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MINIREVIEW

Is *Staphylococcus lugdunensis* Significant in Clinical Samples?

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ABSTRACT The implication of coagulase-negative staphylococci in human diseases is a major issue, particularly in hospital settings wherein these species often act as opportunistic pathogens. In addition, some coagulase-negative staphylococci such as *S. lugdunensis* have emerged as pathogenic bacteria, implicated in severe infections, particularly, osteoarticular infections, foreign-body-associated infections, bacteremia, and endocarditis. *In vitro* studies have shown the presence of several putative virulence factors such as adhesion factors, biofilm production, and proteolytic factors that might explain clinical manifestations. Taken together, the clinical and microbiological data might change the way clinicians and microbiologists look at *S. lugdunensis* in clinical samples.

KEYWORDS virulence, osteoarticular infections, protease, biofilm, endocarditis, *Staphylococcus lugdunensis*

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Staphylococcus lugdunensis has emerged since the 1990s as a distinctive coagulase-negative staphylococcus (CoNS), implicated in a wide range of severe infections. This bacterium produces a large variety of putative virulence factors. Until recently, the tools used to identify staphylococci at the species level relied on phenotypic methods such as coagulase identification, which helped distinguish *S. aureus* from other staphylococci. Consequently, the epidemiology of CoNS, which is generally considered less pathogenic than other staphylococci, remained unclear. The identification of coagulase activity refers to two distinctive molecular activities that aim to convert soluble fibrinogen into insoluble fibrin. The first activity involves a free coagulase that leads to prothrombin activation and, ultimately, the conversion of fibrinogen into fibrin. The second activity involves a bound coagulase or clumping factor that matches with two distinctive proteins in *S. aureus*, clumping factor A and B, that directly convert fibrinogen into fibrin. However, some CoNSs, for example *S. lugdunensis*, *S. schleiferi*, and *S. sciuri*, may produce a bound coagulase that is distinctive from the *S. aureus* bound coagulase but with similar activity (1). Other CoNSs such as *S. pseudointermedius*, *S. intermedius*, *S. hyicus*, *S. delphini*, and *S. lutrae*, produce a free coagulase (1). Phenotypic identification of CoNS is thus challenging, but the implementation of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) has provided laboratories with a fast and cost-effective identification tool (2). The production of coagulases and various phenotypic properties of CoNS are no longer obstacles for performing a more systematic identification of CoNS at the species level. This is opportune because CoNSs are emerging as major causes of opportunistic and nosocomial infections. In this context, some CoNSs have emerged as putative virulent species, mainly in retrospective and epidemiological studies, in addition to case reports, with limitations because of the methodology of the analyses (3). The concept of virulence involves two distinctive factors: the clinical severity of infections and *in vitro* production of virulence factors. The causative link between these two factors remains unknown for CoNS, but it has been extensively explored for *S. aureus*. Most CoNSs such

as *S. epidermidis* produce various molecular factors, including cytotoxins and adhesion factors that are involved in pathogenicity and help these commensal bacteria become pathogenic (4). *Staphylococcus lugdunensis* is a specific CoNS with an unusual pathogenicity that recent clinical and *in vitro* studies have partially explained. These new results will change how microbiologists and clinicians interpret the positivity of clinical samples for this bacterium. Similar to other known CoNSs, *S. lugdunensis* is a commensal bacterium that colonizes the skin. This review examines the current clinical and microbiological research to understand why *S. lugdunensis* appears different from other CoNSs. We also attempt to derive conclusions regarding how microbiologists and clinicians should consider this bacterium when a positive sample is obtained in the laboratory.

