Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania

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**Abstract**

*Staphylococcus* (\textit{S.}) \textit{aureus} is an opportunistic pathogen with the ability to invade and persist in unprofessional phagocytes. Romania is amongst the countries with the highest prevalence of methicillin-resistant \textit{S. aureus}, with level of incidence about 70\% from all isolated \textit{S. aureus} strains. The objective of this study was to analyse and compare the virulence patterns of isolated methicillin resistant \textit{S. aureus} strains. **Materials and methods.** The study was realized on the 144 MRSA strains isolated from nasal secretions and nosocomial infections between 2011-2014, from patients of the Emergency Institute for Cardiovascular Diseases "Prof. Dr. C.C. Iliescu", Bucharest. The strains were identified using conventional methods and confirmed using the Vitek 2 System analyzer. The presence of some virulence factors was tested phenotypically using specific substrata and also at molecular level, by simplex and multiplex PCR. **Results and discussions.** The comparative analysis of the distribution of virulence factors depending on the isolation site revealed that at least one virulence factor is expressed in all samples. The most virulent strains were isolated from blood cultures and the peritoneal fluid. **Conclusions.** This study has provided evidence that \textit{S. aureus} strains express different virulence patterns according to isolation sources.

**Keywords:** MRSA, virulence patterns, molecular analyses

1. **Introduction**

\textit{S. aureus} is an opportunistic pathogen with the ability to invade and persist in unprofessional phagocytes: fibroblasts, osteoblasts and different types of epithelial cells. The infectious potential of this bacterium is determined by a large number of cell-associated and extracellular virulence factors, some of which are implicated in the adhesion process and others in the bacterial invasion (Holban & al. [8]). The evolution of the infectious process is influenced by the host’s immunity, strain virulence and resistance to antibiotics.

The intracellular presence of \textit{S. aureus} in host tissues has been recently highlighted, suggesting the ability of the microorganism to avoid host defense mechanisms and to persist in host cells (Garzoni and Kelley [4]; Sendi and Proctor [22]; Zautner & al. [27]).
The ability of *S. aureus* to adhere to tissues is due to a wide array of cell-wall-associated factors, which include microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), molecules that play an important role in *S. aureus* pathogenesis, allowing its transition from the bacteremia stage to infections such as endocarditis or osteomyelitis (Bur & al. [1]). The MSCRAMMs group includes protein A, the fibronectin-binding proteins (*fnbA* and *B*), collagen-binding protein (*cna*), elastin-binding protein, clumping factors A and B (*ClfA* and *ClfB*). These virulence determinants are important for the invasiveness and development of invasive *S. aureus* infections (Sabat &. al. [21]). Fibrinogen is the most abundant host protein in endothelial lesions. Among fibrinogen-binding proteins expressed by *S. aureus* on bacterial cells, there are clumping factors A and B (*ClfA*, *ClfB*), *fib* and *fnbpA* and *fnbpB* which are responsible for *S. aureus* binding of fibrinogen, promoting adherence to cell surfaces. The *ClfA* factor is expressed during the bacterial growth, whereas *ClfB* is present only during the early logarithmic phase. Studies conducted on invasive infections, such as endocarditis showed that a large percentage of the *S. aureus* isolated strains possessed the *fnbA* and *fnbB* adhesins (Peacock & al., [19]). Other studies have associated the clumping factor A with endocarditis cases determined by *S. aureus* in animal models, mainly rats and rabbits (Josefsson & al. [10]; Vernachio & al. [25]). This virulence factor has been shown to prevent phagocytosis by leucocytes (Higgins & al. [6]).

MRSA strains isolated from tissue infections are characterized by the presence of the Panton-Valentine leukocidin which causes tissue necrosis (Vandenesch & al. [24]; Lo & al.[13]; Hesje & al. [5]).

Romania is amongst the countries with the highest prevalence of methicillin-resistant *S. aureus*, with level of incidence of about 70% of all *S. aureus* isolated strains (Monecke & al. [16]; DeLeo & al. [3]; Pardo & al. [18]).

The **objective** of this study was to analyze and compare the virulence patterns of methicillin resistant *S. aureus* strains isolated from different clinical specimens.

