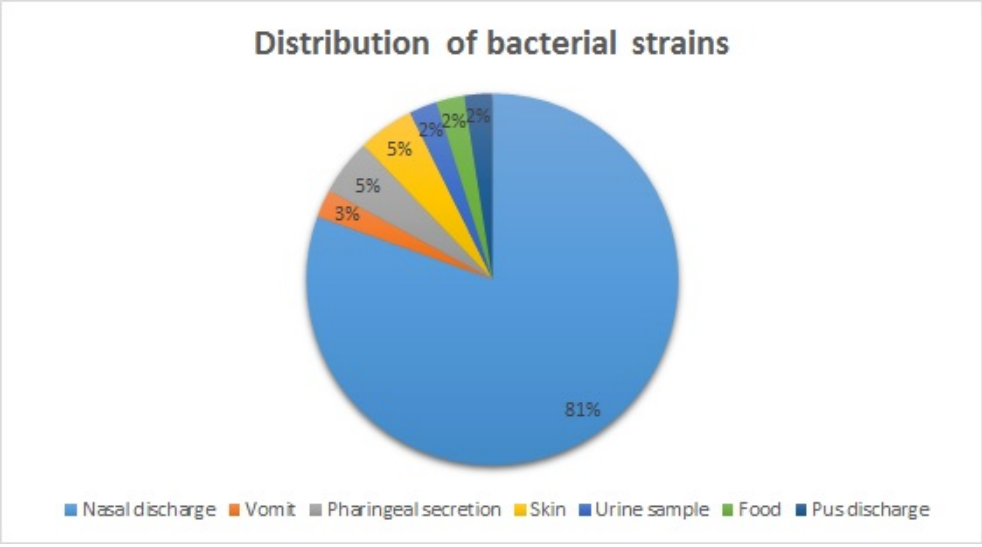

Figure 2. Distribution of bacterial strains between source of isolation. The generality of the strains stem from nasal discharge 81%, presenting that this bacteria appears mostly in the respiratory tracts. A few isolates stem from pharingeal secretion, 5%, skin, 5%, food, 2%, pus discharge, 2% and urine sample, 2%.

We could not identify methicillin-resistant *S. aureus* strains.

Conclusions

Using molecular biology methods only 33% of the strains were confirmed as *S. aureus*. The other are probably coagulase-negative staphylococcus strains. It is difficult to make distinction between the coagulase-positive and coagulase-negative strains, because using classical methods we can not see the slight difference which can be seen with molecular methods. For example: using PCR.

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