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Review article

The quorum sensing system of accessory gene regulators in Staphylococcus aureus

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Abstract

The pathogenicity of *Staphylococcus aureus* is profoundly affected by the quorum sensing mechanism which is regulated by the accessory gene regulator (*agr)* system. This system composed of 4 groups of genes which govern more than 70 genes, including 23 that are recognized as virulence factors genes. These genes enhancing the synthesis of extracellular toxins such as (haemolysins, proteases, enterotoxins, etc.) and decrease the production of cell surface-correlated proteins (microbial surface substances realising adhesive matrix molecules related to biofilm formation). So up-regulation of agr genes promotes dispersion while down-regulation results in excessive biofilm thickness and lack of structure. This increases the virulence of bacteria.

The control of virulence factors by *agr* is crucial for the development of illness. Each section of the genome plays a role in a variety of traits within the population of bacteria, increasing the chances of survival in various settings. *Agr* serves as a fundamental quorum sensing mechanism, enabling bacteria to modulate gene expression based on the density of the population.

Aim of this review

The objective of this article is to elucidate the quorum sensing system mediated by the accessory gene regulator in *Staphylococcus aureus* and to explore its connections to various virulence factors and the process of biofilm formation.

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Introduction

S. aureus is an important pathogen causing a wide spectrum of infections. The organism usually colonizes the skin and mucous membrane of humans and several animal species. Multiple body sites can be colonized in humans; however, the anterior nares of the nose are the most frequent carriage site for *S. aureus*. [1]

Pathogenecity of *S. aureus* is regulated by various factors, one of them is the accessory gene regulator (*agr*) system which constitutes a critical component in the regulation of *S. aureus* pathogenicity. Several reports suggested that the role of accessory gene regulator *(agr)* in *S. aureus* virulence is sophisticated. Accessory gene regulator dysfunction causes changes in the expression of genes and has global effects on bacterial pathogenicity.[2]

It is anticipated that around 70 genes are under the regulatory influence of the *agr* system, with 23 identified as virulence factors. The activation of the *agr* system facilitates the transition of the bacterium from a non-pathogenic, adhesive commensal to a more invasive and aggressive pathogen. The virulence factors regulated by *agr* can be categorized into two distinct classes: the first class encompasses factors that play a role in host attachment and evasion of the immune response, while the second class consists of genes involved in the synthesis of exoproteins associated with invasion and toxin generation.[3]

Because RNAIII decreases surface adhesin expression and enhances the synthesis of capsules, toxins, and proteases, *Agr* deficiency has been linked to increased biofilm development.[4]

The genes that govern *S. aureus* pathogenicity and invasive infections by
various virulence factors. The *agr* operon, comprising the genes *agr*A, *agr*B, *agr*C, and *agr*D, regulates various virulence factors, including exfoliative toxins (ETs), toxic shock syndrome toxin (TSST-1), and staphylococcal enterotoxins (SEs).[5]

Moreover, the sequences of the *agr*C (auto inducing peptide) and *agr*D (cyclic AIP) genes allow *S. aureus* to be divided into 4 distinct groups (*agr* I, *agr* II, *agr* III, and *agr* IV). It is said that different *agr* kinds have distinct characteristics and are more common in different geographic locations; as a result, identifying the most common varieties in each area may be useful.[6]

Staphylococcus aureus

Morphology:

S. aureus is a facultative anaerobic, Gram-positive coccal (round) bacterium also known as "golden staph". It is nonmotile and does not form spores and appears as (grape-like clusters) when viewed through a microscope, and has large, round, golden-yellow colonies, often with hemolysis, when grown on blood agar plates. *S. aureus* reproduces asexually by binary fission and is mediated by *S. aureus* autolysin, In its absence or targeted inhibition, the daughter cells remain attached to one another and appear as clusters.[7]

Pathogenesis:

S. aureus is one of the most common bacterial infections in humans and are the causative agents of multiple human infections, including infective endocarditis, bacteremia, skin and soft tissue infections (e.g., impetigo, folliculitis, carbuncles, furuncles, cellulitis, scalded skin syndrome, and others), septic arthritis, osteomyelitis, prosthetic device infections, pulmonary infections (e.g., pneumonia and empyema), gastroenteritis, meningitis, toxic shock syndrome, and urinary tract infections. Depending on the strains involved and the site of infection, these bacteria can cause invasive infections and/or toxin-mediated diseases.[8]

Mechanisms of host immune evasion include the production of an antiphagocytic capsule, sequestering of host antibodies or antigen masking by protein A, biofilm formation, intracellular survival, and blocking chemotaxis of leukocytes. Binding of the bacteria to extracellular matrix proteins and fibronectin in infectious endocarditis is mediated by bacterial cell wall-associated proteins such as fibrinogen-binding proteins, clumping factors, and teichoic acids.[9]