### 2. Materials and methods

144 methicillin resistant *S. aureus* strains (MRSA) isolated from nasal secretions and nosocomial infections (peritoneal fluid, ocular secretions, blood cultures and wounds) between 2011-2014, from patients of the Institute for Cardiovascular Diseases "Prof. Dr. C.C.Iliescu", Bucharest were analyzed. The isolation sources presented in this paper are representative for *S. aureus* infection pathology and epidemiological analysis according to the literature data. The prevalence of the infections caused by *S. aureus* varies by site and geographic area. The identification of the strains was based on conventional tests (coagulase Oxoid kit) and the Vitek 2 System analyzer.

**Phenotypic analysis**

The strains were tested for the **virulence factors expression** using specific substratum according method described by Lazar et. al.: haemolysins, CAMP factor, lecithinase, lipase, caseinase, gelatinase, hydrolysis of esculin, amylase, DN-ase (Lazar & al. [12]). In order to determine the presence of hemolysins, the strains were spotted on blood agar plates with 5% sheep blood. After 24h of incubation at 37°C the halo around the strains represented a positive test. Agar medium supplemented with 2.5% yolk, gelatin and Tween 80 respectively, were used to determine the expression of lecithinase, gelatinase and lipase. After 3 days of incubation at 37°C the presence of the three afore mentioned virulence factors was determined by the presence of a clear precipitation area around the strains spot. For the caseinase activity a medium containing 15% soluble casein was used. The DNA agar medium
was used for the determination of DNA-se presence. After maxim 72h of incubation at 37°C, the HCl solution (1N) was added on the surface of the medium. A clear halo around the strain was considered a positive reaction. The presence of amylase was determined on a media with starch substrate. After incubation up to 3 days at 37°C the presence of a precipitation around the strains was regarded as a positive result.

For aesculin hydrolysis the medium with Fe $^{3+}$ citrate was used and inoculated by spotting and incubated for 24h at 37°C. A black precipitate around culture due to esculetol released under the action of beta-galactosidase was considered as positive reaction.

The adherence pattern to the cellular substratum was determined by using the Cravioto’s adapted method (Lazar [11]). The HeLa cell monolayers were washed three times with phosphate buffered saline (PBS) and 1 ml of fresh medium without antibiotics was added to each well. Suspension of MRSA strains from bacterial mid-logarithmic phase cultures grown in nutrient broth was adjusted to $10^8$ cells/ml and 1 ml was used for the inoculation of each well. The inoculated plates were incubated for 2 h at 37°C. After incubation, the monolayers were washed 3 times with PBS, briefly fixed in cold methanol (3 min), stained with Giemsa solution (1:10) for 20 min. The plates were examined microscopically to evaluate the adherence index and patterns. The adherence index was expressed as the ratio between the number of the eukaryotic cells with adhered bacteria and 10$^4$ eukaryotic cells counted on the microscopic field. The adherence patterns were defined as: localized adherence (LA) when tight clusters of microorganisms were noticed on the HeLa cell surface, aggregative adherence (AA) when a microbial stacked brick pattern characterize the attachment, diffuse adherence (DA) when the bacteria adhered diffusely, covering the whole surface of the cell (Mihai & al. [16]).

Molecular analysis

The first step in the genotypic study of the strains was the extraction of the DNA. For every strain we obtained a bacterial suspension in purified water, after which we added 500 μl TEG (TRIS, EDTA, glucose) and lysozyme mixture (Table 1). The protocol used consisted of the following steps: i) incubation at 37°C for 30 minutes; ii) centrifugation at 800 RPM for 4 minutes; iii) elimination of the excess liquid; iv) resuspension in PCR water; v) incubation at 100°C for 10 minutes; vi) thermal shock by bringing the samples in a short amount of time to a temperature of -80°C.

**Table 1.** Composition of TEG solution

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TRIS</td>
<td>0.5M (121.14 g/mol)</td>
<td>60.7 mg</td>
</tr>
<tr>
<td>EDTA</td>
<td>0.5M (292.24 g/mol)</td>
<td>186.1 mg</td>
</tr>
<tr>
<td>Glucose</td>
<td>1M (180.16 g/mol)</td>
<td>180.16 mg</td>
</tr>
</tbody>
</table>

