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Prevalence of *agr* specificity groups among *Staphylococcus aureus* strains isolated from different clinical specimens patients with cardiovascular surgery associated infections

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ABSTRACT

Staphylococcus aureus is an extremely versatile pathogen responsible for a large spectrum of human diseases, ranging from skin infections and food poisoning to life-threatening nosocomial infections. *S. aureus* pathogenicity is a complex process involving a diverse array of virulence factors that are coordinately expressed during different stages of infection by a network of virulence regulators. The accessory gene regulator (*agr*) is one of these virulence regulators, being a quorum-sensing system activated by a bacterial-density-sensing peptide (autoinducing peptide - AIP). Different *S. aureus* strains produce AIPs with distinct structures, and strains can be grouped on this basis since they will activate the *agr* response of strains within the same group and inhibit the *agr* response of strains from different groups by competitive inhibition.

The aim of this study was to investigate the potential associations between each *agr* group and a certain type of clinical sample by studying 88 *S. aureus* strains isolated from different clinical specimens from patients admitted in the Emergency Institute for Cardiovascular Diseases, Bucharest. A multiplex PCR was used to determine the *agr* groups among analyzed strains. The obtained results showed that *agr* III was the most prevalent group among the tested strains. The *agr* I was associated with strains isolated from blood cultures, whereas *agr* III prevailed among strains isolated from respiratory tract secretions. Although the obtained results do not allow us to establish a direct relationship between the *agr* group and type of *S. aureus* infection, but we can speculate that the preferential association between certain *agr* alleles and virulence factors may favour a more efficient activation of the virulence potential.

Letters in Applied NanoBioSci New Open Access Journal

Keywords: *S. aureus*, *quorum-sensing, virulence genes regulators, invasive infections*

1. INTRODUCTION

S. aureus is an extremely versatile human pathogen responsible for a broad range of nsocomial and community-acquired infections due to an impressive array of extracellular and cell-wall-associated virulence determinants that allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host [1]. The coordinated expression of *S. aureus* virulence factors is regulated by a complex network including the quorum-sensing (QS) system *agr* and the well

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characterized virulence gene regulators [2]. The *agr* (accessory gene regulator) locus consists of two divergent transcription units RNAII and RNAIII driven by two promoters, P2 and P3, respectively. The P2 transcript, RNAII, contains four genes: *agrA*, *agrB*, *agrC* and *agrD*. The sensor, AgrC, and the response regulator, AgrA, comprise the two component system that responds to auto-inducing peptide (AIP). This peptide is encoded by *agrD,* being posttranslationally modified and secreted by AgrB [3]. The synthesis of the effector molecule RNAIII is dependent on *agr* activation and driven by the P3 promoter of the *agr* system [3]. Under conditions of high autoinducer concentration, e.g., high bacterial density, RNAIII up-regulates the expression of post-exponentially synthesized extracellular virulence factors (toxins, hemolysins, proteases, lipases) and down-regulates the expression of cell-surface-associated proteins [4,5]. This conversion from a tissue-adhering to a tissue-damaging and phagocyte-evading phenotype is thought to be important for in vivo pathogenesis and the development of invasive infection [6]. Also, RNAIII carries the *hld* gene that codes for delta- hemolysin. Micro-array studies have revealed that 104 genes are upregulated and 34 genes are downregulated as a result of QS, representing ~5% of the genome [7]. A polymorphism in the amino acid sequence of the AIP and of its corresponding receptor AgrC divides *S. aureus* strains into four major groups [8]. Each group is characterized by a specific pheromone amino acid sequence, in which only the thiolactone structure (or lactone structure, in one case) is conserved. AIP-I and AIP-IV differ by only one amino acid and function interchangeably [9]. Within a given group, each strain produces a peptide that can activate the *agr* response in the other member strains, whereas the AIPs belonging to different groups are usually mutually inhibitory. The inhibitory activity of these *agr* groups represents a form of bacterial interference that influences virulence gene expression. Different in vivo studies have shown that *agr* mutants appear to have diminished virulence in several animal infections models, including intramammary infections, arthritis in mice, and endocarditis in rabbits [10]. Recent studies have shown the existence of a strong association between the *agr* types and certain of *S. aureus* diseases [11]. Thus, strains associated with generalized exfoliative syndromes belonged to *agr* group IV, the vast majority of menstrual toxic shock strains belonged to *agr* group III, bullous impetigo strains were associated with *agr* groups II and IV, and suppurative infections (endocarditis) belonged to *agr* groups I and II [12]. In this study we analyzed by PCR the *agr* specificity groups in order to establish a possible relationship between a specific group and the clinical origin of *S. aureus* strains.