S. LUGDUNENSIS IDENTIFICATION AND MICROBIOLOGICAL ISSUES HAVE BEEN SOLVED

Staphylococcus lugdunensis grows under aerobic conditions on common media such as blood agar and causes intense beta-hemolysis. The traditionally used tube test and slide agglutination test are now obsolete due to their low sensitivity and specificity. Rapid latex and hemagglutination assays have been developed more recently based on the detection of clumping factor, protein A, and capsule types 5 and 8. Nevertheless, these innovative tools fail to distinguish *S. aureus* from *S. lugdunensis* efficiently, because *S. lugdunensis* might produce a bound coagulase (clumping factor) and yield positive results in up to 65% of cases (5). At the biochemical level, *S. lugdunensis* can be differentiated from other staphylococci based on showing positivity in pyrrolidonyl arylamidase and ornithine decarboxylase reactions in more than 90% of cases (6). Various manual and automated biochemical test systems are still used in laboratories for the identification of bacteria, including staphylococci. Regarding *S. lugdunensis* identification, despite its biochemical peculiarities, the reliability of these systems is too low and the accuracy of identification ranges from 70% to 90% depending on the system used and the performance of additional tests (7, 8). In an effort to improve the identification rate, various molecular methods, particularly real-time PCR assays targeting some conserved genes such as *gyrA*, *gap*, *sodA*, and *rpoB* or *16S* and *23S* ribosomal DNA, have been successfully proposed (6, 9, 10). Identification by nucleic acid-based approaches appears to be a gold standard for *S. lugdunensis*, but the implementation of proteomic methods, particularly MALDI-TOF MS, in routine clinical laboratories has allowed a rapid, cost-effective, and reliable identification of bacteria, including staphylococci (2). The growing number of MALDI-TOF MS spectra included in manufacturers' databases has allowed high levels of sensitivity and specificity, approximately 100%, to be achieved and markedly changed the way we look at CoNS, particularly *S. lugdunensis* (3). More recently, it appears that MALDI-TOF MS is a reliable tool to identify *S. lugdunensis*, among other bacteria, directly from blood cultures (11, 12). This revolution in the usual laboratory workflow might directly impact patient management and prognosis in the context of bacteremia, as recently reported for *S. aureus* bacteremia (13).

S. LUGDUNENSIS VIRULENCE: GROWING EVIDENCES FROM CLINICAL STUDIES

According to the literature, *S. lugdunensis* colonizes 30% to 50% of patients (14, 15). Three studies precisely analyzed *S. lugdunensis* colonization and detected inguinal colonization in 22% to 39% of patients, followed by axillary colonization in 19.8% to 20% of patients and nasal colonization in 9.3% to 17.9% cases (14–16).

Retrospective clinical studies. Until recently, most studies that emphasized the role of *S. lugdunensis* in clinical settings were retrospective analyses that mainly described its role in skin and soft tissue infections. Some reports also described the occurrence of bacteremia and endocarditis owing to this bacterium. Liu et al. reviewed the literature regarding endocarditis and showed that *S. lugdunensis* was absent at the portal of entry in 45% of cases and occurred on native valves in 80% of cases, with a global mortality rate of 39% (17). Overall, even if mortality rates vary between studies,

severe valvular lesions are a common finding. Although retrospective data have to be carefully interpreted, they intrigue clinicians, because the results reported in the literature suggest that the mortality rate of *S. aureus* endocarditis is approximately 20%, whereas the global mortality rate of CoNS endocarditis is approximately 12% (18, 19). The occurrence of bone and joint infections appeared recently in retrospective studies describing prosthetic joint infections, particularly knee joint infections (20). Argemi et al. (3) and Douiri et al. (21) showed in two recently published studies that 40% of all clinical samples that tested positive for *S. lugdunensis* were obtained from patients with proven infections, particularly bone and joint infections. This infection rate confirmed the status of this *Staphylococcus* species retrospectively, and we further confirmed these results through a prospective clinical trial.

Prospective clinical studies. We recently published the first and currently only prospective study (named VISLISI) (5). In this monocentric clinical trial, all bacteriological samples that yielded positive results for *S. lugdunensis* were systematically screened during a 3-year period. We provided evidence that 37.2% of the 347 strains isolated originated from infected patients, particularly from those with bone and joint infections (34.6%). We also showed that the inoculation of pediatric blood culture bottles with joint fluids, tissue specimens, and sonicated prosthetic materials could significantly improve the diagnostic rate of these infections, as previously shown in prospective cohort studies (22). This prospective clinical trial once again confirmed, but with a strong methodological background, the virulence of *S. lugdunensis* at the clinical level. At the microbiological level, it appears that this bacterium might also produce a range of putative virulence factors that could explain these clinical findings.

