

Supplemental Analyses

Further description of the data set used

156 species, representing 3 domains, 8 phyla, 12 classes, 22 orders, 39 families, and 58 genera, were identified among the specimens included in the analysis. The proportion of foot infection specimens with the 25 most prevalent specimens is shown in Table 1. The full list of all the various identifications made and their respective frequencies is provided in Supplemental Table A.

The total number of specimens at each body site meeting inclusion and exclusion criteria were as follows:

Supplemental Table 1: Source and number of specimens included in the analysis.

Specimen Source Location ^a	Unique Specimens
other soft tissue or fluid	2,527
foot soft tissue or fluid	440
foot bone	287
leg soft tissue or fluid	175
other bone	144
sacral bone	82
leg bone	37
sacral soft tissue or fluid	22
spine bone	19

^aOther refers to specimens obtained from soft tissue or bone locations other than the foot, leg, sacrum, or spine

The median age of all patients was 68 (25-75% interquartile range of 61 to 74). Patients from whom foot infection specimens were obtained were older, with a median of 70 and 25-75% interquartile range of 64 to 75

Justification for analyzing foot bone specimens together with foot soft tissue and fluid specimens

The proportion of specimens with polymicrobial results are similar in foot bone versus foot soft tissue or fluid specimens.

Supplemental Table 2: Number and proportion of soft tissue and bone specimens from the foot yielding polymicrobial results, foot soft tissue or fluid vs. foot bone.

	foot bone (N=287)	foot soft tissue or fluid (N=440)	p value
Polymicrobial			0.682
No	69 (24.0%)	100 (22.7%)	
Yes	218 (76.0%)	340 (77.3%)	

The number of isolates per polymicrobial specimen is comparable in soft tissue or fluid versus bone of the foot:

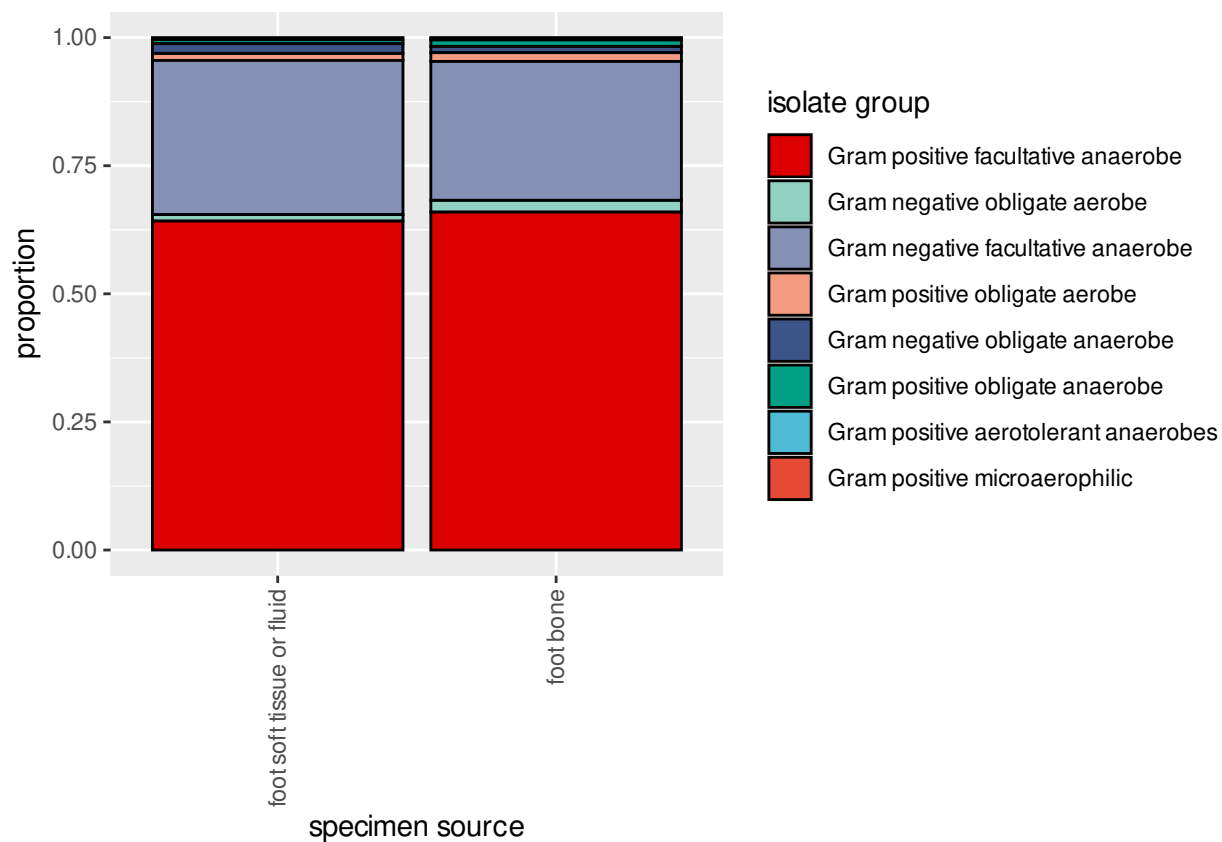
Supplemental Table 3: Number soft tissue and bone specimens from the foot with two or more species isolated.

	foot bone (N=218)	foot soft tissue or fluid (N=340)	p value
Number of Species Isolated			0.551
2	107 (49.1%)	137 (40.3%)	
3	64 (29.4%)	120 (35.3%)	
4	31 (14.2%)	55 (16.2%)	
5	11 (5.0%)	19 (5.6%)	
6	4 (1.8%)	7 (2.1%)	
7	1 (0.5%)	1 (0.3%)	
8	0 (0.0%)	1 (0.3%)	

The number of isolates per specimen also did not differ significantly per Wilcox rank-sum comparison ($p = 0.142$).