2. EXPERIMENTAL SECTION

2.1. Samples and strains. The study was performed on 88 *S. aureus* strains isolated from various clinical specimens from patients with cardiovascular surgery associated infections, hospitalized in the Emergency Institute for Cardiovascular Diseases Prof. Dr. C.C. Iliescu, Bucharest. The strains were identified by help of API microtests and the automatic Vitek II system. The source of isolation for the analyzed strains is presented in Figure 1.

2.2. DNA Extraction. Genomic DNA was extracted from the analyzed clinical isolates and used as templates in PCR experiments. Few colonies of each strain from mannitol salt agar were inoculated into 5 ml of BHI (Broth Heart Infusion) and grown overnight at 37°C with shaking. From the bacterial suspensions obtained DNA extraction was performed by using Wizard® SV Genomic DNA Purification System kit (Promega, USA) according to the manufacturer ′s recommendations. Genomic DNAs were stored at 4 °C until their use as templates in PCR assays and subsequently at −20 °C.

2.3. PCR Assay. The characterization of the *agr* operon in the analyzed *S. aureus* strains was performed by PCR multiplex using specific primers for the detection of *agr* groups. The *agr* locus in *S. aureus* has been shown to be polymorphic, because it consists from highly conserved and hypervariable regions among *S. aureus* strains, and can be divided into four distinct genetic groups. The *agr* specificity groups in the analyzed *S. aureus* strains were identified by PCR amplification of the hypervariable domain of the *agr* locus using oligonucleotide primers specific for each of the four major specificity groups.

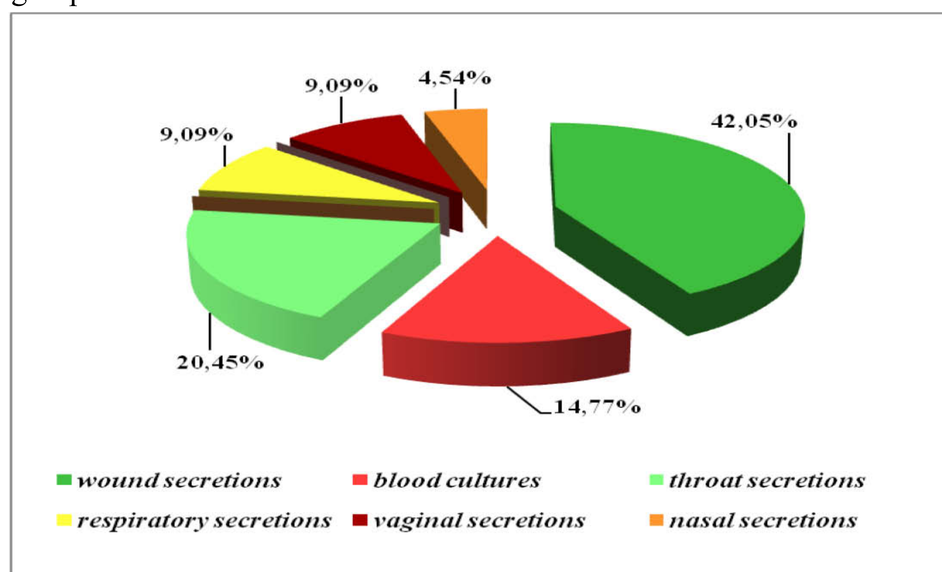

Figure 1: The clinical origin of the analyzed *S. aureus* strains.

Thus, we used in all reactions a forward primer, pan-*agr* corresponding to the conserved sequences from the *agrB* gene, and four reverse primers, each specific for amplification of a single *agr* group based on *agrD* or *agrC* gene nucleotide polymorphism [13].

Table 1: The primer sequences used for amplification of the hypervariable region of *agr* locus in the analyzed *S. aureus* strains.

Primers	Sequence (5′ - 3′)	Amplicon size (bp)
agr I	5'-GTCACAAGTACTATAAGCTGCGAT-3'	440 bp
agr II	5'-GTATTACTAATTGAAAAGTGCCATAGC-3'	572 bp
agr III	5'-CTGTTGAAAAAGTCAACTAAAAGCTC-3'	406 bp
agr IV	5'-CGATAATGCCGTAATAC CCG-3'	588 bp
pan-agr	5'-ATGCACATGGTGCACATGC-3'	

Table 2: The conditions used for the amplification of *agr* gene.