The sequences of primers and amplification protocols followed the indications provided by Miheirico et. al. (2007) and Zhang et. al. (2005) (table 2). The assays were carried out by using the Corbet Thermal Cycler. The results of the PCR reactions were analyzed by migration on electrophoresis gel (5 μl agarose gel 1.5 %). The gels were loaded with 5 μl of primers stained with ethidium bromide (10 μ g / ml). The presence or absence of the virulence genes was determined using UV light comparing with the bands obtained with the specific molecular weight marker (100bp, I Lader Bench Top 100bp DNA) (Strommenger & al. [23]).
Virulence patterns of *Staphylococcus aureus* hospital strains isolated in Bucharest, Romania

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Dimension of amplicon (bp)</th>
<th>Nucleotide sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>bbp</td>
<td>BBP-1</td>
<td>575</td>
<td>5'-AACTACATCTGACTCAACAAACAG-3'</td>
</tr>
<tr>
<td></td>
<td>BBP-2</td>
<td></td>
<td>5'-ATGTGTTGTAATAACACCACATCATCT-3'</td>
</tr>
<tr>
<td>ebpS</td>
<td>EBP-1</td>
<td>186</td>
<td>5'-CATCCAGAACAATCGAAGAC-3'</td>
</tr>
<tr>
<td></td>
<td>EBP-2</td>
<td></td>
<td>5'-CTTAACAGTTACATCATGTTTTATCTTTG-3'</td>
</tr>
<tr>
<td>fnbB</td>
<td>FNBB-1</td>
<td>524</td>
<td>5'-GTAACAGCTAATGGTCGAATTGACT-3'</td>
</tr>
<tr>
<td></td>
<td>FNBB-2</td>
<td></td>
<td>5'-CAAGTTCGATAGGAGTACTATGTT-3'</td>
</tr>
<tr>
<td>fib</td>
<td>FIB-1</td>
<td>404</td>
<td>5'-CTACAACACTCATAATGCCGCACAG-3'</td>
</tr>
<tr>
<td></td>
<td>FIB-2</td>
<td></td>
<td>5'-GCTTCTGTAAGCACCTTTCTTCCAC-3'</td>
</tr>
<tr>
<td>clfA</td>
<td>CLFA-1</td>
<td>292</td>
<td>5'-ATGGGCGTGCTGCTCGTTGCT-3'</td>
</tr>
<tr>
<td></td>
<td>CLFA-2</td>
<td></td>
<td>5'-CGTTCTTCCGTAAGTTTTGGGCAC-3'</td>
</tr>
<tr>
<td>clfB</td>
<td>CLFB-1</td>
<td>205</td>
<td>5'-ATCATTAGGATAAAGTGGGGGCAAC-3'</td>
</tr>
<tr>
<td></td>
<td>CLFB-2</td>
<td></td>
<td>5'-CTCGCACTGTTTGGTCACCAC-3'</td>
</tr>
<tr>
<td>fnbA</td>
<td>Forward</td>
<td>1362</td>
<td>5'-CAACACAGCAATATAG-3'</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td></td>
<td>5'-CTGTGTGGTATTATTACCAGTTC-3'</td>
</tr>
<tr>
<td>cna</td>
<td>Forward</td>
<td>X560</td>
<td>5'-AGTGTTTACTAATACTG-3'</td>
</tr>
<tr>
<td></td>
<td>reverse</td>
<td></td>
<td>5'-CAGGATAGATGGTTTTA-3'</td>
</tr>
<tr>
<td>luk-PV</td>
<td>luk-PV-1</td>
<td>443</td>
<td>5'-ATCATTAGGATAAAGTGGGGGCAAC-3'</td>
</tr>
<tr>
<td></td>
<td>luk-PV-2</td>
<td></td>
<td>5'-GCTGCACTGTTTGGTCACCAC-3'</td>
</tr>
<tr>
<td>hlg</td>
<td>hlgC</td>
<td>937</td>
<td>5'-CCCAATCCGTATTTAGGAAATAGC-3'</td>
</tr>
<tr>
<td></td>
<td>hlgB</td>
<td></td>
<td>5'-CCATAGACGTAGCAACGATGAT-3'</td>
</tr>
<tr>
<td>tst</td>
<td>tst1</td>
<td>476</td>
<td>5'-CATCTACAACCAAGATAATAAAAGG-3'</td>
</tr>
<tr>
<td></td>
<td>tst2</td>
<td></td>
<td>5'-CATTGTTATTCTCCAAATACCAACCGG-3'</td>
</tr>
</tbody>
</table>

The detection of the specific virulence genes was performed by three simplex PCR and three multiplex PCR assays (Table 3). The information obtained was used to compare the prevalence of specific genes amongst nosocomial strains isolated from different sources.