The distribution of organisms by Gram staining and metabolism was also similar in soft tissue and fluid versus bone specimens of the foot:

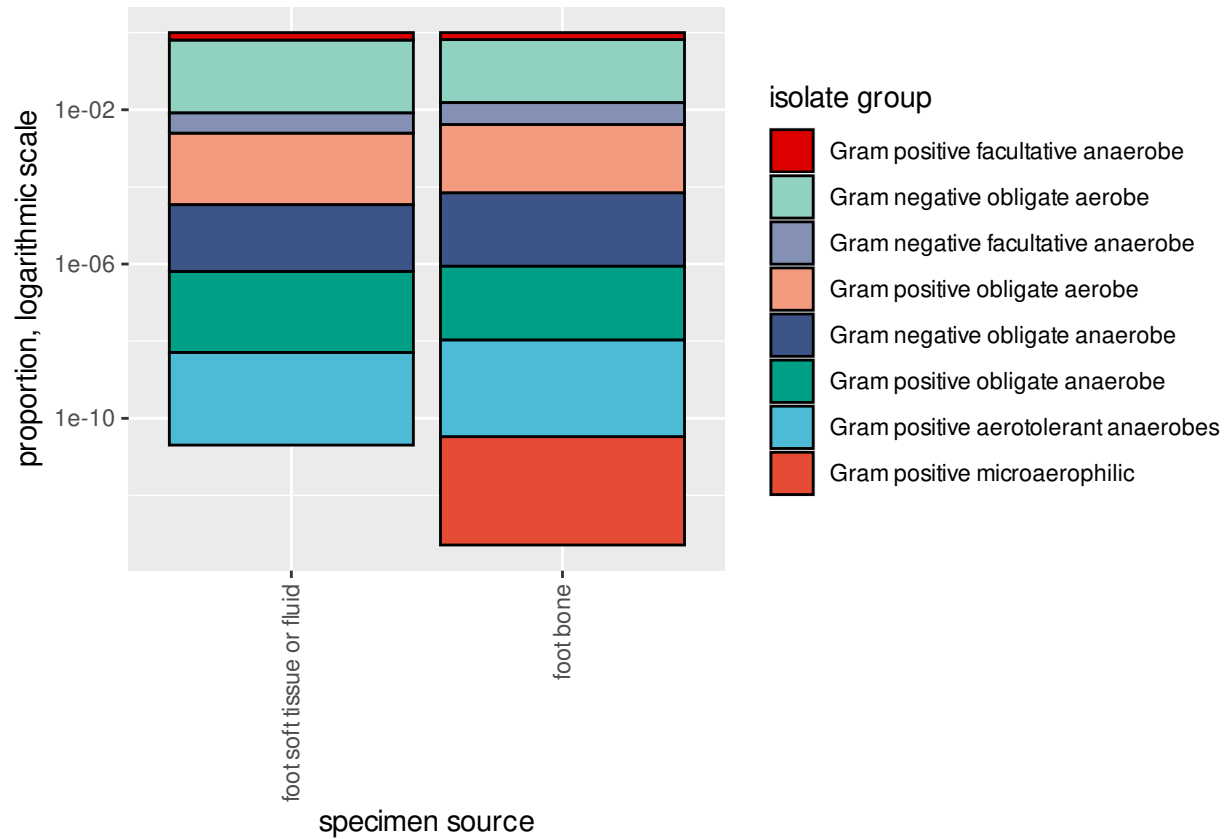
Supplemental Figure 1: Proportion of isolate groups, foot soft tissue or fluid vs. foot bone; lin-



ear scale.

The same data shown on a logarithmic scale more clearly shows rarer groups:

Supplemental Figure 2: Proportion of isolate groups, foot soft tissue or fluid vs. foot bone; loga-



rithmic scale.