Initial denaturation	No. of cycles	Denaturation in each cycle	Annealing	Primers extension	Final extension
95 °C, 2 min	25	95 °C, 1 min	55 °C, 30 sec.	72 °C, 30 sec.	72 °C, 5 min

The *agr* specificity groups were identified by the expected product sizes. In the PCR assay, the *agr* gene was amplified on a Corbett instrument using necessary components provided by Fermentas (DreamTaq™ Green PCR Master Mix kit). The sequence of specific primers used in PCR reactions, and the molecular size of the amplicons are presented in Table 1. The parameters for the

amplification cycles used in PCR are presented in Table 2. PCR products were separated in a 1.5% agarose gel for 1 h at 100 V, stained with ethidium bromide and detected by UV transillumination. For amplification products whose sizes ranged between 500 and 600 bp PCR we performed two separate tests using DNA samples and primers for *agr* group II, respectively *agr* group IV.

3. RESULTS SECTION

The analysis of the PCR results showed that all analyzed strains possess *agr* gene, which means that have functional quorum-sensing system (Fig. 2).

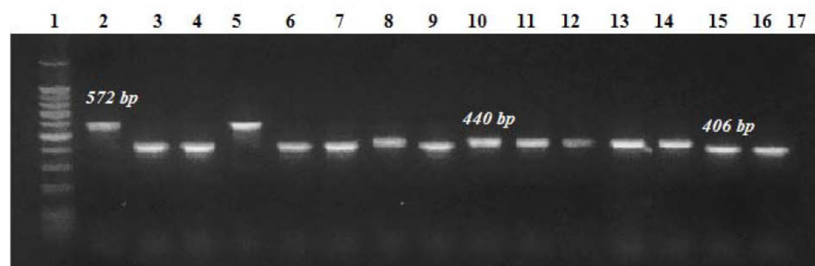

Figure 2: Gel electrophoresis of amplification products of *agr* gene in DNA samples from *S. aureus* analyzed strains. Line **1** - DNA ladder 100 bp (Promega); **2**–1**6**— DNA samples from *S. aureus* strains; 1**7**—negative control (pure water).

The *agr* III group was the predominant group, being present in 54 strains, followed by *agr* I in 25 strains (Fig. 3). The results of the two separate PCR assays allowed us to confirm that 6 strains belong to group *agr* II and 3 strains belong to group *agr* IV.

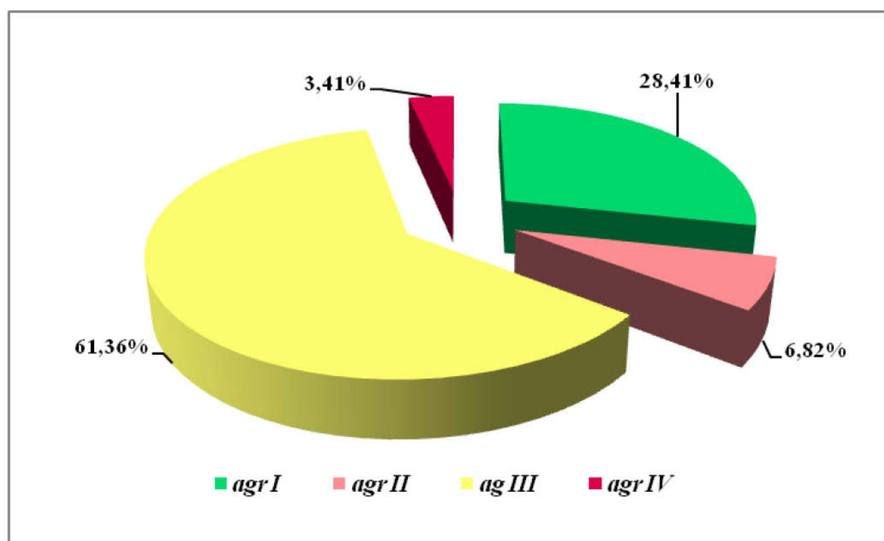

Figure 3: The percentual distribution of *agr* groups among analyzed *S. aureus* strains.

The *agr* III was the predominant group, being present in strains isolated from all types of clinical samples. A specific relationship between the type of clinical sample and *agr* group was found. Thus, the *agr* I was prevalent among *S. aureus* strains isolated from blood cultures, whereas *agr* III had prevailed among strains isolated from respiratory tract specimens. The strains isolated from vaginal secretions have had only *agr* II and *agr* III groups (Figure 4).

