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## **Virulent *Staphylococcus lugdunensis* with limited genetic diversity in hidradenitis suppurativa lesions**

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***To the editor,***

Hidradenitis suppurativa (HS) is an inflammatory skin disease characterized by recurring painful, deep-seated inflammatory nodules, abscesses, sinus tracts, and scarring in the intertriginous areas.<sup>1</sup> Bacteria may be involved in the pathogenesis of HS via follicular dysbiosis in the initial stages, and via biofilm in chronic sinus tracts.<sup>2</sup> *Staphylococcus lugdunensis*, a coagulase-negative staphylococcus (CNS), has been cultured from hidradenitis suppurativa nodules and abscesses. This points towards a role of *S. lugdunensis* in the aggravation and secondary colonization of HS lesions.<sup>3,4</sup> *S. lugdunensis* has also been associated with biofilm-driven infections in different tissues.<sup>5</sup> Therefore, we compared the *in vitro* growth, antibiotic susceptibility and biofilm-forming capabilities of *S. lugdunensis* strains from HS lesions with those of healthy controls and a reference strain.

This study has been approved by the ethical board and the data protection agency of Denmark. Informed consent was obtained from all participants. The *S. lugdunensis* strains were cultured from punch biopsies and swabs from active HS lesions. The controls comprised two strains from one healthy participant and the ATCC 49576 control strain. All strains were typed as described previously.<sup>6</sup> Rifampicin, tetracycline, clindamycin and doxycycline, commonly used to treat HS, were included in the experiments. Genetic relatedness of the *S. lugdunensis* strains was investigated with Pulsed-Field Gel Electrophoresis (PFGE)<sup>7</sup> and analysed using BioNumerics software (version 5.0).

*S. lugdunensis* was cultured in 12 out of 26 patients (46%). All strains showed growth in liquid medium *in vitro*, however after 6 hours the HS lesional strains and the control strain grew faster than the two strains from a healthy individual (HS versus healthy  $p < 0.0354$ ; control versus healthy  $p < 0.0299$ ). All strains were strong biofilm producers, but the healthy strains produced less biofilm when compared to HS lesional strains and the control strain. Clindamycin resistance was observed in 41.6% of the strains (Table 1). Rifampicin was superior to clindamycin, doxycycline, and tetracycline in both growth-inhibition and biofilm eradication ( $p < 0.05$ , Fig. 1). Five clusters of genetically closely related strains were identified, mostly in pairs. A high similarity was seen within the clusters especially for the two healthy strains.

The specific pathway by which bacteria are involved in the pathogenesis of HS needs further elucidation. Differences in the functional characteristics of *S. lugdunensis* strains from HS patients were identified in our study. The faster growth curves of the lesional HS and control strains than healthy strains, indicate a more pathogenic virulent status of the lesional *S. lugdunensis* strains.<sup>8</sup> Nonetheless, the biofilm forming capacities were identical. The high clindamycin resistance rate of 41.6% has previously been reported in more than 10% of the *S. lugdunensis* strains.<sup>9</sup> Doxycycline appeared particularly effective at inhibiting the growth of *S. lugdunensis*, which may suggest that doxycycline could be used in the management of HS when CNS are present.

All tested antibiotics significantly eradicated biofilm in the four tested strains. Interestingly, this also occurred in strain number 4, which showed resistance against tetracycline, doxycycline and clindamycin. Resistance to clindamycin is mostly

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caused by a mutation whereby the receptor for the antibiotic is modified.<sup>10</sup> Our findings in strain 4 imply that the efficacy of clindamycin in biofilm degradation is independent of its bactericidal activity. The close genetic relatedness of the strains, with five highly similar clusters, indicates that the number of specific strains that are involved in HS pathogenesis is limited.

A major strength of this study is the carefully optimized culture conditions for our experiments. A limitation is that by culturing the biopsies, and not using molecular bacterial sequencing such as 16S ribosomal RNA techniques, we possibly missed some bacterial strains.

In conclusion, we show that *S. lugdunensis* strains were more frequently resistant to antibiotics used to treat HS, and showed an increased biofilm production. Based on our current findings, it is conceivable that these characteristics may foster HS disease activity. A larger sample size across different HS phenotypes and anatomical regions with bacterial genome sequencing is needed for a better picture of the role of *S. lugdunensis* in HS.

#### **Conflicts of interest**

None of the authors has any conflict of interest to declare.

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### Legend for figure

**Figure 1.** Eradication of bacterial biomass in preformed biofilms exposed to rifampicin (0.0625 µg/mL), tetracycline (0.25 µg/mL), clindamycin (0.25 µg/mL) and doxycycline (0.125 µg/mL) for 24 hours. The graphs are showing HS strain number 4 (**a**), HS strain number 5 (**b**), the healthy control strains (**c**) and the laboratory control strain (d). The graphs are normalized to the growth controls (no stimuli added), which is depicted as 100% of biofilm formation. Statistical significance is indicated with asterisks \*  $p < 0.05$ , (GraphPad Prism USA).

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## Table

**Table 1.** Antimicrobial susceptibility and biofilm production pattern for all strains.

<i>S. lugdunensis</i> strains	MIC (µg/mL)				Antibiotic use	Biofilm production
	Rif	Tet	Clinda	Doxy		
Strain 1 (biopsy)	0.03	0.25	<b>&gt;128</b>	0.13	None	Strong
Strain 2 (biopsy)	0.06	0.25	0.50	0.13	None	Strong
Strain 3 (biopsy)	0.03	0.25	<b>&gt;128</b>	0.13	None	Strong
Strain 4 (biopsy)	0.06	<b>64</b>	<b>&gt;128</b>	<b>4</b>	Clindamycin lotion	Strong
Strain 5 (biopsy)	0.06	0.13	0.50	0.13	None	Strong
Strain 6 (biopsy)	0.03	0.25	0.25	0.13	Clindamycin lotion	Strong
Strain 7 (biopsy)	0.06	0.13	0.25	0.25	Unknown	Strong
Strain 8 (biopsy)	0.01	0.25	<b>&gt;128</b>	0.13	None	Strong
Strain 9 (biopsy)	0.03	0.50	<b>&gt;128</b>	0.13	Clindamycin lotion	Strong
Strain 10 (swab)	0.06	0.50	0.50	0.25	None	Strong
Strain 11 (swab)	0.06	0.50	0.50	0.25	None	Strong
Strain 12 (swab)	0.06	0.25	0.25	0.25	None	Strong
Healthy control (biopsy)	0.06	0.25	0.25	0.13	None	Strong
Healthy control (swab)	0.06	0.25	0.25	0.13	None	Strong
Laboratory control strain	0.06	0.13	0.25	0.13	None	Strong

Rif, rifampicin; Tet, tetracycline; Clinda, clindamycin; Doxy, doxycycline

Notes: Resistance is indicated in bold with an underlining. (a) received topical clindamycin treatment prior to collection the biopsies/swabs, while (b) has an unknown antibiotic treatment history.



