Clinical Effectiveness of a Biofilm Disrupting Surgical Lavage in Reducing Bacterial Contamination in Total Knee Arthroplasty Revision Surgery in Known Cases of Prosthetic Joint Infection

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Background

Biofilm-based infectious diseases represent up to 80% of all infectious diseases. Biofilms are structured communities of bacteria encased in a protective Extracellular Polymeric Substances (EPS) matrix. This EPS matrix serves as a physical barrier against antimicrobial or even host immune agents. Biofilms can excrete up to 800 new molecules within hours of attachment/clustering, which are an important component of biofilm formation and stability. Due to the nature of the EPS matrix of biofilm, the bacteria encased within the biofilm are more resistant to conventional antimicrobial treatments. Thus, to combat rising antimicrobial resistance, it is vital to incorporate biofilm-disrupting technology in the use of surgical lavage.

Objectives

The objective of this 40-patient study was to demonstrate a reduction in fluid cell counts in aspirate acquired from primary or Stage 1 revision total knee arthroplasty patients diagnosed with prosthetic joint or surgical site infections using a biofilm-disrupting surgical lavage.

Methods

Subject population was drawn from patients undergoing the first stage of a 2-stage revision for Prosthetic Joint Infection (PJI). Bactisure™ Wound Lavage was used at the end of the procedure prior to closure, then followed by saline lavage. 3 mL fluid cultures were obtained from deep in the surgical wound both before the use of Bactisure Wound Lavage and after the saline lavage. Cell counts were compared before and after articular irrigation. The WBC cell count served as a proxy for particulate and cellular matter in the articular wound. Plate counting was performed to determine the bacterial colonization of the surgical site and DNA analysis was used to identify the bacteria. Subjects were followed for 90-days to evaluate recurrence of PJI.

Results

The data demonstrate reduced bioburden and bacterial count within the surgical site after use of the surgical lavage. There was a 2.3 log reduction in white blood cells in all patients and a 3.8 log reduction in bioburden in patients with countable bacteria prior to washing. For patients which completed the study, the 90-day infection rate was 12.9%.

Key Words

Biofilm, infection, total knee arthroplasty, wound wash, wound gel

Introduction

Total joint arthroplasty (TJA) is one of the most common surgical procedures performed in the US and worldwide (Merx, et al., 2003) (Lohmander, et al., 2006). Over 1 million total hip and total knee replacement (arthroplasty) procedures are performed each year in the U.S. (Kremers, Larson, Crowson, & Kremers, 2015). Periprosthetic Joint Infection (PJI) affects 1-2% of total joint arthroplasty patients and remains one of the most serious complications of TJA. While rare, the condition incurs substantial morbidity and costs, and a significant portion of sufferers will bear consequences for the remainder of their lives
(Aggarwal, Rasouli, & Parvisi, 2013). By 2020, the predicted cost for infected revision procedures may reach as high as $1.6 billion (Aggarwal, Rasouli, & Parvisi, 2013) (Kurtz, Lau, Watson, Schmier, & Parvisi, 2012).
Bacteria spend their existence cycling between planktonic and biofilm form. However, a vast majority, approximately 90%, of bacteria naturally form biofilms (Petrova & Sauer, 2012). Unlike planktonic bacteria, bacteria in

biofilms are shielded within a protective Extracellular Polymeric Substances (EPS) matrix, which provides both a mechanical and chemical barrier to make them more resistant to attack. Moreover, bacteria within biofilms proliferate more slowly, reducing their susceptibility to antibiotics that target cell replication machinery (Malone, et al., 2017). Biofilm components have been demonstrated to modulate macrophage behavior, further inhibiting immune attack (Roilides, Simitsopoulou, Katragkou, & Walsh, 2015). Consequently, biofilms are more tolerant to antimicrobial agents, disinfectants, and host immune defenses. Bacteria in biofilms can demonstrate up to 1000-fold more resistance to antimicrobial agents than planktonic bacteria (Malone, et al., 2017). According to the US National Institutes of Health, biofilms are present in over 80% of microbial infections in humans and can affect every organ system, including the skin, and attach to any surface

(National Institutes of Health, n.d.). Therefore, incorporating biofilm disrupting technology in treating infections is not only an advantage—it is a necessity.

