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Staphylococcus aureus virulence phenotypes among Romanian population

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ABSTRACT

S. aureus is an important pathogen, especially due to the emergence of strains that are resistant to most antibiotics used against bacterial pathogens (MRSA). The aim of this study was the analysis of virulence patterns in corelation with the infection site for 100 MRSA strains isolated between 2011-2013, in the Laboratory of the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C.Iliescu", Bucharest. The identification of *Staphylococcus* strains was done with conventional tests, API STAPH tests and Vitek 2 Compact Sistem. The virulence patterns were determined with cellular substrata and specific enriched mediums. The results of this study showed that 95% of the *S. aureus* strains possessed at least one of the virulence factors and the most virulent strains were isolated from nasal secretions. **Conclusions.** *S. aureus* is the most versatile bacterial pathogen because of its rich repertoire of virulence factors which are expressed in a coordinated manner, explaining its ability to cause a wide spectrum of infections in humans. Moreover, the MRSA strains show an increasing frequency in the hospital environment worldwide. The present study showed that he most expressed virulence factor present in 88% of the strains was the hydrolysis of esculin, suggesting the important role played by esculetol in the evolution of *S. aureus* infections. Given the high incidence of invasive infections with methicillin-resistant *S. aureus,* finding new therapeutic strategies to combat staphylococcal infections is a priority at an international level.

Keywords: *MRSA, soluble virulence factors, adhesion to cellular substratum*.

1. INTRODUCTION

The *Staphylococcus* genus belongs to the *Micrococcaceae* family, comprising of facultative anaerobic, immobile Gram-positive cocci [1; 2]. The species of *Staphylococci* most frequently isolated in the invazive infections are: *S. aureus*, *S. epidermidis* and *S. saprophyticus* [3; 4]. *S. aureus* is an important pathogen, especially due to the emergence of strains that are resistant to most antibiotics used against bacterial pathogens [5; 6]. *S. aureus* colonizes and infects immunocompromised patients as well as immunocompetent individuals [7]. The nostrils are considered the major reservoir for *S. aureus* infections [8]. Other sites of colonization for *S. aureus* are the: skin, perineum, axillae, vagina and gastrointestinal tract [9; 10]. Although a frequent member of the normal microbiota, under certain conditions *S. aureus* can cause opportunistic infections [11; 12]. The ability of *S. aureus* to cause a wide spectrum of infections in humans is due to a rich repertoire of virulence factors expressed [13-15]. Romania is one of the countries with the highest prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) in the world, ranging from approximately 30% up to 65% [16-20].

2. EXPERIMENTAL SECTION

In this study there were analyzed 100 methicillin resistant *S. aureus* strains (MRSA) selected from a collection of isolates obtained from nasal carriage and nosocomial infections between 2011-2013, in the Microbiology Laboratory of the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C.Iliescu", Bucharest. The identification of the strains was based on conventional tests, the API STAPH bioMerieux test and the Vitek 2 Compact Sistem bioMerieux. The coagulase test was performed with Slidex Staph- kits bioMerieux. API STAPH is a standardized system used for the identification of *Staphylococcus, Micrococcus* and *Kocuria* genera by miniaturized biochemical tests and the electronic ApiWeb database, for identification. The kit contains 20 microtubes with dehydrated substrates, which in contact with the bacterial suspension during the incubation, change color triggered by the bacterial metabolism. The VITEK 2 Compact System uses a bacterial database containing specific biochemical details. For Gram-positive bacteria the identification card contains total of 43 tests based on the activity of metabolic enzymes used to identify the microorganism in 2 to 8 hours. The analysis of the virulence pattern was centered on the expression of the following virulence factors: haemolysins, lecithinase, amylase, lipase, caseinase, gelatinase, hydrolysis of esculin, DN-ase and the CAMP factor. The strains were seeded on mediums with specific substrata (agar medium enriched with 1% Tween 80 of the lipase virulence factor; agar medium enriched with 1% esculin and iron citrate, which highlights the presence of hidrolysis; agar medium supplemented with DNA to highlights expression of DN-ase factor; agar medium supplemented with 15% caseinase and 3% gelatin to highlight

presence of caseinase factor; agar medium enriched with 1% starch to highlight the presence of amylase), an the method followed the principle stated by Lazar et. al.[21]. The study of bacterial adherence to the cellular substratum was performed using the Cravioto's adapted method.