<table>
<thead>
<tr>
<th>Gene</th>
<th>PCR assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>bbp</td>
<td>Multiplex</td>
</tr>
<tr>
<td>ebpS</td>
<td>Multiplex</td>
</tr>
<tr>
<td>fnbB</td>
<td>Multiplex</td>
</tr>
<tr>
<td>fib</td>
<td>Multiplex</td>
</tr>
<tr>
<td>clfA</td>
<td>Multiplex</td>
</tr>
<tr>
<td>clfB</td>
<td>Multiplex</td>
</tr>
<tr>
<td>luk-PV</td>
<td>Multiplex</td>
</tr>
<tr>
<td>hlg</td>
<td>Multiplex</td>
</tr>
<tr>
<td>fnbA</td>
<td>Simplex</td>
</tr>
<tr>
<td>cna</td>
<td>Simplex</td>
</tr>
<tr>
<td>tst</td>
<td>Simplex</td>
</tr>
</tbody>
</table>

3. RESULTS

The analysis of the distribution of virulence factors has revealed that from the 144 analyzed strains, 108 of them (75%) were positive for the production of lecithinase, 100 (70%) presented aesculin hydrolysis, 90 strains (62%) expressed lipase, 70 strains (49%) presented the gelatinase, 66 strains (46%) expressed the amylase factor, 58 strains (40%) were found positive for the production of caseinase, 35 of the strains (25%) were positive for Romanian Biotechnological Letters, Vol. 20, No. 3, 2015
DN-ase and also 35 of them (25%) expressed the CAMP factor. Regarding the haemolysis type 85% of the analyzed strains presented β type haemolysis, while the remaining 15% possessed type haemolysis.

The comparative analysis of the distribution of virulence factors depending on the isolation sources revealed that all strains expressed at least one virulence factor, and only two strains expressed all the virulence factors, the most strains presenting between 6-8 virulence factors. Further analysis showed that 66 strains isolated from nasal secretions were positive for the production of lecithinase, 58 expressed aesculin hydrolysis, 56 presented the lipase factor, 40 expressed gelatinase, 35 presented the amylase factor, 32 were found positive for the production of caseinase, 15 were positive for DN-ase and 14 expressed the CAMP factor (fig. 1).

In regard to ocular secretions the distribution of the virulence factors was the following: 5 strains expressed lecithinase, 8 were positive for aesculin hydrolysis, 7 presented the lipase factor, 6 presented the gelatinase factor, 7 expressed the amylase factor, 6 were found positive for the production of caseinase, 5 presented the DN-ase factor and 4 expressed the CAMP factor (fig. 1). In the case of the strains isolated from wounds 25 presented lecithinase, 24 expressed aesculin hydrolysis, 15 were positive for the lipase factor, 14 expressed the gelatinase factor, 12 presented the amylase factor, 10 expressed the caseinase factor, 6 presented the DN-ase factor and 7 expressed the CAMP factor (fig. 1). Blood cultures were characterized by the presence of lecithinase and lipase in 6 cases and of aesculin hydrolysis, the lipase factor, the gelatinase factor, the amylase factor, the caseinase factor, the DN-ase factor and of the CAMP factor in 5 cases (fig. 1).

The strains isolated from peritoneal fluids expressed the lecithinase and lipase factors in 6 cases, the aesculin hydrolysis, gelatinase, amylase, caseinase and CAMP factors in 5 cases, the presence of DN-ase factor was detected in only 4 cases (fig. 1).

Most tested strains produced lecithinase and esculinase. Iron is an essential component for growing and virulence of the microorganisms. In the extracellular medium iron is not accessible for bacteria, therefore siderophores (such as transferrin) are necessary to acquire it.

It was proved that iron may be fixed by esculentol, so esculinase has an important role in ensuring Fe uptake necessary for the activation of bacterial genes and expression of some virulence factors (Chifiriuc & al. [2]).

