think of infection in terms of bioburden, which refers to the presence of bacteria in a wound and the number of microorganisms that contaminate an object" and subdivided bioburden into 4 categories: (1) contamination-bacteria within a wound without host reaction, (2) colonization-bacteria within the wound that multiply or initiate a host reaction, (3) critical colonization-bacteria that multiply to cause a delay in wound healing, often with increased pain but not with an acute host reaction and (4) infection-bacteria that multiply and cause a host reaction [15]. It seems logical that the presence of greater numbers of bacteria would correlate with the presence and severity of a periprosthetic shoulder infection. The results of this systematic review point out the paucity of available information, knowledge and understanding of the role of quantitative culture in the evaluation and management of shoulder PJI.

The limited data available suggests that standard fluid and tissue cultures are better than sonication cultures for diagnosis of shoulder PJI. However, there is insufficient experience and study of this technique to make definitive evidenced based recommendations. From a practical standpoint sonication is not readily available in all institutions. However, it seems that if sonication is used the quantitative culture results should reported.

New culture independent techniques and assays employed to identify the presence of bacteria including polymerase chain reaction, next generation sequencing and labeling techniques hold promise to aid both in the actual diagnosis of shoulder PJI as well as reduce the time to diagnosis. Nevertheless, the results of culture remain an important means to identify and characterize pathogenic microorganisms, to determine antibiotic susceptibility and to confirm the results of culture-independent methods. Previous experience demonstrates that the actual presence of bacteria does not always correlate with clinical manifestations of infection and that a number of pathogen and host factors must be considered in the diagnosis and management of shoulder PJI.

In summary, the results of prior studies in other specialties suggest that determining bacterial load with semi-quantitative and quantitative culture assessment in shoulder arthroplasty is of value in the evaluation and management of cases in which PJI is suspected. The application of these semi-quantitative and quantitative culture results to the evaluation of a failed shoulder arthroplasty requires (1) a standardized approach to harvesting specimens (source, number and technique), (2) using standardized culturing protocols designed to detect the presence of *Cutibacterium*, (3) standardized approach to reporting of the semi-quantitative or quantitative results and (4) documentation of the semi-quantitative or quantitative results of control specimens from the OR that have not been in contact with the patient.

REFERENCES

[1] Kallstrom G. Are quantitative bacterial wound cultures useful? J Clin Microbiol. 2014;52:2753–2756. doi:10.1128/JCM.00522-14.

[2] Mook WR, Klement MR, Green CL, Hazen KC, Garrigues GE. The incidence of Propionibacterium acnes in open shoulder surgery: a controlled diagnostic study. J Bone Joint Surg Am. 2015;97:957–963. doi:10.2106/JBJS.N.00784.

[3] MacNiven I, Hsu JE, Neradilek MB, Matsen FAI. Preoperative skin-surface cultures can help to predict the presence of Propionibacterium in shoulder arthroplasty wounds. JBJS Open Access. 2018;3:e0052. doi:10.2106/JBJS.OA.17.00052.

[4] Laboratory Quality Assurance Program. n.d. https://www.cps.sk.ca/imis/CPSS/Programs_and_Services/Laboratory_Quality_Assurance.aspx?LabQualityCCO=Laboratory%20Quality%20Assurance%20Program%20Overview (accessed August 31, 2018).

[5] Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clin Microbiol Rev. 2001;14:244–269. doi:10.1128/CMR.14.2.244-269.2001.

[6] Ahsan ZS, Somerson JS, Matsen FA. Characterizing the Propionibacterium load in revision shoulder arthroplasty: a study of 137 culture-positive cases. J Bone Joint Surg Am. 2017;99:150–154. doi:10.2106/JBJS.16.00422.

[7] Hsu JE, Neradilek MB, Russ SM, Matsen FA. Preoperative skin cultures are predictive of Propionibacterium load in deep cultures obtained at revision shoulder arthroplasty. J Shoulder Elbow Surg. 2018;27:765–770. doi:10.1016/j.jse.2018.01.021.

