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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. Bacteria may be involved in the pathogenesis of HS via follicular dysbiosis in the initial stages, and via biofilm in chronic sinus tracts. 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. S. lugdunensis has also been associated with biofilm-driven infections in different tissues. 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. 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) and analysed using BioNumerics software (version 5.0).

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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. 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. 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
caused by a mutation whereby the receptor for the antibiotic is modified. 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.
References

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).
Table

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

<table>
<thead>
<tr>
<th>S. lugdunesis strains</th>
<th>MIC (µg/mL)</th>
<th>Antibiotic use</th>
<th>Biofilm production</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rif</td>
<td>Tet</td>
<td>Clinda</td>
</tr>
<tr>
<td>Strain 1 (biopsy)</td>
<td>0.03</td>
<td>0.25</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Strain 2 (biopsy)</td>
<td>0.06</td>
<td>0.25</td>
<td>0.50</td>
</tr>
<tr>
<td>Strain 3 (biopsy)</td>
<td>0.03</td>
<td>0.25</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Strain 4 (biopsy)</td>
<td>0.06</td>
<td>64</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Strain 5 (biopsy)</td>
<td>0.06</td>
<td>0.13</td>
<td>0.50</td>
</tr>
<tr>
<td>Strain 6 (biopsy)</td>
<td>0.03</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Strain 7 (biopsy)</td>
<td>0.06</td>
<td>0.13</td>
<td>0.25</td>
</tr>
<tr>
<td>Strain 8 (biopsy)</td>
<td>0.01</td>
<td>0.25</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Strain 9 (biopsy)</td>
<td>0.03</td>
<td>0.50</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Strain 10 (swab)</td>
<td>0.06</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Strain 11 (swab)</td>
<td>0.06</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Strain 12 (swab)</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Healthy control (biopsy)</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Healthy control (swab)</td>
<td>0.06</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Laboratory control strain</td>
<td>0.06</td>
<td>0.13</td>
<td>0.25</td>
</tr>
</tbody>
</table>

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.