Caseinase is a proteolytic enzyme that hydrolyzes casein, a protein abundant in milk. Gelatinase presents also large-spectrum proteolytic activity. Numerous studies have shown that proteases produced by pathogenic organisms may contribute to the severity of the clinical symptoms of an infection.
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Amylase is an enzyme produced by numerous microorganisms, capable of hydrolyzing starch. This enzyme is involved in breaking the complex sugars to monosaccharides, which provide the C source for multiplication, necessary for colonization of any type of tissue, but also for the microbial persistence.

Lecithinase, lipase, and haemolysins are pore forming toxins that cause pores in the cell membrane, allowing the dissemination of infection.

The presence of all these enzymes proves the pathogenic potential of these strains which allows them to damage the host tissue and to disseminate.

All tested strains had the capacity to adhere to the cellular substrate, the adherence pattern being different between strains.

The aggregative pattern predominated, being found in 90% of the analyzed strains. The adherence index was also different between strains, from 40% to 100%, the most strains showing an adherence index of over 80%. The correlation between the adhesions patterns and infections sites showed that the aggregative pattern was encountered in 85 strains isolated from nasal secretions, 8 strains from ocular secretions, 25 strains from wounds and 6 strains from blood and peritoneal fluid cultures (fig. 2). Regarding the localized pattern it was found in 54 strains obtained from nasal secretions, 10 from ocular secretions, 24 from wounds and 6 strains from blood and peritoneal fluid cultures (fig. 2). The diffuse pattern was seen in 23 of the strains isolated from nasal secretions, 3 from ocular secretions, 7 from wounds and 5 strains from blood and peritoneal fluid cultures (fig. 2).

The molecular analysis using PCR methods showed that only 1 strains presented the *tst* gene (fig. 4), 103 strains possessed the *ebpS* gene (fig. 5, 6), 108 strains presented the *fib* gene and 141 strains expressed both the *clfA* and *clfB* genes (fig. 7, 8). No strain expressed the *fnbA, bbp, cna, luk-PV, hlg* genes. These results regarding the presence of the *ebpS, fib, clfA* and *clfB* genes highlight the importance of the adherence stage in the development of the invasive infections determined by *S. aureus* regardless of the infectious sources.

The analysis of the virulence genes expression according to the isolation site pointed out the fact that the *ebpS, fib, clf* genes were expressed by the majority of the strains studied. So in the case of the *ebpS* gene, it was encountered in 60 strains isolated from nasal secretions.
secretions, 10 from ocular secretions, 23 from wounds and 5 from blood and peritoneal fluids cultures (fig. 3).

The fib gene was found in 64 strains isolated from nasal secretions, 8 from ocular secretions, 25 from wounds and 5 from blood and 5 from peritoneal fluids cultures (fig. 3).

Regarding the clf genes (A and B), they were expressed by 87 strains isolated from nasal secretions, 13 from ocular secretions, 29 from wounds and 6 from blood cultures and peritoneal fluids (fig. 3).

![Figure 3. Distribution of virulence genes according to isolation site.](image)

**Figure 3.** Distribution of virulence genes according to isolation site.

![Figure 4.](image)

**Figure 4.** Ethidium bromide-stained 1.5% agarose gel showing the amplified products of tst gene. Lines 1 Gene Ruler 100bp (Fermentas), MRSA strains 1-MRSA strains 27.
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**Figure 5.** Ethidium bromide-stained 1.5% agarose gel showing the amplified products of *ebpS* and *bbp* genes. Lines 1 Gene Ruler 100bp (Fermentas), MRSA strains 1-MRSA strains 27.

**Figure 6.** Ethidium bromide-stained 1.5% agarose gel showing the amplified products of *ebpS* and *bbp* genes. Lines 1 Gene Ruler 100bp (Fermentas), MRSA strains 28-MRSA strains 44.

**Figure 7.** Ethidium bromide-stained 1.5% agarose gel showing the amplified products of *fnb*, *fib*, *clfA* and *clfB* *ebpS* and *bbp* genes. Lines 1 Gene Ruler 100bp (Fermentas), MRSA strains 1-MRSA strains 27.

**Figure 8.** Ethidium bromide-stained 1.5% agarose gel showing the amplified products of *fnb*, *fib*, *clfA* and *clfB* *ebpS* and *bbp* genes. Lines 1 Gene Ruler 100bp (Fermentas), MRSA strains 28-MRSA strains 44.

