BIOFILM-PRODUCING GROUP A STREPTOCOCCAL INFECTIONS: MANAGEMENT AND TREATMENT

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Streptococcus pyogenes, or Group A Streptococcus (GAS), is a common colonizer of the upper respiratory tract in humans, that represent the only natural host. The most frequent pathological manifestations of GAS are mild suppurative throat and skin infections, with a worldwide estimate of 727 million cases per year. The cost for the health care system is rather heavy because, even though the infections are normally mild and self limiting, antibiotic treatment is suggested, first to relieve discomfort but also to minimize transmission and reduce complications. In fact, in susceptible hosts, GAS infections may lead to life-threatening complications such as sepsis, necrotizing fasciitis and toxic shock, or debilitating sequelae such as rheumatic fever, glomerulonephritis or tics.

The antibiotic of choice for streptococcal infections still remains penicillin. The ability of penicillin and related antibiotics (e.g., amoxicillin) to kill group A streptococci has not changed in more than 50 years. Up to now, there has never been a report of a group A streptococcus grown from a person resistant to this class of antibiotics. Thus, it appears that, in nature, Group A strep are unable to acquire resistance to penicillin.

Other possible therapeutic choices, particularly in case of allergic reactions to b-lactams, include macrolides, although macrolide resistance has showed an increasing trend in the last decades, with resistance rates which vary considerably in different countries. They range around 10%, but may reach up to almost 30% in some part of Europe, with marked regional variations in resistance rates (Table 1).

Table 1. Incidence of macrolide resistance in different countries

Year of publication	Country	Incidence of macrolide resistance (%)
2000	Belgium	10
2002	Finland	16.5 down to 8.6
2007	Canada	42
2007	Italy	26 down to 18
2008	Portugal	26 down to 13
2008	Denmark	3
2009	USA	3.5-4.5 (down from 9%)
2010	Norway	3.4
2010	France	1990 to 2003 - from 6 to 24

Despite the availability of an antibiotic which should be universally effective, *S.pyogenes* infections may fail to respond to antibiotic therapy leading to persistent throat carriage and recurrent infections. Kuhn and colleagues (2001) and Conley *et al*. (2003) examined a cohort of patients (104 and 99 patients respectively) with GAS throat infections, paired for age, sex and other parameters. In both cases one third of infections failed to respond to penicillin therapy.

Having established that group A strep does not carry the genetic determinants for resistance to penicillin, it is clear that such failures must have different explanations.

Recently, Pichichero and coworkers (2007) reviewed a list of factors possibly implied in such failures. Among these, of importance the carrier state which often depends on recurrent exposure; lack of compliance or a too early start of therapy during the infection, which may result in a deficient immune response. Also poor tonsillar penetration of the antibiotic used for treatment; presence of other microorganisms able to produce penicillinase, thus destroying the antibiotic before it came in contact with GAS, may represent important factors. An elegant study published in Lancet a few years ago (2001), suggested that especially macrolide-resistant GAS may be equipped with molecules favouring cell penetration (bacteria are often found intracellularly especially in peritonsillar abscesses); as penicillin does not penetrate epithelial cells, GAS would be protected. In light of more recent studies, also biofilm should be added to the list (Baldassarri *et al*., 2006). It is known that biofilm may certainly contribute to phenotypic resistance to anti-infective agents. Also what has been known as "tolerance" until now may in fact be an aspect of the resistance conferred by biofilm. In stationary-like phase (such as in biofilm), in which cell wall synthesis is minimal, penicillin may be ineffective (Eagle effect), also because several PBPs of GAS are lost when the bacterium enters the stationary phase *in vitro*.

