NSU Florida
NOVA SOUTHEASTERN UNIVERSITY

Mako: NSU Undergraduate Student Journal

Volume 2 Article 2

Fall 12-1-2008

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Maria E. Cevallos
Nova Southeastern University

Running head: *STREPTOCOCCUS PYOGENES*

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Maria E. Cevallos

Faculty Advisor: Jason A. Rosenzweig, Ph.D.

Nova Southeastern University

Division of Math, Science and Technology

Abstract

Streptococcus pyogenes is one of the most medically relevant genera of Gram positive bacteria. *S. pyogenes* belongs to Group A streptococcus (GAS), the most pathogenic streptococci. *S. pyogenes* is exclusively a human pathogen that is known for its ability to cause a wide array of infections ranging from superficial pharyngitis and impetigo to invasive life threatening conditions including septicemia, necrotizing fasciitis, and streptococcal toxic shock syndrome. The dissemination of *S. pyogenes* within a human host is dependent upon virulence factors aimed at host immune system evasion leading to infection. More specifically, *S. pyogenes*' virulence factors are required for bacterial dissemination, adherence, colonization and host-cell entry. Other virulence factors enable the evasion of phagocytosis, and survival within the phagocyte. The plethora of virulence factors explains the variety of *S. pyogenes* infections and its recent epidemiological resurgence world-wide. Uncovering the molecular mechanisms of *S. pyogenes* pathogenesis is crucial in helping scientists develop new therapies for this human health threat. In the following review, the most potent virulence and virulence-associated factors of *S. pyogenes* will be discussed along with their mechanisms of action.

Streptococcus pyogenes: A versatile human pathogen

Streptococcus pyogenes is one of the most important Gram positive human pathogens (Abbot et al, 2007). *S. pyogenes* is exclusively a human pathogen whose primary habitat is skin and throat tissues, areas providing for maximal growth and efficient transmission of progeny to new hosts (Bessen et al, 2005). This pathogen is notorious for its ability to cause a wide array of human diseases ranging from superficial skin infections of the throat and skin to systemic diseases. In the case of superficial infections, the organism does not disseminate from the original infection site and does not become life-threatening. In sharp contrast, invasive *S. pyogenes* infections pose a serious threat to human life because of their keen ability to dissipate through the bloodstream into other tissues and organs. (Terao et al, 2008) Invasive infections of *S. pyogenes* account for approximately 500,000 deaths each year world-wide (Carapetis et al, 2005) (Terao et al, 2008).

Superficial diseases caused by infection of *S. pyogenes* include pharyngitis, impetigo and cellulitis (Jalava et al, 2004). Pharyngitis, also known as strep throat, is an infection of the upper respiratory tract while impetigo and cellulitis are skin infections (Sumby et al, 2008). Currently, patients with pharyngitis have a 30% rate of antibiotic treatment failure (Lembke et al, 2006). The prevalence of pharyngitis and impetigo each depend on the climatic conditions and the specific *S. pyogenes* strain colonizing in the host (Bessen et al, 2005). Certain *S. pyogenes* serotypes have strong preferences for certain infection sites. Since the microenvironments in the throat and skin are so different, the signal transduction pathways which control gene expression at these sites may also differ for throat versus skin strains (Bessen et al, 2005). For example, epidemiological studies indicate that the *S. pyogenes emm* D strain mostly causes skin infections and rarely causes pharyngitis (Bessen et al, 2005) while the *emm* E strain affects both skin and

throat tissues (Bessen et al, 2005). Although these superficial infections are not considered life threatening, it is important to understand that they may develop into dangerous medical conditions if left untreated (Sumby et al, 2008). More specifically, when superficial infections are left untreated, they may develop into severe infections that invade other tissues and organs (Terao et al, 2008). For instance, in some cases where pharyngitis is left untreated, severe acute rheumatic fever may develop (an invasive *S. pyogenes* infection) (Sumby et al, 2008).