3. RESULTS SECTION

The results of the virulence patterns showed that 95% of the *S. aureus* strains possessed at least one of the virulence factors. Only two strains expressed all virulence factors, 5 strains expressed seven factors, while 8 strains possessed only one virulence factor. In terms of the distribution of virulence factors it was revealed that 53 strains were positive for the production of lipase, 88 strains were positive for the esculin hydrolysis, 34 strains were positive for DN-ase, 18 strains were positive for the production of caseinase, 66 for the production of amylase, 67 strains were positive for the production of lecithinase, 65 strains produced the gelatinase factor and 18 strains were positive for the presence of the CAMP factor highlighting the vast array of virulence factors (Fig 1-2). The study of the types of haemolysis showed that 70% of the strains possessed β type haemolysis, while 30% exhibited Y type haemolysis. The analysis of the distribution of the virulence factors pointed out the isolates from the nasal secretions of being the most virulent strains. In the case of the adhesion patterns the results showed that all strains had adhered to the cells, more than half possessing two adhesion patterns. 90% of the analyzed strains exhibited the aggregative adhesion pattern, followed by the localized pattern found in 70% of strains and the diffuse pattern in 30 % of strains (Fig. 3).

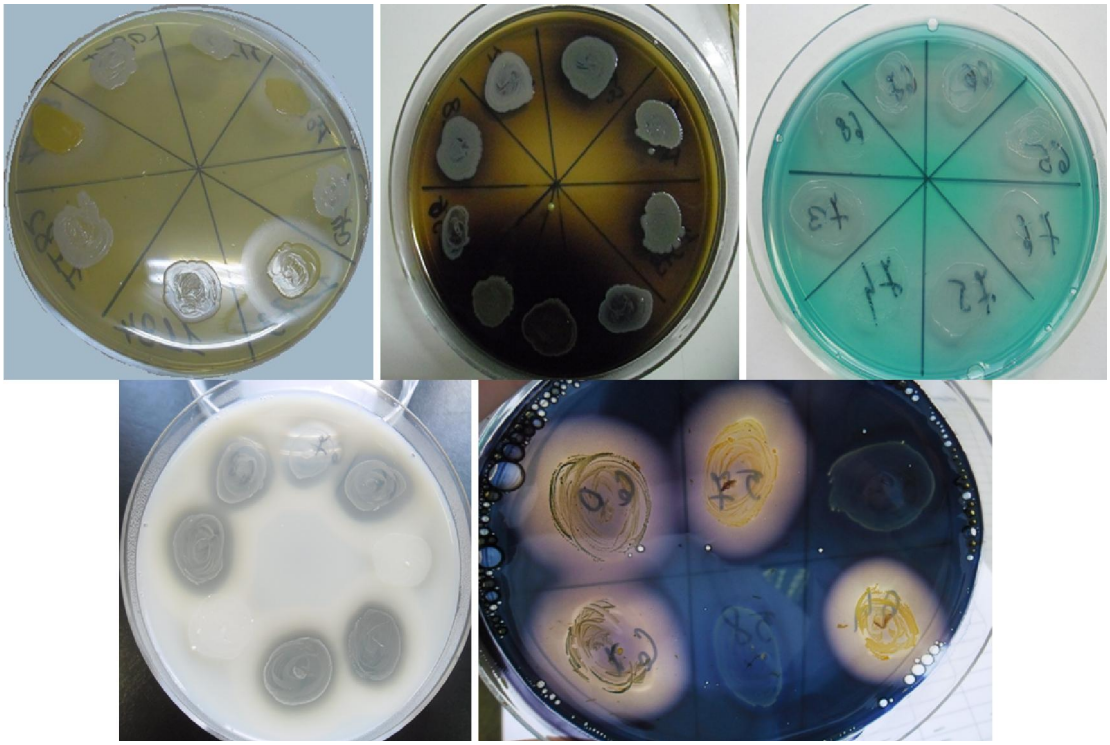

Figure 1. Appearance growth of *S. aureus* strain on selective media with specific substrata. Top row - Left: yellow = lipase positive strain; white= lipase negative strain. Center: esculin hidrolysis = poor growth; lack of hydrolysis = strong growth on selective medium. Right: DN-ase pozitive strains=strong growth; DN-ase negative strains = poor growth; Bottom row- Left: clear zones around the colonies= positive for casein hidrolysis; Right: yellow strain = amylase production used to test for amylase production by flooding with iodine.