[8] Hsu JE, Somerson JS, Vo KV, Matsen FA. What is a "periprosthetic shoulder infection"? A systematic review of two decades of publications. Int Orthop. 2017;41:813–822. doi:10.1007/s00264-017-3421-6.

[9] Matsen FA, Butler-Wu S, Carofino BC, Jette JL, Bertelsen A, Bumgarner R. Origin of propionibacterium in surgical wounds and evidence-based approach for culturing Propionibacterium from surgical sites. J Bone Joint Surg Am. 2013;95:e1811-e1817. doi:10.2106/JBJS.L.01733.

[10] Carli AV, Bhimani S, Yang X, Shirley MB, de Mesy Bentley KL, Ross FP, et al. Quantification of peri-implant bacterial load and in vivo biofilm formation in an innovative, clinically representative mouse model of periprosthetic joint infection. J Bone Joint Surg Am. 2017;99:e25. doi:10.2106/JBJS.16.00815.

[11] Esteban J, Gadea I, Pérez-Jorge C, Sandoval E, García-Cañete J, Fernandez-Roblas R, et al. Diagnosis of spacer-associated infection using quantitative cultures from sonicated antibiotics-loaded spacers: implications for the clinical outcome. Eur J Clin Microbiol Infect Dis. 2016;35:207–213. doi:10.1007/s10096-015-2531-6.

[12] Grosso MJ, Frangiamore SJ, Yakubek G, Bauer TW, Iannotti JP, Ricchetti ET. Performance of implant sonication culture for the diagnosis of periprosthetic shoulder infection. J Shoulder Elbow Surg. 2018;27:211–216. doi:10.1016/j.jse.2017.08.008.

[13] Trampuz A, Piper KE, Hanssen AD, Osmon DR, Cockerill FR, Steckelberg JM, et al. Sonication of explanted prosthetic components in bags for diagnosis of prosthetic joint infection is associated with risk of contamination. J Clin Microbiol. 2006;44:628–631. doi:10.1128/JCM.44.2.628-631.2006.

[14] Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, et al. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J Clin Microbiol. 2009;47:1878–1884. doi:10.1128/JCM.01686-08.

[15] Kravitz S. Infection: are we defining it accurately? Adv Skin Wound Care. 2006;19:176.

2.2. DIAGNOSIS: CULTURE TECHNIQUE

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QUESTION 1: What is the optimal culture technique (e.g., culture medium, days of incubation) in evaluating patients for shoulder periprosthetic joint infection (PJI)?

RECOMMENDATION: Current evidence suggests that culture of tissue samples for the diagnosis of shoulder PJI is best performed using both aerobic and anaerobic conditions. For solid culture media, diagnostic accuracy may be improved by using enrichment media. Fourteen days is the most common culture duration cited.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

RATIONALE

PJI of the shoulder is a common indication for revision surgery [1]. The organisms that are most commonly responsible include *Staphylococcus* and *Cutibacterium acnes* (formally *Propionibacterium acnes*). Culture techniques and interpretation of culture results for the former are well established, but *C. acnes* is a ubiquitous skin commensal in humans. Therefore, the distinction between it being a contaminant versus pathogen is challenging. This is complicated by the fact that *C. acnes* is often associated with few local or systemic signs of inflammation and is often slow to grow in the laboratory. Defining the optimal culture technique for diagnosis of shoulder PJI is, therefore, important. However, even if this were achieved, cultures are likely to yield a proportion of false positive results, and, therefore, the inclusion of a confirmatory test in the diagnostic pathway is critically needed for the interpretation and corroboration of culture results. There are three main variables relating to culture conditions for the diagnosis of shoulder PJI.

Duration of Culture

In order to optimize detection of all organisms, including *C. acnes,* in upper limb PJI, most authors advise prolonged incubation, although the ideal duration has yet to be established. An incubation time which is too short may limit the sensitivity; an incubation time which is too long results in the isolation of non-diagnostic isolates or contaminants, thereby limiting the specificity.

Zappe et al. [2], in a retrospective analysis of 139 cases of PJI, suggest that *Cutibacterium* associated infection occurs at a frequency comparable to many other pathogens and that the median time to culture positivity is 8 days. They advise that tissue samples should be incubated for 14 days.