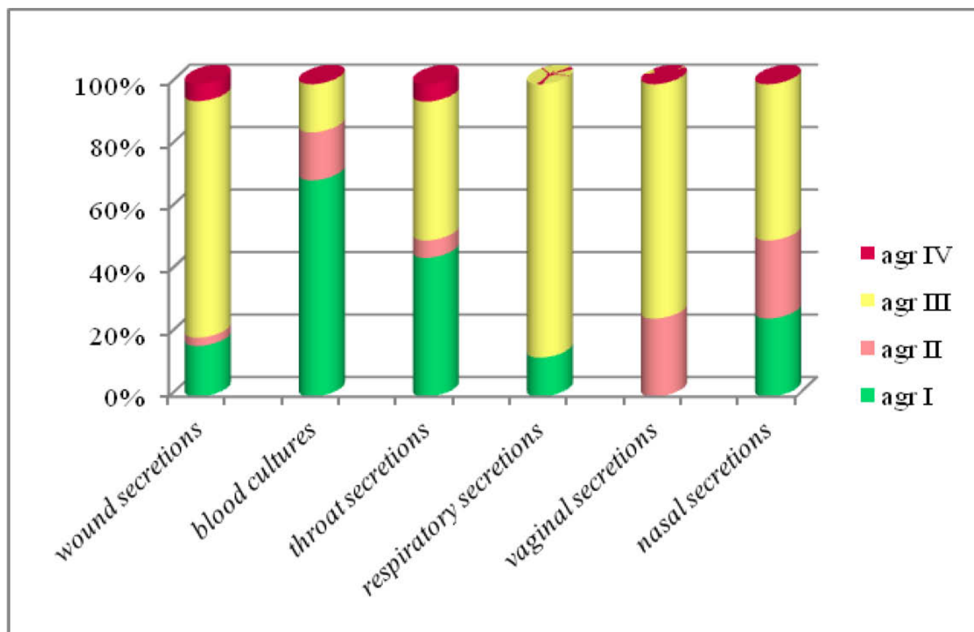

Figure 4: The distribution of *agr* groups among *S. aureus* strains isolated from different clinical samples.

The strains analyzed in this study was selected from a collection of strains isolated from different clinical specimens from patients admitted in the Emergency Cardiovascular Disease Institute of Bucharest. All strains, excepting those isolated from vaginal secretions were derived from patients with infections associated with cardiovascular tissue and prosthetic devices, that are an important cause of discomfort, disability and severe evolution leading to high mortality rates [14]. These infections involve biofilm formation, being very challenging due to resistance of bacteria from biofilm to both host immune responses and available chemotherapies [15].

The QS mechanism is responsible for the coordinated regulation of the expression of virulence factors during infection. *S. aureus agr* is a 3-kb locus showing highly conserved and hypervariable regions among *S. aureus* strains. The sequence of the hypervariable region was used as target of PCR amplification for defining *agr* groups [16,17]. During this study we have investigated the prevalence of *agr* specificity groups among *S. aureus* strains isolated from different clinical specimens in order to find a specific relationship between the type of clinical sample and the *agr* group. The *agr* III was the predominant group, being present in strains isolated from all types of clinical samples, but had prevailed among strains from respiratory tract and wound secretions. Also, we found that the majority of the strains isolated from blood cultures belong to *agr* I, whereas the strains from vaginal secretions belong to *agr* II or *agr* III groups. The specific association between the type of the clinical sample and the *agr* groups was also reported by other researchers that found a strong association between the *agr* group, and disease type [12]. Although the obtained results do not allow us to establish a direct relationship between the *agr* group and the type of infection, we can speculate that the preferential association between certain *agr* alleles and certain virulence factors, may favour a more efficient activation of strains' virulence. As many *S. aureus* strains are becoming multidrug resistant, the search for new therapeutic approaches became stringent. Different studies have shown that *agr* mutants are less virulent comparative with their wild counterparts, thus QS could become an ideal target for the inhibition of *S. aureus* virulence. Also, taking into account that QS is not involved in the bacterial growth, QS inhibition should not yield a strong selective pressure for the development of resistance.

4. CONCLUSIONS

The results of PCR analysis showed that all analyzed strains, regardless of the source of isolation harbour the *agr* gene, showing that they have a functional QS system. Also, we have found a specific association between the *agr* groups and the type of clinical sample. The *agr* I is being predominant in isolates from blood cultures, *agr* II in vaginal secretions, and *agr* III in respiratory tract and wound secretions, which shows the importance of monitoring this regulation system of virulence factors in *S. aureus* strains. Further studies should be carried out on a high number of strains isolated from each type of clinical specimen, and also from different types of clinical syndromes in order to statistically validate these specific associations.

5. ACKNOWLEDGEMENT

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