

EDITORIAL



Novel insight into the pathogenicity of *Streptococcus gallolyticus* subsp. *gallolyticus* belonging to the *Streptococcus bovis*/*Streptococcus equinus* complex

Konstantinos Papadimitriou

Laboratory of Dairy Research, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens, Greece

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The implication of members of the *Streptococcus bovis*/*Streptococcus equinus* (SBSEC) complex to human health is proving to be of increasing importance and research on this field is gaining momentum. With the term SBSEC, that is becoming more and more popular, we actually refer to one of the six main groups of species within the *Streptococcus* genus, also known as the *S. bovis* group [1]. SBSEC includes primarily non-hemolytic Lancefield group D commensals. For many years, members of the SBSEC were recognized and perhaps treated as lower grade pathogens found mostly in the gastrointestinal tract of humans and animals [2]. However, the association of *S. bovis* and *S. bovis* related isolates with bacteremia, infective endocarditis (IE), colorectal cancer (CRC) and meningitis in humans was early realized [3]. Today, the pathogenicity of certain SBSEC members is even more evident while case reports broaden the spectrum of diseases they may cause. Obviously, understanding the mechanisms of virulence is necessary to combat these underestimated pathogens.

Rather unexpectedly, one of the main obstacles in this effort is the current taxonomic status of the SBSEC. As reviewed by Dekker and Lau over the past three decades several studies have appeared in the literature proposing important alterations in the taxonomy of this group of streptococci [4]. It has been suggested that taxonomy within the SBSEC has been complicated and sometimes contradicting. Currently, the most common SBSEC taxonomic scheme emerging from the relevant literature includes seven species and subspecies [1,4,5], i.e. *S. equinus*, *Streptococcus infantarius* subsp. *coli* (former *S. bovis* biotype II/1), *S. infantarius* subsp. *infantarius* (former *S. bovis* biotype II/1), *Streptococcus alactolyticus*, *Streptococcus gallolyticus* subsp. *gallolyticus* (former *S.*

bovis biotype I), *S. gallolyticus* subsp. *pasteurianus* (former *S. bovis* biotype II/2), and *S. gallolyticus* subsp. *macedonicus*. Of note, in this scheme *S. bovis* is considered a later heterotypic synonym of *S. equinus*, while *S. gallolyticus* includes three subspecies i.e. those of *gallolyticus*, *pasteurianus* and *macedonicus*. The scheme is not universally embraced by researchers in the field [4,6], and perhaps most importantly it is not officially acknowledged by the International Committee on Systematics of Prokaryotes (ICSP). For the aforementioned reasons, the situation is highly problematic for researchers but also for the clinical practice [6,7]. Obviously, accurate delineation of clinical isolates to a taxonomic scheme that captures the biodiversity within the SBSEC as correctly as possible is a prerequisite for correct pathogen identification and treatment, as well as, for valid epidemiological studies. In several instances, collections of clinical isolates still include strains vaguely characterized as *S. bovis* that certainly need to be correctly reclassified to reveal meaningful clinical information [7–9].

It should be emphasized, that relatively recently strains of *Streptococcus macedonicus* (*S. gallolyticus* subsp. *macedonicus*) and *Streptococcus infantarius* (*S. infantarius* subsp. *infantarius*) have been systematically isolated from milk and fermented dairy products around the world. Genomic analysis of both *S. macedonicus* and *S. infantarius* have indicated evolutionary traits similar to benign dairy lactic acid bacteria and a diminished pathogenic potential due to absence of known virulence factors [10,11]. Nevertheless, strains of *S. macedonicus* and *S. infantarius* have been also associated with disease and their close phylogenetic relatedness to virulent SBSEC strains is unquestionable. Thus, a food related path of transmission of certain members of