Bactisure Wound Lavage (BWL) solution (Next Science Ltd, Jacksonville, FL; distributed by Zimmer Biomet), administered via pulsed (jet) lavage has been developed for removal of planktonic and biofilm bacteria from the articular joint space. BWL is a mixture of surfactants, chelating agents, and salts to disrupt and dissolve contaminants, indicated to clean debris (including microorganisms) from the wound. BWL deconstructs EPS matrix, exposing the bacteria to antibiotics, the body's normal defense systems, and even removal via lavage.

Objective

The primary objective of this study was to demonstrate a reduction in contamination in the surgical sites of revision total knee arthroplasty patients diagnosed with Prosthetic Joint Infection by White Blood Cell (WBC) Counts before and after irrigation with BWL.

The secondary objectives of this study are twofold. The first is to demonstrate a reduction in the bacterial bioburden in revision total knee arthroplasty patients diagnosed with Prosthetic Joint Infection by comparing bacterial counts (Colony Forming Units per mL) before and after irrigation with BWL. The second is to demonstrate a reduction in the 90-day Prosthetic Joint Infection reinfection rate, as identified by International Consensus / Musculoskeletal Infection Society (ICM/MSIS) criteria.

Methods

A 40-patient multicenter prospective clinical trial was designed for patients either 1) undergoing the first stage of a 2-stage revision for knee PJI or 2) those undergoing irrigation and debridement with component retention (DAIR) following TKA with the PJI occurring within 30 days of the primary procedure or 1 year with primary implant retention. Infection status was determined using a combination of ESR, CRP, albumin/total leukocyte count, joint aspiration, leukocyte esterase strip, synovial culture, and/or the Synovasure® Alpha Defensin test.

BWL was performed at the end of the procedure, prior to closure, and then followed by saline lavage. 3 mL fluid cultures were obtained from deep in the surgical wound both before the Bactisure and after the saline lavage. White blood cell (WBC) counts were obtained from the fluid as a surrogate marker for bioburden. Plate counting was performed to determine the bacterial colonization of the surgical site and DNA analysis (PathoGenius Laboratories, Lubbock, TX) was used to identify the bacteria.

Subjects were monitored per standard practice for 90 days post-procedure to evaluate infection status.

Results

The patient demographics for the 40 patients enrolled in the study are demonstrated in Table 1. The average subject age was 68.7 +/- 7.8 years, the average BMI was 34.1 +/7.7, and the average days from PJI diagnosis to surgery was 24.2 +/- 49.7 days. 53.5% of patients were on antibiotics for PJI at the time of surgery. Operative data are summarized in Table 2.

45 patients were initially enrolled; of those, five (5) did not undergo surgery for various reasons unrelated to the study two (2) of these failed to be properly marked "study completed" by the site monitor. Of the 43 patients completing the study, five (5) had incomplete follow-up data (Table 3).

40% of the patients in the study were enrolled for an irrigation and debridement with component retention, while 60% had a first stage of a 2-stage revision as can be seen in figure 2. The ASA classification for these joints were 20.0% for class 2, 65% for class 3, and 15% for class 4. 95% of the patients received antibiotics at discharge and the length of stay was 9.0 +/-6.4 days on average.

Data was obtained on all 40 patients for WBC counting (Figure 1). There was a substantial (>99%) reduction in the WBC counts, (2.3 log reduction, 3.9 +/- 1.2 log to 1.5 +/- 1.0 log, p-value <0.01).

Plate count data was obtained for 37 patients. There was a dramatic decrease in the number of colony forming units in the surgical site (Figure 2). For patients with positive cultures, the average plate count decreased by 99.98% (3.8 log), with the log CFU of bacteria being reduced from 4.6 +/- 1.3 log to 0.8 +/- 1.6 log (p-value < 0.01). For the entire population, the average plate count decreased by 99.08% (2.0 log), with the log CFU of bacteria being reduced from 2.5 +/- 2.4 log to 0.4 +/- 1.2 log (p-value < 0.01).

Pre-lavage, 20 of 37 samples were culture positive; post-lavage, 33 of 37 were culture negative. For those patients with a positive culture in the pre-lavage test, 80% had no countable bacteria in the post-lavage test (p-value < 0.01).