Within the past twenty years, there has been a staggering resurgence of *S. pyogenes*-related infections (Walker et al, 2005), most of which are invasive (Phelps & M, 2007). The increase in frequency of invasive infections is an indication of this pathogen's ability to evolve in order to survive and proliferate in new environments (Phelps & M, 2007). These systemic infections must escape the host's immune defenses and survive in the plasma in order to disseminate to other tissues (Phelps & M, 2007) (Terao et al, 2008). Invasive infections caused by *S. pyogenes* include streptococcal toxic shock syndrome (STSS), sepsis (Bessen et al, 2005) (Walker et al, 2005), rheumatic fever, glomerulonephritis and necrotizing fasciitis (Sumby et al, 2008) (Walker et al, 2005). STSS is characterized by hypotension, tissue destruction and multiple organ failure (Pahlman et al, 2008). In STSS, *S. pyogenes* spreads quickly to other tissues (Pahlman et al, 2008), and, if not treated promptly, multiple organ failure may occur (Tsai et al, 2006). Mortality rates of up to 50% have been reported for patients afflicted with STSS (O´Brien et al, 2002) (Phelps & M, 2007). Sepsis is another serious medical condition that occurs when bacteria spread to the bloodstream and other tissues. The spreading of bacteria into the bloodstream often leads to systemic inflammation.

Rheumatic fever is an inflammatory disease caused solely by the Group A Streptococcus (GAS). It may involve the heart, brain, joints and skin and is most commonly found in children.

Glomerulonephritis is characterized by inflammation of the small blood vessels in the kidneys. *S. pyogenes* is one of the most common causes of necrotizing fasciitis. This is a rapidly progressing disease in which infected subcutaneous tissue results in the severe destruction of fascia and adipose tissue (Bisno & DL, 1996). Patients with necrotizing fasciitis have a 25% mortality rate (O´Brien et al, 2002) (Phelps & M, 2007), and these invasive infections often develop into septic and systemic infections, making these infections extremely dangerous to the host (Terao et al, 2008). Many of these severe infections result from the release of exotoxins and the expression and interaction of various virulence factors.

Most superficial infections heal without the use of antibiotics, indicating that the body's defenses are often able to eradicate *S. pyogenes* without treatment (Eliasson et al, 2007). At the same time, however, the ability of some superficial infections to become invasive because of a lack of treatment is also indicative of the potency of *S. pyogenes* against the human immune system. The establishment of infection indicates that the pathogen has been able to evade the host immune system to some degree. There are a plethora of virulence factors, each with different functions that come together to produce the unique and versatile pathogenic properties that *S. pyogenes* employs to carry out the aforementioned task. In order for a pathogen to cause an infection, it must first adhere to the host epithelial cells. For this purpose, various extracellular proteins (Phelps & M, 2007) and virulence factors are employed for adhesion. Once *S. pyogenes* has colonized the host it has the ability to evade the host immune defenses, allowing it to proliferate and cause infection (Phelps & M, 2007). Virulence factors provide bacteria with the tools necessary for immune evasion, thus playing a vital role in *S. pyogenes* infections. Each virulence factor serves a unique function in the pathogenicity of the bacteria while promoting survival within the host. Throughout this review, the most potent *S. pyogenes* virulence factors

and various virulence-associated factors as well as their mechanisms of action will be discussed.

M protein

S. pyogenes cell-surface components are often vital for adherence to human epithelial cells (Hasty et al, 2006). Laminin and collagen-binding proteins, various fibronectin-binding proteins and the M protein virulence factor are all involved in mediating adhesion to human cells (Abbot et al, 2007). There are many types of M proteins; they are distinguished from each other through a numbering system, such as M1 protein or M2 protein. M protein has many properties that contribute to the pathogenesis of *S. pyogenes* including: adherence to host epithelial cells (Eliasson et al, 2007), antiphagocytic properties (Courtney et al, 2006) (Pahlman et al, 2008) and mediating internalization of *S. pyogenes* into host cells (Abbot et al, 2007). M protein is encoded by the *emm* gene (Phelps & M, 2007) and is positively regulated by Mga (multigene activator) (Pahlman et al, 2008). More than 150 different M proteins have been characterized thus far, with some types being more prevalent in certain diseases (Pahlman et al, 2008). M protein has also been used to classify the GAS. M proteins have a helical coiled coil structure with a highly variable amino terminus which is used for classifying the GAS into over 100 *emm* types (Courtney et al, 2006).