MICROBIOLOGICAL STUDIES THAT STRENGTHEN CLINICAL EVIDENCE

Several studies described the occurrence of putative virulence factors in *S. lugdunensis*. In 1997, Donvito et al. described the hemolytic properties of this bacterium that were then linked to the presence of a delta-like hemolysin encoded by a gene that was found in the non-*agr* locus named *slush* (23). Since the publication of this study, several other virulence factors have been characterized.

Adhesion factors. Similar to *S. aureus*, *S. lugdunensis* produces a fibrinogen-binding protein linked to the bacterial cell wall that acts as a clumping factor (24). Fibrinogen-binding proteins have been involved *in vitro* and in animal models in the occurrence of *S. aureus* endocarditis and persistent bacteremia (25). We also showed in the VISLISI clinical trial that the production of a clumping factor was strongly associated with the occurrence of bacteremia. *Staphylococcus lugdunensis* produces various other adhesion proteins that belong to a group of molecules, called microbial surface components recognizing adhesive matrix molecules, that covalently link the bacterial membrane through LPXTG motifs and the action of a sortase enzyme (26). This bacterium also produces a von Willebrand factor-binding protein and functionally displays high binding capacities to various extracellular matrix components, such as fibronectin, collagen, vitronectin, laminin, and human IgG (27). The functions of these adhesion factors are not limited to extracellular matrix molecule binding; these factors have numerous other functions, such as immune evasion and biofilm formation (25).

Biofilm. *Staphylococcus lugdunensis* is a biofilm-producing bacterium with some specific properties. Frank and Patel showed that it frequently forms biofilms, but unlike the matrix of biofilms formed by other CoNSs, that of biofilms formed by *S. lugdunensis* is not made of poly-*N*-acetylglucosamine and is instead mainly proteinaceous, even when an *ica* locus has been identified in this species (28). Recent reports have also described the role of the autolysin atlL in biofilm formation and the role of a novel locus, *comEB*, in DNA-dependent biofilm formation (29). In a secondary analysis of the VISLISI clinical trial, we showed evidence using the BioFilm ring test (Biofilm Control, Saint-Beauzire, France), which is a new test to evaluate the kinetics of biofilm production, with respect to all the 28 strains found in osteoarticular infections producing biofilms within 6 h after culturing (30). The relationship between osteoarticular infections and biofilm formation has been described previously for *S. aureus* and might also

be linked to *S. lugdunensis* as a causative agent (31). Perhaps this could explain why all patients infected with *S. lugdunensis* had previously undergone surgical interventions. Antibiotic susceptibility of biofilm-embedded bacteria has not been studied with *S. lugdunensis*, but several *in* vitro studies have demonstrated that *S. aureus* and Gram-negative bacteria display lower antibiotic susceptibility when they are grown in a biofilm than that when grown in a plankton, although the potency of some antibiotics, such as rifampin and linezolid, appear to be less impaired (32). New pharmacodynamic parameters, for example biofilm bactericidal concentration or minimal biofilm inhibitory concentration, are probably needed to more accurately evaluate the antibiotic susceptibility of the bacteria in a biofilm (33).

Proteolytic activity. We recently described a previously unknown novel protease, named lugdulysin, that might also be implicated in osteoarticular infections (5). This Zn^{2+}-dependent protease is similar to hyicolysin, another metalloprotease found in *S. hyicus*, a CoNS isolated from pigs presenting with exudative epidermatitis. In addition to hyicolysin, lugdulysin is possibly a member of the M30 family of proteases (according to MEROPS database). Lugdulysin still needs further chemical and structural characterization, but its implication in pathogenicity remains coherent with previous reports regarding metalloproteases (34). Cassat et al. characterized the role of aureolysin, another metalloprotease secreted by *S. aureus* strains that might play a role in osteomyelitis (35). Aureolysin acts as a conductor for the virulence repertoire of *S. aureus* to modulate bone remodeling. The involvement of metalloproteases in human diseases relies on their capacity to remodel the extracellular matrix, as observed in osteomyelitis, tumor invasion and metastasis, and inflammatory vascular diseases.