The first indications that also *S.pyogenes,* besides other streptococcal species such as *S.salivarius* or *S.bovis*, was able to produce biofilm came from histological observations of structured communities present in human or animal model lesions (Akyiama *et al*., 2003; Neely *et al*., 2002; Hidalgo-Grass *et al*., 2004)

Biofilm formed *in vitro* is possibly less organized compared to the classical one, such as that produced by *Staphylococcus epidermidis*. Curiously, it appears that GAS start forming biofilm at the extremities of the bacterial chains, a character suggesting a "specialization" of terminal cells that has been already observed for GAS in cell attachment (Molinari *et al*., 2000).

As for the mechanism possibly involved in biofilm formation and regulation (Figure 1), it has been suggested that GAS starts as biofilm, colonizing the mucosa of the upper respiratory tract, then external stimuli may operate on a transcriptional regulator such as srv (which regulates several virulence factors), which in turn affect production/expression of the cysteine protease speB which degrades protein and DNA, which are integral part of the biofilm, releasing cells for colonization of distant sites (Doern *et al*., 2009; Sumby *et al*., 2006; Walker *et al*., 2007).

Alternatively damage by speB is perceived as signal inducing mutation in covS, another regulator, which would repress speB, induce sdaI (a dnase) and again leading to degradation of protein and DNA and cell releasing.

A number of molecules have been suggested to be in relation with biofilm production: such as M protein, or analogous of the M protein (Courtney *et al*. 2009), the product of the gene *has*A (hyaluronic acid capsule) which is not required for biofilm formation in static system, but it may be needed for aggregation and biofilm maturation (evaluated in flow conditions).

As is well known, in general biofilm-embedded cells are more resistant to anti-infective agents compared to planktonic cells of the same culture.

Conley and colleagues (2003) reported that while no strains of 50 from pharingits were resistant to penicillin, only one to the combination of penicillin and rifampin and 7 to rifampin alone, a large percentage of the 30 strains for which the minimal biofilm eradication concentration was determined where non susceptible to the antibiotic tested, including penicillin.

Besides being "insensitive" to penicillin treatment, we observed that penicillin at subMIC concentration may stimulate an increment in biofilm formation (Figure 2).