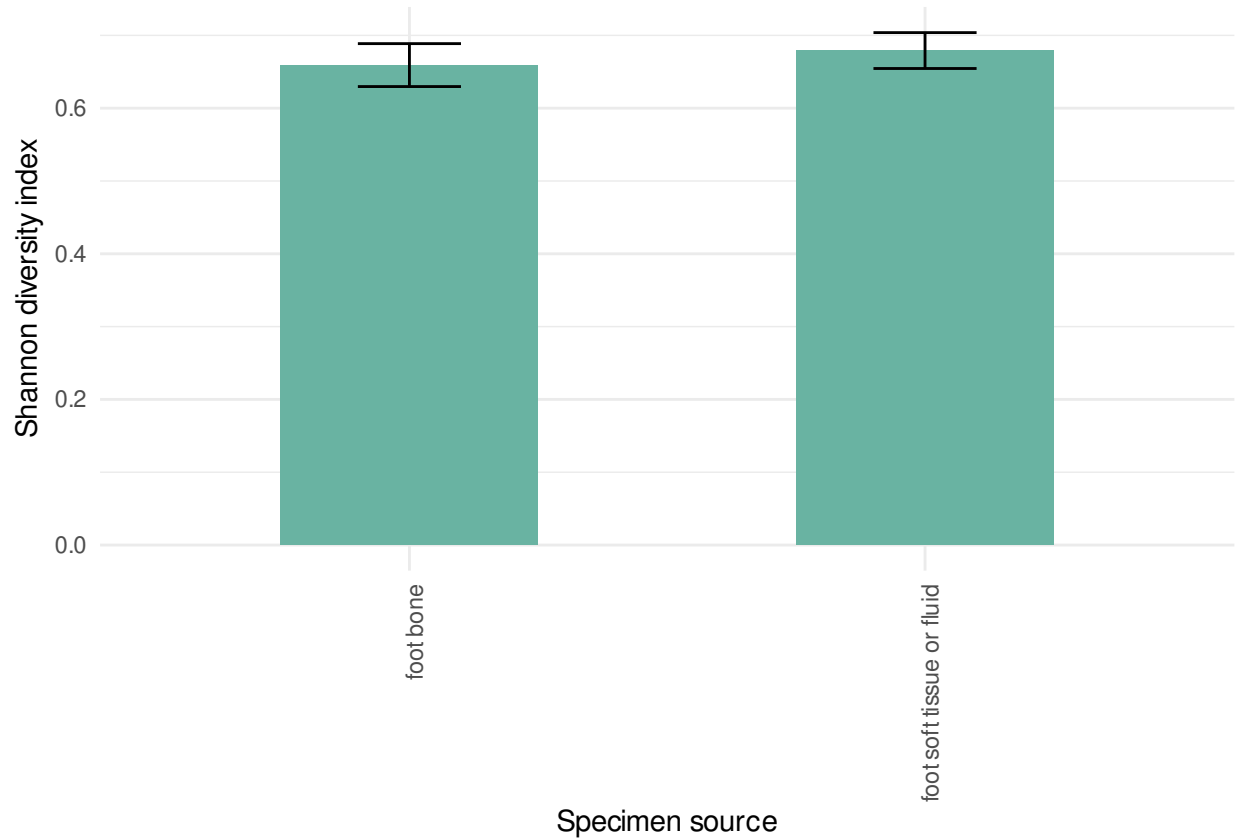
These are the same data shown in tabular format:

Supplemental Table 4: Number and proportion of isolates with various Gram staining patterns and metabolic categories, foot soft tissue or fluid vs. foot bone.

	foot bone (N=646)	foot soft tissue or fluid (N=1011)	p value
Gram Staining and Metabolism Category			0.367
Gram negative facultative anaerobe	175 (27.1%)	304 (30.1%)	
Gram negative obligate aerobe	15 (2.3%)	13 (1.3%)	
Gram negative obligate anaerobe	8 (1.2%)	19 (1.9%)	
Gram positive aerotolerant anaerobes	2 (0.3%)	4 (0.4%)	
Gram positive facultative anaerobe	426 (65.9%)	649 (64.2%)	
Gram positive microaerophilic	1 (0.2%)	0 (0.0%)	
Gram positive obligate aerobe	11 (1.7%)	14 (1.4%)	
Gram positive obligate anaerobe	8 (1.2%)	8 (0.8%)	

The Shannon diversity index was comparable for soft tissue and fluid versus bone specimens from the foot:

Supplemental Figure 3: Shannon diversity index for foot soft tissue or fluid vs. foot bone.



An analysis of variance comparison showed no significant differences in the Shannon diversity index for foot soft tissue or fluid versus foot bone (p-value of 0.606).

Finally, no significant differences were found in the prevalence of specific organisms of interest in soft tissue or fluid versus bone specimens of the foot:

Supplemental Table 5: Relative Frequency of Species of Interest, Foot Soft Tissue or Fluid vs. Foot Bone.

	foot bone (N=295)	foot soft tissue or fluid (N=463)	p value
Genus ±Species			0.663
Candida	9 (3.1%)	18 (3.9%)	
Corynebacterium	67 (22.7%)	85 (18.4%)	
Escherichia coli	28 (9.5%)	55 (11.9%)	
Pseudomonas aeruginosa	28 (9.5%)	43 (9.3%)	
Staphylococcus aureus	110 (37.3%)	182 (39.3%)	
Staphylococcus, coagulase-negative species	53 (18.0%)	80 (17.3%)	

Justification for analyzing foot specimens separate from specimens from other locations

Specimens from other locations (including sacrum, leg, spine, and other soft tissue and bony sites) differed from foot specimens in many ways. First, the proportion of specimens with polymicrobial results was much lower in non-foot specimens compared to foot specimens:

Supplemental Table 6: Number and proportion of soft tissue and bone specimens from the foot yielding polymicrobial results, foot vs. other sites.