Iron metabolism. Iron plays a crucial role in bacterial metabolism and growth. *Staphylococcus lugdunensis* is the only CoNS that carries a complete operon dedicated to iron capture and metabolism that encodes the iron-regulated proteins, IsdB, IsdC, IsdJ, and IsdK (36). It is the only CoNS that has a captation system similar to that of *S. aureus*.

Virulence factor regulation. *Staphylococcus lugdunensis* also bears an *agr* locus and produces an RNAIII-like RNA molecule that is distinct from the delta-like hemolysin activity that relies on a different locus (*slush*). The role of *agr* is crucial in *S. aureus*, and a similar locus has been described in other CoNSs, such as *S. epidermidis*, that also bear different regulation loci such as the LuxS/AI-2 system that contributes to the quorum sensing machinery. These systems are now well understood in *S. aureus*; however, the *agr* locus still needs further characterization in *S. lugdunensis*.

Clinical manifestations and their putative correlations with virulence factors are illustrated in Fig. 1.

Antibiotic susceptibility. *Staphylococcus lugdunensis* remains remarkably sensitive to most antibiotics, particularly beta-lactams, contrary to other CoNSs. Fosfomycin is the only antimicrobial with highly variable results and a resistance level of >50% depending on the study. This resistance is mostly because of the presence of the gene *fusB*. Beta-lactamase production is also variable depending on the study, with production reported in anywhere from 0% to 70% of the strains. However, this phenotype is not unusual among staphylococci, and methicillin resistance still appears to be marginal in *S. lugdunensis*, although only some strains have the *mecA* gene associated with methicillin resistance. In the largest collection of bacteria tested for the presence of methicillin resistance and the *mecA* gene, Kleiner et al. found that 3% of 36 strains tested were oxacillin resistant and displayed the *mecA* gene (37). According to American (Clinical and Laboratory Standards Institute) and European (European Committee on Antimicrobial Susceptibility Testing) guidelines, *S. aureus* and *S. lugdunensis* have identical clinical breakpoints, higher than those of other CoNSs (38). These two species with oxacillin MIC values of >2 mg/liter are mostly methicillin resistant due to the presence of the *mecA* gene. The corresponding MIC for other CoNSs is >0.25 mg/liter, because a lower breakpoint correctly classifies most CoNSs with the *mecA* gene whereas it overcalls resistance for *S. lugdunensis* (39). It is of interest to note that

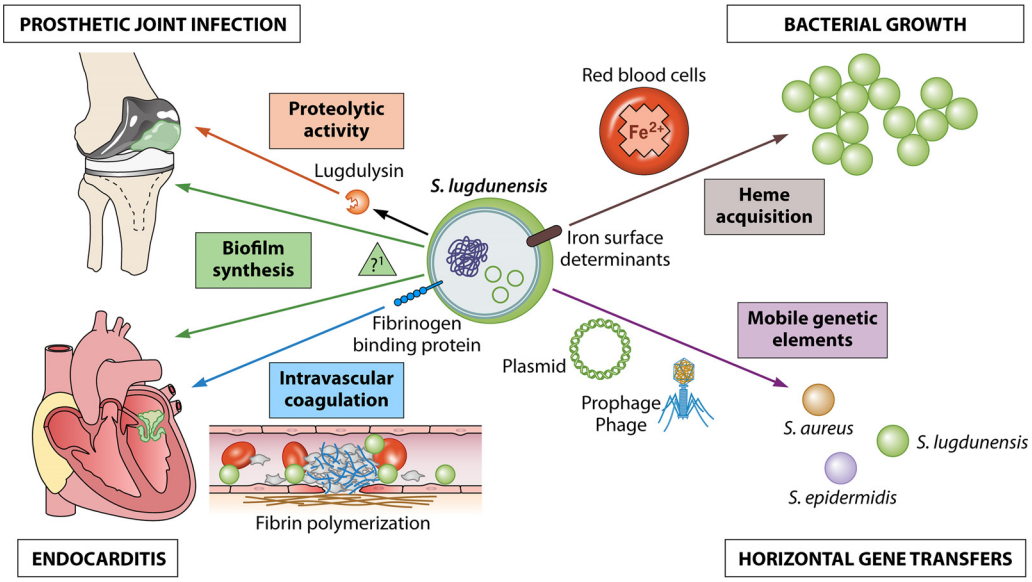

FIG 1 Clinical and bacteriological roles of the main putative virulence factors identified in *S. lugdunensis*.

cefoxitin has appeared as a more reliable indicator to detect methicillin resistance, and once again, *S. aureus* and *S. lugdunensis* share similar breakpoints. Cefoxitin MICs of >4 mg/liter predict methicillin resistance and the presence of the *mecA* gene.