The ability of M proteins to allow *S. pyogenes* to adhere to its host is vital for active infection. M1 protein contains binding sites for host fibrinogen, immunoglobulin G and albumin (Eliasson et al, 2007). Also, M1 protein binds to host cells through glycosaminoglycans (GAGs) and sialic acid (Eliasson et al, 2007). Frick et al. 2000 found that the presence of M1 protein alone enhances the adherence of *S. pyogenes* to tonsil epithelium (Frick et al, 2000). M1 protein can be released sporadically or specifically by *S. pyogenes* through streptococcal exotoxin B (SpeB) (Eliasson et al, 2007). This suggests that M protein has the ability to affect host cells

from a distance (Eliasson et al, 2007). In lymphoid cells, M proteins have been shown to enter the nucleus where they interfere with eukaryotic replication (Eliasson et al, 2007). Furthermore, M protein triggers the internalization of *S. pyogenes* into host cells by binding to host fibronectin (Abbot et al, 2007), and also promotes the survival of *S. pyogenes* within host cells (Eliasson et al, 2007). M1 is also antiphagocytic (Pahlman et al, 2008) by virtue of its ability to bind to fibrinogen in human blood thus preventing the deposition of complement protein C3b on *S. pyogenes* (Courtney et al, 2006). C3b is a protein of the complement that binds to the surface of bacteria to aide the opsonization process whereby bacteria are more efficiently phagocytosed (Terao et al, 2008). Overall, M protein is a versatile virulence factor that has multiple properties that promote *S. pyogenes* colonization and survival in its host.

Streptococcal pyrogenic exotoxin B

Another well characterized *S. pyogenes* virulence factor is the cysteine protease, Streptococcal pyrogenic exotoxin B (SpeB). SpeB is a zymogen (proSpeB) that, once matured, degrades aggregated proteins and cleaves GAS virulence factors and other biologically important molecules (Cole et al, 2007). Terao et al. 2008 observed SpeB mediated degradation of the complement protein C3b in sera from STSS patients (Terao et al, 2008). This degradation provides mechanisms for the evasion of phagocytosis; thus, it is a vital mechanism for the invasion of host tissues by *S. pyogenes* (Terao et al, 2008).

HtrA is a chaperone protein found in Gram negative bacteria that functions to degrade proteins that have been misfolded. A homologue of HtrA has been found in Gram positive bacteria (Cole et al, 2007). Using an HtrA deleted strain (Δhtra), Cole et al. 2007 found that HtrA does not activate proSpeB *in vitro*, and, therefore is only indirectly involved in the maturation of SpeB (Cole et al, 2007). However, HtrA is important for the optimally efficient maturation of

proSpeB (Cole et al, 2007). Furthermore, HtrA may influence the secretion of supplementary factors required for SpeB maturation (Cole et al, 2007).

SpeB is responsible for the specific release of M protein from the surface of *S. pyogenes* (Eliasson et al, 2007). The release of virulence factors, such as that of M protein, is sometimes crucial for the evasion of host immune defenses. For instance, since M protein induces host production of the antibacterial peptide MIG (Eliasson et al, 2007), it is vital for this virulence factor to be released from the surface of the bacterium in order to prevent a host immunity response on the bacterial cell. Also, in the Δ*htra* mutant, due to an accumulation of proteins, the cell has attenuated its virulence and was hyper-sensitive to certain environmental factors such as high temperatures (Cole et al, 2007). Thus, SpeB plays a role in the optimal virulence of *S. pyogenes*, as well as its resistance to thermal, oxidative and osmotic pressures (Cole et al, 2007).