	foot (N=727)	non-foot (N=3006)	p value
Polymicrobial			< 0.001
No	169 (23.2%)	1671 (55.6%)	
Yes	558 (76.8%)	1335 (44.4%)	

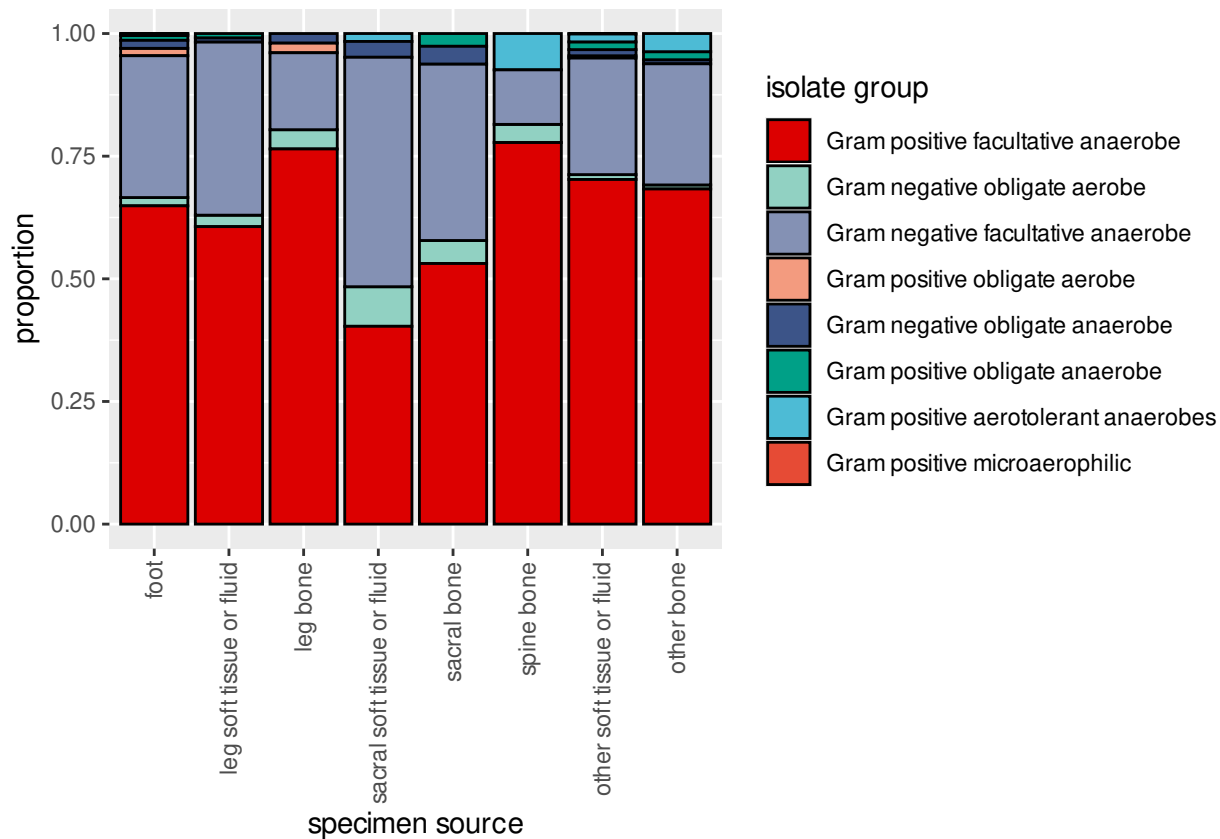
Among specimens with polymicrobial results, more isolates were found in foot specimens than in non-foot specimens:

Supplemental Table 7: Number of specimens from foot vs. other sites with two or more species isolated.

	foot (N=558)	non-foot (N=1335)	p value
Number of Species Isolated			0.026
2	244 (43.7%)	680 (50.9%)	
3	184 (33.0%)	399 (29.9%)	
4	86 (15.4%)	189 (14.2%)	
5	30 (5.4%)	50 (3.7%)	
6	11 (2.0%)	16 (1.2%)	
7	2 (0.4%)	1 (0.1%)	
8	1 (0.2%)	0 (0.0%)	