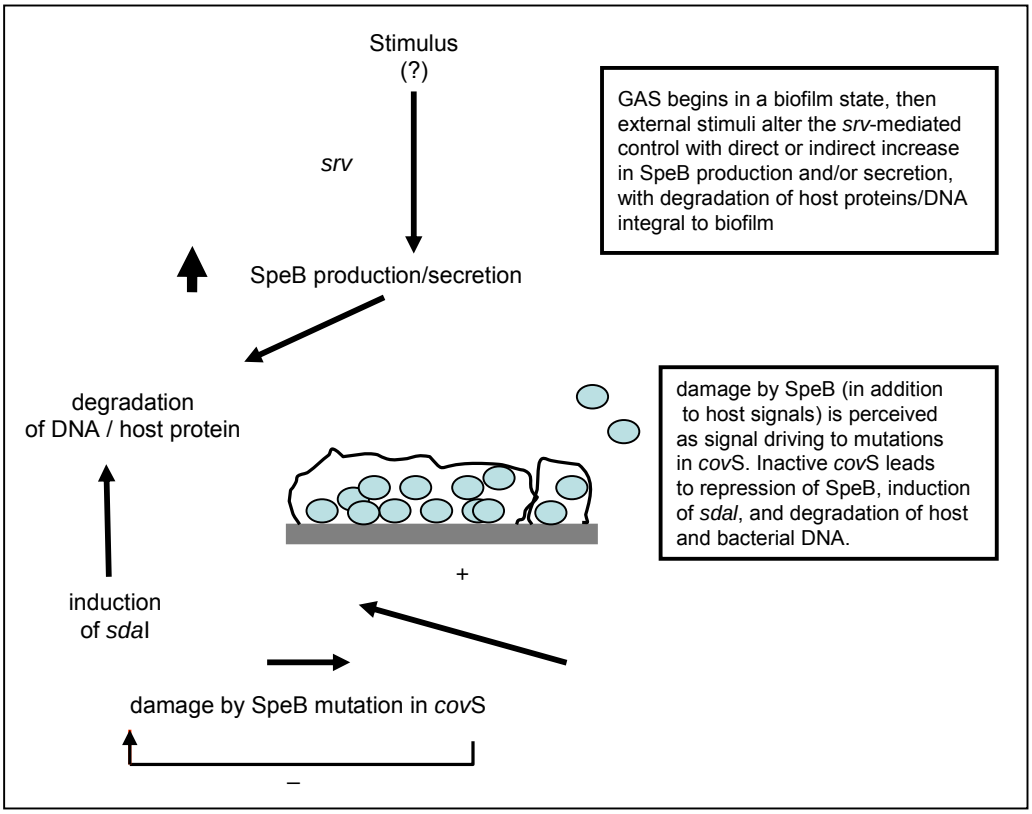

Figure 1. Possible mechanisms involved in biofilm formation by GAS

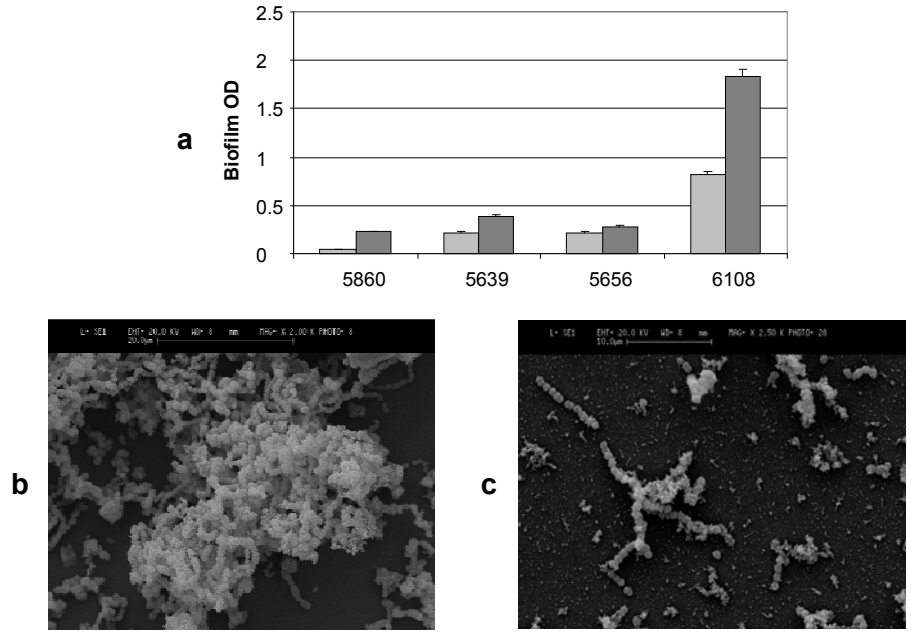

Figure 2. Biofilm OD of four GAS isolates after growth in plain medium (light grey bars) or in the presence of penicillin at ½ of the MIC (dark grey bars) (a).
Scanning electron microscopy of cells grown in presence of penicillin (b) or in plain medium (c)

Analogous effect could be observed for erythromycin, tested on susceptible strains.

We found that such effect was all the more common depending on the antibiotic resistance pattern of the isolates. In fact, those carrying the *erm* gene, which codes for macrolide resistance through methylation of the target site, where less susceptible to the presence of subMIC penicillin, while those carrying genes coding for efflux pumps, were more easily affected.

In the most recent years compounds other than antibiotics have been taken into consideration for their possible action on biofilm-producing microorganism, in general, and on GAS. Among those cationic peptides (CAMPs) have received some attention, as well as other natural compounds such as plant extracts or coral-associated actynomycetes that have been found to interfere with quorum sensing signals and biofilm formation, without effect on growth rates (Limsuwan & Voravuthikunchai, 2008; Nithyanand *et al*., 2009; Rasooli *et al*., 2008).