Thus, despite its occurrence in nosocomial infections and its colocalization on the skin with other CoNSs and *S. aureus* that is commonly methicillin resistant, *S. lugdunensis* does not seem to share resistance genes through horizontal genetic transfer. Nevertheless, recent genetic reports have emphasized the occurrence of various mobile genetic elements.

SPECIFIC GENETIC FEATURES

Staphylococcus lugdunensis was fully sequenced in 2010 by Tse et al. (40). Since then, 19 genome assembly and annotation projects have become currently available. The genome lengths from the various strains of this bacterium range from 2.5 to 2.6 Mb with a GC content of 33.7% to 33.9%. The closest related species is *S. haemolyticus*, with 78.3% homology of the coding sequences, followed by *S. aureus* with 77.8% homology (41). The genes encoding virulence factors that we cited previously have been identified in all strains. One distinctive feature of this CoNS is the presence of several mobile genetic elements (MGE).

Identification of MGE. We recently identified several plasmid and prophage sequences through the whole-genome sequencing of seven strains obtained from the VISLISI clinical trial (42). We did not identify any virulence or resistance genes in these MGE, but we did find several homologies to some previously described prophages and plasmids, particularly pVISLISI_3 that has 100% homology to pRIVM6519_1, a plasmid first identified in *S. aureus*. This result suggests the occurrence of horizontal genetic exchange between these two species, characterized by their clinical virulence. We also found full nucleotide similarities between the plasmids pVISLISI_1, pVISLISI_2, and pLUG_10 from *S. lugdunensis* and SAP108B from *S. epidermidis*. With respect to the four prophage sequences, we showed that ϕSL2 to ϕSL5 shared 25% to 44% of the putative encoded proteins with stB12 from *S. hominis* and PH15 from *S. epidermidis*.

TIME TO CHANGE MICROBIOLOGICAL AND CLINICAL CRITERIA OF INTERPRETATION

The Infectious Diseases Society of America (IDSA) was the first to consider *S. lugdunensis* as a different CoNS in its 2015 guidelines for the diagnosis management of osteomyelitis in adults (43). IDSA does not recommend a bone biopsy in patients with

