Are we really sterile? Bacterial Contamination Rates During Anterior Cruciate Ligament Reconstruction: A Laboratory Analysis of Surgical Gloves

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INTRODUCTION: Anterior Cruciate Ligament (ACL) reconstruction is among the most frequent procedures performed in orthopaedic surgery. The rate of post-operative septic arthritis is low overall with reports around 1%. However, due to the morbidity caused by post-operative infection, including the need for subsequent surgery, arthrofibrosis, cartilage injury, and pain, it is imperative orthopaedic surgeons understand potential sources of bacterial contamination that may occur during ACL reconstructions and increase the risks for post-operative infection. Recent literature has described the phenomenon of subclinical bacterial colonization of soft tissue grafts and the development of biofilms. This colonization has been implicated as a potential contributor to graft failure, as prior studies have isolated bacterial DNA in failed ACL graft samples taken at the time of revision surgery, and in the right host environment, could progress to symptomatic intra-articular infection. The purpose of this study was to assess for the presence of bacterial contamination on surgical gloves at various timepoints throughout an ACL reconstruction and to evaluate for any differences based on the type of graft being utilized. We hypothesized that despite best efforts to maintain sterility bacterial contamination would be present.

METHODS: This study assessed bacterial contamination of gloves in 16 ACL reconstructions (8 bone-patellar tendon-bone autograft; 8 hamstring autograft). Key clinical timepoints identified for sample collection were: 1) After patient draping, 2) After graft harvest, 3) After graft preparation, and 4) Prior to graft passage. At these times, the primary member of the surgical team performing each task pressed their gloved fingers onto a sterile Tryptic Soy Agar (TSA) plate. Outer gloves were then removed and replaced with fresh sterile gloves to proceed with surgery. TSA plates were incubated for 48 hours at 37°C and 5% CO2. Plates were then checked for bacterial growth. Tissue trimmings from the autograft were also collected prior to the antibiotic soaking of the graft. The tissue was incubated in Tryptic Soy Broth for 24hrs at 37°C. After 24 hours, a 100-microliter aliquot of the growth media was used to inoculate a TSA plate and incubated for 24hrs to assess the presence of bacterial growth. Colonies, if present, were then transferred to TSB broth using a sterile loop. They were then incubated for 24hrs and spun down to form cell pellets, and they were frozen for subsequent 16s rRNA sequencing to evaluate for bacteria. A negative control plate was included for each case. The negative control plate was set out and transported with the experimental plates.

RESULTS: Samples were collected from sixteen ACL reconstruction cases, accounting for eight bone-patellar tendon-bone grafts and eight hamstring grafts. Eight of the sixteen cases (50%) produced at least one sample with bacterial growth. Nine agar plates out of the Ninety-six (9%) collected were positive for bacterial growth and accounted for three different time points. One plate of the sixteen (6%) after graft harvest had one colony forming unit (CFU). One plate out of the sixteen (6%) collected after graft preparation had one CFU. Seven plates of forty-eight (15%) demonstrated growth prior to graft passage. Of the seven plates with bacterial growth collected prior to graft passage five plates had one CFU, one plate had three CFUs, one plate had four CFUs. Three out of the sixteen tissue samples (19%) were positive for bacterial growth. No negative control plates were found to have bacterial growth. Fischer exact testing was performed to compare rates of contamination at each timepoint and between graft types. Although there was no statistically significant difference between time points or graft types, the presence of any bacterial growth on a subset of the agar plates and tissue samples collected may be clinically relevant. It indicates the presence of detectable bacteria during ACL reconstruction surgeries at a higher rate than the post-operative septic arthritis rate.

DISCUSSION AND CONCLUSION: These results indicate that despite attempted maintenance of a sterile surgical field, some bacterial contamination was detected in 50% of cases. 16s rRNA sequencing will allow for bacterial species identification. The low level of bacterial contamination detected during this study are of uncertain clinical significance, as no patients developed overt septic arthritis after surgery. Longitudinal follow-up of positive cases could elicit clinical implications of detecting bacteria at the time of surgery, without overt post-operative septic arthritis, including monitoring for graft failure, which could be a result of subclinical graft colonization which has been previously described in the literature.

Table 1. Total Positive Cases

	Total Cases	Positive Cases	Percent Positive
Hamstring Graft	8	3	38%
BTB Graft	8	5	63%
Total cases	16	8	50%

Table 2. Positive Samples per Timepoint

Time Point	Total plates	Positive Samples	Percent Positive	CFU per plate
After Patient Draping	16	0	0%	-
After Graft Harvest	16	1	6%	1 CFU
After Graft Prep	16	1	6%	1 CFU
Prior to Graft Passage	48	7	15%	1 CFU (5x) 3CFU (1x) 4CFU (1x)
Tissue Sample	16	3	19%	-