SpyCEP

SpyCEP was recently recognized as a GAS virulence factor by Sumby et al. 2008. This protein serves as a general chemokine protease, inactivating interleukin 8 (IL-8) protease, GCP-2 (human chemokines granulocyte chemo tactic protein-2) and GROα, a growth related oncogene (Sumby et al, 2008). IL-8 recruits neutrophils to the site of infection while GCP-2 and GROα are made by the tonsillar epithelial cells (Sumby et al, 2008). Neutrophils are a major component of the innate immune system and are directly involved in killing bacteria primarily through phagocytosis (Sumby et al, 2008). Thus, SpyCEP reduces phagocytic killing of *S. pyogenes* primarily through the cleavage and inactivation of IL-8 (Sumby et al, 2008). SpyCEP's ability to cleave human chemokines provides *S. pyogenes* with yet another immune evasion mechanism. Additionally, SpyCEP functions to sequester antibodies through their Fc region in order to prevent antibodies from binding to *S. pyogenes*, thereby preventing antibody-mediated

phagocytosis (Sumby et al, 2008).

The transcription of the spyCEP gene is directly regulated by the covR/S system (Sumby et al, 2008) (discussed at length near the end of this review), and its location on the surface of *S. pyogenes* is important for its chemokine degradation activity (Sumby et al, 2008). For instance, in an upper respiratory infection, SpyCEP helps to secure the colonization site despite the flow of saliva (Sumby et al, 2008). The mechanism through which SpyCEP is processed is similar to that of M protein. The main difference, however, is that SpeB does not appear to be involved in the cleavage of SpyCEP, as it does in the cleavage of M protein. The mechanism through which SpyCEP is cleaved has yet to be identified (Sumby et al, 2008).

Streptolysin S

Streptolysin S (SLS), the functional product of the sagA operon, is another virulence factor involved in the sequestering of iron from host cells (Salim et al, 2007). SLS lyses erythrocytes as a means of acquiring intracellular iron (Salim et al, 2007). Erythrocytes store abundant amounts of iron in hemoglobin molecules (Salim et al, 2007). Thus, the lysing of erythrocytes provides *S. pyogenes* with the highest possible levels of intracellular iron. SLS is also a cytolysin involved in inflammation, tissue injury and killing of phagocytes (Salim et al, 2007). In fact, S. *pyogenes* have been shown to escape the phagocytic vacuoles of polymorphonuclear leukocytes and dissipate into the cytoplasm where they were then able to multiply (Medina et al, 2003). However, it has not been demonstrated that SLS could play some mechanistic role.

Virulence-associated Factors

*sia*A and *sagA*

Iron is an essential nutrient, required for the survival of pathogenic bacteria (Salim et al,

2007). In fact, when bacteria are deprived of iron they are unable to colonize their human hosts (Bates et al, 2003). The exact effects of decreased iron levels on *S. pyogenes* are poorly understood; however, it is known that low iron levels widely affect gene expression (Bates et al, 2003). For example, in low iron levels there is a decreased transcription of the *emm6.1* gene that encodes the antiphagocytic M6 protein (Bates et al, 2003). Since iron is essential for full virulence of *S. pyogenes,* the mechanisms for the acquisition of iron are extremely important. It is often difficult for bacteria to attain this nutrient because it is located intracellularly and sequestered by host iron-binding proteins, such as hemoglobin and transferrins (Salim et al, 2007). Both *siaA* and *sagA* are involved in iron acquisition (Salim et al, 2007).