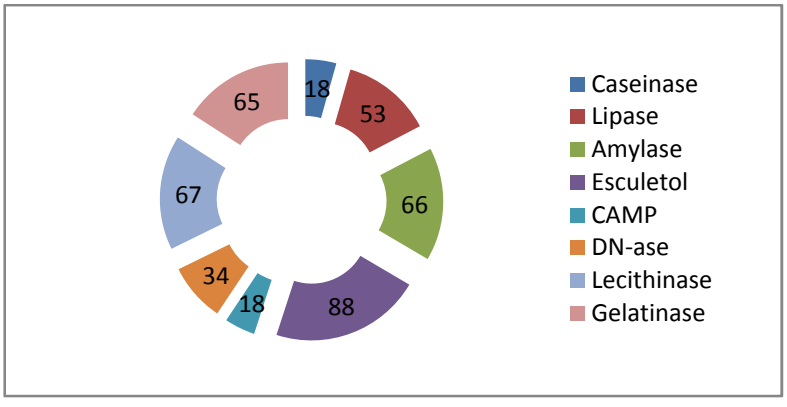

Figure 2. Graphic representation of the distribution of soluble virulence factors in the analyzed strains.

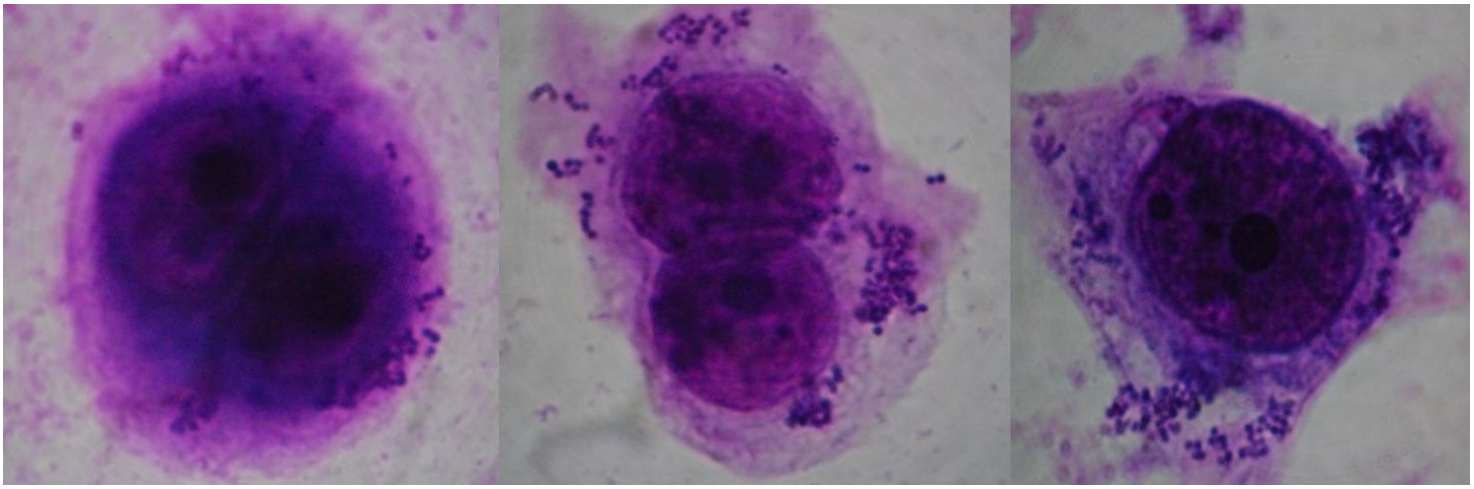

Figure 3. Type of adherence pattern of studied *S. aureus* strains: diffuse adhesion pattern (left), aggregative adhesion pattern (center), localized adhesion pattern (right).

4. CONCLUSIONS

S. aureus is the most versatile bacterial pathogen because of its rich repertoire of virulence factors which are expressed in a coordinated manner. Due to its ability to cause a wide spectrum of infections in humans and increased resistance to antibiotics *S. aureus* has become the subject of a number of studies on its implication in hospital infections. The MRSA strains show an increasing frequency in the hospital environment worldwide. The present study aiming to establish the cell associated and soluble virulence factors profiles conducted on 100 MRSA strains showed that all the strains presented at least one of the virulence factors. The most expressed virulence factor present in 88% of the strains was the hydrolysis of esculin and the less expressed factor was caseinase in 18% of the strains suggesting the important role esculetol plays in the evolution of *S. aureus* infections. 70 % of strains studied presented β-type haemolysis and 30% exhibited Y-type haemolysis suggesting the decreased activity of *S. aureus* haemolysins on human erythrocytes. The study of the adhesion patterns showed that all strains adhered to the cell membrane, exhibiting more than one adhesion pattern. It was observed that the most virulent strains were the ones isolated from nasal secretions. Given the high incidence of invasive infections with methicillin-resistant *S. aureus* finding new therapeutic strategies in combating staphylococcal infections is a priority at an international level.

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