Schäfer et al. [3] likewise suggested that prolongation of the incubation period was associated with an increase in the proportion and diversity of positive samples. They recommended an incubation period of up to 14 days based especially on late recovery of aerobic gram-positive rods and *Cutibacterium* species.

Similarly, Butler-Wu et al. [4] estimated the median time to positivity using standard bacteriological methods to be 6 days with a range of 2-15.

Based on such studies, many authors advise a minimum incubation period of 14 days [5–8] while some advise at least 21 days [1,9].

However, prolonged incubation of cultures increases the risk of generating false positive results due to sample contamination and, therefore, may adversely affect the specificity of the test. A retrospective study by Frangiamore et al. [10] suggested that, amongst 46 cases, median time to *C. acnes* growth in the probable true-positive group was 5 days as compared to 9 days in the probable contaminant group ($p = 0.002$).

Peel et al. [11] demonstrated that, in 117 cases of proven PJI as defined by the Infectious Disease Society of America (IDSA) criteria, the median time to positivity using blood culture bottles was around 24 hours. Extending anaerobic incubation beyond 7 days yielded a diagnosis of PJI in only five additional subjects who fulfilled the IDSA diagnostic criteria and anaerobic blood culture bottles detected pathogen growth more rapidly than agar or thioglycolate broth.

Minassian et al. [12] prospectively analyzed 332 revision arthroplasty patients whose surgical samples were processed using both blood culture bottles and conventional media. Amongst 66 who had microbiologically confirmed PJI, 65 cases were identified as culture positive within 3 days and one at day 8.

Anaerobic and Aerobic Culture

PJI caused by strictly anaerobic pathogens is rare but mandates careful selection of antimicrobials for optimal therapy. While *C. acnes* is an anaerobic organism, many strains are aerotolerant and Butler-Wu et al. [4] suggested a significant and clinically important improvement in yield by using aerobic and anaerobic culture conditions. Peel et al. [11], however, suggest little advantage of prolonged aerobic cultures specifically for the diagnosis of *C. acnes* but reported benefit from extended anaerobic culture.

Choice of Culture Medium

Conventionally, the laboratory diagnosis of PJI has relied upon culture of tissue specimens on solid media (agar) and broth cultures. Unless they become visibly turbid, the latter are terminally subcultured onto agar to detect any non-visible growth in the broth. This is time consuming, cumbersome and provides no advantage over automated techniques.

Butler-Wu et al. [4] analyzed the accuracy of *C. acnes* PJI diagnosis in 198 revision arthroplasty procedures using four different culture media (blood agar, chocolate agar, Brucella agar and brain-heart infusion (BHI) broth). They found that recovery of *C. acnes* from blood agar was exclusively associated with the presence of infection (16 specimens), but all specimens positive for growth of *C. acnes* on blood agar were also positive for growth on at least one additional culture medium. BHI yielded the highest number false positive results and Brucella agar yielded the highest number of true positive results. They suggest that isolation of *C. acnes* from clinically proven infected cases were 6.3 times more likely to have two media positive for growth as compared to unproven cases of infection ($p = 0.002$).

Hughes et al. [13] prospectively compared conventional culture media and blood culture medium in 849 separate specimens from 178 patients undergoing arthroplasty revision. They estimated the sensitivity and specificity of blood culture medium to be 87% and 98% respectively. By comparison, the sensitivity of direct plates and cooked meat broth culture were 39% and 83%

Motwani et al. [14] found that, in 60 cases of pediatric septic arthritis caused by any organism, incubation of clinical samples in BACTEC blood culture bottles, as compared to conventional agar plates, increased the yield from 42% to 71%.

A prospective study of 369 adults by Peel et al. [11] similarly showed that use of blood culture bottles improved bacterial yield in comparison to conventional agar and broth culture (92.1% versus 62.6%, respectively).

REFERENCES

[1] Fink B, Sevelda F. Periprosthetic joint infection of shoulder arthroplasties: diagnostic and treatment options. BioMed Res Int. 2017;2017:4582756. doi:10.1155/2017/4582756.