**DISCUSSIONS**

The obtained results regarding the phenotypic analysis of the virulence patterns are in accordance with those provided by other studies conducted in our country on *S. aureus* strains isolated from genital infections, blood cultures and broncho-alveolar secretions and suggest that different virulence factors are expressed depending on the infection site (Holban & al. [7]; Cotar [2]). The analysis of the infection sites in connection with the presence of various virulence patterns pointed out that the most virulent strains were isolated from blood cultures.
and the peritoneal fluid while the less virulent strains were isolated from ocular secretions, the results being confirmed by other studies, suggesting the existence of a coordinated and controlled expression of virulence factors in strains to determine acute or chronic infections in the host body (Holban & al. [7]). The results regarding the adhesion patterns of the studied strains are in accordance with those obtained by Holban et. al. in a study including 103 *S. aureus* strains isolated from nosocomial infections, in which all strains adhered to the cellular substrate with an index that exceeds 80% in 55% of the cases, and that the most prevalent adhesion pattern was the aggregative one. The results are explainable taking into account that the ability of *S. aureus* to adhere to the cellular substrate is essential for establishing both in the normal microbiota as well as for initiating an infectious process.

Our findings reveal the high prevalence of the ebpS and clf genes are strengthen by the results presented in studies done by Paniagua-Contreras et. al. on bacterial pathogens isolated from catheter devices and Rasmussen et. al. on 134 MSSA strains isolated from hospital environment.

Paniagua-Contreras et. al. observed a 100% prevalence of the clfA gene and a 85.4% prevalence of the ebpS gene amongst the isolated *S. aureus* strains (Paniagua-Contreras & al. [17]). Rasmussen et. al. reported in his study that clfA, clfB, ebpS, fib genes were present in all isolates regardless of the infection site (Rasmussen & al. [20]). Other studies that are in accordance with our results were carried out by Jochmann et. al. and Machuca et. who worked on 48 *S. aureus* strains isolated from atopic dermatitis cases and healthy individuals, and found that the fib gene was expressed in 66.6% of the cases and ebpS gene in 70% of the cases (Jochmann & al. [9]). Machuca conducted a study on 53 MRSA strains isolated from the hospital environments in Colombia which showed that 89% of the strains carried the clfA gene, and 87% carried clfB gene (Machuca & al. [14]). In contrast, both studies showed a large prevalence of the fnbA gene (Jochmann & al. [9]).

A study conducted by Wang et. al. on 60 *S. aureus* strains isolated from blood cultures in Tianjin pointed out the fact that similar to our findings the luk-PV and tst genes were not present amongst the virulence genes encountered (Wang & al. [20]).

The ocular strains have proved to be less virulent, explaining by the fact that these isolates have fewer interactions with the host immune response, so for bacteria is not necessary to express a large arsenal of virulence factors.

In contrast, the most virulent strains proved to be those isolated from blood cultures and peritoneal fluid due to the necessity to avoid the immune cells of the host, to disseminate to other organs for escape from the immunity effectors, and to transform the host tissue into nutrients in order to survive.

**CONCLUSIONS**

*S. aureus* is capable of causing a wide spectrum of infections by making use of its arsenal of virulence factors which are expressed in certain stages of the infection. The increasing frequency of MRSA strains isolated in hospital environments from various sites has made the study of virulence patterns associated with this pathogen a subject of interest in the world today.

Our findings regarding the expression of virulence patterns showed that all the analyzed strains regardless the isolation source presented at least one virulence factor. The most prevalent virulence factor found was a pore forming toxin (lecithinase), involved in the dissemination of infection.
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The genotypic study pointed out that a large array of genes were expressed, all of them encoding the information for virulence factors implicated in the adhesion process, the most prevalence of them being the *clfA* and *clfB* genes, which were present in 141 strains (99% of the cases). The huge difference between the number of genes codifying for virulence factors involved in adherence and those codifying for factors expressed in the latter stages of infection underlies the importance of the adhesion to the host tissues for the initiation of an infectious process, but also for the colonization of the upper respiratory tract in healthy individuals.

This study has provided evidence that *S. aureus* strains express different virulence patterns according to clinical sources. The most virulent strains were found to be the ones isolated from blood cultures and peritoneal fluids due to the need to overcome the responses of the host body to the infections and for succeeding to trigger an infectious process.

**Acknowledgements**

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