Also, Staphylococcal superantigens (TSST-1 or toxic shock syndrome toxin 1) are important virulence factors in infectious endocarditis, sepsis, as well as toxic shock syndrome. Pneumonia

infections are associated with the bacterial production of PVL (Panton-Valentine leukocidin), Protein A and alpha-hemolysin, such infections are more common following influenza virus infection as well as a diagnosis of cystic fibrosis. Prosthetic device infections are often mediated by the ability of *S. aureus* strains to form biofilms as well as communicate using quorum sensing in a bacterial cell density-dependent manner.[9]

Quorum-sensing (QS)

Definition:

QS is a way enabling bacterial cells to communicate chemically with each other in response to extracellular signaling molecules known as autoinducers. [10]

Importance:

It permits groups of bacteria to alter their behaviors in response to changes in the population density and species composition of the vicinal community. [10]

Autoinducers of Gram-positive bacteria like **staphylococci** use oligopeptides to communicate. [11] Through these chemical signaling molecules, Gram-positive bacteria form communication circuits to control different ranges of bacterial characteristics, such as virulence, conjugation, competence, antibiotic production, sporulation, motility, and biofilm formation. [12]

Nearly 80% of microbial infections are related to biofilm formation, which is controlled by QS, It has been shown that, compared to free single cells, bacteria that are found in biofilms are more resistant to antibiotics, environmental pressure, and the host immune system. [13]

S. aureus agr quorum-sensing

The staphylococcal *agr* quorum sensing (QS) system consists of two transcriptional units, RNAII and RNAIII, which encode four genes: *agr*A, *agr*B, *agr*C, and *agr*D. RNAII is produced as a single transcript in the order of *agr*BDCA, while the transcriptional units are oriented in opposing directions. *Agr*D serves as the propeptide for the QS signal and autoinducing peptide (AIP) of the system, which is subsequently processed by the membrane-bound export protein *agr*B into AIP before being released from the cell.[14]

AIP, which is comprised of seven to nine aminoacyl residues, stimulates *agr*C, which in turn causes trans-autophosphorylation, which in turn activates *agr*A, the response regulator. *Agr*A sustains the activation of its own expression by binding the *agr* promoter areas, which are P2 for RNAII and P3 for RNAIII, once it has been activated. Because of this, the ensuing positive feedback loop may swiftly and significantly change gene expression in cells with different densities, setting apart the *agr* operon as a traditional QS system. The *agr* operon is frequently variable amongest *S. aureus* strains, leading to the classification of four *agr* types (I-IV). These types are distinguished from one another by differences in the amino acid sequences of the AIP and by the magnitude and timing of *agr* activation.[15]

S. aureus agr regulation:

One of the most well-researched bacterial two-component regulatory systems is the *S. aureus agr* regulation. Many repressors and inducers regulate the expression of *agr*, and some of them regulate one another to create further regulatory loops. Staphylococcal accessory regulator A (SarA), a regulator mainly responsible for regulating the production of secreted proteins including exoproteases, is one inducer of optimum *agr* expression.[16]

σB, an established regulator of biofilm development, has been intricately associated with *agr* regulation, thereby reinforcing the relationship between *agr* regulation and the metabolic condition of *S. aureus*. infection. Furthermore, it was discovered that the relationship between *agr*A and DNA is regulated by an oxidation detecting mechanism built into the *agr* system.[17]

The *agr* system orchestrates the suppression of bacterial growth in conjunction with the enhancement of virulence factors, aligning with the metabolic state during *S. aureus* infections. This system operates simultaneously with various regulatory proteins and adapts to fluctuating environmental conditions. Notably, σB, a recognized regulator of biofilm development, has been closely linked to the regulation of the *agr* system. Moreover, it was discovered that the *agr* system's built-in oxidation sensing mechanism regulates how *agr*A and DNA interact.[18]

*Agr*A not only promotes RNAII production, which in turn up-regulates *agr* through auto-feedback, but it also up-regulates RNAIII dependent virulence genes and phenol soluble-modulin (PSM) cytolysin genes, which increase S. aureus pathogenicity. *Agr*A directly binds to the psm α and psm β promotors to control PSM expression, independent of RNAIII. Seven PSM peptides, ranging in length from around 20 to 45 amino acids, have been identified in S. aureus (PSMα1-4, PSMβ1-2, δ-toxin). Certain PSMs may both stimulate and destroy neutrophils; these are mostly those encoded in the psm α operon.[19]