CONTACT Konstantinos Papadimitriou  kpapadimitriou@aua.gr  Laboratory of Dairy Research, Department of Food Science and Human Nutrition, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece. **CONTACT** Konstantinos Papadimitriou
Comment on: Julia Isenring, et al. *Streptococcus gallolyticus* subsp. *gallolyticus* endocarditis isolate interferes with coagulation and activates the contact system. VIRULENCE, 2018 VOL. 9, NO. 1, 248–261 <https://doi.org/10.1080/21505594.2017.1393600>

SBSEC should not be excluded until we gain a better understanding of the pathogenicity and epidemiology of the complex. In a recent hospital-based unmatched case-control study conducted in Kenya, *S. infantarius* was found elevated in patients with CRC whereas the consumption of African traditional fermented dairy products was not identified as a risk factor for the disease [12]. Further research is warranted, since several African traditional fermented dairy products may be a major source of *S. infantarius* reaching populations of up to 10^8 cfu per milliliter of product.

Despite the fact that SBSEC has been characterized as an emerging pathogenic complex for humans and animals [5], the relevant studies revealing molecular mechanisms of virulence for the complex are truly scarce. *S. gallolyticus* subsp. *gallolyticus* (*S. gallolyticus*) is becoming the model organism for the SBSEC most probably due to the frequency of its involvement in bacteremia, IE and CRC. A number of studies have been concerned with the ability of this bacterium to adhere to host cells, a property that is necessary to colonize the host and initiate infection. *S. gallolyticus* carries a number of genes coding for “microbial surface component recognizing adhesive matrix molecules” (MSCRAMM) and other adhesive proteins that can bind to components of the extracellular matrix (ECM) of host cells [13]. Genomic sequences of *S. gallolyticus* support the existence of three pilus gene clusters assigned as *pil1*, *pil2* and *pil3* [13–15]. The first virulence factor experimentally identified in *S. gallolyticus* was the Pil1 pilus [16]. Pil1 was responsible for binding to collagen, affected biofilm formation and played an important role in the initial attachment and colonization stage of IE in a rat model. Furthermore, intestinal colonization by *S. gallolyticus* in murine model was found dependent on Pil3 pilus, which assists bacterial attachment by binding to colonic mucus [17]. In a later study, the repertoire of Pil3 host ligands was expanded to human stomach mucins and human fibrinogen [18]. Pil3 seems to be the only pilus present in the other two subspecies of *S. gallolyticus* (i.e. *pasteurianus* and *macedonicus*) and in *S. infantarius* suggesting variability among SBSEC members to bind to ECM [11].

Interestingly, there is an important number of cases for which it can be supported that bacteremia and/or IE may be manifestations of CRC involving SBSEC like *S. gallolyticus* [2,19]. *S. gallolyticus* may be disseminated to the bloodstream through (pre)malignant lesions in the gut and then bind to the collagen-rich surfaces of cardiac valves. Paracellular translocation of *S. gallolyticus* across malignant intestinal epithelium in the absence of a major immune response has been suggested [20]. However, it is not clear if SBSEC members may be the cause or a consequence of CRC [18]. In a recent study by Kumar et al., it

was demonstrated for the first time that *S. gallolyticus* promotes proliferation of CRC cells in a β -catenin dependent manner [21]. This effect was established in both cell culture and a CRC mouse model. The upstream events by which *S. gallolyticus* targets the Wnt/ β -catenin signaling path, whose dysregulation is central to the development of CRC, remain to be elucidated. Contact or close proximity of *S. gallolyticus* cells to CRC cells seems to be necessary. Notably, not all CRC cells are responsive to the influence of *S. gallolyticus* suggesting the involvement of host factors in the interaction. These findings make research on the pathogenicity of *S. gallolyticus* even more urgent.

