In Vitro Assessment of Virulence Traits in Cutibacterium acnes Shoulder Periprosthetic Joint Infections

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INTRODUCTION: Cutibacterium acnes (C. acnes) is a frequent isolate in shoulder periprosthetic joint infections (PJI), yet its clinical significance remains controversial due to its indolent behavior and common presence in skin flora. Distinguishing pathogenic strains from contaminants is critical, especially as culture positivity alone may not portend a diagnosis of PJI. Prior studies have proposed that in vitro traits, such as hemolysis, aerotolerance, rapid growth, and antibiotic resistance, may reflect greater virulence, though most studies included only 30–50 isolates. Our ongoing study aims to evaluate these traits in a larger cohort and assess their associations with infection classification using the 2018 International Consensus Meeting (ICM) criteria.

METHODS: This is a two-arm study combining in vitro testing of C. acnes isolates with retrospective chart review. Twenty-nine isolates were collected, each from a unique patient undergoing revision shoulder arthroplasty or aspiration. Isolates were tested for growth rate under anaerobic conditions (time to visible colony detection), hemolysis on Brucella blood agar, aerotolerance via candle jar, and antibiotic susceptibility. Interpretive categories were applied using Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints. Corresponding clinical data were reviewed to determine patient classification on the 2018 ICM criteria for shoulder PJI. Statistical comparisons were conducted using Fisher's exact test and Welch's t-test in R version 4.5.0. A P value <0.05 was considered to be statistically significant.

RESULTS: Of the 29 patients, 22 (76%) were classified under the 2018 ICM definition as definite shoulder PJI, 1 as probable, and 6 as unlikely infection. Hemolysis was observed in 16 isolates (55%) but was not associated with ICM-based infection classification (P = 1.0). Aerotolerance was observed in 14 isolates (48%) and was significantly more common among those who did not meet ICM criteria for definite infection (6 of 7, 86%) versus those who did (8 of 22, 36%) (P = 0.035).

Using the CLSI breakpoint (MIC >2 µg/mL), 3 of 29 isolates (10%) were categorized as not susceptible to clindamycin. These isolates demonstrated significantly faster growth, with a mean time to detection of 2.00 days vs 2.23 days for susceptible isolates (P = 0.01; 95% CI: 0.06–0.40). Hemolysis was observed in 2 of 3 non-susceptible isolates and 14 of 26 susceptible isolates (P = 1.0), while aerotolerance was present in 1 of 3 non-susceptible isolates and 13 of 26 susceptible isolates (P = 1.0). No isolates were resistant to vancomycin (>2 µg/mL) or doxycycline (≥ 16 µg/mL) using CLSI breakpoints.

Using the EUCAST breakpoint (MIC > 0.25 µg/mL), 5 of 29 isolates (17%) were categorized as resistant to clindamycin. These resistant isolates demonstrated significantly faster growth than susceptible isolates, with a mean time to detection of 2.00 vs 2.25 days (P = 0.01, 95% CI: 0.06–0.44). Hemolysis was observed in 3 of 5 resistant isolates (60%) compared to 13 of 24 susceptible isolates (54%) (P = 0.66), and aerotolerance was present in 3 of 5 resistant isolates (60%) versus 11 of 24 susceptible isolates (46%) (P = 0.39). No isolates were resistant to vancomycin (MIC > 2 µg/mL) based on EUCAST breakpoints (Table 1).

DISCUSSION AND CONCLUSION: In this preliminary cohort, hemolysis, despite its proposed role as a virulence marker in prior studies, did not show significant association with definite infection classification or antibiotic resistance under either breakpoint definition. Interestingly, aerotolerance was more common among isolates from patients who did not meet ICM criteria, suggesting a possible association with less clinically significant presentations. This difference was statistically significant and raises the possibility that aerotolerant strains may represent contaminants or less virulent phenotypes.

Prior studies have reported clindamycin resistance in C. acnes ranging from 2.5% to 31%, with hemolytic strains more often associated with resistance and confirmed infection. In contrast, our preliminary data showed no significant association between hemolysis and infection status or resistance using either breakpoint system. Although our findings diverge from prior reports, they align with earlier suggestions that faster growth may reflect increased virulence. These results require confirmation in our full dataset of 200 isolates. As shoulder arthroplasty volume continues to rise, improved understanding of C. acnes virulence and its correlation with clinical PJI criteria is essential. Final results may clarify whether traits like hemolysis, growth rate, or aerotolerance should guide diagnostic or therapeutic decisions in suspected C. acnes PJI.

	MIC for isolates taken from the shoulder (n=29)					
Antibiotic	**MIC_{50} (µg/mL)**	**MIC_{90} (µg/mL)**	**EUCAST Breakpoint (µg/mL)**	**CLSI Breakpoint (µg/mL)**	**% Not Susceptible (EUCAST)**	**% Not Susceptible (CLSI)**
Clindamycin	0.064	2.2	S ≤ 0.25; R > 0.25	S ≤ 2; I = 4; R ≥ 8	5 (17%)	3 (10%)
Vancomycin	0.38	0.5	S ≤ 2; R > 2	S ≤ 2	0 (0%)	0 (0%)
Doxycycline*	0.094	0.15	—**	S ≤ 4; I = 8; R ≥ 16	—	0 (0%)
Rifampin	0.006	0.012	—	—	—	—
Cefazolin	0.094	0.19	—	—	—	—

* Doxycycline breakpoints are not published for anaerobes. CLSI tetracycline breakpoints were substituted.
** No interpretive standards from the Clinical and Laboratory Standards Institute (CLSI) or EUCAST, and therefore, the percentage of resistant strains cannot be determined.