[2] Zappe B, Graf S, Ochsner PE, Zimmerli W, Sendi P. Propionibacterium spp. in prosthetic joint infections: a diagnostic challenge. Arch Orthop Trauma Surg. 2008;128:1039–1046. doi:10.1007/s00402-007-0454-0.

[3] Schäfer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin Infect Dis. 2008;47:1403–1409. doi:10.1086/592973.

[4] Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA, et al. Optimization of periprosthetic culture for diagnosis of Propionibacterium acnes prosthetic joint infection. J Clin Microbiol. 2011;49:2490–2495. doi:10.1128/JCM.00450-11.

[5] Parvizi J, Gehrke T, International Consensus Group on Periprosthetic Joint Infection. Definition of periprosthetic joint infection. J Arthroplasty. 2014;29:1331. doi:10.1016/j.arth.2014.03.009.

[6] Fink B, Makowiak C, Fuerst M, Berger I, Schäfer P, Frommelt L. The value of synovial biopsy, joint aspiration and C-reactive protein in the diagnosis of late peri-prosthetic infection of total knee replacements. J Bone Joint Surg Br 2008;90:874–8. doi:10.1302/0301-620X.90B7.20417.

[7] Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Löhr JF. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? Clin Infect Dis. 2004;39:1599–1603. doi:10.1086/425303.

[8] Nodzo SR, Boyle KK, Bhimani S, Duquin TR, Miller AO, Westrich GH. Propionibacterium acnes host inflammatory response during periprosthetic infection is joint specific. HSS J. 2017;13:159–164. doi:10.1007/s11420-016-9528-2.
[9] Pottinger P, Butler-Wu S, Neradilek MB, Merritt A, Bertelsen A, Jette JL, et al. Prognostic factors for bacterial cultures positive for Propionibacterium acnes and other organisms in a large series of revision shoulder arthroplasties performed for stiffness, pain, or loosening. J Bone Joint Surg Am. 2012;94:2075–2083. doi:10.2106/JBJS.K.00861.
[10] Frangiamore SJ, Saleh A, Grosso MJ, Alolabi B, Bauer TW, Iannotti JP, et al. Early versus late culture growth of Propionibacterium acnes in revision shoulder arthroplasty. J Bone Joint Surg Am. 2015;97:1149–1158. doi:10.2106/JBJS.N.00881.
[11] Peel TN, Dylla BL, Hughes JG, Lynch DT, Greenwood-Quaintance KE, Cheng AC, et al. Improved diagnosis of prosthetic joint infection by culturing periprosthetic tissue specimens in blood culture bottles. MBio. 2016;7:e01776–e01715. doi:10.1128/mBio.01776-15.
[12] Minassian AM, Newnham R, Kalimeris E, Bejon P, Atkins BL, Bowler ICJW. Use of an automated blood culture system (BD BACTEC™) for diagnosis of prosthetic joint infections: easy and fast. BMC Infect Dis. 2014;14:233. doi:10.1186/1471-2334-14-233.
[13] Hughes HC, Newnham R, Athanasou N, Atkins BL, Bejon P, Bowler ICJW. Microbiological diagnosis of prosthetic joint infections: a prospective evaluation of four bacterial culture media in the routine laboratory. Clin Microbiol Infect. 2011;17:1528–1530. doi:10.1111/j.1469-0691.2011.03597.x.
[14] Motwani G, Mehta R, Aroojis A, Vaidya S. Current trends of microorganisms and their sensitivity pattern in paediatric septic arthritis: a prospective study from tertiary care level hospital. J Clin Orthop Trauma. 2017;8:89–92. doi:10.1016/j.jcot.2016.09.001.

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QUESTION 2: Should *Cutibacterium acnes* (formerly known as *Propionibacterium acnes*) isolated in samples from the shoulder be sub-typed?

RECOMMENDATION: *Cutibacterium acnes* isolated in samples from the shoulder should not be routinely sub-typed.