Bacterial DNA results were obtained for 38 patients. Figure 3 is a heat map of the bacteria present pre- and post-lavage. 79% of the Pre-Wash samples had culturable bacteria, compared to 74% for the post-wash samples. It is not surprising to find bacterial DNA in these samples, as PCR cannot distinguish between viable and unviable bacteria. There was an increase in the average number of cultured bacteria in the post-wash samples compared to the pre-wash samples (4.7 vs. 3.3), but it was not statistically significant.

The following bacterial genera were found in over 15% of patients: Staphylococcus (53%), Escherichia (42%), Cutibacterium (37%), Corynebacterium (21%), Acinetobacter (18%), Pseudomonas (16%) and Streptococcus (16%).

As shown in Table 3, 81.4% of the study patients completed the study according to protocol. Of the patients who completed the study, 87% completed the 90 day follow-up without signs of an infected joint, and four (10% of the total population) presented with the appearance of an infected joint. The intra-operative bacterial DNA sample was not collected for one of the of these four patients, and this same patient did not have bacterial identification available at the 90-day time point.

In those patients deemed infected at the 90 day follow-up, the bacterial DNA detected either pre or post wash were Acinetobacter, Staphylococcus, Pseudomonas, Pelomonas, Anaerococcus, or Corynebacterium. There was no apparent correlation between the initial bacterial species detected and 90-day infection status. The dominant pathogens identified in these same individuals at the 90 day time point were Candida albicans, Proteus mirabilis, and Prevotella bivia; however, as none of these were in the Pathogenius screening, it is impossible to determine if these were returning or new pathogens.

There was likewise no apparent correlation between initial ASA grade, surgical type (I&D vs Stage 1), or diagnostic criteria and 90-day infection status. Relevant data on the four infected individuals is summarized in Table 4.

Discussion

The bioburden present in the joint after washing with BWL was greatly reduced, as evidenced by the substantial white blood cell count reduction and by the bacterial count data. Indeed, the bioburden reduction as measured by white blood cells was greater than 99% (2.3 log). This was consistent with the plate-count data for the entire population, which demonstrated a 99% reduction in bacteria (2.0 log).

The reduction in bacteria for those patients which had a bacterial count pre-lavage was even more dramatic, with the BWL reducing the bacterial burden within these patients by 99.98% (3.8 log). More importantly for these patients, 80% of them had no viable bacteria in their samples after lavage. This reduction in bacterial burden suggests that these patients will have a reduced chance of a continuing infection.

There was a small but not statistically significant increase in culturable bacteria after the wash, which may indicate liberation of bacteria from the biofilm. Further study will attempt to enumerate the relative rates of biofilm-encapsulated bacteria liberation vs. destruction by the product.

From the DNA testing, the use of BWL had a signal towards a reduced number of cultured bacteria in these joints after treatment. The high prevalence of Staphylococcal, Pseudomonal, and Streptococcal bacteria align well with the infection rates of these bacteria in PJI. The effectiveness of the BWL in removing and eliminating bacteria from the surgical site cannot be inferred from this data, as DNA typing will identify both viable and non-viable bacteria.

The 12.9% PJI rate post-wash correlated well with the plate count data as 15% (6 out of 40) patients in the study had a plate-count value after wash. Although data on revision complications due to infection are limited, a study by Wu et al reported the complication rate for 2-stage revisions of 79.1% (range 33.3%-100%) (the composite success rate for infection control in 2-stage revisions) (Wu, Gray, & Lee, 2014). This infection rate looks at infection rates beyond the 90-day timeframe but is indicative of poor outcomes.

Conclusions

The use of Bactisure Wound Lavage prior to closure significantly reduces the bioburden and bacterial count within the surgical site. There is a wide range of bio-diversity present within the cultured bacteria within the wound, with 79% of the wounds having culturable bacteria. There was a profound reduction in the recoverable bacteria after the application of the Bactisure Wound Lavage, with only 10% of individuals bearing a new or continuing infection at the end of the 90-day observation period.