Additionally, various elements of bacterial physiology seem to be regulated without the influence of RNAIII. Nevertheless, the predominant *agr*-dependent regulation of virulence in *Staphylococcus aureus* is mediated by the interaction of the regulatory RNA, RNAIII, with numerous target genes. Notably, the hld gene, responsible for encoding the δ PSM toxin, is included within RNAIII. Beyond the synthesis of δ-toxins, RNAIII has been linked to the upregulation of several leukocidins, enterotoxins, and α-toxins, as well as the degradation of exoenzymes, including serine and cysteine proteases and lipases. Furthermore, RNAIII plays a role in regulating surface binding proteins and protein A, which is known for its immune evasion properties, aligning with the hypothesis that surface binding proteins are crucial for establishing infection when bacterial cell density remains low.[20]

However, investigations of clinical strains have not definitively established that surface binding proteins are not regulated by *agr*. Moreover, metabolic enzymes have been identified as being under the regulation of RNAIII, indicating a potential equilibrium between toxin regulation and diminished metabolic activity, a concept further supported by the independent regulation of metabolic enzymes by RNAIII. It is believed that RNAIII primarily influences gene expression through an antisense base-pairing mechanism and the action of the transcriptional repressor protein gene Rot.[20]

Biofilm formation and agr function

It has been closely linked to *agr* function in a number of *S. aureus* strains; up-regulation promotes dispersion while down-regulation results in excessive biofilm thickness and lack of structure. The direct facilitators of those actions that are *agr*-controlled are the detergent-like PSM peptides. LuxS/AI-2 is a significant QS system that is present in staphylococci and is widely distributed in bacteria. This mechanism up-regulates the PSM peptides that facilitate biofilm dissociation and structure, seemingly acting in accordance with *agr* in the control of biofilm development.[21]

Immunization against α-toxin, an *agr* regulated toxin, resulted in a decreased development of cutaneous abscesses, a typical CA-MRSA infection. In similar fashion, *S. aureus* strains with *agr* removed showed reduced infectivity in animal models of necrotising pneumonia and endocarditis.[22]

The enhanced capacity of *agr* mutants to generate significantly large, albeit disorganized, biofilms implies that mutations resulting in a compromised *agr* system may contribute to the effective colonization and sustained infection by *Staphylococcus aureus*. Research indicates that *agr* mutations can accumulate in *S. aureus* during in vitro passage, leading to diminished production of virulence factors. In investigations of *agr* dysfunction in clinical isolates, strains exhibiting *agr* defects were linked to hospitalization, familial transmission, and chronic infection. Furthermore, the correlation between a defective *agr* system and a heightened occurrence of chronic endocarditis suggests that *agr* dysfunction confers a survival advantage for *S. aureus* in certain infections. Additionally, the selection pressure exerted by antibiotics appears to promote the loss of *agr* function, potentially elucidating the diminished efficacy of certain antibiotic treatments against specific *S. aureus* infections. It is important to recognize that the survival benefit associated with reduced or absent *agr* activity may be compromised when introduced into naïve hosts, which could account for the lack of success of HA-MRSA strains in community environments.[23]

Targeting S. aureus agr quorum-sensing

The investigation of naturally occurring and chemically modified autoinducing peptides (AIPs) alongside *agr*-inhibiting molecules is underway as a potential strategy to concurrently inhibit the

expression of numerous virulence genes regulated by the *agr* system in *Staphylococcus aureus*. Within *S. aureus*, four distinct *agr* subgroups exist, each generating unique AIPs characterized by different amino acid sequences, yet all possessing similar thiolactone ring structures.[24]

The AIPs function as activating agonists for the *agr*C histidine kinase within their respective subgroups; however, they frequently suppress the *agr* activity of other subgroups. Notably, the AIPs from subgroups I and IV stand out due to their significant similarity and absence of cross-inhibition. The variability in the composition and length of the N-terminal amino acid residues that constitute the N-terminal "tail" of *S. aureus* AIPs is pronounced, even as the size of the C-terminal thiolactone ring remains conserved. Each of the four AIPs exhibits differences at nearly every position, with the exception of the cysteine residue, which is essential for the formation of the thiolactone-bonded ring. The phenomenon of AIP cross-inhibition has led to extensive research into both natural and modified AIPs as potential agr inhibitors.[25]

Conclusion

The review reveals the definition and importance of quorum sensing system which is an important communication system in *Staphylococcus aureus*, This communication is done by a group of genes called accessory gene regulator system by producing autoinducing peptides. These peptides regulate pathogenesity of bacteria either by activating *agr* system so increase pathogenesity of bacteria or deactivating agr system so decrease pathogenesity of bacteria and increase liability for biofilm formation.

Recommendations and how they could be helpful to patients

More researches are advised to be done on different autoinducing peptides types as an *agr*-inhibiting molecules as potential ways of simultaneously inhibiting the production of the considerable number of *agr*-controlled virulence genes so decrease pathogenesity of bacteria causing an infection in patients.

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