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Appendix

Understanding Common Pathogens and Choosing the Right Clinical Isolate

Selecting and culturing the appropriate strain of bacteria is critical when attempting to model periprosthetic joint infection (PJI). Genetic and molecular differences among strains of the same pathogen can substantially influence experimental results and the generalizability of conclusions, especially if the antibiotic resistance profile or knowledge of the strain's genome remains poorly understood. One example of pertinent strain differences is found with *Staphylococcus epidermidis*. Of the 2 strains for which genome information is available, the ATCC12228 strain does not produce biofilm[129], while the clinical isolate RP62A does[130]. Furthermore, *S. epidermidis* strains can become more virulent through acquisition of mobile genetic elements in the in vivo environment[131]. Interestingly, the most commonly encountered sequence type of *S. epidermidis* is also the most invasive of all, although its genome has yet to be sequenced[132].

Staphylococcus aureus has many commercially available strains[133,134] whose full description is beyond the scope of this review. With regard to orthopaedic infection studies, several distinct strains have been investigated (RN1, Newman, COL, SH1000, and UAMS-1)[135], and most notably USA300, a methicillin-resistant *S. aureus* (MRSA) strain of epidemic proportions within the United States[136]. SH1000, UAMS-1, and USA300 produce unique patterns of biofilm proliferation and formation over time in a smooth pin PJI model[137]. The same authors deleted the *agr* locus of the UAMS-1 strain, turning off its "quorum sensing" ability, which stopped bacterial colonies from leaving the biofilm, leading to negative in vivo culture and an apparently nontoxic animal. This study provides insight into how alteration of understood mechanistic pathways can lead to predictable changes in bacterial function and possible therapeutic targets. The finding is especially interesting, considering that UAMS-1 is

among the most virulent of *S. aureus* strains, equivalent to MRSA strains that secrete potent exotoxins and use efflux pumps to clear antibiotics[138-141].

Other bacteria, although less frequently encountered clinically, have different biological traits that must be considered when incorporating them into a model. Gram-negative bacteria, specifically *Escherichia coli*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*, are responsible for 10% of clinical PJI[82] and can produce aggressive biofilms. Compared with gram-positives, gram-negative bacteria utilize different surface proteins to adhere to implants[142] and incorporate palmitate acyl chains into their biofilm, which counteract the inflammatory response and diminish antimicrobial potency[143]. Furthermore, gram-negative bacteria have a high tendency to develop multidrug-resistant clones over time, making their eradication a formidable task[144-146]. Another bacterium that has been implicated in PJI is *Propionibacterium acnes*, a gram-positive facultative anaerobe that normally colonizes human skin and is becoming an increasingly recognized cause of PJI. Strains of *P. acnes* are divided into 3 types and several subtypes on the basis of features of hemolysis and DNA repair[139,140]. Type-IB and type-II phylotypes have been most commonly associated with PJI, but a correlation between the *P. acnes* genomic phylotype and PJI has yet to be identified[82,141,142]. The ability of *P. acnes* to form a biofilm is not phylotype-dependent and forms at 19 to 96 hours after inoculation in vitro. However, in vivo biofilm takes longer, as many as 7 days on titanium-based devices, to reach what otherwise occurs in 24 hours with staphylococcal species[143]. Although *P. acnes* creates a biofilm similar in appearance to other bacteria, its surface layer has been shown to be distinctively resistant to antibiotic penetration[144]. This resistance is strain-specific[145,146], illustrating the importance of not only choosing a type of bacteria but also investigating individual strain aspects when developing a model.