In the current issue of Virulence, Isenring and colleagues present important novel information concerning interactions of *S. gallolyticus* within the host that may contribute to the initiation of IE [22]. IE is a thromboinflammatory disease and thus its symptoms are affected by the interplay of the coagulation system and the pathogenic microorganisms [23]. Coagulation can be initiated either by extrinsic or intrinsic pathways. The human contact system is an intrinsic coagulation pathway [24]. It includes a cascade starting by the activation of coagulation factor XII (FXII) on a foreign surface. Active FXII converts prekallikrein to plasma kallikrein (PK). PK then releases bradykinin (BK) after degrading high molecular weight kininogen (HK). BK induces a number of responses like inflammation, pain, fever, vasodilation, etc. In the commented study, the authors demonstrate that *S. gallolyticus* is able to survive and grow in citrated human blood at similar levels to *Streptococcus pyogenes* AP1 employed as a positive control. *S. gallolyticus* was also found significantly more resistant to phagocytosis and killing by mouse J774 macrophages compared to *S. pyogenes* AP1. Using *S. gallolyticus* mutants $\Delta pil1$, $\Delta pil3$, $\Delta term$ (overexpressing Pil1) and $\Delta cpsD$ (presumably perturbing capsule formation) it was shown that these genes do not influence survival in blood. Expression of *pil1* and *pil3* in the *S. gallolyticus* wild-type (wt) seems to negatively affect survival in macrophages while a proper capsule may provide protection to an extent. *S. gallolyticus* wt and mutants were all able to trigger coagulation of blood. In the $\Delta pil1$ mutant the onset of coagulation was a bit delayed suggesting interactions of Pil1 with components in blood involved in coagulation. Prothrombin time (PT) and activated partial thromboplastin-time (aPTT) were measured in human plasma incubated with the bacterial strains to evaluate the extrinsic and the intrinsic coagulation pathways, respectively. Results indicated that only aPTT was significantly altered suggesting FXII activation. Furthermore, increased aPTT times for the $\Delta term$ and $\Delta pil3$ mutants indicate interaction of Pil1 and Pil3 with components of the intrinsic coagulation

pathway. Indeed, *S. gallolyticus* wt was able to activate the contact system since activity of FXII and PK could be detected at the surface of the bacterial cells using a chromogenic assay. Activation of FXII and PK was found decreased in $\Delta pil1$ and $\Delta pil3$ but increased in $\Delta cpsD$. Pilus like structures covered by plasma proteins could be visualized by scanning electron microscopy (SEM) on the surface of *S. gallolyticus* wt and the $\Delta cpsD$ strain but such aggregates were observed to a lower extent in the $\Delta pil1$ and $\Delta pil3$ mutants. *S. gallolyticus* wt and all of its mutants analyzed here were able to bind and degrade HK on their cell surface as revealed by immunoblotting of eluted human plasma proteins with an anti-HK polyclonal antibody. The efficiency to absorb and degrade the HK was highest in the *S. gallolyticus* wt. These findings could be correlated with the amounts of bradykinin released from the bacterial surface. The amount released was highest from *S. gallolyticus* wt but it was significantly decreased in all mutants showing lowest values for $\Delta pil1$ and $\Delta pil3$ strains. With the help of recombinant Pil1 proteins it was demonstrated that the adhesin molecule (GALLO2179) binds FXII selectively but not PK or HK. In contrast, the major pilin (GALLO2178) did not bind to any of these factors. The adhesion of Pil1 adhesin to FXII was also verified by the microscale thermophoresis (MST) and surface plasmon resonance methods. In several of the experiments described in the study of Isenring et al., *S. infantarius* commensal strains were also included and behaved differently from *S. gallolyticus*. For example, some of the strains of *S. infantarius* exhibited decreased survival in human blood and during phagocytosis by J774 macrophages. Most *S. infantarius* strains exhibited increased aPTT but did not affect PT also suggesting activation of the intrinsic coagulation pathway. In the absence of Pil1, *S. infantarius* does not bind FXII or PK. Given the central role of blood coagulation in IE, the activation of the human contact systems by *S. gallolyticus* may be an important virulence mechanism. Further investigation of these properties in other members of the SBSEC apart from *S. gallolyticus* is required to address pathogenicity of the complex as a whole.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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