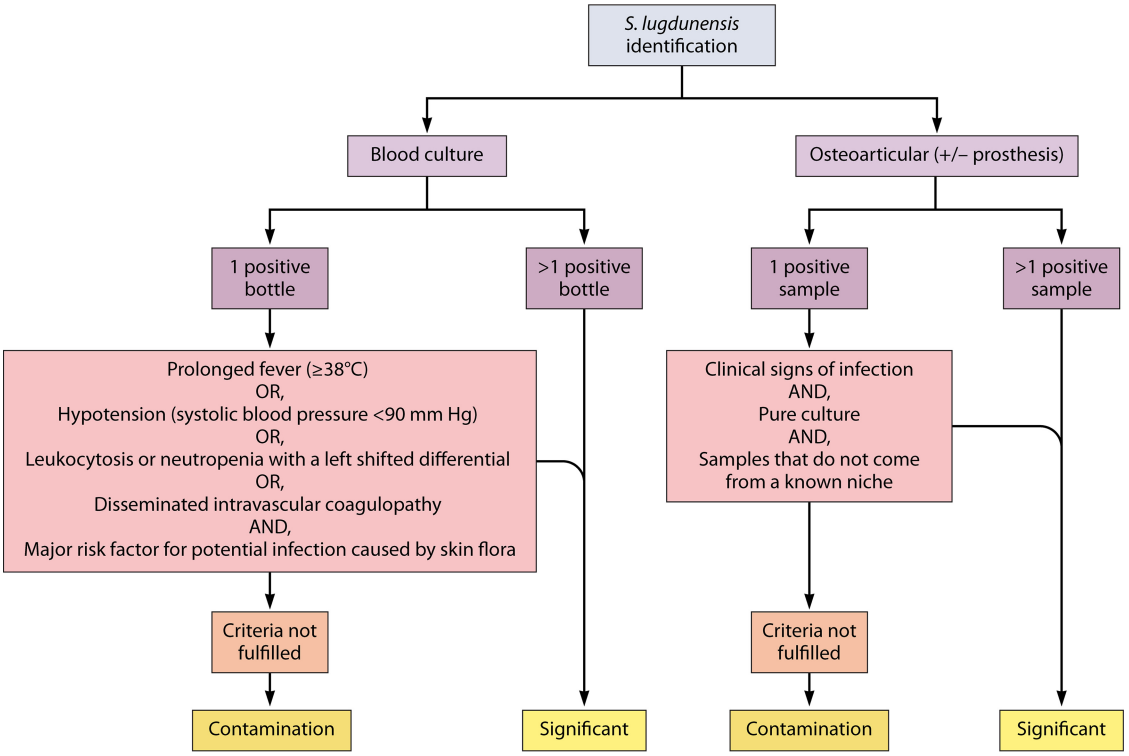

FIG 2 Clinical significance of microbiological samples with *S. lugdunensis* identification in blood cultures and osteoarticular samples. Major risk factors for potential infection caused by skin flora are: long-term intravascular catheterization, peritoneal dialysis, hemodialysis, or extensive postsurgical infections with CoNS. *S. lugdunensis* known niches are inguinal and axillary.

suspected osteomyelitis when *S. aureus* or *S. lugdunensis* infection has been established based on positive blood cultures. In the same year, the European Society of Cardiology published guidelines for the management of endocarditis and emphasized the role of *S. lugdunensis* in destructive infectious endocarditis, unlike other CoNSs. These results are clearly supported by growing clinical evidence, and the causative role of the virulence factors described in this review is likely, even if *in vitro* and animal models are lacking to model this relationship. At the same time, the European Manual of Clinical Microbiology still advises that two positive samples for CoNS are required in a clinical sample to consider it significant, because contamination and colonization remain frequent (44). In contrast, only one *S. aureus*-positive sample in blood cultures or a bone sample is enough to be considered pathological. Clinical and microbiological evidences are now concordant enough to consider that *S. lugdunensis* cannot be regarded as a regular CoNS. Fadel et al. demonstrated in a retrospective analysis that 45% of 29 patients with a single *S. lugdunensis*-positive blood culture did indeed have bacteremia (45). The authors used the criteria published by Souvenir et al. (46), which have proven useful to determine the clinical significance of blood culture positivity for CoNS. Regarding bone, joint, and prosthetic joint infections, the most informative data emerged from the VISLISI clinical trial (5). Among 28 patients with proven infections, 25% had only one positive sample. In this trial, infection was considered to have been proven in cases with only a single positive sample if three criteria were fulfilled, namely, there were clinical signs of infection, a pure culture, and a sample that did not come from a known niche for this organism.

Thus, we propose that *S. lugdunensis* be considered pathogenic in deep clinical samples, such as blood cultures or bone and articular samples, even if only one sample is positive but in pure culture, at least until any other diagnosis has been proven. However, we would advise caution when interpreting single positive samples in skin and soft-tissues or within a known niche of this bacterium (inguinal, axillary, and nasal).

In addition, the positivity of two deep samples is sufficient to consider the bacterium clinically significant. Those aspects are summarized in Fig. 2.

CONCLUSION

Frank et al. were the first to show that *S. lugdunensis* appeared to be different from other CoNS; since then, clinical, microbiological, and genetic evidences continue to distinguish *S. lugdunensis* from other CoNSs (6). It is now time to change how clinicians and microbiologists interpret the positivity of clinical samples of *S. lugdunensis*, particularly blood cultures and osteoarticular samples. This bacterium may not show a high virulence level similar to *S. aureus*, but its virulence is higher than that of all other CoNSs. The mechanisms linking the identified virulence factors with the clinical observations remain to be elucidated.

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