SiaA (also referred to as HtsA) is involved in binding human hemoproteins as an iron scavenger (Bates et al, 2003), (Salim et, al 2007). The expression of *sia*A is upregulated under low iron levels (Salim et al, 2007), allowing *S. pyogenes* to increase its iron levels by increasing the binding of human hemoproteins. The *sag*A gene (also referred to as *pel*) encodes a bacteriocin-like peptide that functions as the structural unit of Streptolysin S (SLS), another *S. pyogenes* virulence factor involved in the iron acquisition (Salim et al, 2007). In fact, SLS is directly involved in the upregulation of *sag*A (Salim et al, 2007). Salim et al. 2007 demonstrated that the expression of *sag*A increases as a result of increased cell density, indicating a quorum-sensing response (Salim et al, 2007). Quorum-sensing molecules alter gene expression as a response to changes in cellular density (Miller & BL, 2001). For instance, as cell density increases, chemical signaling molecules reach a threshold concentration leading to a change in gene expression (Miller & BL, 2001). SagA is also indirectly involved in the regulation of several virulence factors including the expression of: M proteins, Sic, and SpeB (Salim et al, 2007).

Sal Y

While *S. pyogenes* employs a plethora of mechanisms for evasion of phagocytes and the immune response, only one mechanism has been identified for the intracellular survival of *S. pyogenes* (Phelps & M, 2007). SalY was first identified in *Streptocuccus salivarius* as a gene in the sal ocus encodeing salivaricin A lantibiotic locus (Phelps & M, 2007). Although most elements in the locus have been conserved, in *S. pyogenes*, structural mutations in the *sal* locus have led to a loss of *S. pyogenes* immunity to lantibiotic and its inability to produce the active lantibiotic (Phelps & M, 2007). An *S. pyogenes* SalY mutant derived from a transposon mutagenesis screen was found to have compromised intracellular survival relative to its isogenic wild type strain (Phelps & M, 2007). Changes undergone by SalY are proposed as an evolutionary adaptation that has allowed *S. pyogenes* to adapt and survive in a new environment (Phelps & M, 2007). Thus, SalY no longer protects *S. pyogenes* from lantibiotic; instead, SalY promotes intracellular survival within human macrophages (Phelps & M, 2007). The function of SalY has provided *S. pyogenes* with a mechanism to escape destruction by a papoose, giving it the ability to persist intracellulary and further evade immune detection. This mechanism clearly demonstrates *S. pyogenes*' ability to adapt to changing environmental conditions.

Biofilms

In addition to secreted toxins and M proteins, *S. pyogenes* have also been shown to form biofilms. A biofilm is a complex accumulation of microorganisms that secrete a protective and adhesive matrix that can form on biotic and abiotic surfaces. Biofilms are often integral components of prokaryotic life (Lembke et al, 2006). The development and maintenance of biofilms is dependent on quorum sensing and on the communication between the species living within the biofilms (Lembke et al, 2006). Although biofilms are not virulence factors, they are

considered virulence associated factors because they are associated with recurrent infections, antibiotic resistance, persistent colonization (Baldassarri et al, 2006) and nosocomial infections (Lembke et al, 2006). Nosocomial infections are often caused by the formation of biofilms on medical devices that have been implanted in humans: intravenous catheters, artificial joints and cardiac pacemakers. These hospital-borne infections may cause chronic and/or acute infections or diseases such as: dental caries, gingivitis, periodontitis, endocarditis and prostatitis (Lembke et al, 2006).

In a recent study, GAS strains removed from patients with penicillin treatment failure formed biofilms *in vitro* (Lembke et al, 2006). GAS infections that form biofilms have much higher antibiotic minimum inhibitory concentrations (MICs) (Lembke et al, 2006), indicating that biofilm forming bacteria require a higher dose of antibiotics to be treated successfully. Some *S. pyogenes* strains have a higher biofilm-forming capacity than other strains. Lembke et al. 2006 demonstrated that biofilm formation is a characteristic of GAS isolates; however, each GAS strain has varying affinities toward different substrates (Lembke et al, 2006). The optimum temperature for growth of a biofilm is 37° Celsius, the temperature of the human body. Also, strains susceptible to macrolide antibiotics produced more biofilms when compared to the strains resistant to erythromycin. Isolates from the *emm6* strain had the highest level of biofilm formation and were most susceptible to macrolides (Lembke et al, 2006). Finally, *S. pyogenes* isolates that invaded host cells with high efficiency were those producing the lowest amount of biofilm, suggesting that the production of biofilm could impede bacterial virulence in some instances (Baldassarri et al, 2006).