Table 1. Preop/Demographic Summary

Variable	Outcome	Summary
Subject Age		68.7 +/- 7.8 [44] (52.0, 68.5, 87.0) 95% C.I. (66.3, 71.0)
Body Mass Index (kg/(m*m))		34.1 +/- 7.7 [45] (23.0, 33.0, 52.0) 95% C.I. (31.8, 36.5)
Days from PJI Diagnosis to Surgery		24.2 +/- 49.7 [44] (0.0, 6.0, 233.0) 95% C.I. (9.1, 39.3)
Blood Test	CRP	88.9% (40/45)
	ESR	77.8% (35/45)
	Albumin/Total Leukocyte Count	33.3% (15/45)
Other Lab Testing	Joint/Synovial Aspiration	84.4% (38/45)
	Leukocyte Esterase Strip	4.4% (2/45)
	Synovial Culture	33.3% (15/45)
	Synovasure	33.3% (15/45)
Antibiotics for Current PJI (Y/N)	Yes	53.3% (24/45)
	No	46.7% (21/45)

Table 2. Operative Information Summary

Variable	Outcome	Summary
Operative Procedure	I And D	40.0% (16/40)
	Stage 1	60.0% (24/40)
ASA Classification	2	20.0% (8/40)
	3	65.0% (26/40)
	4	15.0% (6/40)
Pre-Lavage: Cell Count		26705.8 +/- 29948.1 [39] (0.0, 14500.0, 105000.0) 95% C.I. (16997.8, 36413.9)
Post-Lavage: Cell Count		223.8 +/- 348.0 [36] (0.0, 87.5, 1417.0) 95% C.I. (106.1, 341.5)
Antibiotics at Discharge (Y/N)	Yes	95.0% (38/40)
	No	5.0% (2/40)
Length of Stay		9.0 +/- 6.4 [40] (3.0, 7.0, 35.0) 95% C.I. (7.0, 11.1)