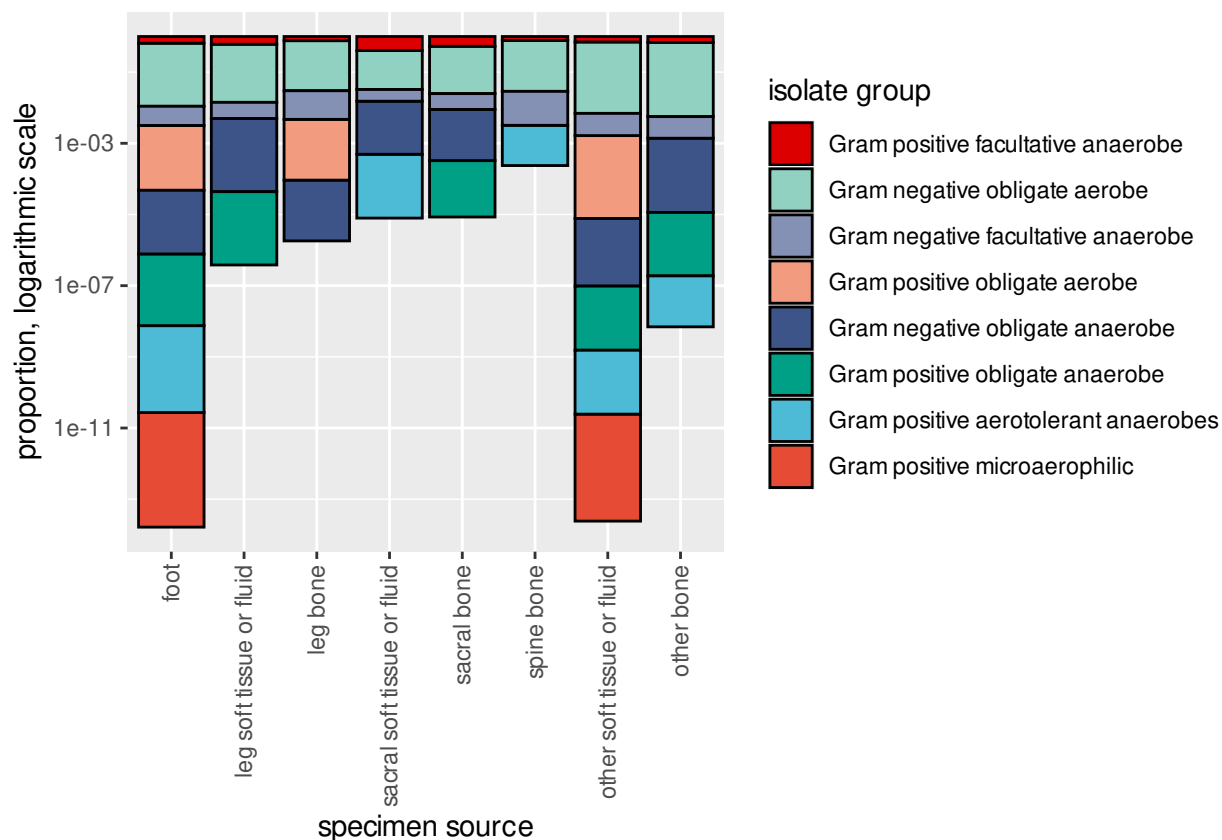
The proportion of Gram negative facultative anaerobes was higher in the foot and sacrum compared to other locations. Most other isolate groups were found in relatively similar order and proportion across sites with some exceptions. Gram positive obligate aerobes were not seen in the sacrum, and neither Gram positive or Gram negative obligate aerobes were seen in the spine. Microaerophilic organisms were rare and seen in foot bone and in non-foot soft tissue only.

Supplemental Figure 4: Proportion of isolate groups, foot vs. other specimen locations, linear



scale.

Supplemental Figure 5: Proportion of isolate groups, foot vs. other specimen locations; loga-



rithmic scale.

Supplemental Table 8: Number and Proportion of Isolates with Various Gram Staining Patterns and Metabolic Categories, Foot vs. Other Locations.

	foot (N=165)	leg bone (N=51)	leg soft tissue or fluid (N=343)	other bone (N=243)	other soft tissue or fluid (N=4054)	sacral bone (N=192)	sacral soft tissue or fluid (N=62)	spine bone (N=27)	p value
Gram Staining and Metabolism Category									< 0.001
Gram negative facultative anaerobe	479 (28.9%)	8 (15.7%)	121 (35.3%)	60 (24.7%)	963 (23.8%)	69 (35.9%)	29 (46.8%)	3 (11.1%)	
Gram negative obligate aerobe	28 (1.7%)	2 (3.9%)	8 (2.3%)	2 (0.8%)	40 (1.0%)	9 (4.7%)	5 (8.1%)	1 (3.7%)	
Gram negative obligate anaerobe	27 (1.6%)	1 (2.0%)	3 (0.9%)	2 (0.8%)	51 (1.3%)	7 (3.6%)	2 (3.2%)	0 (0.0%)	
Gram positive aerotolerant anaerobes	6 (0.4%)	0 (0.0%)	0 (0.0%)	9 (3.7%)	65 (1.6%)	0 (0.0%)	1 (1.6%)	2 (7.4%)	