Lipoteichoic acid

Lipoteichoic acid (LTA) represents a Gram-positive pathogen-associated microbial

pattern (PAMP) that is recognized by the innate arm of the host's immune system. It is thought to represent the Gram-positive counterpart to the Gram-negative endotoxin lipopolysaccharide (LPS) since both molecules are membrane components that share many biological activities. These biological activities include adherence to animal cells, stimulation of alternative complement pathway, stimulation of non-specific immunity (innate immunity), nephrotoxicity and mitogenicity (Hasty et al, 2006). This extracellular component activates the host innate immune system through toll-like receptor 2. LTA is also able to bind to host components: surfactant protein D, ficolin, LPS-binding proteinand plasma lipoproteins. These interactions may activate, inhibit or have no effect on LTA activity in the host (Hasty et al, 2006).

Pili

Although pili are not necessarily a virulence factor, they have important adhesive properties that allow bacteria to adsorb to host cells and promote the initial stages of an infection (Abbot et al, 2007). Pili consist of one major unit known as a shaft which contains many other small subunits on its surface. Gram positive bacteria are known to produce large amounts of pili on its surface. Interestingly, pili-mediated adherence to the host cell is not dependent on host fibronectin or type 1 and 4 collagen (Abbot et al, 2007). In fact, pili have a high specificity when adhering to the host and have been shown, in some cases, to be involved in the internalization of bacteria into host cells (Abbot et al, 2007). The *Spy128* gene encodes for the major subunit of pili while the *Spy125* and *Spy130 genes* encode for minor subunits. As it turns out, however, pili are not vital for adherence to the host since there are other cellular components and virulence factors through which *S. pyogenes* can adsorb to the host.

Regulation of virulence factors

Up to this point, a variety of virulence factors and virulence associated factors have been

described. Now, the various mechanisms of virulence gene regulation will be discussed. GAS strains have the ability to grow in many conditions within the host (Dalton et al, 2006). Thus, these strains contain mechanisms that afford them the opportunity to survive in adverse environments such as high temperatures and low pH (Dalton et al, 2006). When bacteria face environmental stress(ors), they often respond by altering gene expression to upregulate certain factors that promote their growth (Dalton et al, 2004). *S. pyogenes* has evolved mechanisms to regulate the expression of virulence factors, some of which respond specifically to changes in the environment (Woodbury et al, 2006). Most bacterial species employ sigma factors of RNA polymerase for the regulation of gene transcription (Woodbury et al, 2006); however, the use of sigma factors in GAS strains is minimal (Dalton et al, 2004). Some of the regulatory systems that have been identified thus far include Mga (multiple gene regulator), RALP (RofA-like protein) and Rgg (RofB). There are also two-component systems: CovR/S, FasBCAX, Ihk/Irr and SilAB (Woodbury et al, 2006). In the GAS genome, it is predicted that there are 13 two-component regulatory systems (Dalton et al, 2004).