Cationic peptides in particular came to our attention, as are major factors for their antibacterial activity on mucosal surfaces. In a small collection of isolates, characterized by different resistance pattern, we evaluated the effect of three different CAMPs. While no difference could be found for indolicidin and polimixin E, i.e. all strains showed the same MIC, and interesting findings was that with nisin. We found that biofilm embedded cells were more susceptible to the action of nisin (Figure 3), a finding in agreement with that the strains with higher MIC (that could grow at higher CAMP concentration) were those producing less biofilm.

A possible cross-resistance to the cationic peptide appeared to be conferred by either the methylation or the efflux pump coding genes, as the majority of the susceptible strains would grow at lower nisin concentration, while it was the other way around for resistant isolates.

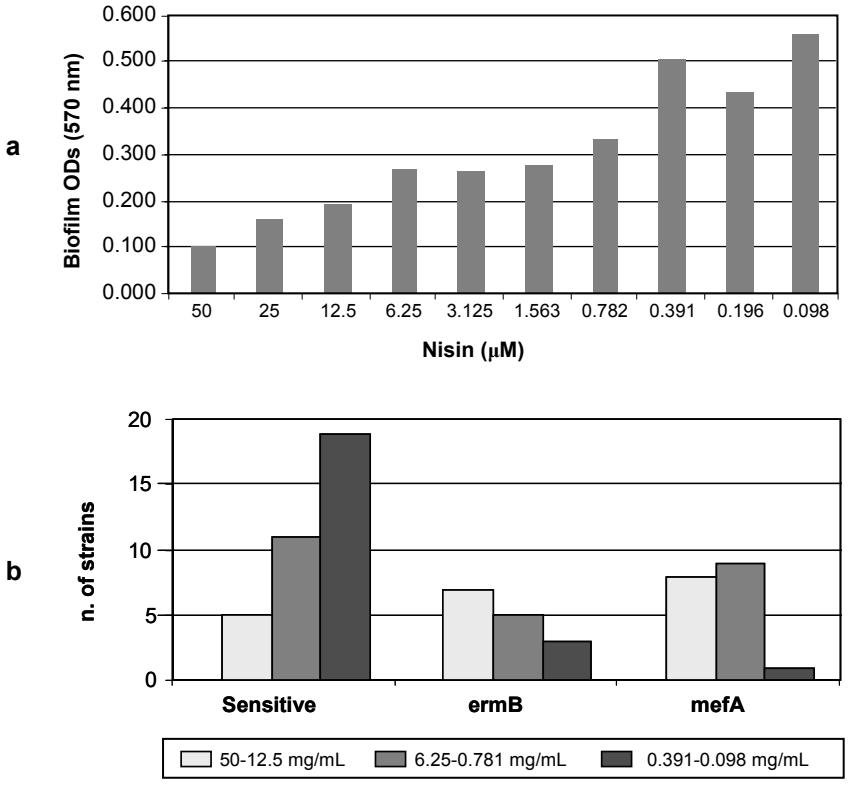

Figure 3. MICs to nisin in a collection of 100 GAS isolates (a), and relationship of MICs to ability to form biofilm (b)

To summarize, clinical practice indicates stabilized procedure for treatment of streptococcal pharyngitis, which are especially important in countries, such as India or Australia, where post streptococcal sequelae such as acute rheumatic fever represent a heavy burden. Thus, long term oral penicillin, erythromycin or clindamycin and vancomycin, are the therapeutic strategies upon which a consensus exists.

However, the best indication to decide the most appropriate therapeutic approach still remains the evaluation of the clinical picture. Also, follow up of the cases should be pursued, for early identification of the carrier state or identification of subjects more prone to recrudescence. Further, knowledge of the local epidemiology as far as resistance rates are concerned is fundamental, to decide appropriate antibiotics alternative to penicillin.

Of fundamental importance remains the pursue of additional information on the mechanism through which antibiotics stimulate biofilm formation and further investigation evaluating substances active on GAS biofilm.

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