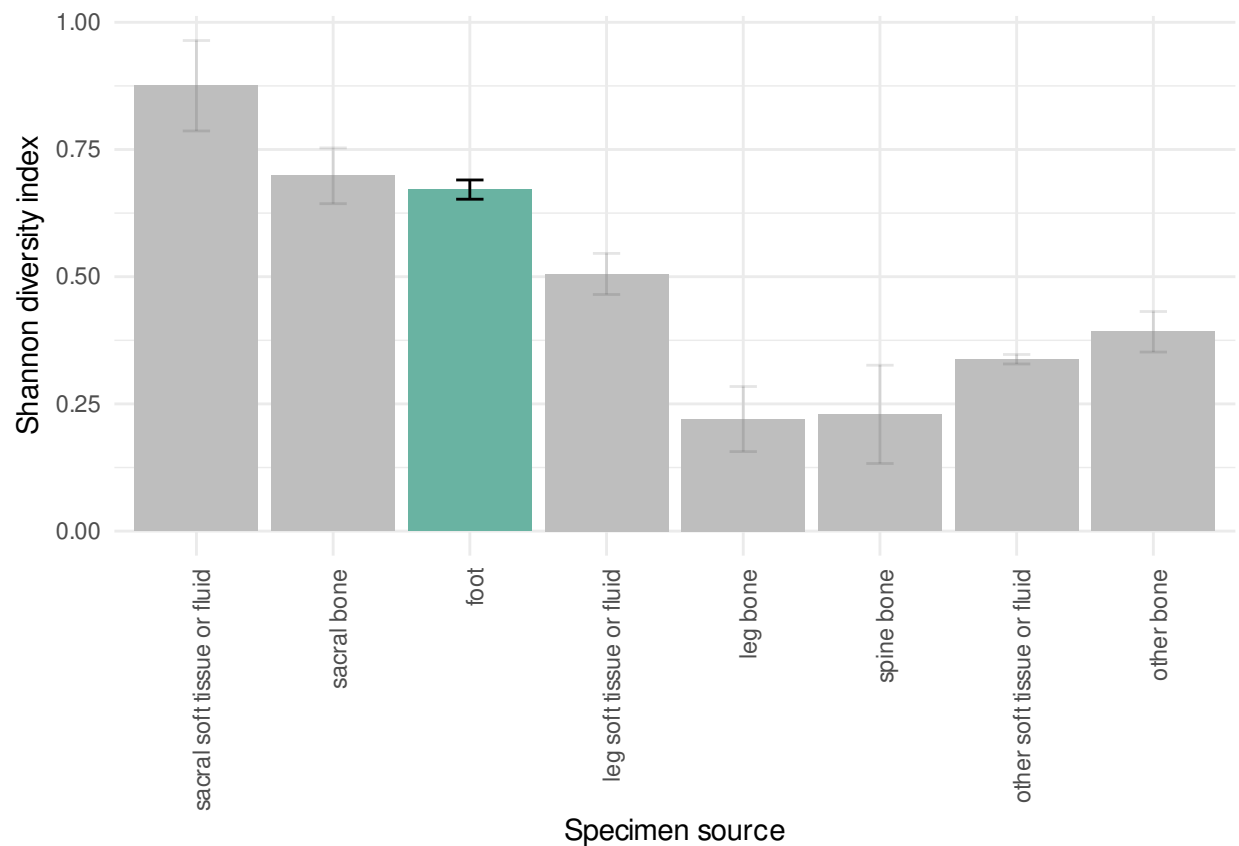
	leg		leg soft	other bone	other soft		sacral	sacral soft	spine	p value
	foot (N=165)	bone (N=51)	tissue or fluid (N=343)		tissue or fluid (N=405)	bone (N=192)	tissue or fluid (N=62)	bone (N=27)		
Gram positive facultative anaerobe	1075 (64.9%)	39 (76.5%)	208 (60.6%)	166 (68.3%)	2848 (70.3%)	102 (53.1%)	25 (40.3%)	21 (77.8%)		
Gram positive microaerophilic	1 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.1%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Gram positive obligate aerobe	25 (1.5%)	1 (2.0%)	0 (0.0%)	0 (0.0%)	19 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
Gram positive obligate anaerobe	16 (1.0%)	0 (0.0%)	3 (0.9%)	4 (1.6%)	64 (1.6%)	5 (2.6%)	0 (0.0%)	0 (0.0%)		

The frequency of species of interest differed significantly among the various sites (Table 2). *Escherichia coli* was found more frequently in sacral samples. The incidence of *Staphylococcus aureus* was highest in the leg and in soft tissue specimens. *Corynebacterium* was found most frequently in bone of the foot, the spine, and the sacrum. The leg and sacrum had high relative rates of *Pseudomonas aeruginosa*.

Supplemental Table 9: Relative Frequency of Species of Interest, Foot vs. Other Locations.

	leg		leg soft	other bone	other soft		sacral	sacral soft	spine
	foot	bone	tissue or fluid		tissue or fluid	bone	tissue or fluid	bone	bone
	(N=758)	(N=35)	(N=187)	(N=112)	(N=2440)	(N=90)	(N=18)	(N=15)	
Genus ±Species									
<i>Candida</i>	27 (3.6%)	1 (2.9%)	1 (0.5%)	6 (5.4%)	95 (3.9%)	1 (1.1%)	1 (5.6%)	0 (0.0%)	
<i>Corynebacterium</i>	152 (20.1%)	5 (14.3%)	27 (14.4%)	19 (17.0%)	271 (11.1%)	18 (20.0%)	1 (5.6%)	3 (20.0%)	
<i>Escherichia coli</i>	83 (10.9%)	1 (2.9%)	14 (7.5%)	8 (7.1%)	186 (7.6%)	19 (21.1%)	6 (33.3%)	1 (6.7%)	
<i>Pseudomonas aeruginosa</i>	71 (9.4%)	3 (8.6%)	29 (15.5%)	7 (6.2%)	214 (8.8%)	19 (21.1%)	6 (33.3%)	2 (13.3%)	
<i>Staphylococcus aureus</i>	292 (38.5%)	20 (57.1%)	88 (47.1%)	50 (44.6%)	1228 (50.3%)	27 (30.0%)	3 (16.7%)	8 (53.3%)	
<i>Staphylococcus, coagulase-negative species</i>	133 (17.5%)	5 (14.3%)	28 (15.0%)	22 (19.6%)	446 (18.3%)	6 (6.7%)	1 (5.6%)	1 (6.7%)	