The most studied of these two-component regulatory systems is the CovR/S system (Dalton et al, 2004). This regulatory system allows *S. pyogenes* to undergo rapid and reversible changes in its gene expression as a response to its environment (Dalton et al, 2004). In this system, CovR is the response regulator which inhibits the transcription of 15% of the *S. pyogenes* genes (Dalton et al, 2004). CovR leads to the direct repression of most of the genes it regulates (Dalton et al, 2006). When CovR is activated it binds to promoters, inhibiting the transcription of the genes associated with those promoters. The phosphorylation of CovR is essential for its activity and ability to bind to promoters (Dalton et al, 2006) (Dalton et al, 2004); this occurs when there are no stressors in the environment. The genes repressed by CovR are

genes that are important to growth under various environmental conditions and include some notable virulence-factor encoding genes (Dalton et al, 2006). It is thought that GAS produces these factors, not to harm the host, but to promote its own growth (Dalton et al, 2006). CovR is known to repress the following operons: hyaluronic acid capsule (*has), ska* (streptokinase), *speMF/sda* (streptococcal DNase), *sagA* (Streptolysin S) and *covR* (Dalton et al, 2006). Streptokinase and Dnase promote the invasion into deeper tissue, while SLS alters the microenvironment, and hyaluronic acid capsule functions as a physical buffer that interferes with the entry of salts and protons into the GAS (Dalton et al, 2006). Thus, these virulence factors may have evolved to help *S. pyogenes* escape harsh environments, grow in harsh environments, or be protected in harsh environments (Dalton et al, 2006). When *S. pyogenes* experiences stress, CovS is stimulated, causing an inactivation of CovR (Dalton et al, 2006). More specifically, when CovS senses stressors in the environment, it inactivates CovR through dephosphorylation. The dephosphorylation of CovR causes CovR to lose its affinity for the promoter and thus CovR releases the promoter, allowing for the virulence gene to be transcribed. CovS is a biphastic protein. It has both phosphatase and kinase activity and is the sensor of the CovR/S regulatory system; however, CovS is not needed for the activation of CovR (Dalton et al, 2006) (Dalton et al, 2004). CovS is located on the surface of *S. pyogenes*, allowing it to respond to environmental signals through autophosphorylation (Dalton et al, 2006). The CovS sensor seems to be stimulated by seemingly unrelated stressors (Dalton et al, 2004). Thus, CovS may function as a general stress response of GAS (13). CovR is activated by phosphorylation (Dalton et al, 2006) (Dalton et al, 2004), but it is not dependent on a phosphate donor (Dalton et al, 2006). One report concluded that in the presence of CovR, CovS is needed for growth of GAS under the following stress conditions: high temperature (40° C), acidic pH (pH 6.0) and increased salt concentrations.

Thus, this system is used as an alternative to sigma factors for growth under environmental stress (Dalton et al, 2006).

Therefore, the inactivation of CovR leads to the transcription of virulence genes needed for growth and survival under harsh environmental conditions. The CovR/S system is able to respond quickly to changing stress levels because there is an accumulation of CovR within the cell after a stress response. Once the stress is relieved, accumulated CovR is activated again and is able to quickly bind to the promoter, repressing the transcription of virulence genes.

Antibiotic Resistance

S. pyogenes is a highly recombinogenic bacterial species (Bessen et al, 2005). High levels of recombination allow for numerous genotype combinations resulting in several independent genetic changes that promote the survival of *S. pyogenes* through increased virulence (Bessen et al, 2005) or the development of antibiotic resistance. The resistance to antibiotics has become a common theme in medicine. There are many different causes for the growing number of antibiotic resistant bacterial pathogens. In the case of *S. pyogenes,* antibiotic resistance is not uncommon. So far, *S. pyogenes* strains have been found to be resistant to the following antibiotics: macrolide, erythromycin, lincosamide, streptogramin B, MLS antibiotics and fluoroquinolone (FQ). This list, however, does not include all of the antibiotics to which different strains of *S. pyogenes* have developed resistance.