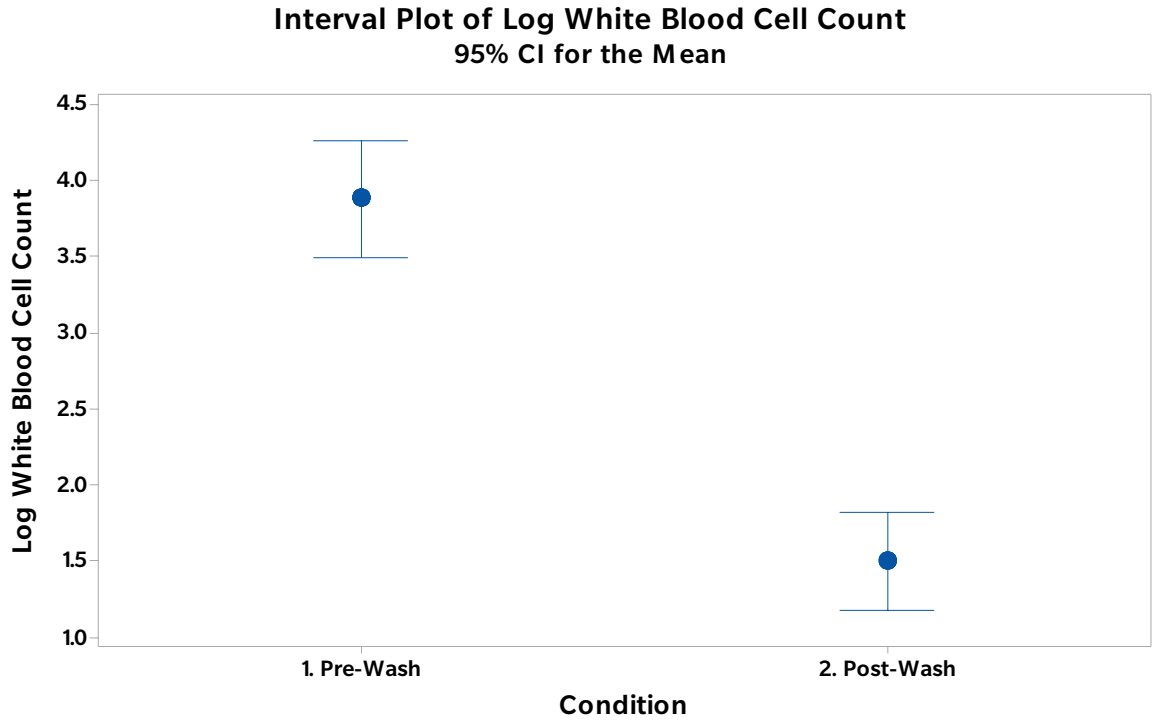

Figure 1. White Blood Cell Counts for Pre - and Post -Wash Samples

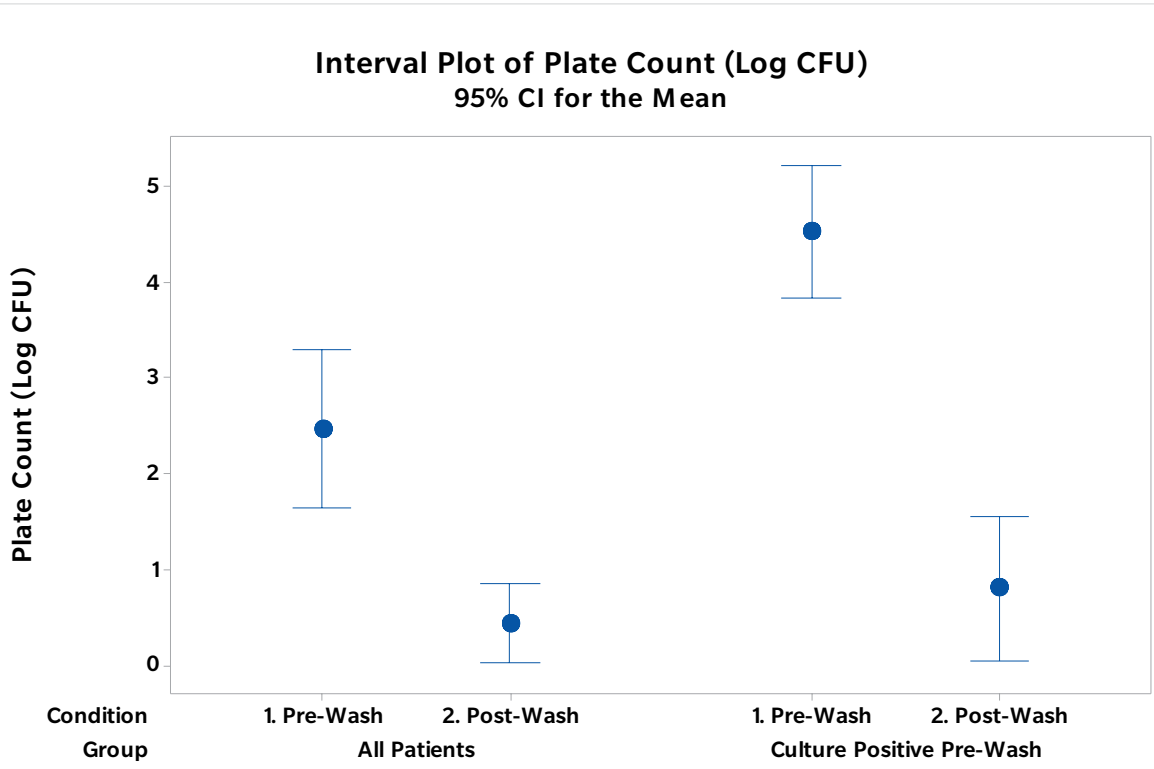

Figure 2. Plate Counts Values for Pre - and Post -Wash Samples

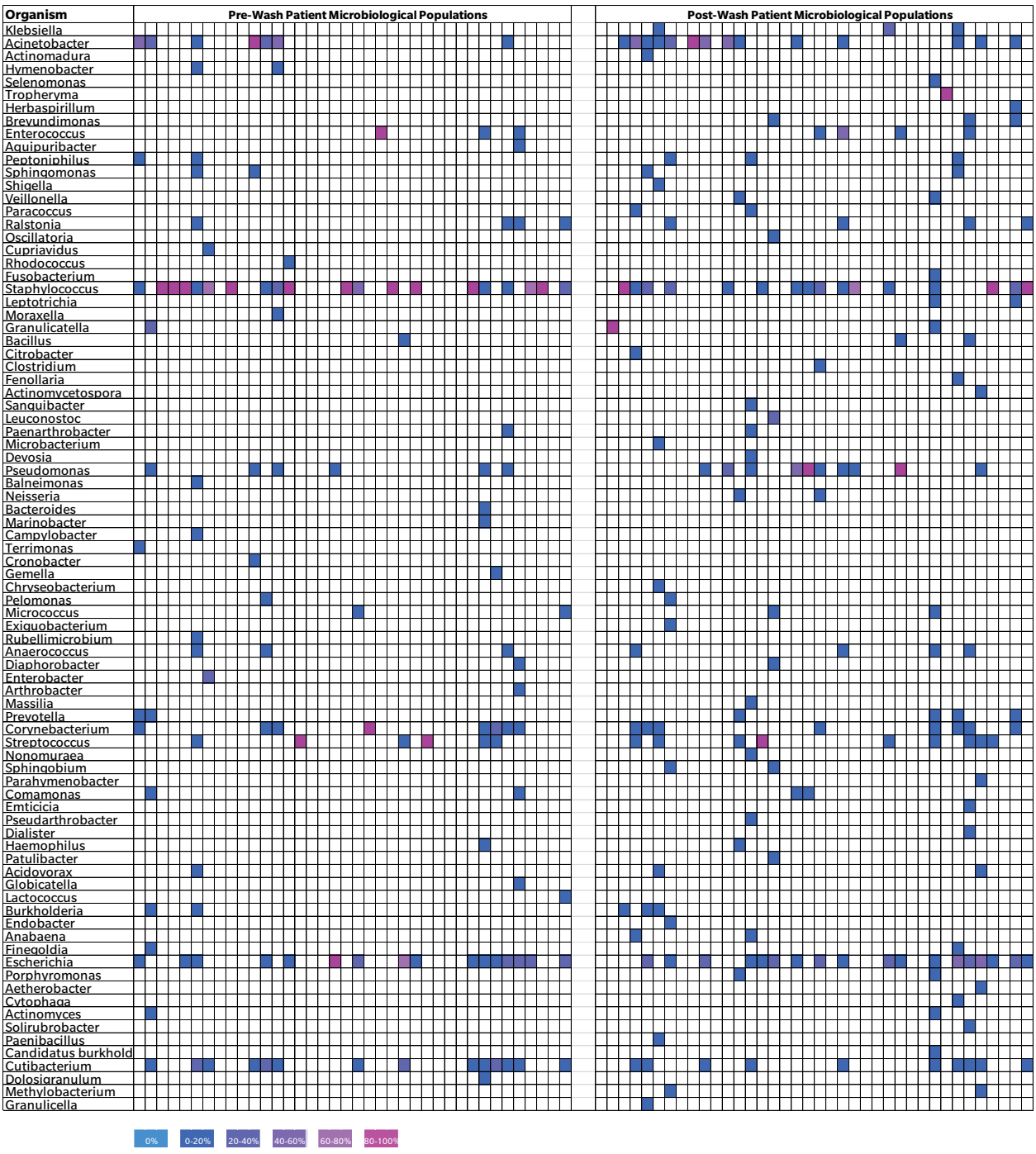

Figure 3. Heat Maps of Genus identification for Pre - and Post -Wash Samples

Table 3. 90 Day FollowUp/Completion Summary

Variable	Outcome	Summary
Does Joint Appear Infected?	Yes	10.0% (4/40)
	No	67.5% (27/40)
	Unable To Assess	22.5% (9/40)
Hospital Re-Admission	Yes	63.2% (24/38)
	No	36.8% (14/38)
Study Completion	Completed Study According To Protocol	81.4% (35/43)
	Lost To Followup	9.3% (4/43)
	Other	9.3% (4/43)

Table 4. Characteristics of subjects with new or continuing infection at 90 days.

Subject	Initial surgery	Initial ASA grade	90-day ASA grade	90-day pathogen
12	Stage 1	4	4	*C. albicans*
19	I & D	3	3	*P. mirabilis*
21	Stage 1	3	3	*P. bivia*
40	I & D	2	No data	No data

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