***Figure 6: Shannon diversity index for foot specimens vs. specimens from other locations.**



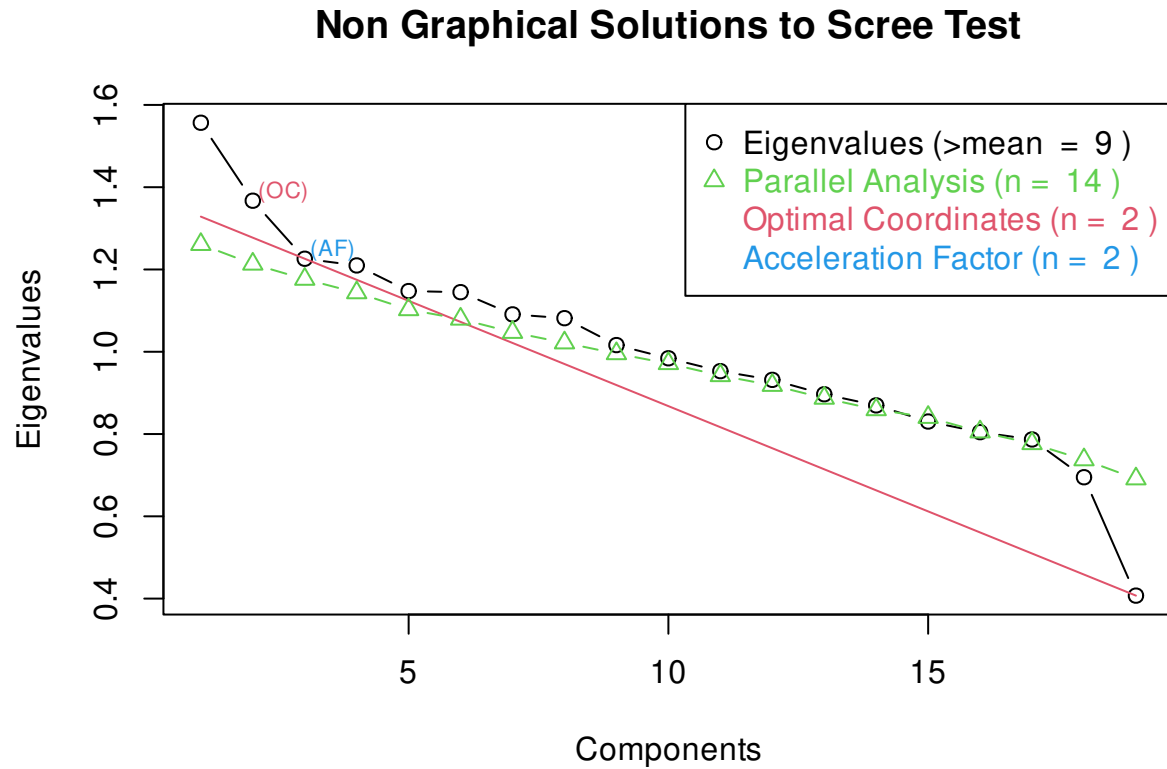
Similarly, significant differences in the Shannon diversity index ($p\text{-value} < 0.00001$) suggested the highest diversity in sacral soft tissue and bone specimens, followed – in order – by foot bone and soft tissue or fluid specimens, leg soft tissue or fluid specimens, spine soft tissue or fluid specimens, and leg bone specimens.

Patterning in Foot Specimens

Please see the separate comma separated data file for the full results of the conditional random fields (CRF) analysis.

A factor analysis was done to identify clusters or patterns of organisms. A Scree test suggested the optimal number of factors was two:

Supplemental Figure 7: Scree plot to identify the optimal number of factors in a factor analysis.