Scientists have been able to identify specific genes in the genome of *S. pyogenes* that are associated with antibiotic resistance: *erm*(B), *erm*(TR) and *mef*(A) **(**Jalava et al, 2004**).** The *erm*B gene is responsible for resistance to macrolides, lincosamide and streptogramin B (Jalava et al, 2004) while the *mef(A)* gene is known for causing resistance to MLS antibiotics and macrolides (Brenciani et al, 2007) (Jalava et al, 2004). The *S. pyogenes* isolates resistant to macrolides,

lincosamide and streptogramin B are phenotypically and genotypically heterogeneous (Brenciani et al, 2007). The resistance associated with the *erm*B gene may be due to an efflux pump or target site modification mechanisms (Brenciani et al, 2007). Efflux pumps are transport proteins that are involved in the extrusion of toxic chemicals, such as antibiotics, away from the cell. The efflux pumps have also been associated with *mef*(A) gene. This gene is associated with a resistance pattern (M phenotype) which is characterized with resistance of MLS antibiotics, only to 14- and 15-membered macrolides (Brenciani et al, 2007).

The primary antibiotic used to treat *S. pyogenes* infections is penicillin (Baldassarri et al, 2006). For individuals allergic to penicillin, macrolides are the next antibiotic of choice; however, the number of macrolide-resistant *S. pyogenes* infections has increased (Jalava et al, 2004). So far, two mechanisms have been identified as the cause of *S. pyogenes* resistance to macrolides: efflux pumps and posttranscriptional target site modification (Jalava et al, 2004). Posttranscriptional target site modifications are mediated by the *erm* gene.

Additionally, increase in resistance to erythromycin (Brenciani et al, 2007) has been associated with the *erm* gene (Jalava et al, 2004) and the presence of *prtF1* gene (Baldassarri et al, 2006). GAS isolates that express the *prtF1* gene produce biofilms at much higher levels than *prtF1* deleted isolates. The biofilm formation in *prtF1* positive isolates is thought to be one of the primary causes of erythromycin resistance. The emergence of FQ resistance is primarily due to point mutations in the quinolone resistance-determining region (QRDR) of the bacterial topoisomerase II enzymes, especially DNA gyrase and topoisomerase IV (Pletz et al, 2006). The QRDR point mutations are attributed to spontaneous mutations, horizontal gene transfer and the spread of resistance genes through cloning (Pletz et al, 2006).

New therapies

S. pyogenes is an extremely versatile pathogen that causes a varietyof diseases ranging from superficial skin infections to systemic diseases. The virulence factors involved in the initial stages of infection such as adherence are often studied as candidates for vaccines (Olive et al, 2006). For instance, M protein is one such possible vaccine candidate (Olive et al, 2006). The structure of M protein contains a highly conserved carboxy-terminal repeat region (Courtney et al, 2006), (Olive et al, 2006). The synthesis of an intranasal M protein vaccine was based on this conserved region of the M protein. However, producing a vaccine that targets such a large region of the M protein like the C-repeat region may cause a human autoimmune response because of the immunological cross-reactivity between host tissues and the antigens of *S. pyogenes* which share some similarity. Therefore, research has become increasingly focused on the conserved regions of the M protein that do not evoke cross-reactive reactions but rather a protective host immune response.

One of the primary entry sites of *S. pyogenes* is the mucosal surface of the upper respiratory tract. At this site, adhesion and colonization of bacteria to the epithelial cells occurs (Olive et al, 2006). One group has evaluated the possibility of creating a vaccine that would prevent the adhesion and colonization to the mucosal layer of the upper respiratory tract. Several studies have supported the use of intranasal immunization since it has promoted reduced pharyngeal colonization and survival of *S. pyogenes* (Bessen & VA, 1988)**.** Olive et al. 2006 synthesized a vaccine formulation that incorporates the conserved C-repeat region of the M protein and the fibronectin-binding protein I (SfbI) and is administered through a lipid core peptide delivery system (Olive et al, 2006). This vaccine elicited a complete protective immune response in immunized mice against the lethal *S. pyogenes* strain NS192 (Olive et al, 2006).

S. pyogenes is a potent human pathogen that possesses many virulence and virulence-associated factors used to infect humans. Although the precise nature of the immune-evasion mechanisms used by *S. pyogenes* are not fully understood, new therapies are being developed to combat this human pathogen.

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