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Treatment of acne patients with isotretinoin increases β-diversity of a putative health-associated strain of *Cutibacterium acnes* within the follicular microbiome of responders.

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Dear Editor, we have read with interest and commend the article by Nolan et al., 2022[1] which reports changes in the diversity of Single Locus Sequence Typing (SLST) A and D-classes of *Cutibacterium acnes* upon treatment with isotretinoin. Most notably, an increase in β-diversity of the D1 sequence type (ST) from *C. acnes* subsp. *acnes* (type I) was found to correlate with a successful clinical response at 20 weeks indicating that intraspecies *C. acnes* changes have potential as biomarkers of treatment-response. While little is known about D-class strains, we wish to highlight evidence from the scientific literature which suggests this lineage may promote skin health. As a consequence, the D1 ST may actively contribute to symptom improvement in responsive patients although this remains to be confirmed.

The D1 ST (ribotype 1), which is unique in genomic composition and biotype, was originally described as the singleton ST27 (based on Multilocus Sequence Typing) in 2010 by Lomholt and Kilian[2], and was the second largest clone identified in their study with 85% D1 isolates recovered from healthy skin. The SLST D class, which currently comprises eight STs, can be found on the cheek, forearm and forehead skin of multiple healthy individuals, but is dominant on the back[3]. Unlike most other type IA organisms, D1 STs (e.g., HL025PA1) carry and express a *deoR* transcriptional repressor gene within the porphyrin biosynthesis operon, and produce pro-inflammatory porphyrins at a low level similar to health-associated *C. acnes* subsp. *defendens* (type II) organisms[4]; they also appear unaffected by vitamin B12 supplementation which can induce acne[5]. D1 also produces less oleate-degrading lipase activity when growing as a biofilm versus the epidemic ST18 clone (SLST A1; ribotype 1) originally associated with moderate-to-severe acne[2].

Recent *in vitro* studies have found that antimicrobially-active staphylococci (AAS) have the potential to target A-class strains of *C. acnes* often associated with acne vulgaris (likely via antimicrobial peptides), and to a lesser extent K and L strains, while co-existing with other strains of *C. acnes* abundant in healthy skin, including those of the D and H classes[3]. Furthermore, compared to healthy skin sites with no AAS, those with AAS show a very marked increase in the relative abundance of D strains (Figure 1). Why this correlation occurs is not understood, but it may reflect an important synergy between the D class and AAS in maintaining skin health via regulation of A-class strain abundance. Interestingly, it has been hypothesised that D strains could suppress and modulate the antimicrobial activity of staphylococci in healthy skin, especially *S. epidermidis*, via interference of their accessory gene regulator (*agr*) quorum-sensing system[3]. Yet, such a mechanism may also benefit and maintain staphylococcal levels as antimicrobial peptides can negatively affect the growth rate of the producing strain[3].

To conclude, current data suggests D-class strains of *C. acnes* play an important role in maintaining skin health. Further work is therefore required to better characterise these strains and their interactions with host cells and other microbiota, especially in the context of isotretinoin response.

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Conceptualization: AMD. *Original draft preparation*: AMD. *Interpretation, review and editing:* AMD, HB, AML. All authors gave final approval for publication and agreed to be accountable for the work.

CONFLITCT OF INTEREST STATEMENT:

The authors declare no conflict of interests regarding this work.

KEYWORDS:

Isotretinoin, *Cutibacterium acnes*, D1 sequence type, B-diversity.

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FIGURE LEGEND:

FIGURE 1. Staphylococci and *C. acnes* co-existence and inhibition profiles. **A.** The mean relative abundances of *C. acnes* SLST classes on skin sites with (+) and without (−) AAS strains are depicted. The presence of staphylococcal strains with antimicrobial activity leads to a decrease in the relative abundance of *C. acnes* A-class strains and a marked increase in D-class strains. **B.** Boxplots of relative abundances of six *C. acnes* SLST classes on skin sites with (+) and without (−) AAS strains [n = 21 (+) and n = 92 (−), respectively] are shown (FDR-adjusted p-value, **p ≤ 0.01. Unpaired Wilcoxon test). Middle lines of boxplots indicate the median. Lower and upper lines represent the first and third quartiles. Whiskers show the 1.5x inter-quartile ranges. Modified figure and legend are from Ahle et.al[3].