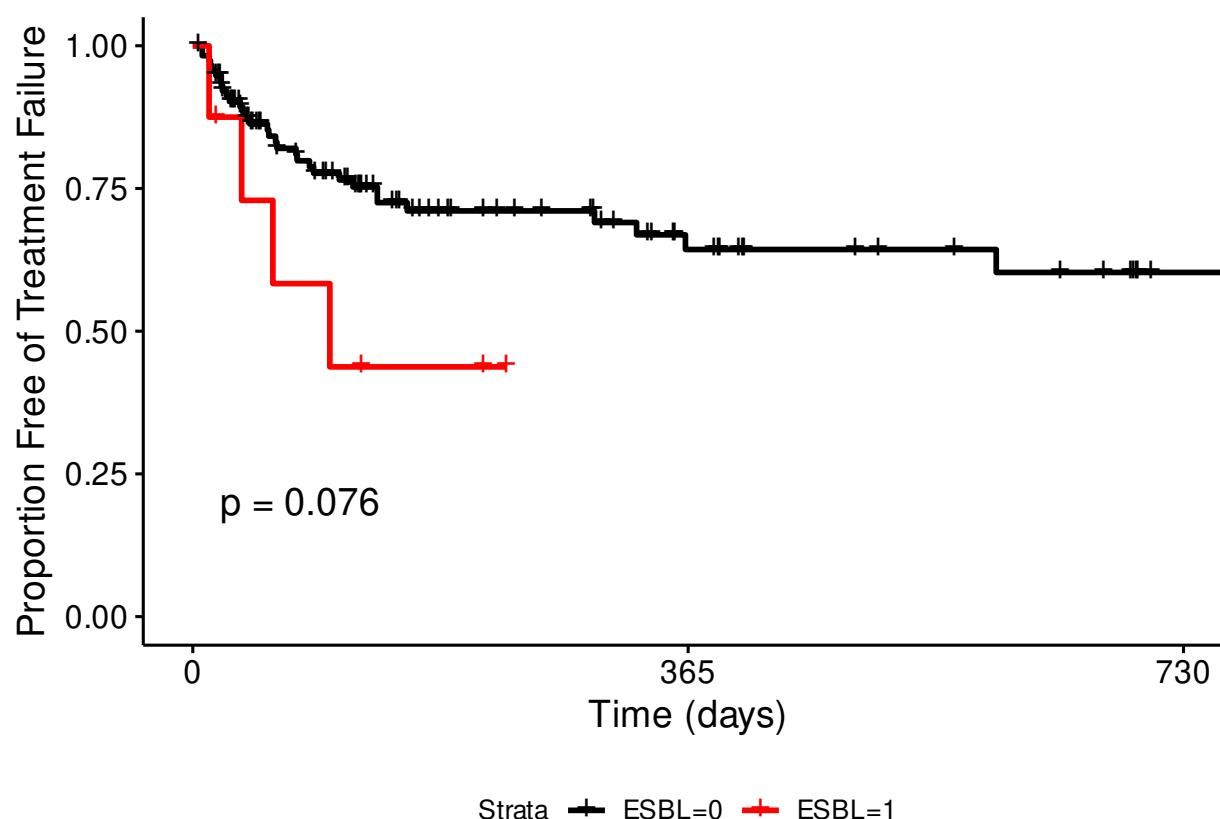


Supplemental Table 10: Factor analysis results.

Bacteria Genus	Pattern A	Pattern B	Communality
Bacteroides	-0.122	-0.186	0.042
Candida	-0.061	0.011	0.004
Citrobacter	-0.003	0.077	0.006
Corynebacterium	-0.057	-0.148	0.022
Enterobacter	-0.113	0.210	0.065
Enterococcus fecalis	-0.021	0.324	0.108
Enterococcus, species not specified	-0.090	0.112	0.024
Escherichia coli	-0.052	0.161	0.031
Klebsiella	0.009	0.262	0.068
Morganella	0.044	0.138	0.019
Proteus	0.014	0.273	0.074
Pseudomonas aeruginosa	-0.064	0.053	0.008
Staphylococcus aureus	1.004	-0.002	0.995

Staphylococcus epidermidis	-0.112	-0.069	0.015
Staphylococcus, coagulase-negative species	-0.263	-0.092	0.069
Stenotrophomonas	-0.130	0.081	0.027
Streptococcus, alpha-hemolytic, species not specified	0.005	0.198	0.039
Streptococcus, group B, species not specified	0.049	-0.104	0.015
Streptococcus, species not specified	-0.028	-0.109	0.012

Supplemental Figure 8: Time to treatment failure in patients with ESBL-producing organisms.



Supplemental Table 11: Multivariate time-to-treatment failure model that included ESBL production

Characteristic	HR	95% CI	p-value	HR	95% CI	p-value
severe or unaddressed peripheral artery disease	9.90	3.72, 26.3	<0.001	9.69	3.61, 26.0	<0.001
insulin-dependent diabetes	4.09	1.55, 10.8	0.004	4.46	1.58, 12.6	0.005
serum albumin <2.8 gm/dL	4.59	1.93, 10.9	<0.001	4.49	1.88, 10.7	<0.001
homeless	5.72	1.68, 19.5	0.005	5.82	1.70, 19.9	0.005
first toe (hallux) location	1.59	0.64, 3.93	0.3	1.57	0.64, 3.88	0.3

Characteristic	HR	95% CI	p-value	HR	95% CI	p-value
first metatarsal location	0.59	0.17, 2.06	0.4	0.62	0.18, 2.17	0.5
fifth toe location	0.38	0.10, 1.48	0.2	0.38	0.10, 1.49	0.2
fifth metatarsal location	0.98	0.34, 2.83	>0.9	0.94	0.32, 2.73	>0.9
uncovered7	3.09	1.25, 7.64	0.014	2.92	1.16, 7.36	0.023
Staphylococcus aureus	0.47	0.16, 1.33	0.2	0.49	0.17, 1.40	0.2
Pseudomonas aeruginosa	8.42	2.58, 27.5	<0.001	8.16	2.46, 27.1	<0.001
Escherichia coli	2.12	0.72, 6.21	0.2	2.14	0.73, 6.28	0.2
ESBL				1.48	0.34, 6.41	0.6