LEVEL OF EVIDENCE: Limited

DELEGATE VOTE: Agree: 100%, Disagree: 0%, Abstain: 0% (Unanimous, Strongest Consensus)

RATIONALE

The survey of the studies was conducted by searching PubMed since January 1, 2000 in the best match sort order with the following query ((Propionibacterium acnes OR Cutibacterium acnes OR P acnes)) AND (strain OR types OR typing OR phylogenetic OR orthopedic infection OR prosthetic joint OR arthroplasty OR shoulder OR implant OR instrumentation) AND (("2000/01/01"[PDat]: "3000/12/31"[PDat]) AND Humans[Mesh]).

Cutibacterium acnes (formerly known as *Propionibacterium acnes* [1]) is a member of the normal human skin microbiota and is associated with various infections and clinical conditions. It is frequently isolated from prosthetic joints (particularly shoulder arthroplasties) and the spine, mainly due to the proximity of these sites to areas of skin rich in pilosebaceous glands, where *C. acnes* reside [2,3].

C. acnes is one of the most frequent microorganisms isolated in shoulder periprosthetic joint infection (PJI). In contrast to the knee and hip joints, *C. acnes* has been isolated in 17.6% to 60% of periprosthetic shoulder infection cases [4–7]. However, its role in pathogenesis has been questioned [8], as up to 60% of patients that grow *C. acnes* from a prosthetic joint have no evidence of acute inflammation in histopathology [9]. Besides that, *C. acnes* has been present in culture specimens during primary shoulder surgery [10–12], and it has been identified as a common contaminant of the surgical field [13]. One possible explanation for these observations is that standard skin surface preparation cannot eliminate *C. acnes* in a high percentage of individuals, thus favoring inoculation from the more superficial dermal structures into the deep tissues during surgery [14].

Within the last 10 years, phylogenetic studies based on single and multilocus gene sequencing, as well as whole-genome analyses have provided valuable insights into the genetic population structure of *C. acnes*, particularly in the context of health and disease. The bacterium has an overall clonal structure, and its isolates can be classified into a number of phylogroups designated types IA1, IA2, IB, IC, II and III [15–17]. These types appear to display differences in associations with specific types of infections and vary in the production of putative virulence determinants, inflammatory potential, antibiotic resistances, aggregative properties and morphological characteristics. However, uncertainty still exists regarding the exact clinical relevance of these phylogroups, as well as the wider issue of whether isolates recovered from different clinical samples are truly representative of infection in all contexts or are simply skin contaminants or passive bystanders within a sample [15].

Since *C. acnes* can be isolated as a pathogen or a contaminant, it can be difficult to interpret clinical significance simply based on its isolation. In addition, subacute and chronic shoulder PJI typically present with low-grade, indolent clinical features and normal laboratory inflammatory markers, which further confounds this distinction [15–17]. Microbial characteristics that indicate whether the isolated *C. acnes* is a likely cause of orthopaedic implant infection versus a colonizing agent would be clinically useful. In a prospective study conducted by Sampedro et al. [18], the phylotype of *Cutibacterium* had no clear association with infection or colonization of failed orthopaedic implants [10]. To date, no clear association between phylotypes and infection/colonization or outcome of infection has been reported [13].

Considering this uncertainty over clinical relevance and utility and considering the high costs and limited availability in clinical microbiology laboratories, we suggest that *Cutibacterium acnes* isolated in samples from the shoulder should not be routinely specified according to phylogroups. Rather, these techniques should be reserved for research purposes. Studies focusing on the determination of phylotypes and identification of virulence factors associated with deep infection should be encouraged, since these tools may become useful to improve diagnosis by means of the development of new techniques to identify target strains that can cause infection [3].

REFERENCES

[1] Scholz CFP, Kilian M. The natural history of cutaneous propionibacteria, and reclassification of selected species within the genus Propionibacterium to the proposed novel genera Acidipropionibacterium gen. nov., Cutibacterium gen. nov. and Pseudopropionibacterium gen. nov. Int J Syst Evol Microbiol. 2016;66:4422–4432. doi:10.1099/ijsem.0.001367.